Unveiling causes for growth retardation in piglets

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Unveiling causes for growth retardation in piglets

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Thesis

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Never forget where you have been. Never lose sight of where you are going and never take for granted the people who travel the journey with you (Susan Gale)

To Carlotía

ABSTRACT

The evolution of hyper-prolific sow breeds has led to a higher number of piglets born per sow per year. This increase in litter size has enlarged the number of light weight (or growth retarded) piglets, increased pre-weaning mortality and heterogeneity at the end of the nursery phase (ten weeks of age). These poorly performing piglets represent a challenge to the swine industry as their presence in the herd has economic and welfare implications. Reducing the heterogeneity at the end of the nursery phase is relevant, as it influences the efficiency of use of the grower and finisher facilities, and/or it reduces penalties for delivering underweight piglets to the slaughterhouse. The focus of this thesis was the end of the nursery phase, as this is the time point where piglets are transferred to the grower and finisher facilities.

The aim of this thesis was to identify and describe the causes of growth retardation in the nursery phase to provide a basis to look for alternative nutrition or management solutions.

The database analysis described in **Chapter 2** provides a phenotypic definition of growth retardation based on the risk factor analysis approach, and describes season of birth, body weight at birth, at weaning and at six weeks of age as the main factors to predict piglet BW at the end of the nursery phase.

Based on the algorithm developed to predict piglets' BW at the end of the nursery phase, our target population was defined as piglets with a birth weight above the mean -2 times the SD from the total population and a predicted BW at the end of the nursery phase below the mean -1 time the SD from the mean of the total population, considered Low Performing piglets **LP**). We aimed to characterize differences between LP piglets and their heavier counterpart (piglets with a predicted BW at the end of the nursery phase above the mean +1 time the SD from the mean of the total population **HP**).

Compared to the HP, the LP piglets grew slower, ate less and were lighter but have an equal gain:feed ratio at ten weeks of age. The LP piglets tended to take more time to touch a novel object and spent more time eating. The LP and HP piglets have an equal macronutrient digestibility, with the exception of NSP. When fed a high fibre diet, both groups have a lower starch and fat apparent total tract digestibility. Yet, the LP piglets have a reduced fermentative capacity, which might place them in disadvantage. Also LP piglets are unable to engage into compensatory gain or compensatory feed intake, as efficiency of nutrient utilization and feed intake per kg $BW^{0.75}$ was unaffected. In terms of skeletal muscle development, the LP piglets have a low muscularity (total fiber number and fiber cross sectional area), which might be of disadvantage for lean mass accretion in further life and for meat quality. The LP piglets exhibit insulin resistance and a lower pancreatic amylase activity, which might be related to the lower performance. Lastly, in the general discussion a comparison of the main findings with literature characterizing growth retarded piglets is presented. Also based on modelled data, the economic impact of growth retarded piglets is estimated from 10 weeks of age until slaughter weight (110 kg BW).

The studies reported in this thesis describe a novel method for selecting growth retarded piglets in the nursery phase and provide insight into possible mechanism for growth retardation in the piglet phase.

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Chapter 1

General Introduction

PROBLEM DESCRIPTION

In order to improve production efficiency, the pig farming sector has enlarged sow prolificacy, aiming to increase the number of slaughtered pigs produced per sow per year (Rutherford *et al*., 2013). Yet, sow hyper-prolificacy has also led to an increase in the within-litter variation in birth weight (Canario *et al*., 2003; Quiniou *et al*., 2002) and a larger number of small sized piglets entering the nursery (Foxcroft *et al*., 2004). In current European pig production one of five piglets born dies before weaning (Wientjes *et al*., 2013). In the Netherlands for the year 2011, from a litter of 14.8 total born piglets 1.1 piglets (~ 7.5%) were born dead; and from 13.7 piglets born alive, 1.8 piglets died before weaning (Wientjes *et al.*, 2013).

1. IUGR = intrauterine growth retarded < -2 SD from the mean of the total population (Birth weight: 0.59 ± 0.1). **2**. Light > -2 SD but < -1 SD from the mean of the total population (Birth weight: 0.93 ± 0.1). **3**. Average > -1 SD and < 1 SD (Birth weight: 1.45 ± 0.2) **4**. Heavy > 1 SD from the mean of the total population (Birth weight: 1.99 ± 0.2) **5**. Heaviest > 2 SD from the mean (Birth weight: 2.39 ± 0.2)

Figure 1.1 Population distribution at birth (n = 20,156 individual records from SRC 2005 - 2010)

According to data of Quiniou *et al*. (2002) small piglets with a birth weight below 1 kg BW represent on average 13% of the total number of born piglets, ranging from 7% in litters with 11 piglets or less to 23% in litters with 16 piglets or more. When analysing an experimental farm dataset from 2005 - 2010 (n = 20,156 individual records; SRC 2013, unpublished[1]). We observed that in this farm, with an average

[1] Nutreco Swine Research Centre, Sint Anthonis, the Netherlands

litter size of 13.6 total born and 12.1 piglets born alive, 10% of the population was born with a low BW (birth weight < 1 kg; Fig. 1.1).

Determining the economic impact of growth retardation, which can result from low birth weight, is not simple since it will vary by farm and country, and it involves many factors that can fluctuate (e.g. cost of facilities, personnel, and cost of feed, market price and demand). After conducting a small survey with swine specialists from Trouw Nutrition International, Nutreco Canada and Trouw Nutrition Mexico[2] in Europe and North America, we could deduce that most piglets in Europe are sold or transferred to the grower and finisher facilities at 8 to 10 weeks of age on average. All specialists agreed that weight was more relevant than age at the time of delivering the piglets either to the slaughter house or grower and finisher facilities. They described that the percentage of piglets that do not reach the required weight at 10 weeks of age varies depending on region and farming conditions. The specialists estimated that the percentage of piglets with growth check ranges between 5 and 20%, with most of them reporting 10%. A global estimate gave a range from 5 to 25% (B. van Gils, personal communication (2012)). In some countries it is common practice to impose a penalty when the piglets are below the required weight at delivery to the fattening unit. In Italy piglets < 15 kg BW are not paid. In Spain there is a deduction on the price paid of 0.75 € per kg below the required weight. Countries like Belgium pay an extra bonus to the farmers when piglets weigh more than the required 23 kg BW or because they deliver a more homogeneous batch to the grower and finisher facilities.

Most literature which reports the effect of within-litter variation on performance assumes that all small piglets belong to the same group, without discriminating within this population (Nissen *et al*., 2004; Quiniou *et al*., 2002; Rehfeldt and Kuhn, 2006). On the other hand, after removing the piglets with a birth weight below 0.75 kg BW from the dataset, Beaulieu *et al*. (2010) found that an increase in litter size resulted in a

[2] Trouw Nutrition International, Putten, the Netherlands; Nutreco Canada, Quebec, Canada; Trouw Nutrition Mexico, Nuevo Leon, Mexico

reduced average birth weight, with no changes in days to market or carcass composition.

According to McMillen *et al*. (2001), the lowest birth weight piglets (below the mean minus two times the SD from the mean of the total population) are considered to be intrauterine growth retarded (**IUGR**). Intrauterine growth retardation is defined as impaired growth and development of the embryo/foetus or its organs during pregnancy (Wu *et al*., 2006). Foetal growth retardation limits neonatal survival and has a 'stunting' effect on postnatal growth resulting from nutrient deprivation (Foxcroft *et al*., 2004). Foetal growth retardation is a result of utero-placental blood flow reduction per foetus which accompanies an increase of litter size (Père and Etienne, 2000) and results in lowered maternal nutrition during gestation (Campos *et al*., 2012).

When browsing literature about growth retardation in piglets, we found two different definitions, IUGR and low birth weight piglets. After a thorough evaluation of most papers, we could deduce that despite the difference in terminology, most authors referred to the same populations (piglets < 1 kg BW at birth). For this reason, in this chapter we refer to them as IUGR piglets. The mechanisms affecting these IUGR piglets are described in Table 1.1.

Table 1.1 Phenotypic characteristics of IUGR piglets (and metabolic changes in humans) that may explain their lower than average gain in postnatal life

Target	Age when measured	Effect	Reference
Organ development	Gestational phase	Brain vulnerable to undernutrition 5 to 10 weeks before farrowing.	Widdowson, 1971
	113-115 days post-conception	Reduced tissue weight in the gastrointestinal tract due to lower cell number.	Xu *et al*., 1994
	14 and 28 days of age	Relative to live weight, the size of stomach, small intestine, caecum and colon are greater for light piglets.	Pluske *et al*., 2003
	Gestational phase	Delayed follicular development in pig ovaries at birth.	Da Silva *et al*., 2003
	Gestational phase	Reprogramming by day 27 to 35 of gestation. Reduction in conceptus by day 50 with compromised placental development of the surviving foetuses.	Foxcroft *et al*., 2006
	5 days of age	Defect in testicular development, germ and somatic cell population.	Smit *et al*., 2013
Small intestine (SI)	Birth	Abnormal gastrointestinal morphology and dysfunction (e.g. necrotizing enterocolitis).	Thornbury, 1993
	14 and 28 days of age	Equal lactase and sucrase activity.	Pluske *et al*., 2003
	Gestational phase	Impaired intestinal	Wu *et al*., 2004

		function and arginine synthesis.	
	1, 7 and 21 days of age	Reduced cellular signalling, redox balance, protein synthesis and proteolysis affecting SI protein expression. Marked alterations of jejunal proteome at birth.	Wang *et al*., 2010
	18 and 28 days post-weaning	Lower SI weight:length ratio due to a thinner tela submucosa and tunica muscularis and a higher secretory capacity, both in the distal jejunum. Gut maturation post-weaning is retarded but unrelated to weaning transition.	Michiels *et al*., 2012
Pancreas	14 and 28 days of age	Lower trypsin activity after weaning.	Pluske *et al*., 2003
	6 to 8 weeks of age	Dysfunction in exocrine and endocrine pancreatic function. Lower insulin and amylase concentration.	Harada *et al*., 2003
Skeletal muscle	Gestational phase	Differentiation in muscle fibers through reduction in myogenic expression.	Tse, 2005
	Market weight	Lower number of myofibers.	Rehfeldt and Kuhn, 2006
	68 days of age	Lower tenderness due to enlarged myofibers.	Gondret *et al*., 2006
	Market weight	Smaller *Longissimus dorsi.*	Rehfeldt *et al*., 2008

	Birth, 21 and 67 days of age	Lower birth weight is associated with lower *Semitendinosus* muscle cellularity. Lower birth weight has a negative effect on postnatal muscle growth and final muscle fiber size and meat quality.	Tristan *et al*., 2009
Carcass composition	Market weight	Fatter carcass and higher muscle lipid content at market weight.	Powell and Aberle, 1980
	68 days of age	No difference in meat drip loss but lower amount and/or activity of proteolytic enzymes (calpain and cathepsin) which affect meat tenderness.	Gondret *et al*., 2006
	Market weight	No difference in backfat depth or *Longissimus* muscle area.	Fix *et al*., 2010
	Market weight	Inter and intramuscular fat tended to be higher in low birth weight piglets.	Beaulieu *et al*., 2010
	Market weight	Equal loin and backfat depths and predicted lean content.	Wolter and Ellis, 2001
	27 days post-weaning	No differences in water, lipid, protein or ash concentration in carcass. Greater gross energy carcass content. Lower empty BW and decreased tissue deposition rate.	Jones, 2012

IGF1	0 and 2 days of age	Growth response is not related to IGF1 concentration.	Ritacco *et al.*, 1997
	Birth until 10 days of age	IGF1 infusion increase protein and fat accretion levels but no its concentration in other tissue. Mal-regulation between growth hormone and IGF1 is responsible for poor nutrient utilization.	Schoknecht *et al.*, 1993
	Birth and one day of age	Inverse correlation between IGF1 concentration and BW.	Bauer *et al.*, 1998
	Market weight	Plasma IGF1 is reduced by 24% compared to higher BW piglets.	Gondret *et al.*, 2005
	2 - 4 hours after birth	Reduced gene expression of mucosal IGF1.	Wang *et al.*, 2005
	21 days of age	Expression level of IGF1 is lower for *L. dorsi,* liver and kidney. No differences in the expression levels of IGF2, IGF1R, IGF2R and IGFBP5.	Chen *et al.*, 2011
	18 and 28 days post-weaning	Lower circulating IGF1 and lower IGF1 receptor in proximal SI.	Michiels *et al.*, 2012
Metabolic changes	Birth and adult age	Thrifty phenotype theory, Syndrome X in adulthood.	Hales and Barker, 1992
	Prepuberal	Insulin resistance for light birth weight children in prepuberal phase.	Hofman *et al.*, 1997

	3 and 12 months of age	Poor pre and postnatal growth is linked with adult obesity and altered glucose tolerance, insulin sensitivity and cardiovascular and endocrine function. Females are predisposed to higher fat deposition and males are prone to less leptin secretion.	Poore and Fowden, 2002
	Adult life	Association between slow growth before birth, accelerated growth in early postnatal life and the emergence of insulin resistance, visceral obesity and glucose resistance in adult life.	Morrison *et al*., 2010; Thorn *et al*., 2011
Behaviour	Lactation phase	Low birth weight piglets have a tendency to drink from the posterior teats. Teat suckling order differences have an effect in future performance by lower gain in later life stages.	Fraser and Jones, 1975
	Lactation phase	No correlation between birth weight and suckling teat.	Kim *et al*., 2000
	Post-weaning phase	Low birth weight piglets have more problems switching from known to unknown configurations.	Gieling *et al*., 2011
	Post-weaning phase	Light piglets tend to pay more visits to the feeder with a lower intake per visit.	Bruininx *et al*., 2001

Performance	Suckling and post-weaning phase	Reduced ADG when compared to heavier piglets.	Gondret *et al*., 2005
	Market weight	Lower than average gain.	Mahan and Lepine, 1991; Rehfeldt and Kuhn, 2006
	Market weight	Lower than average gain and longer time to reach market weight.	Wolter and Ellis, 2001
Nutrient digestibility	Post-weaning phase	Lower dry matter, gross energy, nitrogen, ash digestibility compared to average weight piglets.	Jones, 2012

This project started under the assumption that undersized piglets are not a homogeneous population. We assumed that growth retardation could occur at many different time points during the nursery phase and it could have different causes (e.g. litter of origin, size of the litter at birth, sow parity number, under nutrition in the pre-weaning or weaning phase). Yet, despite the cause, the outcome was similar, low BW at the end of the nursery phase. Even so, gaining more insight into the factors responsible for the weight difference in the nursery phase could help us understand the fate of these piglets and design the proper nutritional and management strategies to maximize the growth performance of these piglets.

Below we describe the factors related to growth retardation in the nursery phase in: **1.** In-utero and maternal effects leading to low birth weight; **2.** Factors related to the birth process. Both providing the same outcome: light piglets at birth or soon thereafter and **3.** Weaning and early post-weaning performance.

1. In-utero and maternal effects leading to low birth weight and relation to future performance

Foetal growth retardation is a result of complex interrelated processes between maternal, placental and foetal compartments. The main **maternal factors** involved are: undernutrition, sows with a low birth weight, parity, preeclampsia, diabetes and renal diseases, and the ability to provide oxygenated blood to the uterine circulation. The **placental factors** may include: abnormal placentation, chronic abruption, infarcts, focal lesions, and quality of placentation. The **foetal factors** are: intrauterine infections, and the capacity of the foetus to deliver the extracted nutrients from the placenta to its tissue (Sankaran and Kyle, 2009).

Sow nutrition during gestation plays a role in piglets' birth weight, myofiber number and liver glycogen concentration. A reduction in DE intake (33.5 MJ/d vs. 9.2 MJ/d) will have a negative impact on these factors (Buitrago *et al*., 1974). In piglets, the uterine crowding (reduction in uterine capacity) occurs before day 35 and the within-litter variation is established on day 27

to 35 of gestation. The reduction in the number of viable conceptuses occurs on day 45 to 50 of gestation. Depending on the uterine crowding, placental development (volume and weight) is modified. Foetal development of the surviving conceptus might be altered if there are not enough placental compensatory mechanisms available (Foxcroft *et al*., 2006). This explains why first parity sows, which have more uterine crowding, have lower birth weight piglets compared to multiparous sows (Wu *et al*., 1989). The porcine placenta develops rapidly between day 20 and 60 of gestation and reaches its maximum size by day 70. The utero-placental blood flow is increased by placental angiogenesis in order to favour the nutrient supply (mainly amino acids (**AA**) and glucose) and gaseous exchange from the sow to the foetuses (Wu *et al*., 2004).

After the foetus is exposed to glucose and AA, its pancreas releases IGF1 and 2, which are the main stimulators for foetal growth (Sankaran and Kyle, 2009). In case there is a dysfunction in the placental vascular system, there will be an abnormal nutrient transfer leading to less nutrient supply for the foetus causing a re-programming and stunting effect by sparing the brain and the axial skeleton. The foetus will try to adapt to this inadequate nutrient supply in order to maximize its chance of postnatal survival. The foetus' immediate response to the reduced nutrient supply will be a catabolic consumption of energy providing substrates. If the undernutrition is prolonged, the foetus will adapt its metabolic rate and alter the hormone production (reduction of IGF1 and its tissue sensitivity; Braems, 2003). If the nutrient deficiency occurs in early gestation, the DNA synthesis rate in the skeletal muscle will be reduced. If it occurs in late gestation, the foetus will suffer from asymmetrical growth and increase its brain:liver weight ratio and there will be a higher foetal adrenal hypertrophy and increased glucocorticoid activity (Sankaran and Kyle, 2009). Hales and Barker (1992) stated that mammals which are deprived of adequate foetal nutrients will suffer from long term consequences such as impaired development of the endocrine pancreas and a higher susceptibility to develop type II diabetes.

2. Factors related to the birth process

Perinatal hypoxia is caused by prolonged uterine contraction leading to oxygen deprivation of the foetus increasing the risk of umbilical occlusion, damage or rupture of the cord. In-utero, hypoxia occurs due to: **a)** umbilical cord flow interruption **b)** partial or complete placenta detachment, altering the oxygen exchange **c)** inadequate placenta perfusion and **d)** improper pulmonary circulation at birth (Flores *et al*., 1996). A pig foetus exposed to hypoxia for 5 min prior to delivery or longer suffers irreversible brain damage (Alonso-Spilsbury *et al*., 2005). Hypoxia can occur in-utero, at the onset of parturition and in the immediate postnatal period. Irrespective the time of occurrence, the blood flow is redistributed to guarantee the function of vital organs (heart, adrenal glands and brain); via chemoreceptors that increase vagal activity to the heart producing bradycardia and increase in sympathic activity, leading to peripheral vasoconstriction and a rise in systemic blood pressure. This goes at the expense of blood flow to non-vital organs (e.g. lungs, intestine, kidney, and muscle; Cohn *et al*. 1974; Oliver Jr., 1965). Hypoxia will promote anaerobic oxidative metabolism, leading to disturbances in the acid-base metabolism causing acidosis. The acidosis can result in cell injury in the heart, kidneys, liver and brain. Acidosis impairs the catecholamine response causing hypotension and impaired capillary blood flow, leading to hypothermia and activation of the coagulation cascade (Alonso-Spilsbury *et al*., 2005; van Kempen, 2007). As piglets have a low amount of brown fat, in case of hypothermia the lipid storage cannot be used as a heat source. The piglet will mobilize free fatty acids but their thermoregulation is mainly due to shivering induced by central or peripheral stimuli (Herpin *et al*., 2002; Lossec *et al*., 1998). Another factor leading to hypothermia is the limited capacity of young piglets for gluconeogenesis which impairs thermogenesis and leads to metabolic acidosis (Svendsen *et al*., 1991).

Hypoxia in-utero or immediately after birth places the young piglets in disadvantage for colostrum intake and makes them prone to suffer from crushing. A lower resistance to cold is highly correlated to a low birth weight and a high within-litter variation (Herpin *et al*., 2002; Le Dividich *et al*., 1991). This leads to a delay in colostrum intake and subsequently less

energy obtained from it (Herpin *et al*., 2002). Piglets < 0.8 kg BW are not able to regulate their temperature due to a lower concentration and activity of respiratory enzymes (succinate dehydrogenase, NADH adiphorase and Cytochrone A) in skeletal muscle but not in vital organ tissue (Hayashi *et al.*, 1987).

The outcome of in-utero problems, maternal factors and difficulties during the birth process is low birth weight piglets. Low birth weight piglets have a reduced average daily gain throughout their productive life (Smit *et al*., 2013); are not able to compensate for their growth check, and will display a different carcass composition at market weight (Rehfeldt and Kuhn, 2006). The variation in weight at birth is amplified at weaning and during the subsequent growth phases (Quiniou *et al*., 2002). Quiniou *et al*. (2002) estimated a three week difference to reach 25 kg BW for piglets with a birth weight of 0.6 kg when compared to piglets weighing 2.6 kg. In agreement with Beaulieu *et al*. (2010) who stated 20 days difference on average to reach the 25 kg BW between the lightest and heaviest piglets from the litter. Birth weight variation accounts for 10.8 and 10.4% of the variation in days to reach market weight for gilts and barrows respectively (Schinckel *et al*., 2010).

3. Weaning and early post-weaning performance

Lower post-weaning performance can occur as a result of a different group composition (mixing of littermates), differences in weaning weight, a poor adaptation to solid feed, among others (Bruininx *et al*., 2001). Piglets with a low BW at weaning will have a lower growth rate, consume less feed compared to normal piglets and will take longer time to reach market weight (Schinckel *et al*., 2009; Wolter and Ellis, 2001). Heavy piglets at weaning can also adapt faster to dry food and consume higher amounts compared to light piglets. This could provide an advantage in terms of growth performance (Dunshea *et al*., 2003; Jones, 2012; Mahan and Lepine, 1991). The faster adaptation to dry food might be related to a more developed gastrointestinal tract when compared to littermates with lower BW at weaning (Pluske *et al*., 2003). Comparing the feed intake pattern of light and heavy piglets at weaning, Bruininx *et al*. (2001) observed that light

piglets paid more daily visits to the feeder but consumed less on each visit when compared to heavy littermates.

Focusing on the batch heterogeneity at the end of the nursery phase in commercial practice, it is difficult to underpin the real cause or combination of causes that led to the growth check. We speculate that by finding early indications of the origin of the growth retardation we can also study possible alternatives to provide these light piglets an opportunity to show their maximum potential.

AIM AND OUTLINE OF THE THESIS

The primary goal of this thesis was to identify and describe the causes for growth retardation in the nursery phase to provide a basis to look for alternative nutrition and management solutions. In order to provide the means to these piglets to be able to display their potential and thus, reduce the heterogeneity in weight at the end of the nursery phase. Figure 1.2 provides a schematic presentation of the outline of this thesis. **Chapter 2** focuses on the phenotypic definition of growth retardation, and on the risk factor analysis to predict piglets' BW at the end of the nursery phase based on a statistical evaluation of three large datasets. With regard to IUGR piglets, we aimed to split low birth weight piglets into various categories, analysing the potential of each category of piglets to compensate for their low birth weight. Our target population was established as piglets with a birth weight above the mean minus two times and below the mean minus one time the SD from the mean of the total population at birth and with a predicted BW at the end of the nursery phase below the mean minus one time the SD from the mean of the total population; **LP** piglets). We aimed to characterize differences between the LP and their heavier counterpart (piglets with a predicted BW at the end of the nursery phase above the mean plus one time the SD from the mean of the total population; **HP**); in terms of performance, body morphology, behaviour, nutrient digestibility and feed intake characteristics (**Chapter 3**). After quantifying their apparent total tract and ileal digestibility differences when providing a highly digestible diet. Their digestive system was

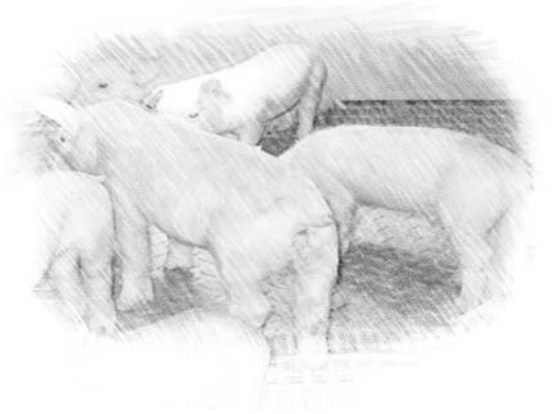
challenged with a low protein, high fibre content diet to determine whether differences in performance between the LP and HP piglets would be enlarged by the low quality protein, high fibre diet fed from six to ten weeks of age (**Chapter 4**). Skeletal muscle histochemistry and gene expression characteristics were studied to determine whether the differences in growth performance between low and high performing piglets would be the result of different skeletal muscle properties (**Chapters 5**). Throughout the studies, we compared the poor performers with their heaviest littermates. In our last study, we focused on a subpopulation of growth retarded piglets (piglets with an average birth weight but with a lower than average gain during the first six weeks of age; **AL**) and determined whether insulin-mediated glucose regulation might explain their growth retardation (**Chapter 6**). In **Chapter 7**, general discussion, the major findings of these studies are discussed. Furthermore, an overview of the main conclusions with their implications is provided.

	Introduction	1
What is a growth retarded piglet	How to predict growth retardation at early age Which subpopulations of growth retarded piglets can increase BW category	2
Similarities/differences compared to heavier littermates in terms of	Performance Nutrient digestibility Behaviour Anatomical characteristics	3
	Nutrient digestibility Immuno-reactive trypsin concentration	4
	Skeletal muscle composition	5
Characterizing a subpopulation of growth retarded piglets	Insulin-mediated glucose regulation Pancreatic enzyme activity	6
	General discussion Main conclusions	7
	List of references	8

Figure 1.2 Schematic presentation of the outline of this thesis

Chapter 2

Analysis of factors to predict piglets' body weight at the end of the nursery phase



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ABSTRACT

In pig production, within-batch variation in body weight (**BW**) gain of piglets during the nursery phase (up to 10 weeks of age) can be high and is of high economic importance. Homogeneity of BW within batches of piglets is important as it influences the efficiency of use of the grower and finisher facilities, and provides an extra value for the fattening farms. In the current study, factors for a low BW at the end of the nursery phase of piglets were determined by analysing datasets from three different swine research centres in the Netherlands and France. The entire dataset contained information on 77,868 individual piglets born in the period between 2005 and 2010. The BW was determined at different time points over the pre- and post-weaning phase; sex, season of birth; litter information (litter size at day of birth and after cross-fostering, number of piglets born alive per litter, number of total born littermates, sow parity number); cross-fostered piglets (yes or no), and pen group size over the post-weaning phase were recorded. A risk factor analysis approach was used to determine factors that predict piglet BW at the end of the nursery phase. The BW at the end of the nursery phase corrected for age was mainly determined by season of birth, birth weight, weaning weight, and BW at 6 weeks of age (all $P < 0.01$). These variables explained approximately 70% of the overall variation in BW at the end of the nursery phase. Litter information did not contribute to explain the BW at the end of the nursery phase ($P > 0.05$). To discard the possibility of intrauterine growth retarded piglets being the reason for the influence of birth weight (**BiW**) as an explanatory factor in the regression model, a further analysis was performed on the effect of this category of piglets on the results of the regression analysis. Overall, it was concluded that piglet's BW at the end of the nursery phase is mainly determined by season of birth, birth and weaning weight and BW at 6 weeks of age. Piglets with a BiW above the mean BiW minus 2.5 times the SD of the mean of the total population have the potential to compensate during the subsequent phases of growth.

Keywords: body weight, performance, pig, prediction, recovery, risk analysis

INTRODUCTION

The development of hyper-prolific pig breeds has led to an increase in the number of piglets born per sow per year. In Denmark, an increase of 0.3 piglets per litter per year was reported from 1992 to 2007 (Bjerre *et al*., 2010). In France, a value of 0.2 piglets per litter per year was found over the period of 1996 - 2007 (Martineau and Badouard, 2009). This increase has led to a greater within-litter birth weight (**BiW**) variation, exceeding 1 kg among the lightest and heaviest piglets in litters of 10 - 15 total born (Foxcroft *et al.*, 2007). The impact of BiW on survival and BW gain in later stages of production remains unclear with some authors stressing negative effects of BiW variation (English *et al*., 1977; Rehfeldt *et al*., 2008) while others report only minor effects (Milligan *et al*., 2001). Low BiW of piglets is associated with low growth rates, longer time to reach market weight and lower carcass quality (Powell and Aberle, 1980; Rehfeldt *et al*., 2008; Smith *et al*., 2007).

Prenatal restriction of uterine space limits postnatal growth (Foxcroft and Town 2004). Consequently, piglets with a very low BiW, referred to as intra uterine growth retarded (**IUGR**), are perceived as a subpopulation that cannot compensate their growth in later life (Wu *et al*., 2006). McMillen *et al*. (2001) defined IUGR piglets as piglets with a BiW below the mean minus two times the SD of the mean. As the number of small piglets in the population increases, it is important to identify the subpopulations with the capacity to compensate their growth in later stages of production. Such information could serve as a basis for customized nutrition or for husbandry practices which can contribute to an improved homogeneity of batches leaving the nursery phase.

This study aimed to identify which factors from birth to end of the nursery could be used to predict piglet BW at the end of the nursery phase from data collected in three research centres. A risk factor analysis approach was applied to the datasets which contained 77,868 individual records in total.

MATERIALS AND METHODS

Piglet performance was considered from birth to the end of the nursery phase (average 23.63 kg BW ± 0.21 SE and 10 weeks of age ± 0.32 SE). The reasons for selecting this time period were the availability of sufficiently large datasets with information from birth until this time point and the need for an early age point to identify piglets falling behind in performance to allow further development of nutritional and management interventions to support these piglets.

The results of a preliminary analysis performed on the three datasets used for this study showed that more than 30% of the variation in the performance at slaughter weight (on average 110 kg BW) was explained by the difference in BW at the end of the nursery phase. The correlation between slaughter weight and weight at the end of nursery phase was over 10% higher when compared to the slaughter weight correlation to weaning weight.

Database information

Datasets from three different research centres in the Netherlands and France were analysed providing a total of 77,868 piglet records. Descriptive statistics of the datasets are provided in Table 2.1.

Dataset A: Swine Innovation Centre "Sterksel", Wageningen University and Research Centre, Sterksel, the Netherlands. Data were obtained from all litters born between 2005 and 2010 and originated from a herd of about 330 crossbred Great Yorkshire x Dutch Landrace sows inseminated with semen from crossbred Dutch Landrace x Great Yorkshire or Tempo sires. During the general operation of the Centre, piglets with a low vitality based on a visual judgement were removed from the litters and euthanized. Boars were castrated at four or five days of age.

Dataset B: Nutreco Swine Research Centre, "Halfweg", Sint Anthonis, the Netherlands. Data were obtained from all litters born between 2005 and 2010 and originated from a herd of 155 Hypor sows inseminated with

semen from Topigs or Hypor sires. During the general operation of the Centre, piglets with a low vitality based on visual judgement were removed from the litters and euthanized. Boars were castrated at four or five days of age.

Dataset C: IFIP Research Centre, Romillé (35), Brittany, France. Data were obtained between 2005 and 2010 from a herd of 168 crossbred Large White x Landrace sows inseminated with semen from Pietrain x Large White sires. During the general operation of the Centre, no piglets were removed from the litters included in this database. Not all the litters produced during this time were followed; the information was obtained from the used batches selected based on requests for trials. Boars were castrated at four or five days of age.

In all three datasets, cross-fostering occurred within 24 to 48 h post-farrowing (in a rate of 5.26, 9.65 and 8.87% respectively from the total population); afterwards piglets suckled by their dam or by a nursery sow. This practice is used to adjust the number of piglets to the number of functional teats.

In the Dataset B, a reduction in the number of piglets that reached the end of the nursery phase was observed. This was likely related to the effect of involvement of piglets in trials up to 7 weeks of age in this research centre. To determine whether this subpopulation would be representative for the complete dataset, normality of BW was checked, using UNIVARIATE procedure of SAS (Version 9.1, 2002), in the complete population and the selected group at birth, weaning and at 6 and 7 weeks of age (data not shown). The results confirmed that this subgroup was representative for the complete population.

Table 2.1 Descriptive statistics of the datasets

Variable	Dataset	N	Mean	SD
Birth weight, kg	A	50954	1.40	0.37
	B	23228	1.44	0.36
	C	3686	1.45	0.37
Pre-weaning mortality[1], %	A	6119	12.01	_

	B	3571	15.38	_
	C	406	11.01	_
ADG pre-weaning, g/d	A	44804	0.24	0.05
	B	19657	0.24	0.06
	C	3280	0.26	0.06
Weaning BW, kg	A	44804	7.73	1.79
	B	19637	6.71	1.61
	C	3280	8.89	1.86
Average age weaning, days	A	44771	26.22	3.27
	B	19646	21.55	2.77
	C	3280	27.78	1.46
BW 1 week post-weaning, kg	A	_	_	_
	B	14228	7.55	1.56
	C	_	_	_
BW 2 weeks post-weaning, kg	A	_	_	_
	B	13514	9.06	1.79
	C	_	_	_
BW at the end of the nursery phase[2], kg	A	36437	22.55	4.63
	B	4883	24.12	4.25
	C	3224	24.23	4.23
Average age at the end of the nursery phase, days	A	36891	63.30	2.65
	B	4894	64.04	2.28
	C	3224	63.69	2.12
Born alive piglets per litter	A	50772	13.80	2.85
	B	23229	13.00	2.93
	C	3687	14.37	3.09
Litter size at farrowing, day 0	A	50772	13.18	2.68
	B	19687	11.98	2.35
	C	3687	14.37	3.09
Litter size day 2 post farrowing	A	47843	11.92	2.27
	B	19689	12.55	2.34
	C	_	_	_
Parity of the sow	A	50772	3.71	2.35
	B	23229	3.42	2.17
	C	3686	3.72	2.47
Percentage of barrows from the male population, %	A	18106	92.93	_
	B	2192	92.55	_
	C	1477	100	_
Percentage of intact males from the male population, %	A	1377	7.07	_
	B	176	7.45	_
	C	_	_	_
Average number of piglets per pen in the post-weaning	A	_	_	_
	B	14317	5.83	4.37

period	C	3687	16.63	8.41

[1] Includes active euthanasia and natural mortality
[2] End of piglet phase corresponded to the transfer of pigs from the nursery to the grower and fattening unit, on average at 10 weeks of age.

Description of variables Included in the analysis

The variables included in the risk factor analysis were: Season: in which the piglet was born (spring, summer, autumn, winter); Sex: male (intact male or barrow), gilt; Birth weight: per piglet in kg; Born alive: number of piglets born alive per litter; Born dead: number of piglets born dead per litter; Total born: total number of piglets born per litter; Litter ID: based on sow number, date of birth and litter size day 0; Cross-fostering: piglet moved to another litter (yes or no) during the first 48 h of age; Cross-fostering weight: BW at the day of cross-fostering in kg; Weaning weight: BW at the time of weaning in kg (age per piglet was identified); BW at 6 weeks of age: in kg; Parity: number of the sow when piglets were born; BW end of the nursery phase: in kg; Age at the end of the nursery phase: in days; Litter size day 0: number of piglets with the sow at the day of birth; Litter size day 2: number of piglets with the sow after cross-fostering; PW group size: number of piglets per pen in the post-weaning phase.

In addition, some new variables were calculated to predict BW of piglets at the end of the nursery phase:

Pre-weaning average daily gain (**ADG**), g/d = [(weaning weight – BiW)/ number of days from birth to weaning]
Relative BiW 1 = [BiW of the piglet / mean BiW of the littermates]
Relative BiW 2 = [(BiW of the piglet – mean BiW of the littermates)/ mean BiW of the littermates]

Statistical analysis

A total of 78,006 records were initially collected from the three research datasets. Normality of BW at each stage was tested using UNIVARIATE procedure of SAS (Version 9.1, 2002). Outliers for the analysed variables (more than four SD below or above the mean) were removed from the datasets. In total 77,868 remaining records were used for the analysis.

Normality was checked per dataset and per year to determine whether data could be merged towards one dataset per centre. Descriptive statistics of all datasets were calculated using MEANS procedure of SAS (Version 9.1, 2002) of which the results are presented in Table 2.1.

The aim of the risk factor analysis was to find explanatory variables which enable prediction of the variance of the dependent variable. Forward (selection) and backward (elimination) stepwise regression (Montgomery and Peck, 1992) were performed using Genstat (Genstat Committee, 2000). Potential risk factors were included and excluded from the regression model in a stepwise approach. When a probability value was below 0.05, factors were retained in the model. Stop criterion for the stepwise regression method was percentage variance accounted for (adjusted R^2) or Mallows C_p (Daniel and Wood, 1980; Mallows, 1973). Piglet was used as the experimental unit.

Analyses were carried out comparable to those described by Brscic *et al*. (2011). The dependent variable was the BW at the end of the nursery phase corrected for age. The explanatory variables were the factors obtained from data registered per piglet on the farm. These were BW measurements at different time points, pen group size over the post-weaning phase, litter information (litter size, born alive, total born littermates, sow parity number) and cross-fostered piglets (yes or no). The variables BiW, weaning weight and BW at 6 weeks of age were transformed into class variables, each with five classes (lightest, light, average, heavy, heaviest) determined by ranges of SD from the mean of the total population (lightest: below -2; light: from -2 to -1; average: from -1 to + 1; heavy: from +1 to +2; and heaviest: above +2). Parity was analysed as actual number (1…8) and transformed into class variables with 4 classes (primiparous, parities 2 to 4, 5 to 6 and 7 or more). Sex variable was divided in two categories, males (including intact males and barrows) and gilts. This approach was taken because the percentage of intact males in Datasets A and B was low compared to barrows and the performance of both groups was not different at the end of the nursery phase ($P > 0.050$; Table 2.1).

For the risk factor analysis, four different regression models were used, as presented in Table 2.2. The difference between the models was the inclusion of different explanatory variables (e.g. pre-weaning growth rate or weaning weight and BW at 6 weeks of age) to determine their effect when added to the other variables described in the database information section.

In the regression models 1 and 2, BW at 6 weeks of age was added as an explanatory variable to quantify its contribution to the variation in the BW at the end of the nursery phase. In regression models 1 and 3, pre-weaning growth was used as explanatory variable instead of weaning weight as used in regression models 2 and 4 to exclude the effects of the contribution of BiW to weaning weight.

To estimate the effect of BiW on the proportion of piglets that have the potential to recover and compensate their performance during their productive life, piglets with a BiW below the population average were divided in seven BiW categories based on SD variation in BW from the mean of the population (< -3 to -3, -3 to -2.5, -2.5 to -2.0, -2.0 to -1.5, -1.5 to -1.0, -1.0 to -0.5, -0.5 to average).

At the end of the nursery phase, piglet weight categories were calculated in the same way and each piglet was thereafter categorized. Each piglet category at birth was compared with its category at the end of the nursery phase to determine whether they remained in the same category, decreased or increased at least one category from birth to the end of nursery phase.

Table 2.2 Factors to predict body weight (BW) at the end of the nursery phase

					Significance of variable, $P < 0.05$								
Regression model	**Dataset**	**N**	**Adjusted R^2**	**Cp[1]**	**Season**	**Sex**	**6 weeks BW**	**Average weaning age**	**Birth weight**	**Pre-weaning ADG[2]**	**BW at Weaning**	**Born alive/litter**	**PW[3] group size[4]**
	A	5306	69.05	10	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.i[5]	----[6]	n.i
1	B	4882	66.60	10	**<0.001**	**<0.001**	**<0.001**	----	*0.069*	----	n.i	**0.039**	----
	C	818	76.98	8	**<0.001**	----	**<0.001**	n.i	*0.050*	**0.014**	n.i	----	**<0.001**
	A	5306	69.08	12	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.i	**<0.001**	n.i	n.i
2	B	4882	59.48	11	**<0.001**	**<0.001**	**<0.001**	----	**<0.001**	n.i	**<0.001**	----	----
	C	818	76.99	8	**<0.001**	----	**<0.001**	n.i	**0.008**	n.i	**0.011**	----	**<0.001**
	A	36402	48.12	8	**<0.001**	----	n.i	**<0.001**	**<0.001**	**<0.001**	n.i	n.i	n.i
3	B	4882	25.64	7	**<0.001**	**<0.001**	n.i	----	**<0.001**	**<0.001**	n.i	----	----
	C	3212	46.93	8	**<0.001**	----	n.i	n.i	**<0.001**	**<0.001**	n.i	----	**<0.001**
	A	5306	41.75	8	**0.003**	----	n.i	**<0.001**	**<0.001**	n.i	**<0.001**	n.i	n.i
4	B	4882	42.03	10	**<0.001**	**<0.001**	n.i	----	**<0.001**	n.i	**<0.001**	----	----
	C	3212	49.64	9	**<0.001**	*0.052*	n.i	n.i	**<0.001**	n.i	**<0.001**	----	**<0.001**

[1] Mallows Cp

[2] ADG = Average daily gain, g/d

[3] PW = Post-weaning

[4] Number of piglets caged in the same pen in the post-weaning phase

[5] n.i. = not included in the model when a similar variable was included (e.g. BW at weaning and pre-weaning ADG)

[6] --- = included in the model; but non-significant variables ($P > 0.05$): born dead, total born, litter size day 0, litter size day 2, cross-fostering, cross-fostering weight, parity number, parity group

RESULTS AND DISCUSSION

Factors to predict BW at the end of the nursery phase

The results of the risk factor analysis are presented in Table 2.2. For the variables that contributed significantly to the prediction of BW at the end of the nursery phase, the size and direction of the effect was analysed resulting in additional regression models, the results of which are presented in Fig. 2.1. The factors that contributed significantly to the prediction of BW at the end of the nursery phase were consistent among datasets. The variable, average age at weaning was only significant in the Dataset A. This was probably caused by the small variation in weaning age throughout the years in the Datasets B and C; as illustrated by the lower SD of this parameter when compared to the Dataset A (Table 2.1). Post-weaning group size in the Dataset C was significant ($P < 0.001$) due to the large difference in numbers (8 or 20 piglets per pen) and variation in the different housing conditions used to accommodate the piglets at this research centre.

Season of birth contributed significantly to the prediction of BW in each of the datasets. As far as the season effect is considered, it can be seen in Fig. 2.1 that the results from the Dataset C differed from those from the Datasets A and B. In fact, the result for the Dataset C reflected the effect of exclusion of piglets from the dataset related to selection and use of piglets for inclusion in experimental research in particular seasons. The effect of season of birth was similar in the Datasets A and B in which data of all litters born were incorporated in the analysis. These two datasets showed a reduction in the BW at the end of the nursery phase in spring and summer. This seasonal effect is in agreement with the results of Sabbioni *et al*. (2010) who stated that piglets born in autumn and winter mature earlier compared to those born in spring and summer. We speculate that the reduction in BW during the summer is related to the stress of an increased environmental temperature. This is in agreement with the results of Safranski *et al*. (2010) who described that under heat stress weaned piglets weighed 0.5 kg less when compared to weaned piglets not exposed to heat stress. Also Quiniou and Noblet (1999) stated that higher temperatures

during the lactation phase reduces milk production and leads to lower BW at weaning.

In Fig.2.1, sex showed a significant effect in the Dataset B. Males reached a higher BW at the end of the nursery phase when compared to gilts. This result is in agreement with those of Poore and Fowden (2004) who reported that at 12 weeks of age, the lighter males at birth are able to catch up with their heavier littermates. The difference in BW remained at 12 weeks of age for each BiW category for the gilts, suggesting an interaction between sex and BiW category. However, we did not observe this interaction in the present study. The increase in weight for the males at the end of the nursery phase disagrees with the study of Wolter and Ellis (2000), who reported that the differences between sexes are only observed when piglets reach the finishing period (50 to 110 kg BW).

An interaction between birth and weaning weight was observed in the Datasets A (P = 0.038) and B (P = 0.048). This interaction was mainly caused by the lightest piglets which lagged more behind at weaning than piglets with higher BiW. After determination of the main variables to predict BW at the end of the nursery phase, predicted means were calculated per dataset for the common explanatory factors. Predicted means were estimated per factor successively (Fig. 2.1) considering only the common factors among datasets (sex, season of birth, BiW, weaning and BW 6 weeks of age).

Figure 2.2 shows the percentage of piglets on each BiW category (based on SD from the mean of the total population) which remained or changed BW category at the end of the nursery phase. Gondret *et al*. (2005) reported that the ADG increase is higher for piglets in a higher BW category during the suckling and post-weaning phase. Powell and Aberle (1980) reported that it takes piglets with a heavier BiW fewer days to reach 25 kg BW. Fix *et al*. (2010); Smith *et al*. (2007) and Rehfeldt *et al*. (2008) reported that a difference in BiW was associated with an increased difference in BW at later stages of growth as a result of improved ADG with increased BiW. This is in agreement with Jones (2012).

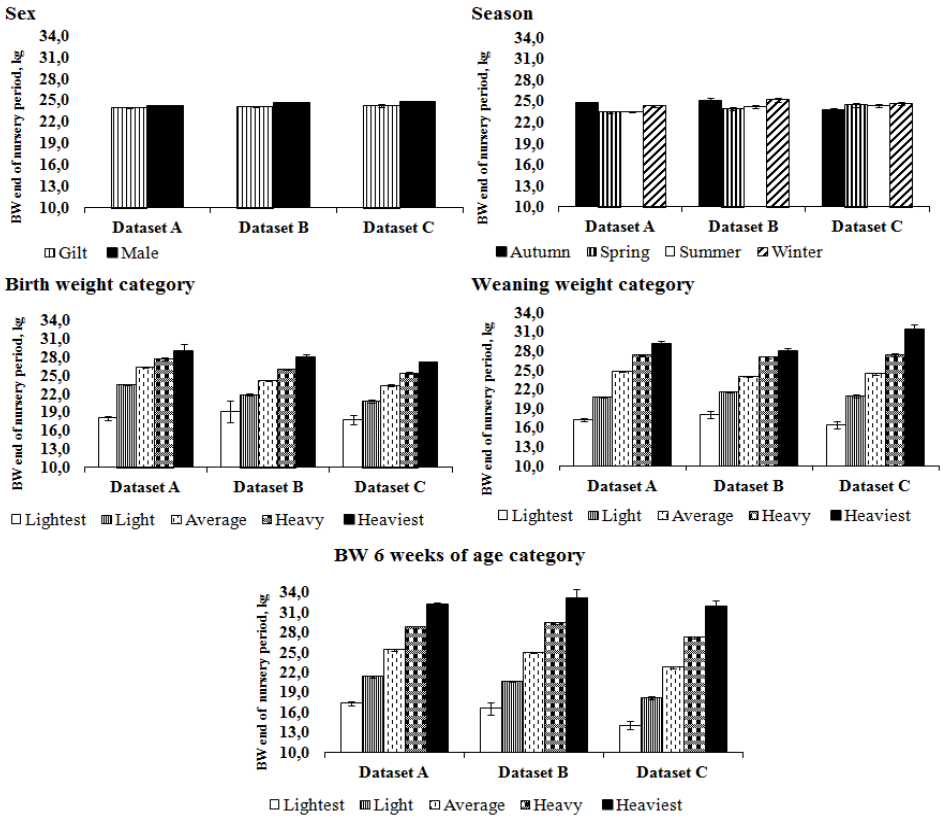
Parity number or parity category (e.g. primiparous vs. multiparous) is noticed by several authors as a clear criterion for piglet performance with piglets from gilts having lower BiW compared to those born from sows (Carney *et al*., 2009; Quesnel *et al*., 2008; Smith *et al*., 2007). In the present study, parity number or category did not explain the end of the nursery BW. We speculate that this may be partly due to the effect of cross-fostering as practiced at the research centres from which the databases were obtained. Due to the limited information to which sow the piglets were cross-fostered this effect could not be quantified.

The effects of the relative BiW 1 and BiW 2 variables (described in the Materials and Methods section) were analysed by substituting BiW for each of them in the four different regression models to compensate for the effect of the mean BiW of the litter. These analyses indicated that there were no differences in the factors that significantly contributed to explain the variation in BW at the end of the nursery phase in the regression models (data not shown). This led us to determine that piglet BiW rather than mean litter BiW was an important factor in the prediction of BW at the end of the nursery phase.

Recovery of growth in piglets with a low BiW

To exclude the possibility that data of extreme low BiW piglets were the sole reason for BiW to appear in the regression model, it was decided to analyse the impact of piglets with a very low BiW in more detail. To this end, the piglets with a lower than average BiW of the whole population (BiW below average) were selectively considered to determine whether the cut-off point, the limit under which piglets are considered IUGR (McMillen *et al*., 2001), would apply for these datasets or not. After the population classified as IUGR had been removed from the dataset, the analysis was performed once more to determine whether BiW would still be a relevant factor to predict BW at the end of the nursery phase. The number of low BiW piglets in each group category (< -3 to -3, -3 to -2.5, -2.5 to -2.0, -2.0 to -1.5, -1.5 to -1.0, -1.0 to -0.5, -0.5 SD to average), was expressed as a percentage of the total population within a dataset (Table 2.3). A similar pattern was observed in all datasets. A steady increase in the

percentage of piglets in each BW category related to the total population and the rise in average BiW per group was observed. In agreement with Milligan *et al*. (2001); Quiniou *et al*. (2002) and Wolf *et al*. (2008), it was found that the lowest chance of survival was for the lightest piglets at birth (Fig. 2.3).



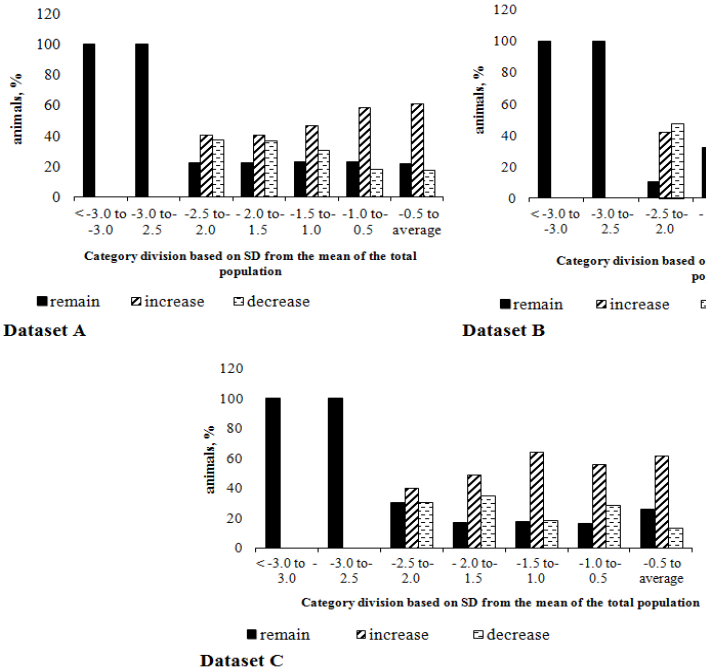
Sex divided in two categories: males (intact males and boars), gilts. Season: calendar season in which the piglet was born. Categories at birth, weaning and 6 weeks of age defined with regard to the predicted mean BW of the population within each dataset as Lightest (<mBW – 2 SD), Light (mBW -2 SD ≤ BW <mBW -1 SD), Average (mBW -1 SD ≤ BW <mBW +1 SD), Heavy (mBW+-1 SD ≤ BW <mBW +2 SD) and Heaviest (mBW +2 SD ≤ BW)

Figure 2.1 Predicted BW (mean ± SD) at the end of the nursery phase for each of the three datasets from common risk factors among datasets

Table 2.3 Percentage of piglets in each BW category at birth related to the total population per dataset

Dataset	Standard deviation category from the mean of the total population[1]						
	< -3 to -3	**-3 to -2.5**	**-2.5 to -2.0**	**-2.0 to -1.5**	**-1.5 to -1.0**	**-1.0 to -0.5**	**-0.5 to average**
A	0.01	0.20	1.67	4.43	9.22	13.51	19.51
B	0.05	0.67	2.14	4.11	7.98	13.84	17.90
C	0.27	0.62	1.22	2.77	6.39	14.45	16.92

[1]Data per row add up to about 50% as the overall data were not completely normally distributed



Group categories based on SD from the mean of the total population. Piglets were followed from birth to end of the nursery phase. A piglet is categorized as “increase” when the piglet has increased in at least one BW category from birth to end of the nursery phase. A piglet is categorized as “decrease” when the piglet has decreased at least one BW category from birth to end of the nursery phase. A piglet is categorized as “remain” when the piglet remained in the same BW category from birth to end of the nursery phase.

Figure 2.2 Percentage of piglets that remain, increase or drop at least one body weight category from their body weight class from birth to the end of the nursery phase

Focusing on mean BiW, piglets in the Dataset A had the lowest weight for the lower group category (< -3 to -3 SD) when compared to the Datasets B and C. However, from the fifth category (-1.5 to -1.0 SD) onwards, the mean BW was higher than that of the other two datasets. In the Dataset C, the increase in BiW with increasing category was lower compared to the Datasets A and B. It appears that piglets in the two lowest categories (< -3 to -2.5 SD at birth from the mean) remained in the same categories (Fig. 2.2). These groups of piglets most probably include the IUGR, i.e. piglets with a permanent setback in growth in the post natal life. These piglets will grow at a slower rate than their average littermates (Allen *et al*., 2004; Widowson, 1971). From the category between the mean -2.5 to -2.0 times the SD onwards, the percentage of piglets that moved towards at least one BW category higher from birth to the end of the nursery phase is higher than the percentage of piglets that remained or decreased categories in the three datasets (Fig. 2.2).

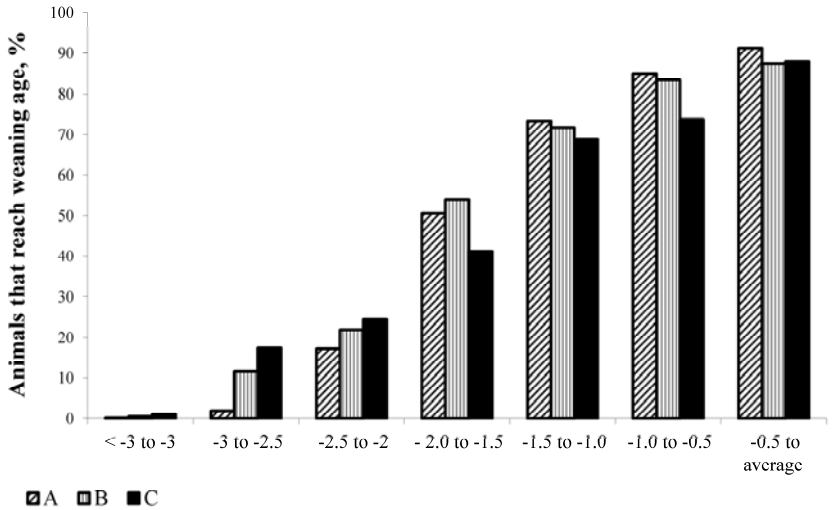
The exception is in categories ranging between -2.5 to -1.5 in the Dataset B where the percentage of piglets dropping one category was larger than the percentage of piglets increasing. No clear explanation was found for this pattern. The increase in group category disagrees with observations of Cole and Varley (2000) that pigs do not have the ability to compensate loss of BW; and that any reduction in BiW will have a detrimental effect on BW gain until slaughter. Our observation that a subpopulation was able to increase categories by the increase on BW indicates that this subpopulation has the potential to improve. This raises a new question on how to identify these piglets and what physiological mechanisms help them thrive.

Piglets that did not reach the nursery phase either died or were removed from the population by the farm manager, mostly due to poor physical condition or low BW. Poor performance is observed in the lowest three BW categories (< -3.0 to -3.0, -3.0 to -2.5, -2.5 to -2.0) with a percentage below 20% of piglets reaching the weaning phase in these categories. It can be observed that the Dataset C had the greatest percentage of piglets reaching the weaning phase in the lowest categories, but above -2.5 to -2.0

BW categories the percentage dropped. The lowest survival rate in the < -3.0 to -3.0 and -3.0 to -2.5 categories was observed in the Dataset A. The percentage of piglets that reached the end of the nursery phase did not differ from the percentage reaching the weaning phase for the Datasets A and C. In the Dataset B, a clear reduction in the number of piglets that reached the end of the nursery phase was observed (Fig. 2.3). This was likely related to the effect of involvement of piglets in trials up to 7 weeks of age in this research centre.

Based on our observations, it appears that a realistic cut-off point for piglets that still have the potential for an adequate growth performance till the end of the nursery phase is above the mean BiW minus 2.5 times the SD from the mean of the total population. It must be realized, however, that the mortality rate of piglets with a BiW close to this cut-off point is still relatively high.

After determining that the cut-off point for the current datasets can best be set at above the mean BiW minus 2.5 times the SD from the mean of the total population, a risk factor analysis was once again performed on the population of piglets above the cut-off point mentioned. No effect was observed due to the low percentage of piglets reaching the end of the nursery phase in the two lightest categories indicating that BiW is still a relevant variable to predict BW at the end of the nursery phase.



Group categories based on SD from the mean of the total population. The figure describes all piglets that were not removed due to active euthanasia or natural mortality from birth to weaning phase

Figure 2.3 Percentage of piglets that reached the weaning phase per BW group category at birth

CONCLUSIONS

The best regression models to explain BW at the end of the nursery phase explained 60 to 70% of the variation with explanatory factors being season of birth, BiW, weaning weight and BW at 6 weeks of age. These factors were consistent among datasets. Interestingly, litter information did not contribute in the regression model.

The present study provides a consistent indication that piglets with a BiW below the mean minus 2.5 times the SD from the mean of the total population have no potential to exhibit an adequate performance in the post natal life under practical farm conditions.

Under current conditions, piglets which deviate no more than the mean minus 2.5 times the SD from the BiW mean of the total population onwards

principally have the potential to compensate in further phases of life. The most interesting population for intervention to support low BiW piglets are piglets with a BiW higher than the mean BiW minus 2.0 times the SD from the mean of the total population onwards.

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Chapter 3

Identifying the limitations for growth in low performing piglets from birth until ten weeks of age

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ABSTRACT

The evolution of hyper-prolific pig breeds has led to a higher within-litter variation in birth weight and in BW gain during the nursery phase. Based on an algorithm developed in previous research, two populations from a pool of 368 clinically healthy piglets at 6 weeks of age were selected: a low (**LP**) and a high (**HP**) performing population and their development was monitored until the end of the nursery phase (10 weeks of age). To understand the cause of the variation in growth between these populations we characterized the LP and HP piglets in terms of body morphology, behaviour, voluntary feed intake, BW gain, and apparent total tract and ileal nutrient digestibility. Piglets were housed individually and were fed a highly digestible diet. At selection, 6 weeks of age, the BW of LP and HP piglets was 6.8 ± 0.1 and 12.2 ± 0.1 kg, respectively. Compared to the LP piglets the HP piglets grew faster (203 g/d), ate more (275 g/d) from 6 to 10 weeks of age and were heavier at 10 weeks (30.0 vs. 18.8 kg BW, all $P < 0.01$). Yet, the differences in ADG and ADFI disappeared when compared per kg $BW^{0.75}$. Assuming similar maintenance requirements per kg $BW^{0.75}$ the efficiency of feed utilization above maintenance was 0.1 g/g lower for the LP piglets ($P = 0.09$).The gain:feed ratio was similar for both groups. Low performing piglets tended to take more time to touch a novel object ($P = 0.10$), and spent more time eating ($P < 0.05$). At 10 weeks, LP piglets had a higher body length and head circumference relative to BW ($P < 0.01$). Relative to BW, LP had a 21% higher small intestine weight; 36% longer length, and relative to average FI, the small intestinal weight was 4 g/kg higher (both $P < 0.01$). Apparent total tract and ileal DM, N and GE digestibility were similar between groups ($P > 0.10$). We concluded that the low performance of the LP piglets was due to their inability to engage compensatory gain or compensatory feed intake as efficiency of nutrient utilization and feed intake per kg $BW^{0.75}$ was unaffected. Low performing piglets tend to be more fearful towards novel objects. The morphological comparisons, increased body length and head circumference relative to BW imply that the LP piglets have an increased priority for skeletal growth.

Keywords: behaviour, digestibility, growth, limitations, piglet

INTRODUCTION

In intensive pig production there is an increase in the number of piglets born per litter. This has enlarged the within-litter variation in birth weight, especially increasing the proportion of low BW piglets (Foxcroft *et al*., 2007). To improve the homogeneity of batches of piglets, we need to understand the causes of variation in body size and growth performance in the nursery phase. By understanding the mechanisms behind growth retardation, it may be possible to adapt farm management, housing conditions, and dietary nutrient supply to more closely meet the specific requirements of this population of piglets.

Based on the analysis of three datasets covering a period of five years, an algorithm was developed for predicting piglet BW at 10 weeks of age by including season of birth, body weight at birth, at weaning and at 6 weeks of age (Paredes *et al*., 2012). Based on this algorithm, 30 Low (**LP**) and 30 High (**HP**) performing piglets were selected from a pool of 368 clinically healthy piglets at 6 weeks of age. The objectives of the current study were **1)** to quantify the differences in performance of piglets in the defined low and high performing group and **2)** to explore phenotypical traits that contribute to explain differences in growth performance during the nursery phase.

MATERIALS AND METHODS

Animal housing and husbandry before selection

The procedures with animals described in the present study were conducted at the Nutreco Swine Research Centre (Sint Anthonis, the Netherlands) and were approved by the Animal Care and Use committee of Utrecht University, the Netherlands.

One day before the expected farrowing date, all Hypor Libra sows (first to fourth parity), were treated with 5 mg of dinoprost (Dinolytic, Zoetis, Capelle aan den IJssel, the Netherlands) to synchronize the farrowing process. The average litter size was 13.5 ± 2.6 SD at birth. All piglets (n = 454) were weighed individually and identified immediately after parturition ended with an ear tag. At birth, all piglets received an intramuscular injection of 15 mg/kg BW of ampicillin (Albipen, Intervet International, B. V., the Netherlands) and a 100 mg intramuscular injection of iron (Serumwerk Bernburg AG, Bernburg, Germany). Cross-fostering was avoided unless the sow did not have a sufficient number of functional teats on the udder to nourish all piglets born alive. In three litters, the heaviest piglet of the litter was moved to a foster sow within 48 h after birth. Piglets with a birth weight below the mean minus two times the SD of the mean of the total population (3.5% of the batch with a birth weight: 0.39 to 0.69 kg; sex ratio 53% males and 47% females) were regarded as intrauterine growth retarded (**IUGR**; McMillen *et al*., 2001; Paredes *et al*., 2012) and were not included in the present study. Males were anaesthetized and castrated on day 5 according to farm procedures. Due to 15.4% of mortality (mainly by crushing and spray legs) and morbidity (post-weaning diarrhoea and coughing), at 6 weeks of age 368 piglets were healthy and available for selection. Piglets remained with their littermates until the start of the study at 6 weeks of age. Piglets were weighed at birth, at day one of age (21.7 ± 0.8 h), at weaning (21.6 ± 1.0 days) and weekly during the post-weaning phase until the end of the study (69.6 ± 1.0 days). Performance data were collected from 6 to 10 weeks of age.

Selection of high and low performing piglets

Based on a data analysis of three different datasets (n = 77,868 individual records) we identified BW at 6 weeks, at weaning and at birth and season of birth as main factors to predict piglet BW at 10 weeks of age (Paredes *et al*., 2012). Applying an algorithm based on these factors, with the exception of season of birth, we selected two groups for the present study (LP and HP piglets) when piglets were 6 weeks of age (41.6 ± 1.0 days). Low performing piglets (n = 30) were selected from a subpopulation of piglets with a predicted 10 weeks BW below the mean minus one time the SD of the population. High performing piglets (n = 30) were selected from a subpopulation of piglets with a predicted 10 weeks BW above the mean plus one time the SD of the population. In addition, piglets selected were healthy based on visual judgement, as described below, clinical blood chemistry, and were balanced for sex and litter of origin. Maximum one piglet per litter was selected in each performance group.

Visual health judgement was conducted two days before start of the study, and all piglets were judged on alertness, appearance of a round belly, presence of nasal and eye secretions, ocular and oral mucosa colour and hair brightness. On day 41 and 69 ± 1.0 of age, 10 mL of blood were collected in serum gel and K-EDTA tubes from the jugular vein for determination of the following clinical chemistry parameters in blood plasma (tubes were centrifuged at 1800 rpm for 10 min at 4° C): glucose, creatinine, sodium, potassium, calcium, phosphate, urea, total protein, albumin, direct and indirect bilirubin, alkaline phosphatase, glutamate dehydrogenase, total protein, glutamic pyruvic transaminase, gamma glutamyl transpeptidase, glutamic oxaloacetic transaminase; creatine kinase, magnesium, chloride, and iron. To discard piglets with signs of inflammation, acute phase proteins (haptoglobin and C-reactive protein) were determined. Clinical blood parameters were determined using standard procedures by Synlab (Augsburg, Germany). Haptoglobin and C-reactive protein concentrations were analysed by Synlab (Augsburg, Germany) using ELISA.

Housing and husbandry during trial

The selected piglets (30 LP and 30 HP piglets) were placed individually in 0.8 m^2 pens and randomly distributed over 3 similar climate-controlled departments with 20 cages each with an equal number of replicates in each department. Piglets had *ad libitum* access to feed and water. During the first 24 h after arrival in the facility lights were on continuously. Thereafter, a 16/8 h light/dark scheme was provided. Piglets were able to interact with each other through the barred pen divisions and were offered toys as environmental enrichment. During the study, one of the LP piglets was euthanized because it was cachectic. All evaluated parameters (creatinine kinase, magnesium, chloride, iron, C-reactive protein, haptoglobin and white and red blood count) were within normal ranges on this piglet.

Feeding

All piglets had access to a commercial creep feed, from day 14 of age until weaning, presented as gruel (2:1 water: feed), and replaced twice a day to stimulate feed intake. From weaning until 10 days post-weaning, all piglets were fed with a highly digestible and palatable commercial pelleted weaner diet. From day 11 to 14 post-weaning, a gradual transition (75:25, 50:50, 25:75 0:100) to a second phase diet (Table 3.1) took place. The second phase diet was formulated to be highly digestible and palatable, and fulfilled the nutrient recommendations provided by National Research Council (**NRC**; 1998). During the last week of the study (9 to 10 weeks of age) 0.3% chromium oxide was included, as indigestible marker, in the diet of a subgroup of 24 piglets for digestibility measurements.

Parameters measured in LP and HP piglets before selection

At day one of age (21.7 ± 0.8 h) all piglets were weighed. From the weight increment between birth and day one of age, the colostrum intake in g, was estimated using the equation from Devillers *et al*. (2004). After weighing the piglets the tail of the piglets was docked at about 75% of its total length. By pressing the docked part of the tail, a drop of blood was placed in the middle of the circle in a pre-treated chromatography filter paper (3MM Chr Filter paper, Whatman, Cat. No. 3030 917), following the

procedure described by the producers of the Piglet Health Indicator (Betervee, the Netherlands). The selected tail blood samples were analysed for IgG concentration by a punch of 4.5 mm from the centre of the blood spot and eluted with an optimized dilution buffer (containing Phosphate Buffered Saline, 10 mM phosphate buffer, 150 mM saline, pH 7.2). All sequential steps of the ELISA were carried out as described in Bianchi *et al*. (1995). At day 7 and 14, the teat order was determined twice per day, i.e. 4 times in total to evaluate the relationship between teat order and vitality of the piglets in later stages of life. The piglet location at the time of massaging the udder and milk ejection was recorded. The measurements were taken one min after the onset of udder massaging. The teats were divided in three regions: anterior: 1 - 2, middle: 3 - 5 and posterior: 6 or more.

Table 3.1 Calculated nutrient composition (%) and net energy (MJ) of the experimental diet[1] (as fed) from six to ten weeks of age[2]

Item	Second phase diet
Crude protein	17.5
Crude fibre	3.1
Lactose	6.0
Net energy	10.7
AID[3] Lysine	1.4
AID Methionine + Cysteine	0.8
AID Threonine	0.8
AID Tryptophan	0.3
Apparent phosphorus digestibility	0.4

[1] Provided the following per kg of diet: Vitamin A 8,000 IU; Vitamin D3 2,000 IU; Vitamin E 30 mg, Pantothenic acid 12 mg; Vitamin K3 1.5 mg; Vitamin B1 1 mg; Vitamin B2 4 mg; Vitamin B6 1 mg; Vitamin B12 20 mcg; Nicotinic acid 20 mg; Folic acid 0.3 mg; Choline Chloride 250 mg; Cobalt 0.15 mg as Basic cobaltous carbonate, monohydrate; Copper 160 mg as Cupric sulphate, pentahydrate; Iron 100 mg as Ferrous sulphate, monohydrate; Iodine 1 mg as Calcium iodate, anhydrous; Manganese 30 mg as Manganese oxide; Zinc 100 mg as Zinc sulphate; Selenium 0.3 mg as Sodium selenite

[2] Based on Centraal Veevoeder Bureau (**CVB**), 2007

[3] AID = apparent ileal digestibility

Parameters measured in LP and HP piglets during the trial

When the piglets were 8 weeks of age they were exposed to a novel object test to determine their degree of fearfulness. An orange object (16 cm size), to which the piglets had not been exposed before, was introduced in the right front side of the cage of each piglet for 3 min. Latencies to approach, touch, and chew the object were measured. Approaching was defined as 2 or more steps directly towards the object. Touching was defined as making contact between object and head, chest or legs of the piglets. At 9 weeks of age, behaviour of all individual piglets was recorded in their pen (home pen observations). Piglets were observed starting at 8:00, 9:15, 10:30, 13:00 and 14:15 h using 4-min instantaneous scan sampling. The Observer XT software package installed on a Pocket Observer 3.1 (Noldus Information Technology B. V., Wageningen, the Netherlands) was used for behavioural recordings. The analysed behaviours were sitting, lying or sleeping, standing, explorative behaviour (nosing, rooting or chewing pen fixtures, floor or enrichment materials), comfort behaviour (rubbing the body against an object, scratching the body with hind leg or stretching part of the body), playing (gambolling, pivoting, jumping, and turning the body axis, rolling, shaking object or shaking head while holding a toy that protrudes from their mouth), eating and drinking.

At 9 weeks of age, 12 piglets per performance group were randomly selected (4 piglets per group per department) for morphometric and digestibility measurements. The selected piglets subsequently received a diet with 0.3% chromium oxide. During the last 3 days of week 10, faeces were collected from these piglets by rectal stimulation once per day obtaining approximately equal amounts per day. Samples were kept at 4° C and pooled over days within piglet, and subsequently mixed to obtain a homogenized sample for determination of dry matter (**DM**), nitrogen (**N**), and gross energy (**GE**) total tract digestibility.

One day before the piglets were sacrificed, body length was determined from the top of the head, from in-between the ears, down to the tail head. In addition, distance from ear to ear and head circumference, from the

most prominent part of the forehead around the widest part of the back of the head, was measured in the selected piglets. Piglets were sacrificed at the end of the trial by an intracardiac injection of pentobarbital sodium (Euthanasol 40% ASTfarma, B. V. Oudewater, the Netherlands), at a dose of 200 mg/kg BW. Liver, kidneys, heart, stomach, gallbladder, brain, spleen and colon were excised and weighed. The length, full and empty weight of the entire small intestine were determined, and ileal digesta (collected from 11 cm from the ileocaecal valve) were collected for determination of DM, N, and GE ileal digestibility.

Faecal and ileal digesta samples were freeze-dried, feed samples were vacuum-dried at 80° C and freeze-dry faeces and digesta samples were dried in a forced air oven at 103° C. All samples were dried to a constant weight according to ISO Standard 6496 (1998b). Following freeze-drying, faeces and ileal samples were ground to pass a 1-mm screen and kept for analysis. Nitrogen content was measured in fresh feed, faeces and ileal digesta according to ISO Standard 5983 (1997). Crude ash content was determined in feed, faeces and ileal digesta. Samples were carefully incinerated in a muffle furnace by slowly increasing the temperature from 20 to 550° C to prevent foaming, and subsequent incineration took place according to ISO Standard 5984 (2002). Gross energy content was analysed in feed, freeze-dried faeces and ileal content using a diabatic bomb calorimetry (model IKA-calorimeter C7000; IKA Werke GmbH and Co. KG, Staufen, Germany) according to ISO Standard 9831 (1998a). All analyses were carried out in duplicate.

Statistical analysis

Piglet growth performance and feed intake were analysed by week and over the 4-week period (6 to 10 weeks of age). For the weekly growth performance and feed intake, differences between performance groups were evaluated including performance group, sex and their interaction as independent variables using the MIXED procedure with the repeated option in SAS (Version 9.1, 2002). For the overall 4-week period differences between performance groups were evaluated including performance group, sex and their interaction as independent variables using the MIXED

procedure in SAS (version 9.1, 2002). From the teat order observations the percentage of piglets that did not change their selected teat during the 2 weeks (1 and 2 weeks of age) was determined. The UNIVARIATE procedure of SAS was used to test residuals for normality with the Shapiro-Wilk test, indicating the normal distribution of the data. A χ^2 test was used to determine whether piglets from the LP or HP piglets would suckle from a different teat order class (i.e. anterior, middle or posterior). The behavioural data obtained in the novel object test were analysed with the MIXED model of SAS after log transformation of the latencies. Differences between performance groups were evaluated including performance group, sex and their interaction as independent variables. For the home pen observations, all data were analysed using the MIXED model of SAS. Behavioural data were analysed as proportions of observations and arcsine-square root transformed when residuals were not normally distributed. Results of behavioural data are presented mentioning untransformed data but indicating the *P* values from analysing transformed data. Clinical chemical blood parameters and concentrations of acute phase proteins were analysed using MIXED models determining the differences between performance groups by week and performance group x week interaction.

RESULTS

Selection criteria

The selection of piglets was done from a pool of 368 piglets at 6 weeks of age, from an original batch of 430 piglets at weaning. A substantial number of piglets were excluded in the immediate post-weaning period since they were showing signs of weakness. The selected piglets were clinically healthy, and remained healthy throughout the study. Values for the clinical chemical blood parameters were within the range of normal values for piglets as described by Kraft and Dürr (2005). Also concentrations of acute phase proteins were within the range for healthy piglets (Biocheck GmbH, 2005). The means per performance group at start and end of the study (6 and 10 weeks of age) of the complete blood chemistry analysis are presented in Table 3.2. The observed BW at 10 weeks of age for LP and HP piglets were similar to those predicted based on the algorithm by Paredes *et al.* (2012). With LP piglets = 19.1 ± 0.7 kg predicted weight vs. 20.2 ± 0.6 kg observed; *P* = 0.910; and HP = 30.0 ± 0.7 kg predicted weight vs. 29.2 ± 0.6 kg observed; *P* = 0.880.

Parameters measured in LP and HP piglets before selection

The estimated colostrum intake at day one of age (21.7 ± 0.8 h) was similar between performance groups (LP = 263 and HP = 311 g; SE = 33.2; *P* = 0.310), both on absolute and when expressed per kg $BW^{0.75}$. Ninety three per cent of the piglets remained suckling from the teat initially selected throughout the first 2 weeks of age. When analysing the distribution of LP and HP piglets drinking in the anterior, middle or posterior teats we observed a tendency for LP piglets to drink mainly from the middle ones, whereas HP piglets drink mainly from the anterior and middle teats (interaction performance group and teat position, *P* = 0.060). Teat selection appeared to be unrelated to BW at 6 and 10 weeks of age (*P* = 0.600 and 0.180, respectively).

Parameters measured in LP and HP piglets during the trial

Low and high performing piglets did not differ in the latency to approach a novel object (LP and HP = 5 s; *P* = 0.700), but HP piglets tended to touch the

object 5 s faster than LP piglets (P = 0.098). The LP and HP piglets did not differ in the duration of the time spent lying, exploring or in any of the other observed behaviours (Table 3.3), except that the LP piglets tended to spend more time eating (P = 0.050). High performing piglets were 17% longer when compared to LP piglets (P < 0.010). Yet, body length per kg BW was 32% higher for the LP piglets (P < 0.010). Head size and diameter were 15% larger for the HP piglets compared to the LP piglets (P < 0.010). When relating the head circumference to BW, though, the ratio was 35% higher for LP piglets (P < 0.010; Table 3.4). Weight of heart, liver and kidneys were higher for the HP piglets (P < 0.010). Relative to the weight of the brain, weights of the heart, kidney and liver were higher in the HP compared to the LP piglets (heart 2.0 vs. 2.6; kidney 2.0 vs. 2.9, and liver 11.4 vs. 15.5 for LP and HP piglets, respectively; P < 0.010). Relative to BW, the weight of the heart tended to be higher in LP piglets (LP = 6.2 and HP = 5.6 g/kg BW; P = 0.070). Relative weight of the liver and kidney did not show differences between performance groups (liver = 36.2 vs. 33.6 g/kg BW and kidney = 6.3 vs. 6.2 for the LP and HP piglets, respectively; P = 0.110 and 0.710).

The empty small intestine was 270 g lighter for the LP piglets and 187 cm shorter compared to the HP piglets (P < 0.010). When expressed relative to BW, the LP piglets showed a 21% higher weight of the small intestine (P < 0.010) and a 36% longer length (P < 0.010). The small intestinal weight relative to the average daily FI over week 6 to 10 was 4 g/kg total FI higher for the LP piglets (P < 0.010) than for the HP piglets. Apparent total tract digestibility of DM (LP = 0.87, HP = 0.87; P = 0.510), N (LP = 0.84, HP = 0.84; P = 0.740) and GE (LP = 0.87, HP = 0.87; P = 0.730) and ileal digestibility of DM (LP = 0.77, HP = 0.79; P = 0.370), N (LP = 0.80, HP = 0.83; P = 0.200) and GE (LP = 0.80, HP = 0.81; P = 0.550) were similar between the LP and HP piglets.

Animal performance

We were able to select for a large contrast in BW at 6 weeks of age between performance groups, with a range between 4.5 and 7.8 kg and between 10.8 and 13.9 kg for LP and HP piglets at 6 weeks of age,

respectively. Performance data are presented in Table 3.5. Average daily gain and ADFI was lower for the LP piglets from 6 to 10 weeks of age ($P < 0.010$) but when expressed per kg $BW^{0.75}$ the differences disappeared ($P = 0.500$ and 0.960). Gain:feed (**G:F**) from 6 to 10 weeks of age was similar between performance groups ($P = 0.190$).

Table 3.2 Blood plasma parameters analysed for Low (LP) and High (HP) performing piglets at 6[1] and 10[1] weeks of age

	Six weeks			Ten weeks			*P* value	
	LP	HP	SE	LP	HP	SE	6 weeks	10 weeks
Serum parameters								
Glucose, mmol/L	6.60	6.60	0.2	6.80	7.00	0.1	0.198	0.355
Creatinine, µmol/L	54.40	55.10	2.9	52.00	57.10	2.1	0.853	*0.092*
Urea, mmol/L	1.20	1.50	0.1	1.70	1.90	0.0	0.133	0.174
Total protein, g/L	45.10	46.20	0.6	53.00	54.10	1.0	0.215	0.438
Serum enzymes								
Alkaline phosphatase, U/L	228.00	216.10	12.2	201.40	188.60	8.2	0.495	0.267
GPT[2], U/L	44.80	43.60	1.9	29.80	32.90	1.5	0.657	0.147
GGT[3], U/L	31.30	27.40	2.6	22.50	27.20	2.4	0.277	0.159
GOT[4], U/L	52.80	60.40	7.9	47.50	59.20	14.0	0.501	0.564
Sodium, mmol/L	142.90	142.80	0.5	140.00	142.60	0.5	0.807	**<0.010**
Potassium, mmol/L	5.00	5.10	0.1	5.20	5.40	0.1	0.958	0.292
Calcium, mmol/L	2.80	2.80	0.0	2.80	2.90	0.0	0.928	0.122
Phosphate, mmol/L	2.30	2.50	0.1	2.90	3.10	0.1	*0.088*	**0.045**
Acute phase proteins in serum								
Haptoglobin, mg/mL	0.70	0.60	0.1	1.00	1.00	0.1	0.751	0.275
C reactive protein, µg/mL	19.10	17.10	2.6	20.10	18.10	2.1	0.247	0.149

[1] Six weeks = 41.6 ± 1.0 days after birth when piglets were individually housed. Ten weeks = 69.6 ± 1.0 days after birth

[2] GPT = Glutamine pyruvate transaminase

[3] GGT = Gamma glutamyl transpeptidase

[4] GOT = Glutamate oxaloacetate transaminase

Table 3.3 Home pen observations[1] of Low (LP) and High (HP) performing piglets at 9 weeks of age

	LP	HP	SE	*P* value
% of observed time				
Playing[2]	2.40	2.70	0.5	0.700
Comfort	0.30	0.20	0.1	0.410
Eating	11.60	9.40	0.8	*0.050*
Drinking	1.30	1.60	0.2	0.320
Eliminating[3]	0.30	0.70	0.1	**<0.010**
Exploring[4]	11.00	12.80	0.9	0.370
Standing + Sitting + Locomotion	12.80	12.50	0.8	0.640
Lying	59.80	59.70	1.6	0.930

[1] Piglets were observed starting at: 8:00, 9:15, 10:30, 13:00 and 14:15 h using a 4-min instantaneous scan sampling

[2] Values are from uncorrected performance group means and *P* values are based on transformed data

[3] Eliminating includes urinating and defecating

[4] Exploring includes rooting, nosing and chewing object

Table 3.4 Piglet morphology and conformation of Low (LP) and High (HP) performing piglets at 10 weeks of age[1]

	LP	HP	SE	*P* value
BW 10 weeks of age, kg	18.80	30.00	0.6	**<0.001**
Morphology, cm				
Head to tail	62.30	73.20	0.9	**<0.010**
Ear to ear diameter	7.30	8.50	0.3	**0.010**
Head circumference	42.80	49.30	0.6	**<0.010**
Relative to BW 10 weeks of age, cm/kg				
Head to tail	3.20	2.40	0.1	**<0.010**
Ear to ear diameter	0.40	0.30	0.01	**<0.010**
Head circumference	2.20	1.60	0.1	**<0.010**
Organ weight, g				
Brain	60.00	70.00	1.4	0.180
Heart	121.70	168.00	7.2	**<0.010**
Kidneys	124.30	188.80	8.1	**<0.010**
Liver	710.00	1010.00	0.04	**<0.010**
Relative to BW 10 weeks of age, g/kg				
Brain	3.20	2.20	0.1	**<0.010**
Heart	6.20	5.60	0.2	*0.070*
Kidneys	6.30	6.20	0.2	0.710
Liver	36.20	33.60	1.1	0.110

[1] BW 10 weeks = BW at the end of the study at 69.6 ± 1.0 days after birth

Table 3.5 Performance for Low (LP) and High (HP) performing piglets from 6 to 10 weeks of age[1]

	LP	HP	SE	*P* value
BW, kg				
Birth	1.15	1.56	0.1	**<0.010**
Weaning	4.70	7.06	0.2	**<0.010**
Six weeks	6.84	12.20	0.1	**<0.010**
Ten weeks	18.83	30.02	0.6	**<0.010**
ADG[2] 6 to 10 weeks				
g/d	434	637	18	**<0.010**
g/kg $BW^{0.75}$/d	63	64	2	0.500
g/kg BW/d	33	30	1	**<0.010**
ADFI[3] 6 to 10 weeks				
g/d	633	908	21	**<0.010**
g/ kg $BW^{0.75}$/d	92	92	2	0.960
g/kg BW/d	49	43	1	**<0.010**
Gain: feed 6 to 10 weeks				
g/g	0.68	0.70	0.01	0.190

[1] Six weeks = 41.6 ± 1.0 days after birth when piglets were individually housed. Ten weeks = 69.6 ± 1.0 days after birth

[2] ADG = average daily gain

[3] ADFI = average daily feed intake

DISCUSSION

Understanding the causes behind growth retardation and finding solutions to provide these piglets a chance to express their utmost potential might help us reduce mortality in the farrowing phase and heterogeneity at the end of the nursery phase. The aims of the present study were to quantify the differences in performance between healthy Low (6.8 ± 0.1 kg BW at 6 weeks of age) and High (12.2 ± 0.1 kg BW at 6 weeks of age) performing piglets, selected based on an algorithm developed previously (Paredes *et al*., 2012). In addition, our second aim was to explore phenotypical traits that contribute to explain the differences in growth performance. Piglets with a very low birth weight, which were considered as IUGR (McMillen *et al*., 2001; Paredes *et al*., 2012) were discarded from this study. Results of muscle fiber analysis of the piglets selected are published elsewhere (Paredes *et al*., 2013). We also aimed to rule out clinical disease as a cause for growth retardation of the selected piglets: the concentrations of acute phase proteins and blood chemistry parameters together with a visual check-up indicated that piglets in both performance groups were clinically healthy during the study.

In the farrowing phase, we focused on colostrum intake and whether piglets would drink always from their selected teat or would switch to another teat during the suckling period. The IgG concentration in blood reflects the amount of antibodies the piglet had obtained from the sow via the colostrum (Damm *et al*., 2002). We aimed to use the IgG concentration as an indication of nutrient intake and relate it to growth in the first 24 h, yet the high variation of this parameter (CV = 68) did not allow us to make this relation. The estimated colostrum intake showed no differences between groups (LP = 263 and HP = 311 g; $P = 0.310$). One explanation for this could be the time lapse from birth to colostrum intake, yet we can only hypothesize as it was not measured. During this study, most piglets continued to suckle from the teat selected in the early postnatal period during the entire suckling phase, in agreement with McBride (1963). The LP piglets tended to suckle from the middle teats whereas the HP piglets tended to suckle from the anterior or middle teats. Kim *et al*. (2000) stated

that piglets occupying the anterior teats during the lactation phase would have a BW gain advantage in later life. We did, however, not observe such advantage in performance from 1 to 10 weeks of age for the piglets drinking in the anterior teats. We did not observe a correlation between teat order and birth weight, in agreement with Kim *et al*. (2000) nor between teat order and weaning weight or performance group (LP or HP piglets).

For the behavioural traits, our findings showed that LP piglets tended to take more time before touching an unfamiliar object. This may be interpreted as a higher level of fearfulness of the LP piglets compared to HP piglets. Gieling *et al*. (2011) suggested that there are cognitive differences between low and normal birth weight piglets as, in their study, low birth weight piglets had more difficulty in performing a spatial memory task after the location of rewards had changed. This suggests that small piglets may have more difficulty in switching from a known situation to a new one. When evaluating the duration of time which both performance groups of piglets spent on different behaviours during the day, it should be noted that the piglets in our study were housed individually, and their behaviour might be different than for grouped-housed piglets. We observed a tendency for a larger proportion of time spent eating for the LP piglets ($P = 0.050$). Feed intake during week 9 was higher for the HP piglets (LP = 794 and HP = 1149 g/d; $P < 0.010$). The estimated eating rate of piglets during the behavioural tests was calculated assuming an average daily amount, determined based on week 9 of age; equal eating patter over 24 h, equal error between groups and equal day/night pattern, such that the relative differences remained. Based on these assumptions, we calculated a substantially lower eating rate in the LP piglets (3.3. vs. 4.9 g/min; $P = 0.020$) compared to the HP piglets. The reduced eating rate for the LP piglets is in line with the findings of Bruininx *et al.* (2001), who stated that lighter piglets after weaning paid more daily visits to the feeder, but that each visit resulted in a lower intake of feed when compared to their heavier counterparts. Our own results evaluating the feed intake behaviour of the two performance groups when provided a commercial diet is in agreement with the results from Bruininx *et al*. (2001) describing

that LP piglets have a lower eating rate when compared to HP piglets ($P < 0.010$).

The anatomical development might be hampered in-utero or in the postnatal period due to factors such as nutrition and maternal environment. For the current study we did not focus on the in-utero phase and we assumed that the piglets selected were not hampered in their pre and postnatal performance in the gestational phase. For this purpose, we excluded the piglets considered as IUGR. The brain is one of the first organs to mature and has the first priority in normal development (Widdowson and McCance, 1960). Brain sparing, a physiological adaptation mechanism from the foetus to increase the delivery of oxygenated blood to the brain at the expense of other organs, has been studied in animal species such as rats (Desai *et al*., 1996), pigs (Ritacco *et al*., 1997; Widdowson and McCance, 1960) and in humans (Winick *et al*., 1970) during periods of maternal nutrient restriction. Our results showed that there were no differences in absolute brain weight between performance groups, indicating that brain development has had priority relative to other organs in LP piglets. When analysing brain weight as per kg BW, LP piglets had a higher brain weight (LP = 3.2 vs. HP = 2.2 g/kg BW; $P < 0.010$). When considering the weights of the different organs excised and expressed as a ratio of brain weight, we found that the size of heart, liver, and kidney and the small intestine were smaller in the LP piglets. In contrast, body length and head circumference related to BW were higher for the LP piglets ($P < 0.010$). The higher head circumference and higher body length when piglets were fed *ad libitum*, provides us an indication of the real intrinsic priorities for these piglets. Body length characteristics, together with the muscle characteristic analysis of these two groups (Paredes *et al*., 2013), which revealed a superior muscularity for the HP piglets in terms of greater total fiber number and fiber cross sectional area clearly indicate that LP piglets have a priority for skeletal development rather than protein deposition and lean mass accretion.

The morphological differences of the LP piglets from the HP pigs were not as marked as those described for IUGR (Sacy *et al*., 2010), but show signs of

similar phenomena. Low performing piglets might be a middle category in terms of anatomical development between IUGR and average weight piglets in the litter. The small intestine of LP piglets was proportionally longer, thinner, but heavier per g of ingested feed when compared to HP piglets, at 10 weeks of age, in line with other studies (Wang *et al*., 2005 in IUGR piglets). These findings led us to speculate that LP piglets had an increased surface to absorb nutrients in the intestinal tract, in agreement with the findings of Han *et al*. (2013) for IUGR piglets.

We did not observe differences in apparent ileal or total tract digestibility of DM, N and GE between LP and HP piglets, at 10 weeks of age, but the longer small intestine of the LP piglets led us to speculate that they would have a higher energy expenditure related to intestinal tissue leading to a lower net energy value, in agreement with the results of Jones (2012). One may expect that once the digestive system is challenged with a lower digestibility diet, differences might appear in apparent nutrient absorption between groups.

When comparing performance data of pigs differing in BW, correcting for the differences in size of piglets is a complicated issue, as some processes are directly proportional to absolute BW and others to metabolic BW. For the latter, different coefficients for BW correction has been proposed (Brody *et al*., 1945). Labussière *et al*. (2011) referred to a different factor for growing pigs, calves and sows. When expressed as a ratio of BW, the differences in ADG reversed, and fractional growth rates in LP piglets were higher compared to HP piglets. This is in line with the findings of others (Poore and Fowden, 2004; Magowan *et al*., 2011). Poore and Fowden (2004) related the increase in fractional growth rate to improved insulin sensitivity early in life after impaired intrauterine growth. When expressed in g/d, ADFI of LP piglets was lower when compared to HP piglets. This was also observed by Ritacco *et al*. (1997). They claimed, however, that regardless of their intake level small piglets grow more efficiently than controls, which implies a higher G:F for the small piglets. We observed no consistent differences in G:F between LP and HP piglets. This is in line with the findings of Jones (2012) who observed no difference in G:F, despite the

differences in ADG between light and heavy littermates selected at weaning. When assuming similar requirements for maintenance per kg $BW^{0.75}$ (Agricultural Research Council (**ARC**), 1981), the efficiency of utilization of feed intake above maintenance can be calculated. When doing so the LP piglets required 3.0 MJ/d whereas the HP piglets required 4.4 MJ/d for maintenance. The calculated feed intake for maintenance for the LP piglets was 213 vs. HP piglets 306 g/d ($P < 0.010$). Calculated feed intake above maintenance was 419 vs. 600 g/d for the LP and HP piglets, respectively ($P < 0.010$). The calculated efficiency of feed utilization above maintenance tended to be lower for the LP piglets (1.0 and 1.1 g/g for the LP and HP piglets, respectively; $P = 0.090$; all calculations based on ARC, 1981). We speculate that when persistent throughout the grower and finisher phases, this lower efficiency for BW gain would not enable LP pigs to catch up with HP pigs.

CONCLUSIONS

Despite differences in the length and weight of the small intestine, ileal and fecal N and GE digestibility were similar between low performing (LP) and high performing (HP) piglets. The lower growth performance of LP compared with HP piglets from 6 to 10 weeks of age is caused by an inability to engage compensatory gain and compensatory feed intake. Differences in feed intake and ADG between LP and HP piglets depended on the way they were expressed. In g/d, LP pigs had a reduced ADFI and ADG. When expressed per kg $BW^{0.75}$, these differences disappeared, and when expressed per kg BW, LP piglets achieved higher rates of ADFI and ADG.

The gain:feed ratio was unaffected, but calculated efficiency of utilising ingested feed above maintenance tended to be higher in HP piglets, suggesting the inability of LP piglets to compensate. The reduced ADFI in g/d in LP piglets coincided with a reduced eating rate. Behavioural comparison between LP and HP piglets indicate that LP piglets tend to be more fearful towards novelty. Morphological comparisons, e.g. increased body length and head circumference relative to BW at 10 weeks of age,

indicate that LP piglets have an increased priority for skeletal growth compared with HP piglets.

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Chapter 4

Challenging low and high performing piglets with a low quality diet in the post-weaning phase

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ABSTRACT

In current swine production there is an increase in the number of light piglets and a higher within-litter variation as a result of sow hyper-prolificacy. We aimed to determine whether differences in performance between low performing (**LP**) and high performing (**HP**) piglets would be enlarged when fed a suboptimal diet (**SO**) compared to a control diet (**CO**) from 6 to 10 weeks of age. Differences in apparent total tract and ileal digestibility were expected as a consequence of a reduced feed intake of LP piglets or due to the high fibre content of the SO diet. A total of 60 piglets (30 LP and 30 HP) were selected from a pool of 470 clinically healthy piglets at six weeks of age, based on an algorithm developed in previous research. Piglets were housed individually. The LP and HP piglets were fed either a CO or a SO diet. The suboptimal diet contained poorly digestible protein sources and had a high NSP content but both diets were formulated to have an equal ratio of SID amino acids to net energy. Their development was monitored until the end of the nursery phase (10 weeks of age). At 6 weeks of age, the BW of the LP and HP piglets was 6.7 ± 0.2 and 12.3 ± 0.2 kg, respectively. Compared with the LP piglets the HP piglets grew faster (173 g/d), ate more (310 g/d) from 6 to 10 weeks of age and were heavier at 10 weeks (32.5 vs. 21.8 kg; all $P < 0.01$). The SO fed piglets had a reduction in gain:feed ($P = 0.01$) but no piglet group x diet interactions were found. Feed intake was not increased in the SO fed piglets, demonstrating that both LP and HP pigs are unable to compensate for a reduced energy content by increasing their feed intake. Starch and fat apparent total tract digestibility were reduced in the SO fed piglets. Apparent total tract and ileal protein digestibility were also reduced in the SO fed piglets. Digestibility of other macronutrients, with the exception of NSP, was unaffected by piglet group. Despite the reduced feed intake, apparent total tract digestibility of sugars in non-glucose polysaccharides, particularly xylose and uronic acid, was reduced in the LP piglets ($P = 0.03$), particularly in the SO fed piglets (interaction $P = 0.05$). At 6 but not at 10 weeks of age there was a lower immuno-reactive trypsin concentration (used as indirect measurement of pancreatic function) in blood plasma of the LP piglets compared to the HP piglets. In conclusion, macronutrient

apparent total tract digestibility is equal for both piglet groups. Yet, the fermentative capacity of LP is reduced which places them in disadvantage for future performance.

Keywords digestibility, growth retardation, high fibre, pig

INTRODUCTION

Litter size has increased strongly in the last decennia. Larger litters, however increase the risk for both intra-uterine as well as postnatal growth retardation of piglets (Foxcroft *et al*., 2007).The consequence is an increase in variation in piglet health and growth performance which poses serious management challenges. Therefore, focusing on the mechanisms behind the growth retardation and possible venues to restore the growth of the light piglets is a target area in swine production.

Our previous studies focussed on low (**LP**) and high (**HP**) performing piglets from six to ten weeks of age. It was demonstrated that LP piglets had a reduced ADG and ADFI when compared to HP piglets, and different small intestine morphology (larger and thinner related to BW), but equal apparent total tract and ileal N, GE, and DM digestibility when fed a high quality diet (Paredes *et al*., 2014). Yet it is still unknown whether the differences in performance of LP and HP piglets in the post-weaning phase would become larger if they receive a lower quality diet (**SO**; low protein, high fibre diet). By providing a SO diet, differences in nutrient digestibility might occur and could explain the observed differences in performance. The objective of the present study was to investigate whether these differences occur and if so, to quantify their effect both in LP and HP piglets fed either a SO or a control diet (**CO**; low fibre, high protein diet) from six to ten weeks of age.

MATERIAL AND METHODS

The procedures with animals described in this study were conducted at the Nutreco Swine Research Centre (Sint Anthonis, the Netherlands) and were approved by the Animal Care and Use Committee of Utrecht University, the Netherlands.

Animal housing and husbandry pre-trial

At birth the average litter size was 13.9 ± 2.7. All piglets born (n = 545) were weighed individually and identified with an ear tag when parturition was completed. Shortly after birth, all piglets received an intramuscular injection of 15 mg/kg BW of ampicillin (Albipen, Intervet International, B. V., the Netherlands) and a 100 mg intramuscular injection of iron (Ursoferran, Trouw Nutrition International, the Netherlands). Birth date, time of birth for each piglet and total duration of parturition were recorded. Cross-fostering was avoided unless a sow did not have a sufficient number of mammary glands to accommodate all her piglets. This was determined based on sow viable nipples, and monitored when the sows entered the farrowing room. In case cross-fostering was needed, the heaviest piglets of the litter were transferred to a foster sow within 48 h after birth. From the selected piglets for the study 10% percent was cross-fostered, following the criteria described above. Piglets with a birth weight below the mean minus two times the SD from the mean of the total population were removed from the study because they were considered intrauterine growth retarded (McMillen *et al*., 2001; Paredes *et al*., 2012). Males were anaesthetized and castrated on day five according to farm procedures. Piglets were selected from a pool of 470 piglets at six weeks of age, coming from 39 litters born in April, 2012 from Hypor Libra sows (first to fourth parity) bred to Topigs P-line sires. Litters remained together until selection. Piglets were weighed at birth, at weaning (25.8 ± 1.1 days) and weekly during the post-weaning phase until the end of the nursery phase (10 weeks of age).

Selection of LP and HP piglets

Based on an algorithm developed in previous research (Paredes *et al*., 2012) 30 LP and 30 HP piglets were selected from a pool of 470 piglets at six weeks of age. Low performing piglets were selected from a subpopulation of piglets with a predicted BW at the end of the nursery phase (10 weeks of age) below the mean minus one time the SD of the population. High performing piglets were selected from a subpopulation of piglets with a predicted BW at the end of the nursery phase (10 weeks of age) above the mean plus one time the SD of the population. In addition, piglets selected were healthy based on visual judgement, as described below and clinical blood chemistry, and were balanced for sex and litter of origin. One piglet per litter was selected in each performance group. The predicted BW at the end of the nursery phase was 19.0 ± 1.0 and 29.6 ± 1.5 kg BW for the LP and HP piglets respectively.

Visual judgement of health was conducted at 40 ± 1.1 days of age, and all piglets were judged on alertness, appearance of a round belly, presence of nasal and eye secretions, ocular and oral mucosa, colour and hair brightness. On days 40 and 69 ± 1.1 of age, 10 mL of blood were collected in serum gel and K-EDTA tubes from the jugular vein for determination of clinical chemistry parameters in blood plasma, as described in Paredes *et al*. (2014). To discard piglets with signs of inflammation, acute-phase proteins (haptoglobin and C-reactive protein) were determined. Clinical blood parameters were determined using standard procedures by Synlab (Augsburg, Germany). Haptoglobin and C-reactive protein concentrations were analysed by Synlab (Augsburg, Germany) using ELISA. Piglet selection was done based on predicted BW, health status (visual judgement and blood chemistry) and sex. Piglets in both groups were considered healthy by visual observation and veterinarian check throughout the study.

Based on the criteria described above, 60 piglets (30 LP and 30 HP piglets) were fed either a high fibre, low protein quality diet (SO), or a low fibre, high protein quality control diet (CO) from six to ten weeks of age.

Housing and husbandry during trial

Piglets were caged individually in 0.8 m^2 pens and randomly distributed over three similar climate-controlled departments with 20 cages each. Piglets had *ad libitum* access to feed and water. During the first 24 h after arrival in the facility lights were on continuously. Thereafter, a 16/8 h light/dark scheme was provided. Piglets were able to interact with each other through the barred pen divisions and were offered toys as environmental enrichment.

Feeding

From day 14 to 21 of age, all piglets were provided with a commercial creep feed presented as gruel (2:1 water: feed) and replaced twice daily to promote feed intake. From day 21 to 31 of age all piglets were fed a commercial weaner diet. From day 32 to 35 of age a gradual transition (75:25, 50:50, 25:75, 0:100) to the CO diet took place to allow all the piglets sufficient time to adapt to the new diet. From six to ten weeks of age, 15 HP and 15 LP piglets were fed the CO diet and 15 HP and 15 LP piglets fed the SO diet. The CO diet was formulated with high protein-quality raw materials (e.g. soy protein concentrate, potato protein) to be highly digestible and palatable and to exceed NRC requirements (1998). The SO diet was formulated with low protein-quality raw materials; equal SID amino acid: NE ratio, at a lower NE level and a higher fibre level (soybean meal, DDGs, canola, sunflower seed meal). The diet composition is given in Table 4.1). Fibrous raw materials were chosen to be high in non-starch polysaccharides (**NSP**).

Feed refusal was measured from six to ten weeks of age, when piglets were individually housed, and the feed intake was corrected by subtracting the amount weighed as refusal.

Evaluated parameters

Immuno-reactive trypsin (**IRCT**) concentration in blood plasma was analysed at six and ten weeks of age after one h of feed withdrawal. Apparent total tract digestibility was measured at the end of the nursery

phase (ten weeks of age). Ileal digesta was collected after euthanasia (procedures described below).

Table 4.1 Ingredient (g/kg feed), nutrient composition (%) and net energy (MJ/kg) of the experimental diets (as fed) from six to ten weeks of age[1]

Ingredient	Control CO[2]	Sub optimal SO[3]
Maize	300.0	300.0
Wheat	260.6	33.1
Soy protein concentrate	110.0	--
Barley	100.0	100.0
Wheat gluten meal	50.0	--
Potato protein	35.7	--
Soya oil	37.8	23.7
Beet pulp	30.0	30.0
Sugar	20.0	20.0
Mono calcium phosphate	12.3	9.1
Vitamin and mineral blend[4]	11.9	11.9
Amino acid blend[5]	11.3	9.7
Sodium bicarbonate	7.5	0.6
Limestone	4.9	2.1
Calcium formiate	3.0	3.0
Citric acid	3.0	3.0
Sodium chloride	2.0	5.0
Sunflower seed meal	--	150.0
Canola (Rapeseed meal)	--	50.0
Soy bean meal	--	98.8
DDGS maize	--	150.0
Calculated nutrient composition		
Crude protein	20.8	20.8
Net energy	10.54	9.36
Crude fat	6.20	6.20
Ratio SID[6] Lysine:Net energy	1.42	1.42
SID Lysine	1.49	1.36
SID Methionine + Cysteine	0.77	0.68

SID Threonine	0.82	0.73
SID Tryptophan	0.25	0.22
Calcium	0.70	0.65
Phosphorus	0.60	0.75
Digestible P, %	0.33	0.29
NSP	298	383

[1] Based on Centraal Veevoeder Bureau (CVB), 2007
[2] CO = control diet
[3] SO = suboptimal diet
[4] Provided the following per kg of diet: Vitamin A 8,000 IU; Vitamin D3 2,000 IU; Vitamin E 30 mg, Pantothenic acid 12 mg; Vitamin K3 1.5 mg; Vitamin B1 1 mg; Vitamin B2 4 mg; Vitamin B6 1 mg; Vitamin B12 20 mcg; Nicotinic acid 20 mg; Folic acid 0.3 mg; Choline Chloride 250 mg; Cobalt 0.15 mg as Basic cobaltous carbonate, monohydrate; Copper 160 mg as Cupric sulphate pentahydrate; Iron 100 mg as Ferrous sulphate, monohydrate; Iodine 1 mg as Calcium iodate, anhydrous; Manganese 30 mg as Manganese oxide; Zinc 100 mg as Zinc sulphate; Selenium 0.3 mg as Sodium selenite
[5] Provided the following per kg of diet: CO diet: DL-methionine, 1.4 g; L-lysine, 7.0 g; L-threonine, 1.9 g; L-tryptophan, 0.5g; and L-valine, 0. 5g. SO diet: DL-methionine, 0. 8g; L-lysine, 6.5g; L-threonine, 1.4g; L-tryptophan, 0.4 g; and L-valine, 0.2 g
[6] SID: standardized ileal digestibility

Parameters measured in LP and HP piglets after selection

From 64 to 70 days of age, chromium oxide (3 g/kg) was included in both dietary treatments as an indigestible marker for digestibility measurements. From day 67 to 69, faeces were collected from each piglet by rectal stimulation once per day, obtaining similar amounts per day. Samples were kept at 4° C and pooled over the 3-day period within piglet, and subsequently mixed to obtain a homogenized sample for determination of apparent total tract digestibility of, DM, N, crude fat, starch and NSP. At the end of the tenth week of age, all piglets were sacrificed by an intracardiac injection of pentobarbital sodium (Euthanasol 40% ASTfarma, B. V. Oudewater, the Netherlands), at a dose of 200 mg/kg BW. After euthanasia, ileal contents (11 cm from the ileocaecal valve) were collected to determine the apparent ileal digestibility of DM, N and starch. Ileal samples were pooled to obtain 7 samples per group x diet to ensure enough sample material was available for the analytical procedures.

Collected faecal and ileal samples were freeze-dried, feed samples were vacuum-dried at 80° C and air-dry faecal and ileal samples were dried in a forced air oven at 103° C. All samples were dried to a constant weight, according to ISO Standard 6496 (1998). Following freeze-drying, faeces and ileal samples were ground to pass a 1-mm screen and kept for analysis. Crude ash content was determined in feed, faeces and ileal content. Samples were carefully incinerated in a muffle furnace by slowly increasing the temperature from 20 to 550° C to prevent foaming, and subsequent incineration took place according to ISO Standard 5984 (2002). Crude fat content was determined after acid hydrolysis in feed and in freeze-dried faeces according to ISO Standard 6496 (1998). The starch content in feed, freeze-dried faeces and ileal content was analysed enzymatically as described by Rijnen *et al*. (2001). Non-starch polysaccharide and uronic acid content were analysed as described by de Vries *et al*. (2014). All analyses were carried out in duplicate. The non-glucose polysaccharides (**NGP**) were calculated by the sum of all carbohydrate residues excluding glucose.

Immuno-reactive trypsin was measured by a two-step competitive ELISA using a 96-well ELISA plate (Nunc International, Roskilde, Denmark) coated with 100 µl porcine cationic trypsin (Novo Chemicals, Bagsvaerd, Denmark). A round-bottomed 96-well plate (Nunc, dilution plate) was loaded with 50 µl plasma samples, diluted 1:10 in ELISA buffer (0.01 M PBS, 0.05% BSA, 0.05% Tween 20) with a trypsin standard series ranging from 2 - 250 ng/mL along with 150 µl of a rabbit antiserum against porcine cationic trypsin (Department of Animal Physiology, Lund University, Sweden) following dilution 1:10000 in ELISA buffer and incubated overnight at 4° C. On the next day, the ELISA plate was washed (0.09% NaCl, 0.05% Tween 20) and 50 µl of ELISA buffer was added to each of the wells along with 150 µl of the samples from the corresponding wells of the dilution plate, and then incubated at room temperature for 1 h. The plate was washed, followed by addition of 200 µl of an alkaline phosphatase-conjugated secondary antibody (porcine anti rabbit IgG, Dako, Denmark) at a 1:5000 dilution. The plate was incubated for 1 h at room temperature and washed before 200 µl of the substrate solution, *p*-nitrophenyl phosphate (Sigma,

St. Louis, USA) was added. A 0.9 mg/mL dissolved in 0.1 M glycine buffer, was added to the wells. The reaction was followed at a wave length of 405 nm and a standard curve was generated. The sample concentrations are interpolated based on the corresponding absorption values.

Statistical analysis

Piglet growth performance and feed intake data collected over the 4-week period (6 to 10 weeks of age) were statistically analysed. For the 4-week period, digestibility data and IRCT plasma concentrations, differences between performance groups were evaluated including performance group, sex, and diet, sex x group and diet x group interaction as independent variables using the MIXED procedure in SAS (version 9.3, 2011). The sex x diet interaction was included in the model, but as it was not significant for any of the evaluated parameters, it was removed from the final model. Clinical chemical blood parameters and concentration of acute phase proteins were analysed using MIXED models determining the differences between performance groups by week and performance group x week interaction.

RESULTS

Selection criteria

The selected piglets originated from a pool of 470 piglets at 6 weeks of age. The selected piglets were clinically healthy, and remained healthy throughout the study. Values for the clinical chemical blood parameters were within the range of normal values for piglets (Kraft, 2005). Also concentrations of acute phase proteins were within the range for healthy piglets of this age (Biocheck GmbH, 2005).

Parameters measured in LP and HP piglets during trial

The LP piglets had a 25% lower ADG compared to the HP piglets during the 4-week period ($P < 0.001$). No interaction between group and sex was observed in ADG. Expressed relative to BW, ADG was 12% higher for the LP piglets from six to ten weeks of age ($P < 0.001$; Table 4.2).

Average daily feed intake was 32% lower for the LP piglets for the 4-week period compared to HP piglets ($P < 0.001$). Total feed intake for the 4-week period expressed as percentage of BW was 0.12 g/kg BW higher for the HP piglets ($P < 0.001$). Total feed intake was 0.11 g/kg BW higher for the SO fed piglets ($P < 0.001$). Gain: feed was 0.06 higher for the LP piglets during the 4-week period ($P < 0.001$). A higher G:F was observed for the CO fed piglets when compared to the SO fed piglets ($P < 0.001$; Table 4.2).

The IRCT plasma concentration was reduced in the LP piglets at 6 weeks of age (LP = 102 vs. HP = 159 ng/mL; $P = 0.007$) but not at 10 weeks of age (Table 4.3). Apparent total tract and ileal protein digestibility was lower for the SO fed piglets regardless the group ($P = 0.240$). There was also a slightly lower fat and starch apparent total tract and ileal digestibility for the SO fed piglets. Other macronutrient digestibility was unaffected by piglet group, with the exception of fibre digestion. Whereas NSP digestion was only numerically lower for LP piglets ($P < 0.100$), NGP digestion was lower for LP piglets ($P = 0.020$), mainly caused by a lower digestion of xylose and uronic acid (Table 4.3). There was a diet interaction effect for the LP piglets (group x diet interaction, $P = 0.060$ and 0.050 respectively).

Table 4.2 Performance of predicted Low (LP) and High (HP) performing piglets from 6 to 10 weeks of age fed a control (CO)[1] or a suboptimal diet (SO)[2]

Diet	**CO**		**SO**			***P* value**		
Group	**LP**	**HP**	**LP**	**HP**	**SE**	**Group**	**Diet**	**Diet x Group**
BW, kg								
Six weeks[3] age	6.65	12.30	6.74	12.30	0.2	**<0.001**	0.929	0.840
Ten weeks[3] age	22.43	32.63	21.15	32.26	0.7	**<0.001**	0.608	0.510
ADG[4] 6 to 10 weeks of age								
g/d	544	701	497	688	20	**<0.001**	0.133	0.429
g/kg $BW^{0.75}$/d	67	73	69	73	3	**<0.001**	0.260	**<0.001**
ADFI[5] 6 to 10 weeks of age								
g/d	670	950	644	985	27	**<0.001**	0.876	0.274
g/kg $BW^{0.75}$/d	85	99	87	105	2	0.552	*0.072*	0.229
Gain:feed 6 to 10 weeks of age								
g/g	0.81	0.74	0.77	0.70	0.02	**<0.001**	**0.014**	0.978

[1] CO = control diet (208 g/kg CP, 10.5 MJ NE and 14.9 g/kg standardized ileal digestible lysine)
[2] SO = suboptimal diet (208 g/kg CP, 9.4 MJ NE and 13.6 g/kg standardized ileal digestible lysine)
[3] Six weeks = 40 ± 1.0 days after birth, when piglets were individually housed. Ten weeks = 60 ± 1.0 days after birth
[4] ADG = average daily gain
[5] ADFI = average daily feed intake

Table 4.3. Immuno-reactive trypsin (IRCT) concentration in blood plasma, apparent total tract and ileal digestibility (%) at the end of the study, ten weeks of age, for Low (LP) and High (HP) performing piglets fed a control (CO)[1] or a suboptimal diet (SO)[2]

Diet	**CO**		**SO**			**P values**		
Group	**LP**	**HP**	**LP**	**HP**	**SE**	**Group**	**Diet**	**Diet x Group**
IRCT[3] 10 weeks of age, ng/mL	177	215	192	204	16	0.131	0.927	0.419
Ileal digestibility, %								
Starch	94.4	95.2	96.5	95.9	0.9	0.908	0.129	0.461
Protein	78.9	82.7	63.8	58.5	2.3	0.770	**<0.001**	*0.070*
Apparent total tract digestibility, %								
Fat	82.9	82.5	81.8	81.7	0.5	0.571	**0.043**	0.718
Starch	99.3	99.2	99.0	98.9	0.1	0.636	**0.016**	0.853
Protein	84.9	84.3	70.6	70.1	0.7	0.411	**<0.001**	0.989
NSP[4]	87.6	87.6	83.0	84.1	4.2	0.243	**<0.001**	0.211
Rhamnose	80.2	80.5	79.6	78.3	0.9	0.589	0.123	0.346
Arabinose	89.6	89.3	81.3	81.8	0.6	0.681	**<0.001**	0.363
Xylose	80.6	81.2	73.1	76.3	0.6	**0.009**	**<0.001**	*0.057*
Galactose	90.9	90.8	85.5	86.9	0.3	*0.052*	**<0.001**	**0.024**
Uronic acid	95.0	95.2	93.8	94.7	0.2	**0.025**	**0.002**	0.115
Mannose	96.9	97.1	96.1	96.0	0.4	0.809	**0.001**	0.547
Glucose	83.2	82.5	80.8	80.7	1.2	0.732	*0.086*	0.827
NGP[5]	88.9	89.1	83.8	85.3	0.3	**0.021**	**<0.001**	*0.052*

[1] CO: control diet (208 g/kg CP, 10.5 MJ NE and 14.9 g/kg standardized ileal digestive coefficient of lysine)

[2] SO: suboptimal diet (208 g/kg CP, 9.4 MJ NE and 13.6 g/kg standardized ileal digestive coefficient of lysine)

[3] IRCT: immune-reactive trypsin concentration in blood plasma after 1 h of food withdrawal

[4] NSP: non-starch polysaccharides carbohydrates are present as polymers

[5] NGP: non-glucose polysaccharides

DISCUSSION

Previous studies comparing LP and HP piglets have demonstrated that both piglet groups have an equal apparent total tract and ileal digestibility of N, GE and DM when fed a high quality diet despite their differences in performance and small intestine morphology (Paredes *et al*., 2014). We hypothesize that when imposing dietary stress to these piglets differences might appear. The aim of the present study was therefore, to investigate to what extent the difference in performance between LP and HP piglets were related to differences in digestive capacity when fed a low quality diet. For this purpose two dietary treatments were provided: a high fibre, low protein quality diet (SO, suboptimal) and a low fibre, high protein quality diet (CO, control) from six to ten weeks of age.

It was hypothesized that when fed the SO diet in the post-weaning phase, differences in performance between LP and HP piglets would be larger than with a high quality diet, as the LP piglets would meet their limitations sooner. The difference in G:F was similar for both diets and no interaction between piglet group and diet were found. No interactions between diet and piglet group were observed in ADG or ADFI, which implies that the higher ADG of the HP piglets is caused by an increased FI and is independent of the diet fed during the post-weaning phase. Jones (2012) described a 150 g/d difference in ADG and 196 g/d difference in ADFI in the post-weaning phase between the lightest and the heaviest piglets selected at weaning. We observed a 157 g/d difference in ADG and a 280 g/d difference in ADFI between LP and HP piglets, which shows a more dramatic difference in feed intake for piglets selected by a combination of birth weight, weaning weight and BW at six weeks of age, rather than just weaning weight. Feed intake was not increased in the SO fed piglets, demonstrating that both LP and HP pigs are unable to compensate for a reduced energy content by increasing their feed intake, unlike older pigs (Souza, 2013). There was a reduction in G:F, corresponding to a reduced digestibility in SO fed piglets. Comparing the diet effect, numerically, CO fed piglets had a 31 g/d higher ADG for the overall period compared to SO fed piglets (P = 0.133). Average daily feed intake was lower for the LP

piglets from six to ten weeks of age. The differences in ADG and ADFI are in agreement with previous characterization of these groups of piglets (Paredes *et al*., 2014) and literature (Rehfeldt and Kuhn, 2006; Ritacco *et al*., 1997).

The IRCT plasma concentration is used as an indirect measurement of pancreatic function (Sandström *et al*., 1986). We had speculated that IRCT plasma concentration of the SO fed piglets would be higher, as a higher pancreatic enzyme secretion might be required to digest low quality raw materials (Wenk, 2001). Yet, there were no differences between diets and no interaction between diet and piglet group. Ileal protein digestibility was lower for SO fed piglets, regardless of performance group and a slightly lower starch and fat digestibility was found. This is in agreement with a previous observation of reduction in pancreatic amylase activity by the LP piglets at 10 weeks of age when compared to HP piglets (P = 0.033; **Chapter 6**). There was a negative effect of dietary fibre content on macronutrient digestibility, in agreement with Noblet and Knudsen (1997). A reduction in apparent total tract energy digestibility is described by Pluske *et al.* (2001) when piglets are fed a high dietary fibre.

The NSP digestion, and more significantly, NGP digestion was lower for the SO fed piglets, particularly in LP piglets (diet x pig group; P = 0.052). This was particularly related to a difference in the digestibility of xylose in the NSP fraction in the SO fed piglets. Both groups of piglets had difficulties fermenting the main xylose source in the diet, being arabinoxylans and xyloglucans, which reduced their apparent total tract digestibility. Yet, the LP piglets showed the lowest xylose digestibility (P = 0.057). Based on the diet composition, we speculate the xylose remaining originated mainly from the DDG arabinoxylan (estimated 50 g xylose from DDGs/kg diet). Galactose total tract digestibility was reduced in the SO fed piglets and was more difficult to digest for the LP piglets (interaction P = 0.024). Uronic acid total tract digestibility was also reduced in the SO fed piglets (P = 0.002) and was lower for the LP piglets (P = 0.025). Whether the digestibility of fibre rich in xylosyl or uronic acid residues might be improved in LP by the use of exogenous enzymes remains to be elucidated.

The HP piglets had a higher colon absolute weight and also when expressed per kg BW (Paredes *et al*., 2014). This fact, combined with the data on the NGP digestibility and the growth performance of LP and HP piglets, it could be speculated that the HP piglets had a higher colon capacity, which allowed them to ferment a higher amount of fibre, and therefore, obtain more net energy for growth from the diet.

As differences were observed in feed intake between LP and HP piglets in absolute sense, one must carefully consider the effect of feed intake on nutrient digestibility. According to Ball and Aherne (1987), in young piglets, restricted feed intake will increase the apparent digestibility of energy and protein. In addition, increased fibre digestion in sows compared with growing pigs, is often attributed to lower feeding level in sows (Le Goff and Noblet, 2001). Apparent total tract digestibility decreases with an increase in feed intake. Our observations were in the opposite direction, especially for LP piglets. The 280 g reduction in ADFI accounted for a 1% difference in NSP digestibility between LP and HP piglets fed the SO diet. Apparently, LP piglets reduce their fibre digestion in an attempt to maintain their performance (ADG drops 47 g/d and ADFI drops 26 g/d between CO and SO fed LP piglets, whereas HP piglets have a 14 g/d decrease in ADG and a 35 g/d increase in ADFI between the CO and SO fed diet). This leads us to suggest that digestive capacity (intake of indigestible material) could be the limiting factor and lead to a reduction of feed intake in the LP piglets.

CONCLUSION

Despite the differences in feed intake, macronutrient apparent total tract digestibility, with the exception of NSP, is equal for LP and HP piglets. A slightly lower apparent total tract digestibility of fibre in a fibre rich diet suggests that intestinal fermentation is less developed in growth retarded piglets. These findings suggest that LP piglets would benefit from a higher protein low fibre diet.

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Chapter 5

Predicted high performing piglets exhibit more and larger skeletal muscle fibers

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ABSTRACT

Postnatal (muscle) growth potential in pigs depends on the total number and the hypertrophy of myofibers in skeletal muscle tissue. In a previous study an algorithm was developed to predict piglet BW at the end of the nursery phase (10 weeks of age) on the basis of BW at birth, at weaning and at 6 weeks of age. The objective of this study was to determine whether the differences in growth performance between low (**LP**) and high (**HP**) performing piglets could be the result of different skeletal muscle properties. Therefore, from a total of 368 piglets (offspring from Hypor sows bred to Topigs sires) two groups with a divergent growth performance were selected at 6 weeks of age: HP (n = 20, predicted BW at 10 weeks of age 26.8 - 30.9 kg) and LP (n = 20, predicted BW at 10 weeks of age 16.0 - 22.9 kg). Piglets were euthanized at 10 weeks of age and samples of the *Semitendinosus* muscle (**STN**) were collected for histochemistry and gene expression analysis using qPCR. At 10 weeks of age, realized BW did not differ from predicted BW in both groups ($P > 0.88$). The HP piglets exhibited greater ADG and ADFI from 6 to 10 weeks of age, and greater BW at birth, 6 and 10 weeks of age ($P < 0.02$) compared with the LP piglets, whereas G:F ratio was similar between groups ($P = 0.42$). Superior growth performance of HP piglets was associated with a 1.27-fold higher IGF1 plasma concentration at 10 weeks of age compared with the LP piglets ($P = 0.04$). The greater weight and muscle cross-sectional area of STN in HP piglets was due to a 1.19-fold increase in total muscle fiber number (**TFN**; $P = 0.01$) and 1.34-fold increase in fiber cross-sectional area (**FCSA**; $P < 0.05$) compared with the LP piglets. The number of myonuclei per fiber tended to be greater in the HP piglets ($P = 0.09$), but the nucleus-to-cytoplasm ratio was unaffected by the performance group ($P = 0.80$). The mRNA expression of PCNA, PAX7, MYF5, and MYOD did not differ between groups ($P \geq 0.33$). However, IGF2 specific mRNA expression tended to be higher in the HP piglets ($P = 0.10$). The greater myofiber number, the higher degree of myofiber hypertrophy, and the increased muscular mRNA expression of IGF2 indicate that HP piglets exhibit a greater capacity for lean accretion and may grow faster until market weight. In conclusion, piglets that were selected for predicted higher BW at

10 weeks of age using a complex selection model had a superior muscularity in terms of greater TFN and FCSA, which may be of advantage for lean mass accretion in further life and for meat quality.

Keywords: growth, IGF, muscle fiber number, pig, skeletal muscle, weight prediction

INTRODUCTION

In the past years, within-litter variation in birth weight of piglets has increased due to a higher number of piglets born per sow per year (Foxcroft *et al*., 2007). However, farmers want to have a homogeneous litter at the end of the nursery phase (10 weeks of age). Body weight at the end of the nursery phase can be accurately predicted using an algorithm based on the data analysis of three datasets (n = 77,868 individual records) were we identified season of birth, BW at birth, at weaning and at 6 weeks of age as main factors for the prediction (Paredes *et al*., 2012).

From literature it can be derived that BW or muscle weight at birth mainly correlates with the number of myofibers formed during prenatal myogenesis (Gondret *et al*., 2006). In contrast, postnatal muscle growth is mostly associated with the hypertrophy of the existing fibers (reviewed by Rehfeldt *et al*., 2000) that in turn is associated with satellite cell activity and myonuclear accumulation (Allen *et al*., 1979). The emphasis to fiber number or fiber size alone could both limit lean mass because of their trait antagonism (Rehfeldt *et al*., 2004). Therefore, it was of interest to determine how the predicted low or high performance in piglets by the model of Paredes *et al*. (2012) would be reflected in muscle microstructure. The muscle development and growth are regulated by the IGF system (Kalbe *et al*., 2008; Oksbjerg *et al*., 2004) and the muscle regulatory factors (**MRF**; te Pas and Soumillion, 2001). Our own studies revealed that IGF2 could play a promoting role both in prenatal myogenesis and postnatal muscle growth (Kalbe *et al*., 2013). The aim of this study was to analyse muscle microstructure as well as mRNA expression of candidate genes for muscle growth and development in predicted low or high performing piglets at 10 weeks of age on the basis of BW at birth, at weaning, and at 6 weeks of age and to determine whether muscle characteristics would play a role in the growth retardation.

MATERIAL AND METHODS

The experimental procedures with animals described in this study were conducted at the Nutreco Swine Research Centre (Sint Anthonis, the Netherlands) and were approved by the Animal Care and Use Committee of Utrecht University, the Netherlands.

Animals

The piglets used for this study originated from 35 litters born in October 2011, from Hypor Libra sows (first to fourth parity) bred to Topigs P-line sires. One day before the expected farrowing date, all sows were treated with an intramuscular injection of 5 mg dinoprost (Dynolitic, Zoetis, Capelle aan den IJssel, the Netherlands) to synchronize the farrowing process. The average number of piglets born alive per litter was 13.5 ± 2.6. All piglets (n = 454) were weighed individually and identified immediately after parturition ended with an ear tag. At the same time piglets received a 0.3 mL intramuscular injection of ampicillin (Albipen, Intervet International, B. V., the Netherlands) and 100 mg iron (Ursoferran, Serumwerk Bernburg AG, Bernburg, Germany). Birth date, individual time of birth of each piglet, and total duration of parturition were recorded. Cross-fostering was avoided unless the sow did not have a sufficient number of functional teats on the udder to nourish all piglets born alive. This procedure was based on sow functioning mammary glands, and this was checked when they entered the farrowing room. In this case, the heaviest piglets of the litter were transferred to a foster sow within 48 h after birth. Males were anaesthetized and castrated on day 5 of age according to farm procedures. Piglets were weaned at 21.6 ± 1.0 days of age and transferred to the nursery facilities were they remained with their littermates until they reached 6 weeks of age. Due to morbidity and mortality in the farrowing room, only 430 piglets remained at weaning.

Selection of high and low performing piglets

Piglets with a birth weight below the mean minus two times the SD from the mean of the total population were excluded from the study. These piglets were considered as intrauterine growth retarded piglets (McMillen

et al., 2001; Paredes *et al*., 2012). From weaning until 6 weeks of age 62 piglets were excluded as they showed signs of weakness according to the evaluation of health status, described in detail in Table 3.2, **Chapter 3**. At 6 weeks of age, 368 piglets were available for selection of the experimental piglets. Based on the algorithm developed by Paredes *et al*. (2012) BW at birth, weaning and 6 weeks of age were included to predict the body weight at the end of the nursery phase (10 weeks of age) in 2 divergent populations of piglets (low performing, **LP,** and high performing, **HP**; each n = 30) balanced for sex and litter of origin.

The two experimental populations were selected when the piglets were 6 weeks (41.6 ± 1.0 days) of age. Piglets were regarded as LP if their predicted BW at 10 weeks of age was between 16.0 and 22.9 kg and HP piglets if their predicted BW at 10 weeks of age was between 26.8 and 30.9 kg. At 10 weeks of age, 20 piglets per group category were randomly selected to be sacrificed with an intracardiac injection of sodium pentobarbital (Euthasol 40%, ASTfarma, B. V. Oudewater, the Netherlands) at a dose of 200 mg/kg BW. The right semitendinosus muscle (**STN**) was excised within 5 min post mortem and its weight, length and circumference were recorded. Samples from the central portion of the muscle mid-belly were snap-frozen in liquid nitrogen and thereafter stored at -80° C for histochemical and gene expression analyses.

Feeding

All piglets had access to a commercial creep feed presented as gruel (2:1, water:feed), replaced twice a day to stimulate feed intake, and supplied from day 14 of age until weaning. From weaning until 10 days post-weaning all piglets were fed with a commercial weaner diet. From day 11 to 14 after weaning a gradual transition (75:25, 50:50, 25:75, 0:100) to a second diet (calculated composition described in Table 5.1) took place. The experimental diet was formulated to meet or exceed requirements of National Research Council (**NRC**, 1998) using highly digestible protein sources.

Table 5.1 Calculated nutrient composition[1] (%) and net energy (MJ) of the experimental diet[1] (as fed) from six to ten weeks of age[2]

Item	Second phase diet
Crude protein	17.5
Crude fibre	3.1
Lactose	6.0
Net energy	10.7
AID[3] Lysine	1.4
AID Methionine + Cysteine	0.8
AID Threonine	0.8
AID Tryptophan	0.3
Apparent phosphorus digestibility	0.4

[1] Based on Centraal Veevoeder Bureau (**CVB**), 2007

[2] Provided the following per kg of diet: Vitamin A 8,000 IU; Vitamin D3 2,000 IU; Vitamin E 30 mg, Pantothenic acid 12 mg; Vitamin K3 1.5 mg; Vitamin B1 1 mg; Vitamin B2 4 mg; Vitamin B6 1 mg; Vitamin B12 20 mcg; Nicotinic acid 20 mg; Folic acid 0.3 mg; Choline Chloride 250 mg; Cobalt 0.15 mg as Basic cobaltous carbonate, monohydrate; Copper 160 mg as Cupric sulphate, pentahydrate; Iron 100 mg as Ferrous sulphate, monohydrate; Iodine 1 mg as Calcium iodate, anhydrous; Manganese 30 mg as Manganese oxide; Zinc 100 mg as Zinc sulphate; Selenium 0.3 mg as Sodium selenite

[3] AID = apparent ileal digestibility

Animal housing and performance

Piglets selected for the study (n = 60) were placed individually in 0.8 m^2 pens and randomly distributed over three similar climate-controlled departments with 20 pens each. Piglets had *ad libitum* access to feed and water. During the first 24 h after arrival to the experimental facility lights were on continuously. Thereafter, a 16/8 h light/dark scheme was provided. Piglets were able to interact through the barred pen divisions and were offered toys to enhance welfare.

Piglet performance was analysed weekly from 6 to 10 weeks of age. Piglets were weighed at birth, at weaning (21.6 ± 1.0 days) and weekly during the post-weaning phase until the end of the study (69.6 ± 1.0 days). Feed intake per piglet was determined daily and calculated on a weekly basis from six to ten weeks of age.

Muscle histochemistry and microscopy

Muscle cross-sectional area (**MCSA**) of STN was calculated from the circumference of the muscle mid-belly. Serial transverse sections of 10 µm were cut at -20° C in a cryostat (Leica, Nussloch, Germany). One section was stained for cytoplasm and nuclei with eosin and haematoxylin, respectively. Another section was exposed to the reaction for NADH-tetrazolium reductase (Novikoff *et al*., 1961) which enables classification into red oxidative, intermediate oxidative and white glycolytic fibers. Fiber type distribution, fiber cross-sectional area (**FCSA**), and myonuclear distribution were determined on 300 muscle fibers per piglet by image analysis (AMBA, IBSB, Berlin, Germany). The estimated total fiber number (**TFN**) was obtained by multiplying the fiber number per unit area by the MCSA of STN muscle. Microscopic analyses were all done by the same person.

RNA isolation, reverse transcription (RT) and qPCR of myogenesis-associated genes

Total RNA was isolated from STN muscle tissue with RNeasy fibrous Mini Kit (Qiagen, Hilden, Germany), as recommended by the supplier. This procedure includes the removal of genomic DNA with RNase-free DNase. The RNA was quantified in a NanoDrop instrument (Peqlab, Erlangen, Germany). Quality of RNA was monitored from randomly selected samples (n = 27) using the Experion Automated Electrophoresis System (Biorad, München, Germany) according to the manufacturer's protocol. All samples were classified by a RNA quality indicator (RQI; 10 = intact RNA, 1 = highly degraded RNA) in the best category ($7 < RQI \leq 10$).

Reverse transcription was carried out with 2 µg of total RNA preparation, a mixture (2:1) of random primer $p(dN)_6$ and anchored-oligo $(dT)_{18}$ primer (Roche, Mannheim, Germany), and Moloney mouse leukemia virus reverse transcriptase (M-MLV RT RNase H Minus Point Mutant, Promega, Mannheim, Germany) in 25 µl of the incubation buffer provided by the supplier, supplemented with deoxy-NTPs (Roche, Mannheim, Germany) and RNasin (Promega, Mannheim, Germany), for 60 min at 42° C. The freshly synthesised cDNA samples were cleaned with the High Pure PCR

Product Purification Kit (Roche, Mannheim, Germany) and eluted in 50 µl elution buffer.

Transcript expression of myogenic factor 5 (**MYF5***)*, myogenic differentiation factor (**MYOD**), insulin growth factor 2 (**IGF2**), paired box 7 (**PAX7**), proliferating cell nuclear antigen (**PCNA**) and TATA box binding protein (**TBP**) genes was measured. For qPCR, 1.25 µl of each purified cDNA sample was amplified in duplicate with the Light Cycler-FastStart DNA Master^{PLUS} SYBR Green I kit (Roche, Mannheim, Germany) in 10 µl total reaction volume. Primer information was described elsewhere (Kalbe *et al.* (2008): IGF2; Rehfeldt *et al*. (2012a): MYF5, MYOD; Erkens *et al*. (2006): TBP and Patruno *et al*. (2008a): PAX7). Primers used to amplify 173 bp from PCNA (GenBank accession no. DQ473295) mRNA were 5'-ACGCTAAGGGCAGAAGATAATGCAG (forward) and 5'-CGTGCAAATTCACCAGAAGGCATC (reverse). All primers were purchased from Sigma-Genosys (Steinheim, Germany) and if possible, were derived from different exons to avoid amplification of residual genomic DNA.

Amplification and quantification of generated products were performed in a Light Cycler instrument 2.0 (Roche, Mannheim, Germany) under the following cycling conditions: pre-incubation at 95° C for 10 min, followed by 40 cycles denaturation at 95° C for 15 s, annealing for 10 s at 57° C (IGF2), 58° C (PAX7), 59° C (TBP) or 60° C (MYF5, MYOD, PCNA), extension at 72° C for 10 s and single point fluorescence acquisition for 6 s in order to avoid quantification of primer artefacts. The melting peaks of all samples were routinely determined by melting curve analysis to ascertain that only the expected products had been generated. Additionally, molecular sizes of PCR products were monitored by agarose gel electrophoresis analysis (not shown). To normalize for variations between individual Light Cycler runs an arbitrarily selected sample were co-amplified as calibrator.

The relative quantification was performed with the Light Cycler software version 4.5 using the quantification module: Relative Quantification – Monocolor. Thereby, the relative expression ratio of the target gene is calculated based on the PCR efficiencies and the crossing point deviation of

an unknown sample versus the calibrator, and expressed in comparison to an endogenous reference gene (TBP) as described by Pfaffl (2001). The TBP expression was not affected by group (P = 0.750) or by sex (P = 0.400).

To calculate the PCR efficiency, routine dilutions of the gene-specific external standard (cloned PCR products) of known concentrations covering 5 orders of magnitude (5×10^{-16} to 5×10^{-12} g DNA) were co-amplified during each run. Sequencing was performed with the automated sequencing system ABI PRISM 310 genetic analyser using the ABI PRISMBig Dye kit (both from PE Applied Biosystems, Weiterstadt, Germany).

Analysis of IGF1 in plasma

One day before piglets were euthanized, blood samples were collected via venipuncture after one hour of fasting. Plasma samples were stored at -20° C before analyses. Plasma IGF1 concentrations were determined using a competitive electro-chemiluminescence immunoassay as described by Rehfeldt *et al*. (2001).

Statistical analysis

The UNIVARIATE procedure of SAS (version 9.1, 2002) was used to test residuals for normality with greater P values for Shapiro-Wilk test indicating the normal distribution of the data. Differences in performance between groups were evaluated using the MIXED procedure in SAS. The statistical model was designed including group (LP or HP), sex, and their interaction (group $\times$ sex) as independent variables. Differences between calculated and real BW at 10 weeks of age were analysed using the GLM procedure of SAS including group (LP or HP) and BW determination (predicted or realized BW). Muscle characteristics (micro-structure and mRNA expression) and plasma IGF1 were analysed using the GLM procedure of SAS, as these data represented a single time point, including group, sex and their interaction as independent variables. The correlation between IGF1 and ADG was assessed by a Pearson correlation test using the CORR procedure in SAS. In all cases, piglet was the experimental unit and differences between means were tested by PDIFF with Tukey

adjustment. Significant differences were identified at $P < 0.050$ and trends at $P < 0.100$.

RESULTS

Performance

There was no difference in predicted and realized BW at 10 weeks of age [LP = 19.1 kg (range 16.0 to 22.9 kg) predicted vs. 19.0 ± 0.8 kg (range 16.0 to 21.9 kg) realized; P = 0.912; HP = 29.2 kg (range: 26.8 to 30.9 kg) predicted vs. 29.8 ± 0.8 kg (range 27.3 to 31.0 kg) realized; P = 0.880]. High performing piglets displayed greater BW than piglets of the LP group at birth and 6 and 10 weeks of age ($P \leq 0.002$; Table 5.2).

Average daily gain and ADFI from 6 to 10 weeks of age were about 1.4-fold greater in the HP piglets compared with the LP piglets ($P < 0.001$). However, no differences in the computed G:F ratio between groups were found (P = 0.417). At birth, female piglets were heavier than the males (P = 0.045), but no sex effect on the other growth performance traits was observed. No significant group × sex interactions were apparent for any performance traits ($P \geq 0.225$).

The concentration of plasma IGF1 was 1.27-fold greater (P = 0.044) in the HP piglets when compared with the LP piglets (Table 5.2). No effects of sex (P = 0.727) and no interaction of sex x group (P = 0.751) were observed. In addition, there was a positive correlation between plasma IGF2 and ADG during the observational period (Table 5.3). This positive correlation was more pronounced in LP piglets (r = 0.585; P = 0.007) than in HP piglets (r = 0.473; P = 0.035).

Structural properties of skeletal muscle

The weight of STN muscle was 1.7-fold greater in HP piglets compared with LP piglets (116.9 ± 4.1 g vs. 69.7 ± 4.1 g; $P < 0.001$). However, relative muscle weight expressed as a percentage of BW was equal for both piglet groups (P = 0.151). High performing piglets had a 58% larger STN MCSA compared with LP piglets and differed in selected microstructural characteristics of STN muscle at 10 weeks of age. Thus, HP piglets exhibited a 20% greater TFN (P = 0.009) and a 34 % larger FCSA (P = 0.004) compared with the LP piglets (Table 5.3). The difference in average FCSA resulted

from nearly equal relative differences in the FCSA of red, intermediate and white fibers. There was a numerically higher number of nuclei per fiber (P = 0.102) in the HP piglets compared with the LP piglets, which was mainly due to differences in red (P = 0.087) and intermediate (P = 0.097) fibers. No differences in the FCSA per nucleus were found, which means there was an unchanged nuclei-to-cytoplasm ratio with increasing fiber size. The performance group of the piglets did not affect the proportions of the fiber types. The sex of the piglets had no influence on any microstructural muscle characteristics examined ($P \geq 0.142$) with the exception of the proportion of red fibers ($P \geq 0.093$), which tended to be greater in castrated males than in females. No interaction of sex x group were detectable ($P \geq 0.163$).

Table 5.2 Body weights, growth performance, and plasma IGF1 concentration of predicted Low (LP) and High (HP) performing piglets from 6 until 10 weeks

	Group			Sex			*P* value	
	LP	**HP**	**SE**	**Castrate**[1]	**Female**	**SE**	**Group**	**Sex**
BW, kg								
Birth	1.2	1.6	0.1	1.3	1.5	0.1	**<0.010**	**0.045**
Six wk[2]	6.8	12.2	0.2	9.5	9.5	0.2	**<0.001**	0.870
Ten wk[3]	19.0	29.8	0.8	25.0	23.8	0.8	**<0.001**	0.280
ADG 6 to 10 wk, g/d	436	629	25	554	510	26	**<0.001**	0.219
ADFI 6 to 10 wk, g/d	635	901	29	793	743	30	**<0.001**	0.229
G:F 6 to 10 wk, g/g	0.68	0.69	0.01	0.69	0.68	0.01	0.417	0.309
Plasma IGF1[3], ng/mL	339	431	31.2	393	378	32.5	**0.044**	0.727

[1] Castrate = castrated males

[2] Six wk = start of the observational period, 41.6 ± 1.0 days of age, when piglets were individually housed

[3] Ten wk = end of the observational period, 69.6 ± 1.0 days of age

Gene expression in skeletal muscle

The mRNA expression of PCNA, PAX7, MYF5, and MYOD did not differ between groups (Table 5.4). However, IGF2 specific mRNA expression was numerically higher in the HP piglets than in the LP piglets (P = 0.101). No effect of sex and no group x sex interaction were observed for the gene-specific mRNA expression.

Table 5.3 Micro-structural characteristics of the right *Semitendinosus* muscle of predicted Low (LP) and High (HP) performing piglets at 10 weeks of age[1]

	Group			Sex			*P* values	
	LP	**HP**	**SE**	**Castrate**	**Female**	**SE**	**Group**	**Sex**
Weight, g	69.7	116.9	4.1	93.0	93.5	4.2	**<0.001**	0.928
Circumference, cm	11.0	13.9	0.3	12.5	12.4	0.3	**<0.001**	0.827
MCSA[2], cm²	9.8	15.5	0.6	12.7	12.6	0.6	**<0.001**	0.912
Total fiber number, thousands	666	799	35	754	712	35	**<0.010**	0.385
Proportion of fiber types, %								
Red	27.6	31.2	2.7	32.6	26.2	2.7	0.330	*0.093*
Intermediate	24.6	25.9	1.8	25.8	24.6	1.8	0.587	0.623
White	47.7	43.2	3.4	42.0	48.9	3.4	0.335	0.142
FCSA[3], µm²								
Red	1155	1527	87	1329	1353	87	**<0.010**	0.837
Intermediate	1643	2244	127	1960	1927	127	**<0.010**	0.849
White	1661	2244	154	1977	1928	154	**0.010**	0.816
Average	1499	2009	122	1738	1770	122	**<0.010**	0.847
No. of nuclei per								
Red fiber	0.7	0.9	0.1	0.8	0.8	0.1	*0.087*	0.803
Intermediate fiber	0.7	0.9	0.1	0.8	0.7	0.1	*0.097*	0.538
White fiber	0.6	0.8	0.1	0.7	0.7	0.1	0.134	0.620
Average	0.6	0.8	0.1	0.7	0.7	0.1	0.102	0.792
Average FCSA per nucleus, µ²	416	409	31	426	399	31	0.861	0.510

[1] 10 weeks = end of the observational period, 69.6 ± 1.0 days of age

[2] MCSA = Muscle cross-sectional area

[3] FCSA = Fibre cross-sectional area

Table 5.4 The mRNA expression of selected genes in semitendinosus muscle of predicted Low (LP) and High (HP) performing piglets at 10 weeks of age[1]

	Group			Sex			*P* value	
Gene[2]	**LP**	**HP**	**SE**	**Castrate**[3]	**Female**	**SE**	**Group**	**Sex**
PCNA	1.09	1.12	0.1	1.15	1.06	0.1	0.814	0.410
PAX7	1.63	1.35	0.4	1.69	1.29	0.4	0.596	0.456
MYF5	0.88	1.05	0.1	1.06	0.88	0.1	0.327	0.295
MYOD	1.35	1.26	0.2	1.16	1.45	0.2	0.716	0.218
IGF2	0.62	0.77	0.1	0.76	0.64	0.1	0.101	0.179

Data are expressed as arbitrary units after normalization by the endogenous reference gene TATA box binding protein (TBP)

[1] 10 weeks = end of the observational period, 69.6 ± 1.0 days of age

[2] PCNA, proliferating cell nuclear antigen; PAX7, paired box 7, MYF5, myogenic factor 5, MYOD, myogenic differentiation factor; IGF2, insulin growth factor 2

[3] Castrate = castrated males

DISCUSSION

The aim of the present study was to determine whether a large difference in piglet performance, low or high, at 10 weeks of age is reflected in skeletal muscle development and phenotype. We focus on the central role of the skeletal muscle as a tissue, because it represents about 50% of body mass in piglets. The question arises, whether muscle growth capacity might be the limiting factor for piglet performance. In addition, it was of interest, whether the LP and HP piglets chosen by the model of Paredes *et al*. (2012) differed in skeletal muscle properties. Previous studies demonstrated the importance of TFN for postnatal growth and carcass and meat quality in pigs (Bee, 2004; Rehfeldt *et al*., 2000). Because the STN is considered a standard muscle for the determination of TFN in pigs, muscle properties were analysed in this muscle.

Animal performance

High performing piglets showed greater ADG and greater ADFI from 6 to 10 weeks of age, resulting in similar computed G:F. The present study represented a subset of piglets (n = 40) from a parallel study (n = 60; Paredes *et al*., 2014). Our observations regarding growth performance are in agreement with the aforementioned study. As shown by Paredes *et al*. (2014), the differences in performance characteristics were not linked to nutrient digestibility.

Superior growth performance of HP piglets was associated with a greater IGF1 plasma concentration, which is in line with the positive correlation of circulating IGF1 with growth rate in pigs (Buonomo *et al*., 1987). On the other hand, a greater plasma IGF1 concentration can also result from a greater feed intake in pigs (Dauncey *et al*., 1990).

Muscle microstructure

The algorithm used for the selection of the experimental piglets (Paredes *et al*., 2012) provides a correct prediction of BW at 10 weeks of age. This selection may also yield information on muscle structure because the groups selected for distinct growth performance also differed in TFN and

FCSA. In addition, enhanced fiber hypertrophy was associated with a proportional increase in the number of myonuclei resulting in unchanged nucleus-to-cytoplasm ratio. In other cases such an enhanced muscle growth may reduce nucleus-to-cytoplasm ratio of myofibers (Rehfeldt, 2007). Remarkably, the HP piglets exhibited both a greater TFN and a greater FCSA. In general, TFN and FCSA are inversely correlated with each other at the end of the intensive growth period, meaning that the growth of individual fibers is slower when a large number of fibers is present and vice-versa (reviewed by Rehfeldt *et al*., 2000). However, both traits correlate positively with MCSA and thus contribute to the accretion of lean mass (Rehfeldt *et al*., 2004). Thus, those piglets not following this trait antagonism and simultaneously exhibiting high TFN and FCSA will grow faster, as realized by our HP piglets.

Fiber number and size are influenced by genetic factors (specie, gender, and breed) and environmental factors such as age and nutrition (reviewed by Rehfeldt *et al*., 2004). Prenatal nutrition is of specific importance, because inadequate nutrient supply in-utero not only retards fetal growth, but also impairs myogenesis. Low birth weight piglets exhibit a smaller TFN than their heavier littermates (Dwyer and Stickland, 1991; Gondret *et al*., 2006; Handel and Stickland, 1987; Rehfeldt and Kuhn, 2006). Although a further increase in TFN occurs during the first postnatal weeks (Bérard *et al*., 2011), these light piglets exhibit a lower TFN throughout life (Rehfeldt and Kuhn, 2006) and display a lower postnatal growth rate (Fix *et al*., 2010; Rehfeldt *et al*., 2008; Wolter *et al*., 2002). Postnatal lean growth depends on the number of fibers and on the degree of fiber hypertrophy. The capacity limit for lean accretion is set by the number of fibers, as this is the pre-requisite for postnatal muscle growth via fiber hypertrophy, which in turn does not continue beyond a certain limit. Once maximum fiber size is achieved, nutritional energy can no longer be used for protein accretion and is deposited as fat instead. This relationship may explain why piglets of low birth weight have deposited more fat and less lean in the carcasses at market weight and exhibit a poorer meat quality (Bee, 2004; Gondret *et al*., 2005, 2006; Rehfeldt *et al*., 2008). Therefore, one important criteria of genetic selection should be birth weight to favour piglets with a good

capacity of muscle growth, even though some light piglets exhibit similar fiber numbers as heavier piglets (Dwyer *et al*., 1993; Gondret *et al*., 2005; Rehfeldt and Kuhn, 2006). In the case that low birth weight was associated with impaired prenatal muscle development, lean accretion until slaughter would be reduced (Rehfeldt *et al*., 2012a, b). In addition, Dwyer *et al*. (1993) stated that birth weight influences growth rate during the early stages of postnatal growth whereas growth from day 70 of age until slaughter depended on TFN. When pigs were categorized within-litter, on the basis of their weight at slaughter, the low and medium weight pigs had similar birth weights and TFN, but the medium weight pigs had greater FCSA (Nissen *et al*., 2004). The pigs with the highest slaughter weight and lean mass exhibited the highest TFN but had similar fiber hypertrophy as medium weight pigs. That study showed that both TFN and a high degree of fiber hypertrophy are the pre-requisite for fast postnatal growth. Our current divergent selection model included birth weight, BW at weaning and at 6 weeks of age and generated 2 groups of piglets with differences in both TFN and FCSA at 10 weeks of age. Our model therefore, may predict differences in growth performance and carcass quality at market age. Taken together, a complex selection model seems to be superior over using only birth weight to predict further growth performance of pigs. It still remains to be investigated whether a predicted BW at 10 weeks of age is an indicator for good carcass quality at market age.

Expression of myogenesis-associated genes

Postnatal muscle growth at the age of 10 weeks is considered to result from hypertrophy by increasing diameter and length of the existing myofibers (Swatland and Cassens, 1972). Hypertrophic processes are attributed to satellite cells which provide new nuclei to growing myofibers (Mauro, 1961). These stem cells are located between the plasmalemmal membrane and the basal lamina of the myofiber (Zammit and Beauchamp, 2001). Beside their anatomic location, quiescent satellite cells are characterized by the expression of PAX7 (Seale *et al*., 2000). Activated satellite cells co-express the muscle regulatory factors MYOD (Yablonka-Reuveni and Rivera, 1994) or MYF5 (Kuang *et al*., 2007) which are markers for myogenic progenitor cells. Our study revealed no differences in the

mRNA expression of PAX7 suggesting that the amount of satellite cells is similar in LP and HP piglets at 10 weeks of age. Other studies revealed conflicting results. Ropka-Molik *et al*. (2011) found greater PAX7 mRNA expression in *Gracilis* muscle of the more muscular Pietrain pigs compared with Polish Landrace at 210 days of age, whereas Wang *et al*. (2012) showed opposite effects comparing satellite cell cultures derived from *Longissimus* muscle of Lantang pigs with those from Landrace pigs. In addition to muscularity, PAX7 expression may be related to muscular maturity, which is suggested from studies of Patruno *et al*. (2008b) who observed differences in PAX7 mRNA expression of myogenic cell cultures derived from STN of pigs at different ages. An equal proliferative activity of satellite cells in muscle of LP and HP piglets is suggested by their comparable PCNA mRNA expression. The PCNA is considered to be a proliferation marker, because the mRNA accumulates only in proliferating cells (Chang *et al*., 1990) such as satellite cells, adipocytes or nerve cells in skeletal muscle tissue. In addition, equal amounts of satellite cells are supported by the similar nucleus-to-cytoplasm ratio between LP and HP piglets. This means that the number of myonuclei increased at the same rate as FCSA in HP piglets.

Another indication of a comparable muscular maturity of the LP and HP piglets in our study is the lack of differences in the MYF5 and MYOD expression. The mRNA expression of both MRF is related to satellite cell activity at postnatal ages (Koishi *et al*., 1995; Patruno *et al*., 2008a; te Pas *et al*., 2000). Therefore, their expression is lower at postnatal than prenatal ages (Patruno *et al*., 2008a). It is difficult to compare our results with other studies, because the expression values are age and muscle (and muscle region) specific. For instance, te Pas *et al*. (2000) analysed MYF5 and MYOD mRNA in adult pigs selected for fast growth or for leanness and found no differences in the mRNA expression in the red region (near bone) of STN, whereas in the white region more MYF5 and MYOD mRNA were detected in fast growing pigs compared with lean selected ones. Therefore, they concluded, that the expression of MRF depends more on the selection for overall growth than for muscle deposition *per se*. In addition, Ropka-Molik *et al*. (2011) detected increased MYOD mRNA in different muscles of Polish

Landrace pigs compared with Pietrain pigs at market weight. Nevertheless, from our mRNA expression analyses of genes encoding satellite cell associated transcription factors, we could not conclude on a different muscle phenotype of LP and HP piglets at 10 weeks of age.

It is known that IGF2 stimulates both proliferation and differentiation of muscle cells (reviewed in Florini *et al*., 1996; Oksbjerg *et al*., 2004). In general, the mRNA expression of IGF2 in porcine skeletal muscle tissue is high in the embryo/foetus and declines in the perinatal/postnatal period (Gerrard *et al*., 1998; Lee *et al*., 1993). Nevertheless, the importance of IGF2 for postnatal skeletal muscle growth in pigs is evident from previous studies, although the underlying mechanisms are not completely understood. A paternally imprinted mutation in the IGF2 gene resulted in an increase in IGF2 mRNA expression (van Laere *et al*., 2003; Stinckens *et al*., 2007). This was associated with increased lean meat percentage related to postnatal muscle hypertrophy due to an increase in muscle fiber diameter and a higher proliferative capacity of satellite cells (van den Maagdenberg *et al*., 2008a, b). Furthermore, Rehfeldt *et al*. (2012b) found reduced IGF2 mRNA expression in *Longissimus* muscle of pigs at market age which originated from gilts fed low (6%) protein diets during gestation. Those pigs exhibited an opposite phenotype compared with that described by van den Maagdenberg *et al*. (2008a, b) because of impaired myogenesis with less myofibers and less lean and more fat in pigs of large litters. In our study, the HP piglets revealed a tendency for more IGF2 mRNA in skeletal muscle than LP piglets. This fits to their heavier STN weight, the greater MCSA, TFN, and number of myonuclei at 10 weeks of age. Examining the skeletal muscle properties of HP piglets together with the knowledge of the IGF2 associated phenotypes, there is evidence that HP piglets will exhibit more lean mass than LP piglets at market age.

CONCLUSION

Piglets selected for predicted low or high performance at ten weeks of age using a complex selection model differed in TFN, FCSA, and myonuclear numbers. As indicated by the greater myofiber number, the higher degree

of myofiber hypertrophy, and the increased muscular mRNA expression of IGF2, the HP piglets possess a greater capacity for lean accretion and are expected to exhibit faster growth until market weight and a greater lean proportion in the carcass. This, however, remains to be investigated.

The unchanged nucleus-to-cytoplasm ratio and mRNA expression of studied genes may suggest that LP and HP piglets seem to grow in a similar manner but on different BW levels from 6 and 10 weeks of age. In addition, how the growth of LP piglets could be stimulated should be investigated. The most reasonable approaches to counteract the incidence of LP piglets could be optimizing the conditions in-utero for the development of muscle and genetic selection for a simultaneous increase of TFN and FCSA.

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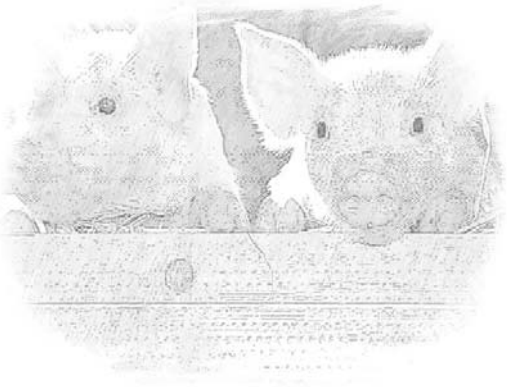
Chapter 6

Glycaemic response of piglets suffering from growth retardation after birth



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ABSTRACT

Growth retardation can occur at different phases in life (e.g. in-utero, in the pre- or early post-weaning phase). Its cause remains unclear; but its long term consequences for humans and livestock are known. For this study we focused on two populations of piglets born with an average weight, but one with lower than average gain (**AL**) compared to piglets with higher than average gain (**AH**) during the first six weeks of age. The aim of this study was to determine if growth retardation early in life affected insulin-mediated glucose tolerance regulation in the nursery phase. Eight AL and eight AH piglets with an average birth weight were selected from a group of 435 piglets at six weeks of age. At the age of 8 to 9 weeks, insulin-mediated glucose tolerance was determined using the oral glucose tolerance test (**OGTT**), oral starch tolerance test, and the hyperglycaemic clamp test. Subsequently, all piglets were sacrificed for determination of pancreatic enzyme activity. The oral starch tolerance test and the hyperglycaemic clamp at 8 mmol/L blood glucose concentration showed that the AL piglets were insulin resistant. The OGTT and hyperglycaemic clamp at 15 mmol/L blood glucose concentration showed that at hyperglycaemia AL piglets were glucose resistant. Pancreatic protein content and amylase activity were lower for the AL piglets compared to the AH group. In conclusion, piglets suffering from growth retardation in the first six weeks of age expressed insulin resistance and had diminished pancreatic amylase activity, which may in part explain their permanent growth retardation during the nursery phase.

Keywords growth retardation, glucose tolerance, insulin sensitivity, pancreas, pig

INTRODUCTION

Growth retardation in piglets' pre- and post-weaning phase is a serious problem in swine production as it increases variation in body weight gain and zootechnical performance in later stages of life. Understanding the causes of the growth check might lead to solutions for growth restoration and to allow the piglets to display their real potential for growth. Our previous work characterizing growth retarded piglets compared to heavier littermates in the nursery phase demonstrated that both groups have an equal nutrient digestibility of DM, GE, N and starch and equal feed efficiency. Growth retarded piglets, though, have a different skeletal muscle structural composition expressed by a lower fibre number and cross sectional area, predisposing them to low lean mass accretion. Growth retarded piglets also eat more frequently and consume smaller meals.

Growth retarded piglets are a heterogeneous population with a similar outcome: a time delay to reach market weight. One subcategory is piglets born with normal body weight (**BW**) but which had a lower than average gain during the first six weeks of age (**AL**). This population occupies the second major population of growth retarded piglets (**Chapter 7**). In-utero growth retardation is related to insulin insensitivity in humans and piglets. The lower insulin sensitivity is related to nutrient redistribution away from skeletal muscle to essential organs compromising growth (Cianfarani *et al*., 1999; Poore and Fowden, 2002). Given the differences observed in skeletal muscle characteristics and feed intake pattern between AL and their heavier counterpart (**AH**) piglets, we aimed to determine whether differences in insulin-mediated glucose tolerance would play a role in the growth check in the AL piglets.

MATERIAL AND METHODS

The experimental procedures applied on the animals described in the present study were conducted at Wageningen UR Livestock Research (Lelystad, the Netherlands) and were approved by the Animal Care and Use Committee of Wageningen UR in Lelystad, the Netherlands.

Animal housing and husbandry pre-trial

The piglets used in this study originated from 30 litters born in March, 2013 from Hypor sows (first to fourth parity) bred to Topigs sires in the Nutreco Swine Research Centre facilities in Sint Anthonis, the Netherlands. The average litter size at birth was 14.5 ± 3.0. All piglets (n = 435) were weighed individually and were identified with an ear tag after parturition was completed. At birth all piglets received an intramuscular injection of 15 mg/kg BW of ampicillin (Albipen, Intervet International, B. V., the Netherlands) and a 100 mg intramuscular injection of iron (Ursoferran, Trouw Nutrition International, the Netherlands). Cross-fostering was avoided unless the sow did not have a sufficient number of functional teats on the udder to nourish all piglets born alive. When needed, the heaviest piglet(s) of a litter were transferred to a foster sow within 48 h after birth. Littermates remained together during the pre- and early post-weaning phase, until the time of selection (six weeks of age). Upon selection, the piglets were transported to the experimental facilities of Wageningen UR Livestock Research in Lelystad, the Netherlands.

Selection of growth retarded and heavier littermates

Piglets were weighed at birth, one, three and five days of age, weaning (28 ± 1.0 days), six weeks of age (41 ± 1.0 days) and before each test (8 and 9 weeks of age). For the selection, BW at birth, weaning and six weeks of age were considered. At each weighing point three categories were made based on SD from the mean of the total population: Low: below the mean minus one time the SD from the mean of the total population at each time point. Average: between the mean minus one and the mean plus one time the SD from the mean of the total population at each time point. Heavy: above the mean plus one time the SD from the mean of the total

population at each time point. Within the piglets that had an average birth weight, 10 AL piglets were selected which were low at weaning and at 6 weeks of age, and 10 AH piglets were selected which were heavy at weaning and at 6 weeks of age. In both experimental groups sex was balanced (50:50 boars: gilts). Twelve littermates were included (6 AL and 6 AH) and 8 piglets from different litters, as no more littermates fulfilling our criteria were available. The weight range at six weeks of age was between 6.2 and 7.6 kg BW for the AL piglets and between 10.5 and 13.4 kg BW for the AH piglets (difference between AL and AH piglets; $P < 0.001$). All selected piglets were considered healthy by visual observation, veterinary check and had no record of previous health problems.

Housing and husbandry during trial

The selected 20 piglets were transported to Wageningen UR Livestock Research in Lelystad and were placed in metabolic cages (160 x 80 cm). Littermates were housed together and the piglets without a littermate were housed individually during the acclimatization period. From the 20 selected piglets, 16 were included in the study (6 litter pairs). The remaining 4 piglets were kept as reserve piglets. After arrival piglets had *ad libitum* access to water. From day one after arrival until catheterization, piglets had *ad libitum* access to feed. Piglets had a 14/10 h cycle of light/dark. During the acclimatization period the piglets were exposed to frequent contact with caretakers to reduce stress and facilitate the handling during the study. After four days of acclimatization to the new facilities, catheterization of the jugular vein and carotid artery took place, following the procedure described by Koopmans (Koopmans *et al*., 2001). After catheterization, piglets were housed individually and fed restrictedly for 2 days (125 g on the day of surgery and 2 meals of 180 g on the second day after surgery). Thereafter, piglets were fed *ad libitum* again. Piglets were offered toys as cage enrichment. The room temperature was 24° C during the first 2 days after arrival, 23° C on day 3 and 22° C from day 4 until the end of the study. Relative humidity was kept on 55%. Temperature and humidity in the experimental room were recorded daily. Piglets' health status was monitored daily.

From weaning (28 ± 1.0 days) until day 31 ± 1.0 of age all piglets were fed a commercial weaner diet. From day 32 to 35 of age piglets had a gradual transition (75:25, 50:50, 25:75, 0:100) to a diet fed during the study (Table 6.1).

Table 6.1 Calculated[1] ingredients (g/kg), nutrient composition (%) and net energy content (MJ/kg) of the experimental diet (as fed) from six to ten weeks of age

Ingredient	g/kg
Maize	300.0
Barley	300.0
Soy bean meal	102.4
Wheat	79.6
Wheat bran	40.0
Soya SPC 60	40.0
Whey powder sweet	30.7
Sugar	20.0
Potato protein	20.0
Soya oil	19.5
Vitamin and mineral blend[2]	11.3
Amino acid blend[3]	10.5
Monocalcium phosphate	8.7
Sodium bicarbonate	5.0
Calcium propionate 98%	5.0
Calcium formiate 99%	5.0
Salt	2.1
Limestone	0.1
Phytase	0.1
Calculated nutrient composition	
Crude protein	17.2
Crude fibre	3.1
Net energy	10.1
SID[4] Lysine	1.1
SID Methionine + Cysteine	0.7
SID Threonine	0.7
SID Tryptophan	0.2
Starch (Ewers)	41.2
Digestible phosphorus	0.3

[1] Calculated based on Centraal Veevoeder Bureau (**CVB**), 2007.

[2] Provided the following per kg of diet: Vitamin A 8,000 IU; Vitamin D3 2,000 IU; Vitamin E 30 mg, Pantothenic acid 12 mg; Vitamin K3 1.5 mg; Vitamin B1 1 mg; Vitamin B2 4 mg; Vitamin B6 1 mg; Vitamin B12 20 µg; Nicotinic acid 20 mg; Folic acid 0.3 mg; Choline

Chloride 250 mg; Cobalt 0.15 mg as Basic cobaltous carbonate, monohydrate; Copper 160 mg as Cupric sulphate pentahydrate; Iron 100 mg as Ferrous sulphate, monohydrate; Iodine 1 mg as Calcium iodate, anhydrous; Manganese 30 mg as Manganese oxide; Zinc 100 mg as Zinc sulphate; Selenium 0.3 mg as Sodium selenite

[3] Provided the following per kg of diet: DL-methionine, 2.2 g; L-lysine, 5.3 g; L-threonine, 1.8 g; L- tryptophan, 0.5 g; and L-valine, 0.9 g

[4] SID = standardized ileal digestibility

Evaluated parameters

In order to determine insulin-mediated glucose tolerance differences three tests were presented to the AL and AH piglets: oral glucose tolerance test (**OGTT**), hyperglycaemia clamp test and oral starch tolerance test (**OSTT**) from 8 to 9 weeks of age.

The OGTT was performed 6 and 7 days after catheterization (8 piglets per day). The hyperglycaemic clamp on day 9, 12 and 13 after catheterization (4, 4 and 5 piglets per day, respectively) and the OSTT on day 15 and 16 after catheterization (7 and 6 piglets per day, respectively). The day previous to each test, the BW of the piglets was determined for the calculation of the amount of glucose and starch to be administered per piglet in both tests. Furthermore, feed was removed from the cages at 16:00 h on the day before each test and returned at the moment of completion of the test. On day 26 piglets were sacrificed by an intracardiac injection of pentobarbital sodium (Euthanasol 40%, ASTfarma B. V., Oudewater, the Netherlands), at a dose of 200 mg/kg BW. After confirmation of death, the complete pancreas was excised and frozen in dry ice for pancreatic enzymology specifically trypsin, amylase and lipase activity and total protein content analysis. Subsequently, a necropsy was performed to identify possible health problems.

Oral glucose tolerance test (OGTT)

At time point 0, 4 g glucose (330 g glucose/L solution) per kg BW was infused intragastrically via a tube to which a 100 mL syringe was connected. Blood was collected at 13 time points (-30, -15, 5, 15, 25, 35, 45, 60, 75, 90, 120, 180 and 240 min relative to the time point of glucose administration). Before infusion, the glucose solution was warmed up to 37° C by placing it in warm water. During the first day of the test it was not

possible to collect a blood sample in one AH piglet at time point 35 min and two blood samples (35 and 45 min) in a second AH piglet.

Hyperglycaemic clamp test

At time point 0, an intravenous infusion with a glucose solution (330 g glucose/L) was initiated at a rate of 0.25*BW (mL/h), preceded by a bolus infusion for a period of 15 min at a rate of 1.00*BW (mL/h) to raise plasma glucose concentrations from baseline to a physiological level, 8 mmol/L. Blood glucose concentration was measured instantly every 10 min. The measured glucose concentration at each time point per piglet was used to adjust the glucose infusion rate to the intended blood glucose concentration. Fifty minutes after the start of the test, the glucose infusion rate was raised to 0.46*BW (mL/h) and adjusted per time point to obtain an intended blood glucose supra-physiological concentration of 15 mmol/L. Hundred minutes after start, an intravenous arginine bolus (1 g L-arginine dissolved in 8 mL of 1 N HCl solution at pH 4) was infused per piglet. Blood samples were subsequently collected 5, 10, 20, 30 and 40 min after arginine infusion and the infusion rate of the glucose solution was adjusted to maintain an intended blood glucose concentration of 15 mmol/L. One AH piglet died during this test, another one could not be used because both catheters did not work properly and a third AH piglet was not used because of signs of cardiac problems.

Glucose clearance from the blood pool per piglet was estimated by dividing the glucose infusion (mol/kg BW/min) by the blood glucose concentration (mol/mL) assuming similar glucose excretion by the kidneys in both groups.

Oral starch tolerance test (OSTT)

The OSTT followed the same procedure as the OGTT, except that a starch suspension was administered instead of glucose solution. At time point 0, a starch suspension containing 297 g native corn starch/L (25% amylose concentration, 450 BU peak viscosity, 4.5 pH and 12% moisture; Roquette, Lestrem, France) was administered intragastrically at a level of 12.1 mL per kg BW. The starch suspension was prepared in warm water and stirred constantly until administered to the piglets. The dose of starch

administered per piglet (3.6 g starch per kg BW) was calculated to provide the same amount of glucose equivalents in the form of a starch suspension compared to the amount of glucose provided in the OGTT (4.0 g glucose per kg BW). Blood was collected at 13 time points (-30, -15, 5, 15, 25, 35, 45, 60, 75, 90, 120, 180, 240 and 300 min relative to the time point of starch suspension administration) in each of the piglets.

During each of the tests described above, 2 mL of blood was collected per piglet per time point of sampling in EDTA tubes. Immediately after collection a drop of blood was placed in a Freestyle Precision test strip in a Precision Xceed blood glucose meter (Abbott Laboratories, IL, USA) to analyse blood glucose concentration. Blood samples were subsequently centrifuged in a cooled centrifuge IEC Centra CLR3-R, model 220/240 (Thermo Needham Heights, MA, USA) at 4° C with 1800 rpm for 10 min such that plasma could be harvested. Plasma was placed in cryotubes stored at -20° C for insulin determination with the Mercodia Porcine Insulin ELISA kit (Mercodia, UP, Sweden). For all the tests the HOMA index as described by Matthews (Matthews *et al*., 1985) and the Quantitative insulin sensitivity check index (**Quicky**) as described by Katz *et al.* (2000) were calculated to assess insulin sensitivity. A higher HOMA index implies a higher insulin resistance and lower Quicky index lower insulin sensitivity. The area under the curve (**AUC**) was calculated in each of the tests as an indirect measure of glucose absorption and total insulin secretion.

Cortisol concentration in blood

Plasma cortisol concentration was determined by radioimmunoassay Coat-a-count cortisol (Siemens Healthcare Diagnostics Inc., CA, USA), in plasma samples from blood collected at 6 time points (-30, -15, 15, 90 and 240 min relative to the time point of glucose or starch suspension administration) in the OGTT and OSTT.

Pancreas enzyme analysis

The unfrozen pancreas tissue was weighed and homogenized in ice-cold 0.2 M Tris-HCl buffer + 0.05 M $CaCl_2$, pH 7.8 in 2 steps (ratio 1/5 weight/volume, using a knife homogenizer followed by further 2-fold

dilution, using a glass homogenizer) for a total dilution 1:10 weight/volume. Two mL of the homogenate was centrifuged at 1800 rpm for 30 min at 4° C and the supernatant was used for analyses of trypsin, amylase, lipase (U enzyme/ g pancreas) and protein content (mg per g pancreas tissue).

Trypsin activity was determined spectrophotometrically with a microplate reader (Pierzynowski *et al*., 1990) after modification from the method of Fritz (Fritz *et al*., 1966); using benzoyl-DL-arginine-4-nitroanilide (BAPNA, Sigma-Aldrich, DP, UK) as substrate. The amylase activity was determined with ethylidene-pNP-G7 as substrate (Infinity Amylase Liquid Stable Reagent; Thermo Scientific, VA, USA). Lipase activity was determined with the Randox lipase kit (Randox Laboratories, ANT, Northern Ireland) using chromogenic substrate 1, 2-o-dilauryl-rac-glycero-3-glutaric acid-(6ı-methylresorufin)-ester. The activity units were recalculated as amount of enzyme that transforms 1 μmol of substrate per minute. The enzyme activities are presented as units per wet pancreatic tissue weight. The protein content of the pancreatic homogenates was determined by the Lowry method (Lowry *et al*., 1951) modified for 96-well microplates (Pierzynowski *et al*., 1990), using purified BSA (Sigma-Aldrich, DP, UK) as standard.

Statistical analysis

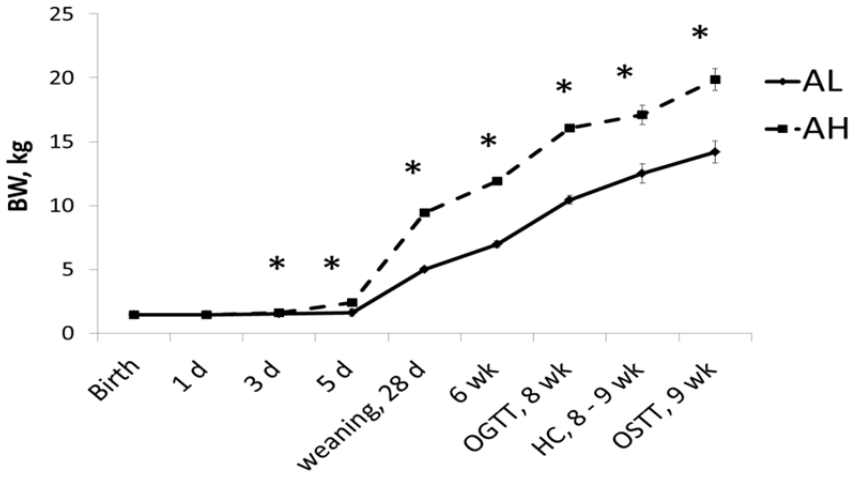
The UNIVARIATE procedure in SAS (version 9.3, 2011) was used to test residuals for normality with $P > 0.05$ for Shapiro-Wilk test indicating a normal distribution of data. In all cases, piglet was the experimental unit and significant differences were identified at $P < 0.05$ and trends at $P < 0.10$. The blood glucose, plasma insulin and cortisol concentrations during the OGTT, hyperglycaemic clamp, and OSTT were calculated with the MIXED procedure with repeated measurements in SAS. The statistical model included group (AL or AH), sex, and their interaction as independent variables per time point. The AUC for the insulin and glucose concentration was calculated with the trapezoidal rule in SAS. Pancreatic enzyme activity and protein content were analysed with the MIXED procedure in SAS including group, sex and their interaction as independent variables.

Glucose infusion rate during the hyperglycaemic clamp was calculated with the MIXED procedure in SAS using the same statistical model as described for blood glucose concentration.

RESULTS

Body weight development

At birth the mean BW of AL and AH piglets was equal (P = 0.975). At weaning the difference in mean BW between groups was 4.45 kg. This difference in BW between groups started at 3 days of age and remained until the end of the study (P < 0.001; Fig. 6.1).

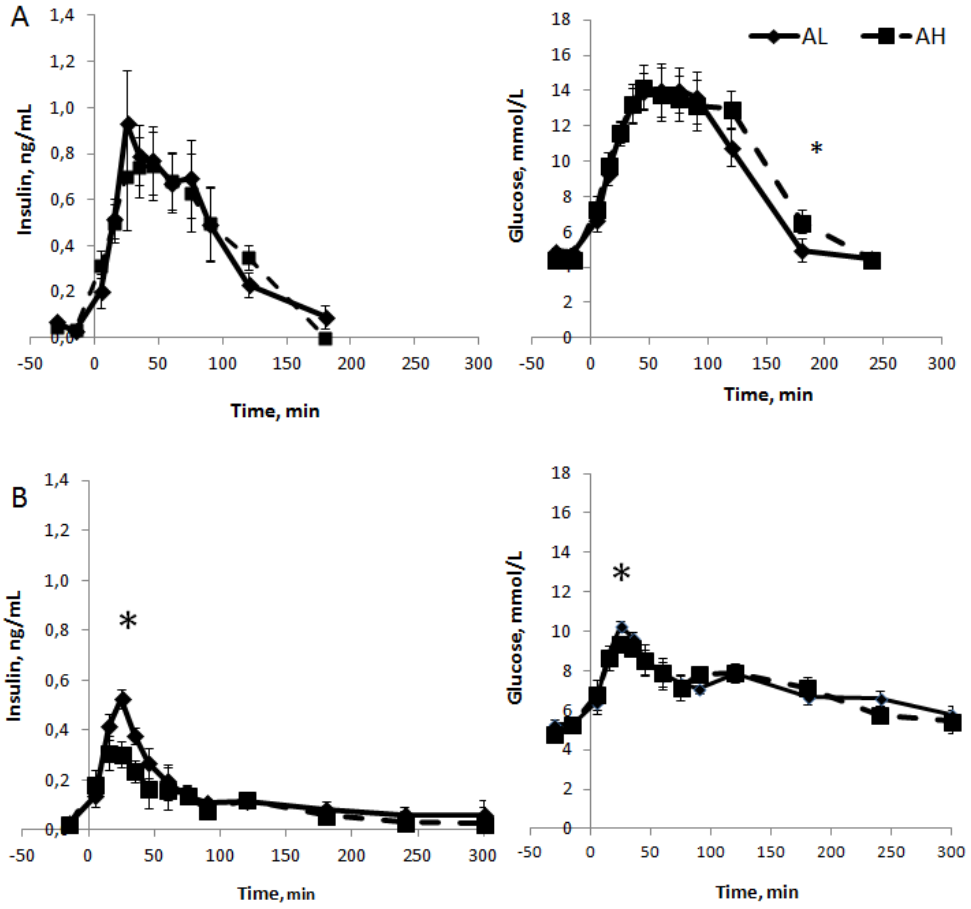


OGTT = oral glucose tolerance test; HC = hyperglycaemic clamp; OSTT = oral starch tolerance test; d = days of age; wk = weeks of age

Figure 6.1 Body weight for piglets selected with an average birth weight but with lower than average gain during the first six weeks of age (AL) or with higher than average gain during the first six weeks of age (AH) from birth until nine weeks of age

Oral tests

The OGTT showed no differences in response in blood glucose concentration between groups (P > 0.100). Except at 180 min after infusion where there was a tendency for a 1.5 mmol/L lower blood glucose concentration in the AL piglets compared to AH piglets (P = 0.095; Fig. 6.2A). No differences in plasma insulin concentration were observed between groups (Fig. 6.2A).

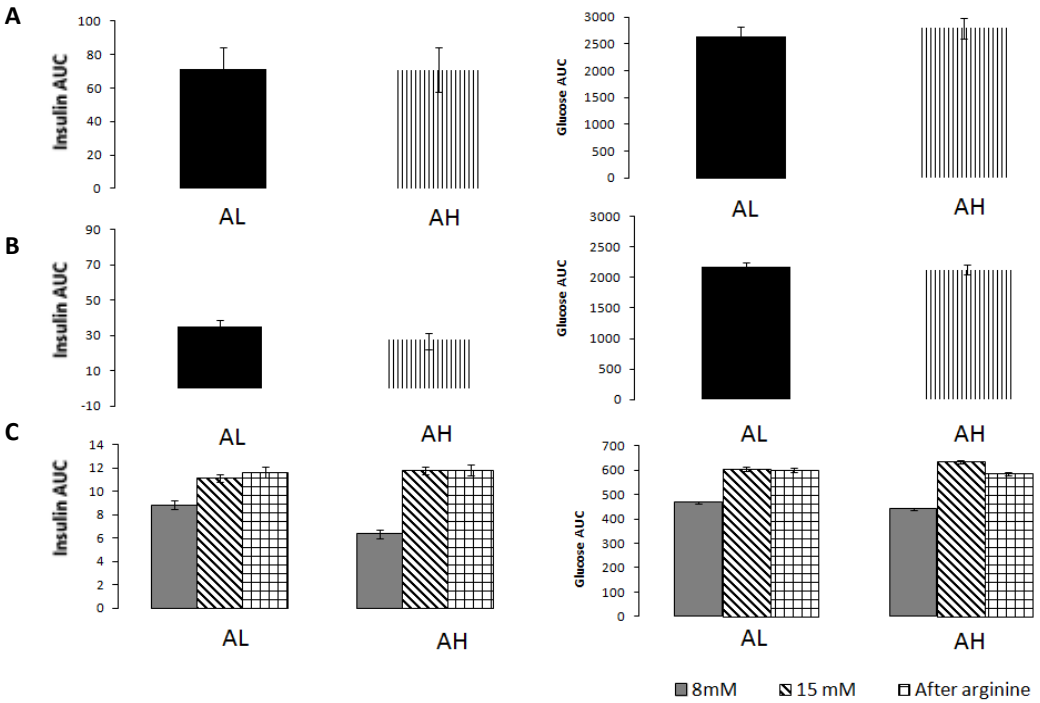


OGTT = oral glucose tolerance test; OSTT = oral starch tolerance test

Figure 6.2 Plasma insulin and glucose concentration after orally administering a dose of 4 g glucose equivalent/kg BW in the OGTT (8 weeks of age) (A) and OSTT (9 weeks of age) (B); as glucose solution or as suspension of native corn starch, respectively, for piglets with an average birth weight but with a lower than average gain during the first six weeks of age (AL) or with a higher than average gain during the first six weeks of age (AH)

The OSTT showed a tendency for higher blood glucose concentration for the AL piglets between 20 and 30 min after infusion (10.3 vs. 9.4 mmol/L; *P* = 0.095). Thirty five min after the starch suspension was provided, there was a higher plasma insulin concentration for the AL piglets (*P* = 0.037). For

blood glucose and plasma insulin concentration, the peak was achieved between 20 and 30 min after infusion, the maximum concentration being higher in the AL piglets (Fig. 6.2B). There were no differences in AUC for glucose and insulin in both the OGTT and in the OSTT test between AL and AH piglets (Fig. 6.3A and B). The plasma cortisol concentration during the OGTT and OSTT displayed two increments in both groups at 15 and 90 min after intragastric infusion of glucose and starch (Fig. 6.4).



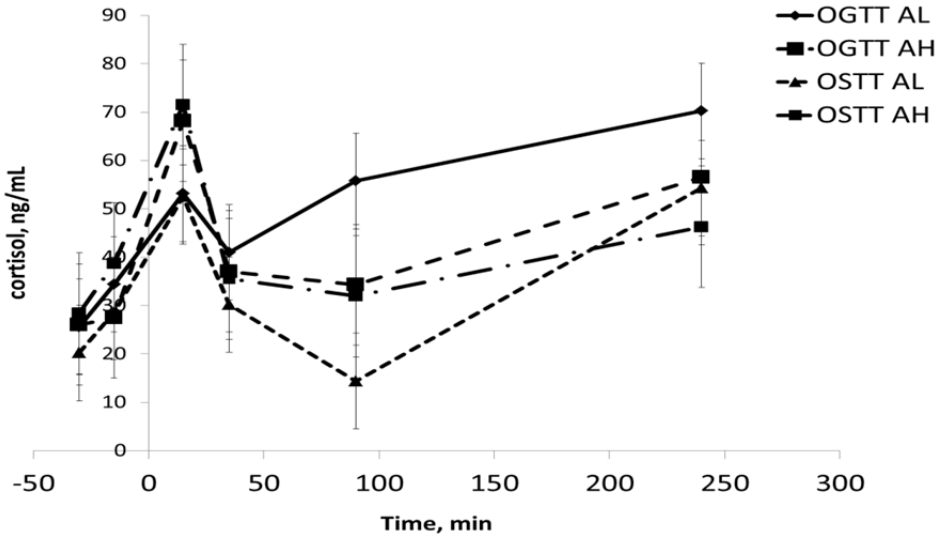
OGTT = oral glucose tolerance test; OSTT = oral starch tolerance test mM = mmol/L

Figure 6.3 Plasma insulin and glucose AUC in the OGTT (8 weeks of age) (A), OSTT (9 weeks of age) (B) and hyperglycaemic clamp (8 or 9 weeks of age) (C) for piglets with an average birth weight but lower than average gain during the first six weeks of age (AL) or higher than average gain during the first six weeks of age (AH)

Hyperglycaemic clamp test

There was a tendency for a higher basal blood glucose concentration for the AL piglets (5.5 vs. 4.3 mmol/L; P = 0.057) compared to the AH piglets (Fig. 6.5A). A lower glucose infusion rate (mmol/kg BW/min) was required

for the AL piglets to reach an 8 mmol/L blood glucose concentration ($P < 0.050$; Fig. 6.5B). No statistical differences in glucose infusion were observed between AL and AH piglets at an intended blood glucose concentration of 15 mmol/L and after intravenous arginine infusion (Fig. 6.5B). Plasma insulin concentration rise came 30 min after infusion and was higher for the AL piglets (0.24 vs. 0.12 ng/mL; $P = 0.043$). Calculated blood glucose clearance was lower for the AL piglets at an 8 mmol/L blood glucose concentration (0.12 vs. 0.21 L/kg BW/min; $P = 0.008$) compared to the AH piglets. There were no differences in calculated glucose absorption and insulin secretion based on the AUC for glucose and insulin between AL and HP piglets (Fig. 6.3C).



OGTT = oral glucose tolerance test; OSTT = oral starch tolerance test

Figure 6.4 Plasma cortisol concentration in the OGTT and OSTT for piglets with an average birth weight but lower than average gain during the first six weeks of age (AL) or higher than average gain during the first six weeks of age (AH)

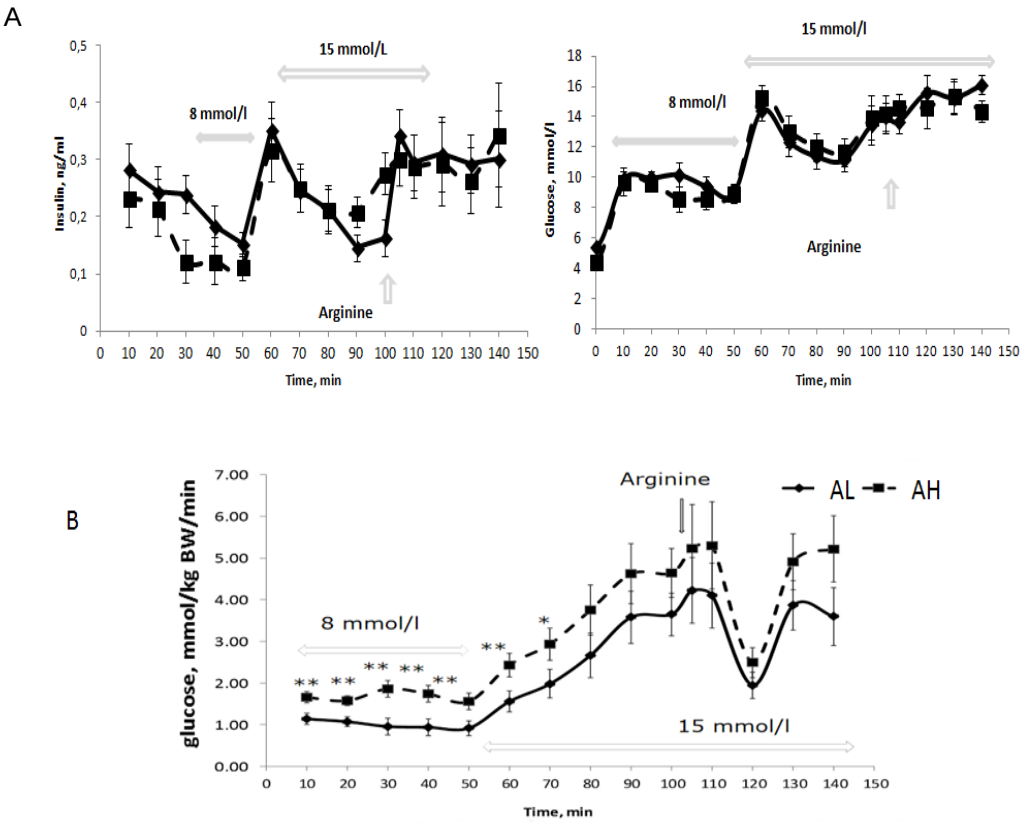


Figure 6.5 Plasma insulin and glucose concentration following an intravenous administration of glucose at two intended blood glucose concentrations (8 and 15 mmol/L) in the hyperglycaemic clamp test for piglets with an average birth weight but with a lower than average gain during the first six weeks of age (AL) or higher than average gain during the first six weeks of age (AH) (A). Intravenous glucose infusion rate to reach the intended blood glucose concentration 8 or 15 mmol/L in AL and AH piglets during the hyperglycaemic clamp test (B).

Pancreatic enzyme activity

There were no differences between groups in pancreas weight relative to BW, nor in trypsin or lipase activity per g pancreas tissue weight (P = 0.984, 0.121 and 0.361; respectively). Protein content (mg per g pancreas tissue weight) and amylase activity per g pancreas tissue weight were lower for the AL piglets compared to the AH piglets (P = 0.002 and 0.033 respectively; Table 6.2).

Table 6.2 Exocrine pancreatic protein content and enzyme activity at 10 weeks of age for piglets with an average birth weight but with lower than average gain during the first six weeks of age (AL) or higher than average gain during the first six weeks of age (AH)

	AL	**AH**	**SE**	***P* value**
Pancreas, g/ kg BW	2.70	2.70	0.2	0.984
Protein (mg/ g pancreas)	2398	3946	278	**<0.010**
Specific enzyme activity (U enzyme/ g pancreas)				
Trypsin	302	460	69	0.121
Amylase	21282	33210	3560	**0.033**
Lipase	1638	2404	596	0.361

DISCUSSION

Growth retardation by average birth weight piglets during the first six weeks of age (AL) represents the second major subpopulation of growth retarded piglets in the nursery phase, after the largest subpopulation of piglets in this group that are born small and remain small during the nursery phase (**Chapter 7**). For humans it is stated that IUGR children are more prone to type II diabetes at later age when compared to normal birth weight children. Diabetes has an effect on nutrient distribution stemming from a reduction in glucose uptake in skeletal muscle which impedes growth (Cianfarani *et al*., 1999). This phenomenon also seems to exist in IUGR piglets (Poore and Fowden, 2004) and could well explain their growth retardation. Persistent insulin resistance due to hypoxia during the birth process is described in normal birth weight children (Camm *et al*., 2011; Cheng *et al*., 1997). No literature describing this phenomenon in piglets is available. As the AL subpopulation is the second largest group of growth retarded piglets in the nursery phase (**Chapter 7**), it is important to understand whether its cause(s) is also linked to insulin-mediated glucose tolerance.

To study this, two groups of piglets were compared; the piglets' birth weight in both groups (AL = 1.43 and AH = 1.44 kg BW; P = 0.975). was considered as average (Smit *et al*., 2013). Equal calculated colostrum intake was observed between AL and AH piglets using the equation described by Devillers (Devillers *et al*., 2004). The estimated colostrum intake is determined as the difference in BW at day one of age and at birth. Despite the assumed equal access to maternal milk, the AL piglets were not able to thrive during the nursery phase, as confirmed by differences in weaning and early post-weaning BW between groups. Insulin-mediated glucose tolerance was monitored in these two populations (AL and AH piglets) from 8 to 9 weeks of age. To evaluate the effect of glucose source, the provision of an oral glucose solution was compared with the supply of a suspension of native corn starch. The provision of glucose equivalents in the OGTT and in the OSTT was similar (4 g glucose equivalents per kg BW). The dose of glucose provided was twice the amount described in literature for an OGTT

in piglets of this age (Blat *et al*., 2012; Poore and Fowden, 2002) aiming to maximize post-absorptive glucose metabolism.

When comparing the OGTT and the OSTT piglets' response one should consider the nature of glucose provision (comparing the oral supply of a glucose solution vs. a starch suspension). When glucose is supplied as a glucose solution it reaches the small intestine more rapidly when compared to the provision of starch which should first be hydrolysed into glucose in the small intestine prior to intestinal absorption. As a result, the rise in blood glucose concentration was higher in case glucose was provided as a glucose solution. As the concentration exceeded the renal threshold (10 mmol/L), there was likely an overflow of glucose to all tissues as skeletal muscle, an important sink for blood glucose, was not able to metabolically handle very high amounts of glucose provided via the blood. At this point the glucose uptake is saturated at the plateau and the influx from the small intestine equals or exceeds the maximum uptake by organs and tissues. This overflow induces metabolic stress to the piglet. After 150 min the glucose concentration returns to basal level indicating that the glucose flux from the digestive tract to the blood is close to zero.

The piglets' response in the OSTT was different from the one in the OGTT. The slower release of glucose from the starch in the small intestine resulted in a slower and longer release of glucose in the blood circulation. As the starch glucose is released at a slower pace, and at a sustained digestion rate (van Kempen *et al*., 2010; van der Meulen *et al*., 1997), it will not lead to a strong glucose peak as observed in the OGTT but instead to a physiological response. The fact that 300 min after infusion the glucose values do not return to baseline level suggests that the digestion of the bolus of starch provided was not completed within the period of measurement. This differs from the OGTT where after 150 min blood glucose concentration had returned to baseline. The former also suggests that piglets in the nursery phase are more able to cope with glucose originating from starch than with glucose orally provided as such. In the OSTT the insulin response to facilitate glucose uptake differed between groups, with a higher insulin peak for the AL piglets, which suggests a

predisposition of the AL piglets to insulin resistance. This feature of insulin resistance makes them to resemble the IUGR children or children suffering from hypoxia during the birth process (Camm *et al*., 2011; Hofman *et al*., 1997; Kaufman *et al*., 2007). The HOMA index was 0.26 for the AL piglets vs. 0.19 for the AH ($P = 0.031$) and the Quicky index was 0.50 for the AL and 0.53 for the AH ($P = 0.070$), in agreement with a predisposition to insulin resistance in AL piglets.

The plasma cortisol concentrations in the OGTT and OSTT displayed points of increase at 15 and 90 min after intragastric dosing of glucose or starch. The first cortisol concentration increase was attributed to the stress induced by the oral administration of the intragastric bolus of glucose solution or starch suspension. The second increase likely reflects metabolic activity due to glucose and starch digestion and metabolism. This second cortisol increase was higher in the OGTT when compared to the OSTT ($P = 0.012$). The observed cortisol response suggests that AL piglets suffered from a higher metabolic stress in the OGTT due to a glucose overflow at hyperglycaemia.

On the hyperglycaemic clamp test the AL piglets needed a lower glucose infusion rate to maintain a blood glucose concentration of 8 mmol/L ($P < 0.010$). This also suggests lower insulin efficiency in the AL piglets, especially because plasma insulin concentration was higher in the AL piglets. Above physiological ranges (at 15 mmol/L blood glucose concentration), there were no differences in glucose infusion between groups, suggesting that the defect in glucose utilization displayed by the AL piglets is present at physiological plasma glucose concentrations and can only be overcome at hyperglycaemia. The latter is a characteristic feature of type II diabetes and is described as glucose resistance. At such high levels of blood glucose, glucose is converted into adipose tissue, a process that likely is not compromised in the AL piglets. This is in line with observations in type II diabetic patients including those linked to IUGR (Rueda *et al*., 2011).

Combined, our data (OGTT and hyperglycaemic clamp at 15 mmol/L) suggest that AL piglets may suffer from both insulin and glucose resistance. It seems that the reduction in glucose utilization can only be overcome at hyperglycaemia (Fig. 6.5A, 15 mmol/L) which might also provoke metabolic stress to the piglets (Fig. 6.4). Calculated HOMA index also displayed differences at 8 mmol/L blood glucose concentration (AL = 0.22 vs. AH = 0.17; P = 0.077). Whereas in the hyperglycaemic clamp test there were no differences in Quicky index between groups (P = 0.932). The AL piglets had a 0.15 mL glucose per kg BW per min lower glucose clearance compared to the AH piglets (P = 0.008) at an 8 mmol/L blood glucose concentration, in agreement with the observation that AL piglets had a lower insulin efficiency.

When combining the information with the skeletal muscle characteristics of growth retarded piglets (lower muscle fiber number and size) compared to heavier littermates, we suggest that at a blood glucose concentration of 8 mmol/L diet (or insulin) induced glucose uptake is mainly done by skeletal muscle for the purpose of lean tissue gain, whereas at 15 mmol/L an important diet induced glucose uptake is done by adipose tissue. Following this hypothesis, we would state that glucose uptake by the adipose tissue is unaffected, while muscle utilization is impaired in growth retarded piglets. The latter, leading to an overflow, resulting in energy going to adipose tissue easier. These findings are in agreement with literature describing a higher content of fat in the carcass in pigs with a longer fattening period at similar body weight at slaughter (Bee, 2004; Rehfeldt and Kuhn, 2006).

There were no differences in trypsin and lipase activity in the exocrine pancreas. Differences were observed in amylase activity between groups, in agreement with Harada *et al.* (2003) who stated that growth retarded piglets have a considerable lower amylase activity when compared to average BW piglets. The authors described that the mechanism behind the lower amylase activity is unknown. One difference, though, when comparing both studies, is that Harada described a lower plasma insulin concentration whereas our AL piglets had no insulin deficiency. As the

endocrine pancreas was challenged in three different tests, one has to carefully interpret the exocrine pancreas activity data, as insulin affects amylase production (Ferrer *et al*., 2000; Pierzynowski and Barej, 1984). One possible explanation for lower amylase activity in the AL piglets is that this is an adaptation mechanism to poor insulin sensitivity. Hypothetically, by secreting less amylase the starch digestion (and as a consequence, gastric emptying) can be slowed down moderating blood glucose and insulin responses (Layer *et al*., 1986). The observation that the AL piglets eat more and a smaller meal are in line with this and implies that growth retarded piglets affect both their physiology and their behaviour with the aim to moderate their blood glucose response after a meal.

Combining the information gathered with the three tests and pancreatic enzyme activity, we speculate that the AL piglets exhibit insulin and glucose resistance. In literature it is mainly stated that low birth weight babies have a higher risk to develop type II diabetes in prepuberal age (Hofman *et al*., 1997) or adulthood (Hales and Barker, 1992) and low birth weight piglets develop it around 12 months of age (Poore and Fowden, 2002). Disorders in insulin-mediated glucose tolerance as a result of a foetal malnutrition and epigenetic reprogramming in the foetal stage are described in literature. In this study we excluded low birth weight piglets and focused only on piglets with a normal birth weight discarding foetal malnutrition and epigenetic reprogramming in-utero (Foxcroft *et al.,* 2006). Yet, the same phenomenon as described for IUGR children and piglets is observed. One explanation for the cause of the insulin resistance could be linked to events during the farrowing process. We did not monitor the piglet birth order or interval. In literature it is described that children suffering from hypoxia during or after birth are prone to insulin resistance (Camm *et al*., 2011; Cheng *et al*., 1997). The key mechanism for the insulin resistance might be the reduced insulin receptor receptiveness with attenuated activity of insulin receptor tyrosine kinase (IR-TK) activity at the post-receptor level (Cheng *et al*., 1997). Whether this switch in insulin sensitivity will remain throughout their life or is a reversible process is still to be determined. Given that growth retardation is deemed permanent for this category of piglets it is likely permanent. As both subcategories of growth

retarded piglets (IUGR and AL) piglets display insulin resistance, from the application point of view, dietary interventions aiming to reduce the insulin resistance might benefit both groups of piglets. Alternatives such as feeding slowly digestible starch to these subcategories of growth retarded piglets in the post-weaning phase might help reverse or circumvent the insulin resistance and promote energy uptake by the skeletal muscle and thus lean tissue growth. Preventing hyperglycaemia may also normalize their feed intake. Combined, this may prevent further growth retardation.

CONCLUSIONS

Piglets with an average birth weight but with lower than average gain during the first six weeks of age displayed differences in insulin-mediated glucose tolerance when compared to piglets born with an average weight and a higher than average gain during the first six weeks of age. At physiological blood glucose concentration, growth retarded piglets required more insulin to regulate blood glucose, implying insulin resistance at skeletal muscle level. At supra-physiological blood glucose concentration, no difference was found between the two groups implying that there is no difference in insulin sensitivity in the adipose tissue. Practically, this means that growth retarded piglets are less apt to grow lean tissue and likely they are adapting their feed intake and feed intake pattern to avoid adiposity, all with negative consequences for overall growth.

ACKNOWLEDGEMENTS

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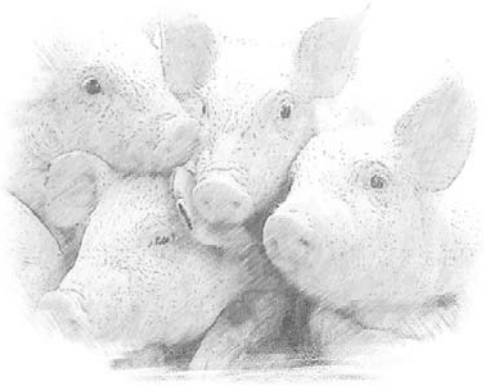
Chapter 7
General Discussion



INTRODUCTION

In swine production, genetic selection in maternal lines has resulted in an increase in litter size. With this increase in litter size, however, within-litter BW variation increased also (Foxcroft et al., 2007; Quiniou et al., 2002; Surek et al., 2014; Fig. 7.1). As a result of the high within-litter BW variation, there has been an increase in the number of piglets with a low birth weight (< 1 kg BW; Quiniou *et al*., 2002). These low weight piglets have a greater risk for higher growth check, morbidity and mortality in the pre- and/or immediate post-weaning phase (Rutherford *et al*., 2013). Whether the growth check suffered by the lighter piglets can be compensated for in later life stages, remains a debate. Some authors describe that piglets exhibit compensatory growth (Gondret *et al*., 2006; Stamataris *et al*., 1991) after a period of growth retardation. Others state, however, that growth recovery is not possible and that these light piglets will remain being the lightest pigs until slaughter (Fix *et al*., 2010; Rehfeldt and Kuhn, 2006). The differences in opinion on whether growth retarded piglets compensate their growth check can partly be explained by the different definition used to describe the growth retarded piglet population.

Growth retardation is defined as failure of an individual to develop at a normal rate of gain for its age, both for height and weight (Mosby, 2009). This definition does not provide insight into its cause(s), consequences or ways for preventing it. In the last years attention for growth retardation has increased in human and livestock science, due to the implications of low birth weight for later life stages. In humans, it has been demonstrated that small for gestational age babies are prone to suffer from syndrome X (a collection of interrelated conditions (e.g. obesity, physical inactivity, etc.) that increase the risk of developing type II diabetes) during adulthood (Hales and Barker, 1992). For livestock, growth retardation has an effect on performance; changes in body composition (higher lipid and lower protein concentration) and a delay in time to reach market weight (Wu *et al*., 2006). In addition, growth retardation leads to management problems (disease susceptibility, slaughter management; Wu *et al*., 2006).

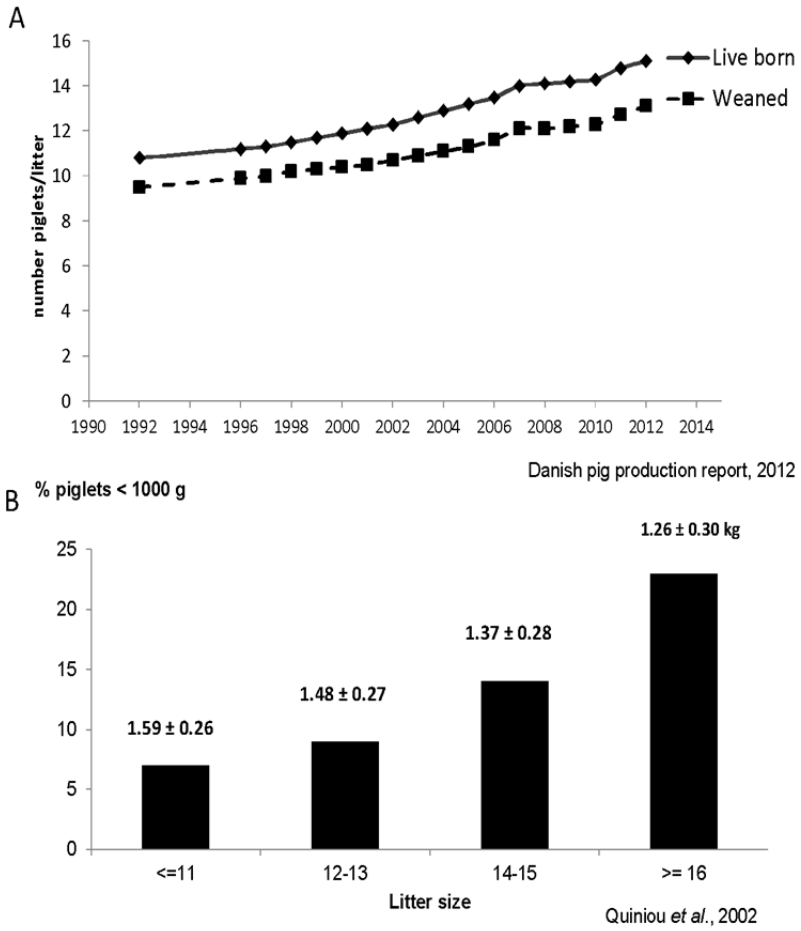


Figure 7.1 Relationship between increase in litter size, within-litter birth weight variation and number of piglets weaned per litter. Development in litter size in Denmark over the last 20 years (A). Body weight variation at birth with increased litter size (number above columns, mean birth weight in kg ± SD within-litter variation; B)

The causes for growth retardation are still debated. One factor is genetics (e.g. under expression of IGF2 gene, over expression of IGFBP1 and 2 gene); mainly related to intrauterine growth retardation (**IUGR;** Sankaran and Kyle, 2009). Intrauterine growth retarded piglets are a population not within the scope of our study as the intervention strategies to avoid their growth check are mainly related to sow nutrition in the gestational phase

(Wu *et al*., 2010). They have a high mortality rate, lack of growth category increase and the efforts needed for their growth restoration outweigh the benefit, as these piglets have incurred in severe growth check (**Chapter 2**). In-utero, nutrient supply is likely the dominant external factor linked to uterine crowding and poor placental development (Foxcroft *et al*., 2006; Sankaran and Kyle, 2009). The birth process can also be a significant stressor leading to hypoxia resulting in hypothermia (Edwards, 2002; Lossec *et al*., 1998). In field conditions, piglets may require days to recover from a difficult birth process. This time hampers their early colostrum intake and makes them weaker and more prone to crushing. Colostrum is the piglets' source of many health promoting compounds including immunoglobulin and growth factor for the intestinal tract. The lack or lower colostrum ingestion can have long term consequences for piglets' health (Le Dividich *et al*., 2005; Le Dividich and Noblet, 1982). After birth, there is competition between littermates around the udder resulting in variation in milk intake, which can reduce the performance for some piglets, especially the weaker/smaller ones (Fraser and Jones, 1975; McBride, 1963). All of these factors (hypoxia, hypothermia, lower colostrum intake, low birth weight), predispose piglets to stress and disease, leading to a setback in performance. For this thesis, the goal was to identify the shortcomings leading to growth retardation in the post-weaning phase, with the ultimate objective to find nutritional or management strategies to help these piglets to fully achieve their growth potential. Hence, this means reducing the heterogeneity in weight at the end of the nursery phase (10 weeks of age). The end of the nursery phase was chosen as end point, as this is the moment when piglets are selected and transported to the finisher barns or sold.

Population selection and subcategories

Low weight piglets are a heterogeneous population with one similar characteristic, low BW compared to their littermates. Different classes of piglets in this group will require different interventions and intervention time points to restore their growth. The first part of this work focused on defining the target population (**Chapter 2**). Using the risk analysis approach on three datasets (n = 77,868 individual piglet records) it was determined

that BW at birth, at weaning, and at six weeks of age, and season of birth were the most significant factors to predict BW at the end of the nursery phase with around 70% accuracy.

The second analysis performed on these datasets aimed to determine the growth response of low birth weight piglets until the end of the nursery phase. The main outcome was that piglets with a birth weight below the mean minus 2.5 times the SD from the mean of the total population remained in the lower side of the curve and did not engage in compensatory gain at the end of the nursery phase. Piglets with a birth weight between the mean minus 2.5 and the mean minus 2.0 times the SD from the mean of the total population sometimes display a degree of growth compensation at the end of the nursery phase, but had a high mortality rate (**Chapter 2**). Therefore, piglets with a birth weight below the mean minus 2.0 times the SD from the mean of the total population were regarded as more prone to be IUGR. This is in agreement with McMillen *et al*. (2001). Consequently, these piglets were excluded from our studies for the reasons stated above.

Based on the light piglet population analysis, the limits for our target population were established for piglets with a birth weight above the mean minus 2.0 times and below the mean minus 1.0 time the SD from the mean of the total population (called throughout the thesis low performing piglets **LP**). Focusing on this population a dataset from 2005 - 2010 (n = 20,156 individual records; SRC 2013, unpublished[3]) was analysed. From this analysis it was concluded that 10% of the piglets born alive fit into this category. The variation in this group increases with time after birth, with some piglets display compensatory gain, some staying in their SD class, while others fall further behind (**Chapter 2**).

The predicted BW at the end of the nursery phase was used to select our target population: Low performing piglets (LP) with a birth weight above the mean minus 2.0, and a predicted end of the nursery weight below the

[3] Nutreco Swine Research Centre, Sint Anthonis, the Netherlands

mean minus 1.0 time the SD from the mean of the total population (Fig 7.2). The most relevant groups of piglets within the LP category were:

1. Piglets with a low birth weight and which remained with a low BW for the rest of the nursery phase (53% of the LP piglets; **LL** = low birth weight, lower than average gain during the post-weaning phase)

2. Piglets with an average birth weight but a lower than average gain during the lactation and post-weaning phase (25% of the LP piglets, **AL** = average birth weight but lower than average gain during the first six weeks of age).

3. Piglets suffering from a growth check after weaning (22% of the LP piglets; Fig. 7.2).

Based on the description above, our target population was selected based on a combination of birth, weaning and BW at six weeks of age. For two of the three in vivo studies, we focused on LP piglets without considering subcategories (**Chapters 3 to 5**) comparing them to their heavier littermates (high performing piglets **HP**. Piglets with a predicted BW at the end of the nursery phase above the mean plus 1.0 time the SD from the mean of the predicted end of the nursery BW). For the last study (**Chapter 6**) the focus was on AL piglets. The AL piglets have not suffered from growth retardation in-utero. As IUGR in humans and animals is described as being born with a lower BW when compared to the mean (Cianfarani *et al*., 1999; Foxcroft *et al*., 2006; Hofman *et al*., 1997; Morrison *et al*., 2010; Oksbjerg *et al*., 2013; Rehfeldt and Kuhn, 2006).

In vivo characterization

Three in vivo studies were set up to explore the phenotypical traits to explain differences in growth performance between LP piglets and their heavier counterpart HP piglets (**Chapter 3 to 5**). Evaluated parameters and age when they were analysed are summarized in Table 7.1. The pre-weaning phase observations were analysed retrospectively.

Pre-weaning phase (birth until 4 weeks of age)

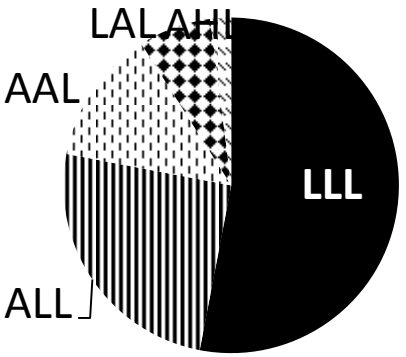


Figure 7.2 Low BW piglet population distribution at the end of the nursery phase based on birth (first letter), weaning (second letter) and six weeks of age (third letter) category. L: low, A: average, H: higher BW

The colostrum intake was estimated based on the equation of Devillers (Devillers *et al*., 2004). In contrast to our expectations, no differences in absolute colostrum intake were observed between LP and HP piglets. In the first study, (**Chapter 3**), the estimated colostrum intake was similar for LP and HP piglets (263 vs. 311 g; *P* = 0.310). In the study described in **Chapter 4,** however, the estimated colostrum intake was reduced in LP piglets when compared to HP piglets (237 vs. 310 g; *P* = 0.053). The observed difference in colostrum intake of the second batch is in agreement with results of Le Dividich *et al*. (2005) and Devillers *et al*. (2007).

After compiling the data of both studies (**Chapter 3 and 4**), 60 individual LP piglet records of estimated colostrum intake were obtained. No differences were observed in colostrum intake between subcategories of growth retarded piglets (*P* = 0.345).

Post-weaning phase

The main observed differences between LP and HP piglets during the post-weaning phase were related to differences in feed intake (pattern and amount). The LP piglets did not engage into compensatory feed intake and, therefore, did not demonstrate compensatory gain. An explanation to why LP piglets have different feed intake characteristics remains to be elucidated. Yet, the findings in this thesis can be used to speculate on possible causes for the reduced feed intake of LP piglets. The main findings are listed below.

1. No differences in apparent total tract and ileal macronutrient digestibility were observed between LP and HP piglets when provided a highly digestible diet (diet composition: 175 g/kg CP, 10.6 MJ NE and 13.5 g/kg AID Lys; **Chapter 3**) or when challenging their digestive system with a low quality protein and high fibre diet (**Chapter 4**).

2. The LP piglets' small intestine weight relative to the total FI from 6 to 10 weeks of age was 4 g per kg higher ($P < 0.001$; **Chapter 3**).

3. The LP piglets spend more time of the day eating when compared to HP piglets. At 9 weeks of age LP piglets' rate of feed intake was 1.6 g/min lower compared to HP piglets (3.3 vs. 4.9 g/min; $P = 0.020$).

Based on the findings described above, the first theory to explain the differences in feed intake between LP and HP piglets is related to *neuroendocrine regulation of feed intake by the gastrointestinal system*. The regulation of feed intake is described as a three-phase component: cephalic, gastric and intestinal, where the three phases are interrelated. This makes it difficult to measure the effect of each component on feed intake separately. The gastrointestinal system is influenced by satiation signals modifying its motility and its secretion, activating satiation signals from the stomach (mechanical distention), small intestine (nutrient content, osmolarity or pH), colon and pancreas (Cummings and Overduin, 2007).

After food is ingested there is gastric distention and release of peptides from enteroendocrine cells. Gastric satiation signals are mainly related to gastric distention by mechanoreceptors. The mechanoreceptors send positive or negative feedback to the brain via vagal and spinal sensory nerves (via glutamate, acetylcholine, nitric oxide, calcitonin-gene-related peptide, substance P and galanin related transcript neurotransmitters) and ghrelin. Gastric emptying regulates the passage of nutrients to the intestine

by adjusting the rate at which the digesta will reach the intestine (Capasso and Izzo, 2008). The positive or negative satiation signal input to regulate feed intake is received by the hindbrain. Leptin and insulin, among others, regulate the vagal and hindbrain response to gastric signals to maintain the long term energy balance. In the intestinal component, food intake regulation is related mainly to nutrient intake rather than amount (Cummings and Overduin, 2007). Its main function is to efficiently digest nutrients and to absorb them with the help of satiation signals (CCK, glucagon like peptide 1, oxyntomodulin, peptide YY, apolipoprotein A-IV) influencing gastrointestinal motility and secretion. We speculate that LP piglets reach satiation faster as a result of a lower gastric distention limit with the aim to prevent suboptimal nutrient digestion. Also, their lower digestive capacity might prevent disturbances in circulating levels of glucose. Small intestine characteristics (heavier/kg FI) leads us to speculate that LP piglets try to adapt their digestive capacity. The difference in fibre fermentation might imply that the LP piglets are at the edge of compromising digestion.

Other factors related to feed intake are:

1. Low weight and muscle cross sectional area in LP piglets, due to a low fiber number and fiber size, thus a limited muscle growth for the LP piglets when compared to HP piglets. These characteristics lead to a disadvantage for lean mass accretion and for meat quality (**Chapter 5**).

2. A lower IGF1 plasma concentration for the LP piglets that can be related to the lower growth and as a result of lower feed intake by this group (**Chapter 5**).

3. Higher insulin resistance at physiological glucose concentrations for LP piglets (**Chapter 6**).

Based on the findings described above, the second theory is that *feed intake is regulated by muscle growth capacity*. The limited muscle

deposition capacity of LP piglets causes them to reach equilibrium between circulating metabolites and satiety at a lower feed intake level. The insulin resistance exhibit by the LP piglets can reduce the sensitivity of the insulin receptor and promote a lower glucose uptake by the skeletal muscle. Also the higher circulating glucose concentration in blood would cause a negative feedback mechanism and lower feed intake for the LP piglets (Schultes *et al*., 2005). Insulin sensitivity problems limit muscle gain as it would reduce the amount of nutrients that can be channelled into muscle synthesis and lead to increased fat deposition. A low glucose flux into skeletal muscle will cause a negative feedback to feed intake regulation while at the same time increasing glucose availability for adipose tissue resulting in an increase in the novo fatty acid synthesis (Gathercole *et al*., 2012).

Table 7.1 Evaluated traits comparing Low performing piglets (**LP**; piglets with a birth weight above the mean minus 2.0 times the SD from the mean of the total population and a predicted end of the nursery weight below the mean minus 1.0 time the SD from the mean of the total population) to high performing littermates (**HP**, littermates with a predicted end of the nursery weight above the mean plus 1.0 time the SD from the mean of the total population)

Parameter	Age	Chapter	Outcome
Health status			
IgG concentration in blood	24 h	3	No differences
Clinical blood chemistry parameters	6 and 10 weeks	3 - 5	No differences
Behaviour			
Teat order test	1 and 2 weeks	3	Tendency to drink from the middle teats for the LP piglets
Fear to novelty (novel object test)	8 weeks	3	Higher fear to novelty for LP piglets
Level of comfort in housing conditions (home pen test)	9 weeks	3	No differences
Time spent eating	9 weeks	3	More time spent eating for the LP piglets
Feed intake pattern characteristics	4 to 10 weeks	3 and 7	Lower rate of feed intake for the LP piglets
Performance			
Calculated colostrum intake	24 h		No clear differences
Growth performance pre-weaning	Birth until 4 weeks	3	Absolute values lower for the LP piglets. Related to BW or $BW^{0.75}$ no differences between groups

Growth performance and feed efficiency	6 to 10 weeks	3	In absolute values lower performance for LP piglets. Related to BW or $BW^{0.75}$ no differences between groups
Feed efficiency above maintenance	6 to 10 weeks	3	Lower efficiency above maintenance for the LP piglets (assuming similar maintenance energy expenditure)
Digestibility			
Immuno-reactive trypsin concentration in blood	6 and 10 weeks	4	No differences
Apparent total tract and ileal digestibility of N, GE and fat	10 weeks	3 and 4	No differences
Pancreatic enzyme activity	10 weeks	6	Lower pancreatic amylase activity and protein content for AL^{1} piglets
Anatomy and morphology			
Skeletal muscle histochemistry and gene expression analysis	10 weeks	5	Lower myofiber number and lower muscle hypertrophy. Lower muscular mRNA expression of IGF2 for the LP piglets
Morphology and conformation	10 weeks	3	Greater body length and larger head circumference related to BW for the LP piglets
Small intestine characteristics	10 weeks	3	Longer and heavier small intestine related to BW for the LP piglets

IGF1 and Insulin

IGF1 concentration in blood	10 weeks	5	Lower plasma IGF1 concentration for the LP piglets
Insulin-mediated glucose tolerance	8 to 10 weeks	6	Higher insulin and glucose resistance for the AL piglets

[1] AL piglets: population of growth retarded piglets born with an average birth weight but with a lower than average gain during the first six weeks of age

Comparison of main findings with previous characterizations of growth retarded piglets

In literature, different approaches have been described for selecting growth retarded piglets to study the effects of growth retardation in future performance. The most described selection method is based solely on birth weight (Gondret *et al*., 2006; Krueger *et al*., 2013; Rehfeldt and Kuhn, 2006; Xu *et al*., 2004); whereas others described growth retarded piglets based on weaning weight or weaning performance (Jones, 2012; Mahan and Lepine, 1991; Wolter and Ellis, 2001). To our knowledge no group has included the three parameters described in our algorithm: birth, weaning and post-weaning weight as criteria to select growth retarded piglets. To determine the impact of the selection criteria on the mechanisms to explain the reduction in growth performance, a comparison between literature and current findings is presented in Table 7.2.

When compared to piglets selected at birth, the main differences are in terms of behaviour and growth performance. Most literature describes a lower feed efficiency for growth retarded piglets; whereas we observed no differences in calculated feed efficiency between LP and HP piglets. We have postulated that the priorities for LP piglets are different when compared to heavier littermates based on differences in body length and head circumference at the end of the nursery phase. It is hypothesised that the LP piglets have a priority for skeletal development rather than protein deposition.

Compared to our selection method for growth retarded piglets to selection at weaning, the main differences are related to nutrient digestibility. Some report lower DM, GE and N digestibility, whereas our LP piglets display equal digestibility when compared to the HP piglets (Table 7.2). Our findings suggest that there are no differences in apparent total tract or ileal digestibility of DM, GE, N, fat between LP and HP piglets.

Table 7.2 Differences in characteristics of growth retarded piglets when identified as growth retarded either at birth, weaning or with a comprehensive equation (this thesis)

Target	Selection criteria	Age when measured	Difference when compared to heavier piglets	Reference
Organ development	**Birth weight**	Birth	Brain vulnerable to undernutrition 5 to 10 weeks before farrowing.	Widdowson, 1971
		113 - 115 days post-conception	Reduced tissue weight in the gastrointestinal tract due to lower cell number.	Xu *et al*., 1994
		Birth	Delayed follicular development in pig ovaries at birth.	Da Silva *et al*., 2003
		5 days of age	Defect in testicular development, germ and somatic cell population.	Smit *et al*., 2013
	Weaning weight	14 and 28 days of age	Relative to live weight, the size of stomach, SI[1] and caecum are greater.	Pluske *et al*., 2003
	Birth, weaning, 6 weeks of age weight	10 weeks of age	Longer body and larger head circumference related to BW. Lower heart, liver and kidney as ratio of brain weight.	Chapter 3
Small intestine (SI)	**Birth weight**	Birth	Abnormal gastrointestinal morphology and dysfunction (e.g.	Thornbury, 1993

			necrotizing enterocolitis).	
		1, 7 and 21 days of age	Reduced cellular signalling, redox balance, protein synthesis and proteolysis affecting SI protein expression. Marked alterations of jejunal proteome at birth.	Wang *et al*., 2010
		18 and 28 days post-weaning	Lower SI weight:length ratio due to a thinner tela submucosa, tunica muscularis and higher secretory capacity in the distal jejunum. Gut maturation post-weaning is retarded but unrelated to weaning transition.	Michiels *et al*., 2012
	Weaning weight and age	14 and 28 days of age	Equal lactase and sucrase activity.	Pluske *et al*., 2003
	Birth, weaning and 6 weeks of age weight	10 weeks of age	Lower SI weight:length ratio. Longer and heavier small intestine related to BW.	Chapter 3
Pancreas	**Weaning weight and age**	14 and 28 days of age	Lower trypsin activity after weaning.	Pluske *et al*., 2003

	Post-weaning	6 to 8 weeks old	Dysfunction in exocrine and endocrine pancreatic function. Lower insulin and amylase concentration.	Harada *et al*., 2003
	Birth, weaning and 6 weeks of age weight	8 to 10 weeks of age	Higher insulin and glucose resistance for the AL^2 piglets. Lower pancreatic amylase activity and protein content for AL piglets. No differences in immuno-reactive trypsin concentration in blood.	Chapter 6
Skeletal muscle	**Birth weight**	Birth, 21 and 67 days of age	Lower birth weight associated with lower *Semitendinosus* muscle cellularity. Lower birth weight has a negative effect on postnatal muscle growth and final muscle fiber size and meat quality.	Tristan *et al*., 2009
		68 days of age	Lower tenderness due to enlarged myofibers.	Gondret *et al*., 2006
		Market weight	Lower number of myofibers.	Rehfeldt and Kuhn, 2006

		Market weight	Smaller *Longissimus dorsi.*	Rehfeldt *et al*., 2008
	Birth, weaning and 6 weeks of age weight	10 weeks of age	Lower myofiber number and lower muscle hypertrophy. Lower muscle mRNA expression of IGF2.	Chapter 5
Carcass composition	**Birth weight**	68 days of age	No difference in meat drip loss but lower amount and/or activity of proteolytic enzymes (calpain and cathepsin) affecting meat tenderness.	Gondret *et al*., 2006
		Market weight	Fatter carcass and higher muscle lipid content at market weight.	Powell and Aberle, 1980
		Market weight	No difference in backfat depth or *Longissimus* muscle area.	Fix *et al*., 2010
		Market weight	Inter and intramuscular fat tended to be higher in low birth weight piglets.	Beaulieu *et al*., 2010
	Weaning weight	Market weight	Equal loin and backfat depths and predicted lean content.	Wolter and Ellis, 2001

		27 days post-weaning	No differences in water, lipid, protein or ash concentration in carcass. Greater GE^3 carcass content. Lower empty BW and decreased tissue deposition rate.	Jones, 2012
IGF1	**Birth weight**	0 and 2 days of age	Growth response is not related to IGF1 concentration.	Ritacco *et al*., 1997
		2 - 4 hours after birth	Reduced gene expression of mucosal IGF1.	Wang *et al*., 2005
		21 days of age	Expression level of IGF1 is lower for *L. dorsi,* liver and kidney. No differences in the expression levels of IGF2, IGF1R, IGF2R and IGFBP5.	Chen *et al*., 2011
		18 and 28 days post-weaning	Lower circulating IGF1 and lower IGF1 receptor in proximal SI.	Michiels *et al*., 2012
		Market weight	Plasma IGF1 is reduced by 24% compared to heavy piglets.	Gondret *et al*., 2005
	Birth, weaning and 6 weeks of age weight	10 weeks of age	Lower plasma IGF1 concentration.	Chapter 5

Metabolic changes (literature in humans and piglets)	**Birth weight**	Birth and adult age	Thrifty phenotype theory, Syndrome X in adulthood.	Hales and Barker, 1992
		Prepuberal	Insulin resistance for light birth weight children in prepuberal phase.	Hofman *et al*., 1997
		3 and 12 months of age	Poor pre and postnatal growth is linked with adult obesity and altered glucose tolerance, insulin sensitivity and cardiovascular and endocrine function. Females are prone to higher fat deposition and males are prone to less leptin secretion.	Poore and Fowden, 2002
		Adult life	Association between slow growth before birth, accelerated growth in early postnatal life and the emergence of insulin resistance, visceral obesity and glucose resistance in adult life.	Morrison *et al*., 2010; Thorn *et al*., 2011
	Birth, weaning and 6 weeks of age weight	8 to 10 weeks of age	Piglets with an average birth weight but lower than average gain in the first six weeks of age display insulin and	Chapter 6

			glucose resistance in the post-weaning phase.	
Behaviour	**Birth weight**	Lactation phase	Low birth piglets have a tendency to drink from the posterior teats. Teat suckling order differences have an effect in future performance by lower gain in later life stages.	Fraser and Jones, 1975
		Lactation phase	No correlation between birth weight and suckling teat.	Kim *et al.*, 2000
		Post-weaning	Low birth piglets have more problems switching from known to unknown configurations.	Gieling *et al.*, 2011
	Weaning weight	Post-weaning	Lighter piglets tend to pay more visits with a lower intake per visit.	Bruininx *et al.*, 2001
	Birth, weaning and 6 weeks of age weight	1 and 2 weeks of age	Tendency to drink from the middle teats.	Chapter 3
		8 weeks of age	Higher fear to novelty.	
		9 weeks of age	More time spent on eating and lower rate of feed	Chapter 3

			intake per visit.	
Performance	**Birth weight**	Suckling and post-weaning	Reduced ADG when compared to heavier piglets.	Gondret *et al.*, 2005
		Market weight	Lower than average gain.	Mahan and Lepine, 1991; Rehfeldt and Kuhn, 2006
	Weaning weight	Market weight	Lower than average gain and longer time to reach market weight but no differences on FE or ADFI.	Wolter and Ellis, 2001
	Birth, weaning and 6 weeks of age weight	6 to 10 weeks of age	Lower performance in absolute values. Related to BW and $BW^{0.75}$ no difference when compared to heavier piglets.	Chapter 3
		6 to 10 weeks of age	Lower feed efficiency above maintenance.	
Nutrient digestibility	**Weaning weight**	Post-weaning	Lower dry matter, GE, nitrogen, ash digestibility when compared to average piglets.	Jones, 2012
	Birth, weaning and 6 weeks of age weight	10 weeks of age	Equal dry matter, GE and ash digestibility. Lower protein total tract and ileal	Chapter 3 and 4

		digestibility when provided a high fibre, low protein quality diet.

[1] SI: small intestine
[2] AL: piglets with an average birth weight but with a lower than average gain during the first six weeks of age
[3] GE: gross energy

The LP piglets are described as an in-between category between IUGR and average piglets. Based on our findings, the degree of retardation of LP piglets is lower when compared to IUGR piglets and can still be reversible. This growth retardation may have been established in-utero, but can also arise after birth. For example, the AL piglets did not suffer from growth retardation in-utero but had a lower than average gain during the first six weeks of age.

In commercial swine farming it is not feasible to determine individual BW of piglets at birth, weaning and six weeks of age or morphological characteristics. Yet, awareness on the different time points and type of intervention needed might shed some light on management or dietary interventions required.

Comparison of light and heavy pigs from 10 weeks of age until slaughter weight (modelling data)

As our studies finished at 10 weeks of age, we can only hypothesize about the effects of growth retardation in further life stages. To this end, the performance of light and heavy pigs was modelled from 10 weeks of age until slaughter weight (110 kg) using the growth model Watson 2.0[4]. Watson consists of four sub-models; animal definition, feed intake, environment and economics. Technical performance in fattening pigs can be calculated using the first three sub-models. The economics sub-model uses the calculations on technical performance to evaluate these from financial perspective. The Watson model can predict feed intake, growth and carcass composition on a daily basis for the average pig in a herd.

[4] Nutreco Canada, Quebec, Canada

Therefore, the pig and environment that is under study should be defined and the feed that will be provided to the pig is imported from a feed formulation software. Hence, the Watson model can be used to support decisions regarding nutrition, management and finance (Ferguson, 2006 and 2008).

The input of the **animal definition** sub-model includes start weight (18 and 30 kg BW for the light and heavy pigs respectively. When mixing light and heavy pigs in the pen, the initial average BW was 24 kg); age at the start of the simulation (10 weeks of age), genotype (hybrid of Hypor libra sows x Topigs P-line sires) and sex (50:50 gilts: castrated males) to predicted feed intake, growth and carcass composition and finally the weight of the pig for which the simulation will terminate (110 kg BW). For the present simulations the data used was obtained from the study described in **Chapter 3**.

The derivate of the Gompertz function was used to determine the deposition of protein (Ferguson, 2006). Protein deposition and weight is the basis of the model and is used for the prediction of growth of lipid, water and ash. The maximum amount of protein deposition (**PDmax**) at one moment in time is dependent on the degree of maturity, which is derived from the current body protein weight as a function of mature body protein weight. The desired lipid deposition is based on the desired fatness of the pig for a certain body protein weight, and follows from the lipid to protein ratio at maturity in relation to mature body protein weight. The deposition of water and ash and ultimately the live weight gain are predicted by its close relation to protein weight or deposition (allometric relations; Ferguson 2006).

The **feed intake sub-model** includes the feed composition (Table 7.3), physical form of the feed (pellet) and feed waste. Feed prices were specified to allow the economic calculations based on current prices on the market (May - June, 2014). The amount of feed was predicted by the model based on the provided input (environment), to reach the animal's growth potential. For both groups (light and heavy pigs) the estimated

growth potential was equal. Actual feed intake is dependent on desired feed intake for protein and may be constrained by the gut volume and environmental factors among others (Ferguson, 2006). The feed bulk is calculated based on feed composition and form. The environmental factors may adjust nutrient requirements and therefore, feed intake and growth calculations.

Four different diet phases (Starter, Grower, Finisher and Pre-slaughter) were provided. The diet change was based on weight rather than age (see Table 7.3 for the BW when each diet was fed). The Starter diet was provided until 30 kg BW. For the heavy pigs this diet was not provided, as the simulations started after piglets have reached this BW. The Grower diet was fed from 30 to 60 kg BW; the Finisher diet from 60 to 80 kg BW and the Pre-slaughter diet from 80 to 110 kg BW. The estimated nutrient composition of these diets is presented in Table 7.3. The nutrient composition was calculated based on CVB values (CVB, 2007).

Table 7.3 Different dietary composition[1] provided from 10 weeks of age until slaughter weight (110 kg BW) for the simulation in terms of performance between light and heavy pigs

Diet	BW range, kg BW	CP[2], g/kg	NE[3], MJ/kg	SID Lys[4], g/kg
Starter	10 weeks weight to 30 kg	176.8	10.2	10.49
Grower	30 to 60	171.3	10.2	9.89
Finisher	60 to 80	155.6	10.2	8.57
Pre-slaughter	80 to 110	142.5	10.2	7.49

[1] calculated g/kg feed of diet (as fed) based on CVB (2007)
[2] CP: crude protein
[3] NE: net energy
[4] SID: standardized ileal digestibility

The **environment** sub-model includes: **a) physical environment** contains the temperature in the stables (for most simulations starting temperature 21° C for the first 15 days and from day 16 until slaughter weight 19° C; for the contrast of summer vs. winter 27° C vs. 15° C respectively). Other conditions: air movement (mild), degree of insulation (average); floor type (partially slated and partially concrete) and relative humidity of the environment (60%). The total heat produced by the pig is calculated as the

sum of the heat produced due to protein and lipid deposition, and maintenance heat production. The effective temperature is calculated based on ambient temperature corrected for rates of air movement, degree of insulation and floor type in the lying area. **b) Social environment** includes wellbeing indicators, mortality rate (2% over the overall period), feeder space (one through feeder per pen) and stocking density (10 pigs in an 11 m^2 pen). The wellbeing status is an indicator of disease pressure and is calculated from the mortality rate, presence and percentage of coughing, manure consistency, air quality and stress.

The **economics** sub-model includes the feed prices, and current market price for meat (May – June, 2014); fixed costs and variable costs of commercial pig facilities. By combining the first three sub-models (animal definition, feed intake, environment) the revenues and fixed, variable and total costs per kg of growth, per pig, per pig place per year and per year are estimated.

As described in the beginning of this section, the aim of modelling the performance of light pigs until slaughter weight was used to estimate the economic impact of growth retardation in further life stages based on common practice farming conditions. The selected conditions were based on commercial practice commonly applied in Europe to manage growth retarded pigs: **1**. mixed vs. individual sex in a pen **2**. Mixed light and heavy vs. segregating pigs by BW at 10 weeks of age **3**. A concentrated diet (0.17 MJ/kg higher than the diets described on Table 7.3 for all phases but maintaining and equal SID Lysine: NE ratio) from 10 weeks of age until slaughter **4**. A concentrated diet until 30 kg and later on commercial diets (Table 7.3).

The evaluated parameters were:

1. Feed consumption (kg/d)
2. Average gain (g),
3. Feed conversion ratio (**FCR**)
4. Days to market
6. Cost/kg of gain

Season effect is described in literature as a cause for delay in growth of pigs (Myer *et al*., 2008; Steyn *et al*., 2012). As the light pigs are already in disadvantage for growth, two simulations were run to determine the impact of summer and winter conditions on days to market and cost/kg gain. The feeding scheme was feeding a concentrated diet until 30 kg BW and from 30 kg BW until slaughter a less concentrated diet. The profit in cost/kg gain from this scheme is observed in the summer (0.11 €/kg gain). During winter, there is a reduction of 2 days to reach market weight, with a 7 g/day higher gain but due to the lower temperature; the pig needs to eat larger amounts and therefore, the FCR is reduced (Table 7.4).

The simulations show that there is an advantage of housing males and females apart for light and heavy pigs (Table 7.5). The negative economic impact of mixed sexes is higher in heavy pigs (1.44 €/pig); whereas for the light pigs it is 1.10 €/pig due to a lower FCR by the females. When housing light and heavy pigs in the same pen, the economic advantage of grouping by sex is 1.20 €/pig (Table 7.5). The difference in days to market between light and heavy pigs is 25 days. This difference is larger than what is described by others (Poore and Fowden, 2004; Rehfeldt and Kuhn, 2006). Separation by group category is more important than by sex. In literature it is described that light pigs have a higher fat deposition when compared to heavier pigs at slaughter (Rehfeldt and Kuhn, 2006). The predicted carcass composition showed slightly higher lipid content for the light pigs, in agreement with literature (data not shown).

One simulation was providing a highly concentrated diet (0.17 MJ/kg higher but equal SID AA to NE ratio as in the diets presented in Table 7.3) to the light pigs from start until slaughter weight aimed to allow for compensatory gain through a higher nutrient intake. A reduction of 4 days to reach market weight was observed. Due to a 21 g/day increase in ADG when compared to a less concentrated diet. Yet, as the cost of these diets is higher when compared to a less concentrated diet there is no economic advantage in terms of cost/kg gain. The main advantage in BW gain is from start until 30 kg BW. Therefore, a simulation was performed providing a

concentrated diet from start until 30 kg BW and then switch to a less concentrated diet until slaughter weight. There was an advantage in reduction in days to market but no difference in reduction in cost/kg gain.

Table 7.4 Simulations using the Watson 2.0 model for light pigs from 10 weeks until slaughter weight (110 kg BW) when fed **1.** Commercial diets **2.** Concentrated diets **3.** A concentrated diet until 30 kg and commercial diets from 30 to 110 kg BW and **4.** Different room temperature (summer 27° C vs. winter 15° C) Assuming high health status, group housing (10 pigs/11 m^2 pen) and a 4-diet scheme (Starter 10 weeks - 30 kg BW, Grower 30 - 60 kg BW, Finisher 60 - 80 kg BW and Pre-slaughter 80 - 110 kg BW)

	Feed consumption, kg/d	**Average gain, g**	**Feed conversion ratio**	**Days to market, d**	**Cost/kg gain**
Commercial diets	1.82	723	2.51	128	0.69
Concentrated diets	1.89	747	2.53	124	0.68
Concentrate start	1.91	748	2.56	123	0.68
Summer (S) vs. Winter (W)	S 1.75; W 2.04	S 729; W 736	S 2.40; W 2.72	S 127; W 125	S 0.66; W 0.77

Table 7.5 Simulations using the Watson 2.0 model for light and heavy pigs from 10 weeks until slaughter weight (110 kg BW) cost of feeding (€/pig) when kept together as mixed groups (light and heavy pigs in the same pen) or apart. Assuming high health status, group housing (10 pigs/11 m^2 pen) and a 4-diet scheme (Starter 10 weeks - 30 kg, Grower 30 - 60 kg, Finisher 60 - 80 kg and Pre-slaughter 80 - 110 kg BW)

	Males (€/pig)	**Females** (€/pig)	**Feed cost** (€/pig) **sex separated**	**Feed cost** (€/pig) **mixed sex**	**Days to market**
Heavy pigs	56.80	55.36	56.08	56.32	103
Light pigs	63.94	62.84	63.39	63.66	128
Heavy and light pigs	60.37	59.17	59.77	60.03	116

IMPLICATIONS AND FUTURE STEPS

Unexpectedly, many of the evaluated parameters (e.g. macro nutrient digestibility and performance per kg $BW^{0.75}$) did not differ between LP and HP piglets. The main problems to overcome by the LP piglets are related to behaviour (fear to novelty), limitations in skeletal muscle growth and to feed intake. Regarding the fear to novelty, we assume that this is an inherited characteristic of LP piglets, as our observations were in individually housed piglets. When following LP and HP piglets in the immediate post-weaning phase, the LP piglets displayed more of a nibbling pattern compared to HP piglets and maintained this until the end of the nursery phase (data not shown). It remains to be elucidated whether this behaviour could be altered in the LP piglets by different feed composition (e.g. slow starch as described by Doty *et al*., 2014) to moderate the blood glucose response after a meal and promote a higher glucose uptake by the skeletal muscle. Whether this difference in feed intake pattern has an effect on piglet performance remains an open question (especially in group housed piglets with limited feeder space). This characteristic might place the LP piglets at a disadvantage. We observed that a high protein quality diet fed in the post-weaning phase (as described in **Chapter 4**) will provide an extra benefit to the LP piglets, as they have more difficulties with their fermentative capacity. The prediction using Watson model indicates that light pigs benefit from an energy dense diet from 10 weeks until 30 kg BW, a period right after our observations.

With proper management, skeletal muscle characteristics might well be restored in LP piglets as satellite cell number is not compromised, implying that fiber muscle accretion could be stimulated (Zammit *et al*., 2006). An early intervention (pre or immediate postnatal) could be considered (e.g. carnitine) to stimulate fiber development and capacity.

Focusing on the insulin insensitivity displayed by the AL piglets and reported in literature for IUGR piglets, an alternative could be to moderate glucose uptake (e.g. by a different glucose source with lower insulin stimulation), aiming to a higher skeletal muscle uptake and reducing the

uptake by adipose tissue. If insulin resistance remains as a persistent problem in the further productive stages of the pigs' life needs to be investigated. Literature describes that lighter piglets have fatter carcass at market weight (Bee, 2004; Rehfeldt and Kuhn, 2006) suggesting that insulin resistance persists in later life stages. Whether this is caused by epigenetic programming and which exact pathway is modified in these light piglets' remains to be elucidated. In humans it is described that IUGR alters insulin sensitivity in a tissue specific way, notably in muscle tissue. After considering the insulin resistance and skeletal muscle characteristics we can hypothesize the same occurs in LP piglets. The observations using the electronic feeding stations (nibbling pattern for the LP piglets in the post-weaning phase) together with the hyperglycaemic clamp response (insulin resistance), suggest that the LP piglets are trying to minimize the damage caused by insulin insensitivity on skeletal muscle through adaptations in their feed intake behaviour.

The proper time of intervention to restore the growth check suffered by these piglets (e.g. pre or early post-weaning) is essential. The two in vivo trials (**Chapter 3 and 4**) demonstrated that with an equal gain per kg BW in the post-weaning phase, the LP piglets did not display compensatory feed intake and therefore, no compensatory gain. Thus, the lack of compensatory gain after six weeks suggests that earlier intervention is needed.

One aspect that has not been addressed so far is what the implications of reversing the growth retardation suffered by the LP piglets would be. In terms of piglet welfare, it is expected to reduce the pre and/or post-weaning mortality and to provide the lighter piglets a better life start. From the farmer perspective, a more homogeneous batch of piglets leaving the nursery phase and at slaughter weight, with the desired lean carcasses. In the long term the social implications of reducing growth retardation must be considered. Especially when focusing on sows and boars designated for breeding purposes. Masking any genetic or epigenetic factors of growth retardation by providing a temporal solution might cause that the non-desired traits are transmitted to future siblings.

From the paediatric research perspective, different subpopulations of growth retarded piglets might allow us to understand the mechanisms behind growth retardation occurring at different time points (e.g. IUGR, due to birth process, postnatal) and their long term consequences. Using the AL piglets as a model for postnatal growth retardation could provide insight into the effects, implications and development of solutions for infants suffering from growth retardation. The data presented in **Chapter 6** suggests that the metabolic problems are the same for IUGR and for AL piglets. Implying that the solution for both categories might be one, which is an advantage from the management perspective.

Future research should be conducted to determine the maintenance requirements and nutrient uptake between different tissues and the role of insulin on them in LP piglets and within LP subpopulations to provide the optimal nutritional solutions to restore growth.

Further knowledge on strategies for reducing the fear to novelty (e.g. more enriched environment) and to determine whether by reducing their growth check their fear to novelty would also be reduced need to be developed. Knowledge on how to restore skeletal muscle composition while reducing fat deposition should be developed. Whether the long term effects of growth retardation and insulin resistance can be reversed in the nursery phase should be further studied.

MAIN CONCLUSIONS

- Growth retardation in the nursery phase cannot be explained only by birth or weaning weight.

- The extent to which the biological mechanisms responsible for the growth retardation are present in piglets depends on the criteria used to select them.

- When growth retarded piglets are selected based on a combination of birth, weaning and six weeks of age BW, there are no differences in apparent total tract and ileal nutrient digestibility of GE, N, and fat compared to heavier piglets in the nursery phase.

- The gain:feed ratio is equal or higher for growth retarded compared to heavier littermates when selected on a combination of birth, weaning and BW at six weeks of age.

- Utilization of ingested feed above maintenance tended to be lower for growth retarded piglets when assuming equal maintenance requirements per kg $BW^{0.75}$.

- Growth retarded piglets do not engage into compensatory feed intake in the post-weaning phase when fed a typical commercial diet.

- Growth retarded piglets have a nibbling eating pattern and spend more time of the day eating when compared to heavier littermates.

- Growth retarded piglets tend to have more fear towards novelty when compared to heavier littermates.

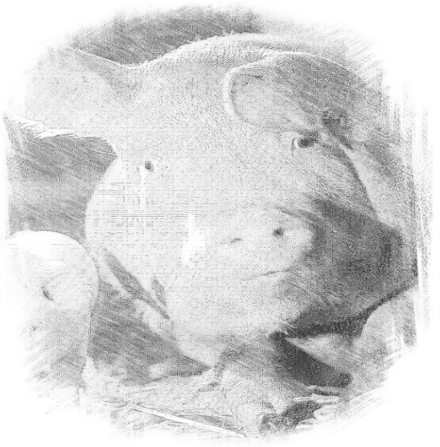
- The fermentative capacity in the digestive tract is less developed in growth retarded piglets when compared to heavier littermates.

- Growth retarded piglets have a lower total muscle fiber number and fiber cross sectional area which creates a disadvantage in terms of lean accretion.

- Piglets suffering from growth retardation in the lactation phase are more prone to insulin resistance in the post-weaning phase.

Chapter 8

List of References



A

Agricultural Research Council. 1981. The nutrient requirements of pigs. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Allen, R. E., R. A. Merkel, and R. B. Young. 1979. Cellular aspects of muscle growth: myogenic cell proliferation. J. Anim. Sci. 49:115-127.

Allen, W. R., S. C. Wilsher, and R. M. Butterfield. 2004. The influence of maternal size on pre- and postnatal growth in the horse: III. Postnatal growth. Reproduction 127:67-77.

Alonso-Spilsbury, M., D. Mota-Rojas, D. Villanueva, J. Martínez, H. Orozco, R. Ramirez, A. Lopez, and E. Trujillo. 2005. Perinatal asphyxia pathophysiology in pig and human: a review. Anim. Reprod. Sci. 90:1-30.

B

Ball, R., and F. Aherne. 1987. Influence of dietary nutrient density, level of feed intake and weaning age on young pigs. II. Apparent nutrient digestibility and incidence and severity of diarrhea. Canadian J. Anim. Sci. 67:1105-1115.

Bauer, R., B. Walter, A. Hoppe, E. Gaser, V. Lampe, E. Kauf, and U. Zwiener. 1998. Body weight distribution and organ size in newborn swine (sus scrofa domestica) - A study describing an animal model for asymmetrical intrauterine growth retardation. Exp. Toxicol. Pathol. 50:59-65.

Beaulieu, A. D., J. L. Aalhus, N. H. Williams, and J. F. Patience. 2010. Impact of piglet birth weight, birth order and litter size on subsequent growth performance, carcass quality, muscle composition and eating quality of pork. J. Anim. Sci. 88(8):2767-2778.

Bee, G. 2004. Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. J. Anim. Sci. 82:826-836.

Bérard, J., C. Kalbe, D. Lösel, A. Tuchscherer, and C. Rehfeldt. 2011. Potential sources of early-postnatal increase in myofibre number in pig skeletal muscle. Histochem. Cell Biol. 136:217-225.

Bianchi, A., H. Moonen, P. van der Heijden, and B. Bokhout. 1995. The use of a double antibody sandwich ELISA and monoclonal antibodies for the assessment of porcine IgM, IgG and IgA concentrations. Vet. Immunol. Immunopath. 44:309-317.

Biocheck GmbH. 2005. Entzündungs- und Belastungsparameter, vol 2. DAP, Leipzig, Germany.

Bjerre, D., T. Mark, P. Sorensen, H. Proschowsky, A. Vernersen, C. Jorgensen, and M. Fredholm. 2010. Investigation of candidate regions influencing litter size in Danish Landrace sows. J. Anim. Sci. 88:1603-1609.

Blat, S., A. Morise, A. Sauret, I. Louveau, K. Macé, I. Le Huëron-Luron, and B. Seve. 2012. The protein level of isoenergetic formulate does not modulate postprandial insulin secretion in piglets and has no consequences on later glucose tolerance. Brit. J. Nutr. 108:102-112.

Braems, G. 2003. Fetal hypoxemia on a molecular level: adaptive changes in the hypothalamic–pituitary–adrenal (HPA) axis and the lungs. Eur. J. Obstet. Gynecol. Reprod. Biol. 110:S63-S69.

Brody, S. 1945. Bioenergetics and growth with special reference to the efficiency complex in domestic animals. Reinhold publishing corporation, New York, NY, USA.

Brscic, M., L. F. Heutinck, M. Wolthouis-Fillerup, N. Stockhofe, B. Engel, E. K. Visser, F. Gottardo, E. A. Bokkers, B. J. Lensink, G. Cozzi, and C. G. van Reenen. 2011. Prevalence of gastrointestinal disorders recorded at post-mortem inspection in white veal calves and associated risk factors. J. Dairy Sci. 94:853-863.

Bruininx, E. M., C. M. van der Peet-Schwering, J. W. Scharama, P. F. Vereijken, P. C. Vesseur, H. Everts, L. A. den Hartog, and A. C. Beynen. 2001. Individually measured feed intake characteristics and growth performance of group-housed weanling pigs: effects of sex, initial body weight, and body weight distribution within groups. J. Anim. Sci. 79:301-308.

Buitrago, J., J. H. Maner, J. T. Gallo, and W. G. Pond. 1974. Effect of dietary energy in gestation on reproductive performance of gilts. J. Anim. Sci. 39:47-52.

Buonomo, F. C., T. J. Lauterio, C. A. Baile, and D. R. Campion. 1987. Determination of insulin-like growth factor 1 (IGF1) and IGF binding protein levels in swine. Domest. Anim. Endocrin. 4:23-31.

C

Camm, E., M. Martin, N. Wright, J. Hansell, S. Ozanne, and D. Giussani. 2011. Prenatal hypoxia independent of undernutrition promotes molecular markers of insulin resistance in adult offspring. FASEB J. 25:420-427.

Campos, P., B. Silva, J. Donzele, R. Oliveira, and E. Knol. 2012. Effects of sow nutrition during gestation on within-litter birth weight variation: a review. Animal 6:797-806.

Canario, L., T. Tribout, J. Gogué, and J. Bidanel. 2003. Estimation of realised genetic trends in French Large White pigs from 1977 to 1998 using frozen semen: farrowing and early lactation periods. Animal 1(7):929-938.

Capasso, R., and A. Izzo. 2008. Gastrointestinal regulation of food intake: general aspects and focus on anandamide and oleoylethanolamide. J. Neuroendocrinol. 20:39-46.

Carney, E., H. Tran, J. W. Bundy, R. Moreno, P. Miller, and T. Burkey. 2009. Effect of dam parity on growth performance and immunity of weaned pigs. Nebraska Swine Reports. pp 30-32.

Centraal Veervoerder Bureau CVB. 2007. Chemical composition and nutritional values of feed ingredients. CVB serie 32. The Hague, the Netherlands.

Chang, C. D., L. Ottavio, S. Travali, K. E. Lipson, and R. Baserga. 1990. Transcriptional and post-transcriptional regulation of the proliferating cell nuclear antigen gene. Mol. Cell. Biol. 10:3289-3296.

Chen, R., Y. Yin, J. Pan, Y. Gao, and T. Li. 2011. Expression profiling of IGFs and IGF receptors in piglets with intrauterine growth restriction. Livest. Sci. 136:72-75.

Cheng, N., W. Cai, M. Jiang, and S. Wu. 1997. Effect of hypoxia on blood glucose, hormones and insulin receptor functions in new born calves. Pediatr. Res. 41:852-856.

Cianfarani, S., D. Germani, and F. Branca. 1999. Low birth weight and adult insulin resistance: the "catch-up growth" hypothesis. Arch. Dis. Child. Fetal. Neonatal. Ed. 81:F71-F73.

Cohn, H., E. Sacks, M. Heymann, and A. Rudolph. 1974. Cardiovascular responses to hypoxemia and acidemia in fetal lambs. Am. J. Obstet. Gynecol. 120:817-824.

Cole, M., and M. Varley. 2000. Weight watchers from birth. New data emphasise the importance of maximising piglet weight from birth onwards. Pig International 30:13-16.

Cummings, D. E., and J. Overduin. 2007. Gastrointestinal regulation of food intake. J. Clin. Investig. 117:13-23.

D

Damm, B., N. Friggens, J. Nielsen, K. Ingvartsen, and L. Pedersen. 2002. Factors affecting the transfer of porcine parvovirus antibodies from sow to piglets. J. Vet. Med. A. 49:487-495.

Daniel, C., and F. Wood. 1980. Fitting equations to data. Rev. Ed. New York: John Wiley and Sons, Inc. United States. pp 458.

Danish Agriculture and Food Council. 2012. Annual report. Pig Research Centre 1st. Ed. Axeltorv, Kobenhaun.

Da Silva-Buttkus, P., R. van den Hurk, E. R. Velde, and M. A. Taverne. 2003. Ovarian development in intrauterine growth-retarded and normally developed piglets originating from the same litter. Reproduction 126:249-258.

Dauncey, M. J., R. A. Shakespeare, B. T. Rudd, and D. L. Ingram. 1990. Variations in somatomedin-C/insulin-like growth factor-I associated with environmental temperature and nutrition. Horm. Metab. Res. 22:261-264.

Desai, M., and A. Crowtheri. 1996. Organ-selective growth in the offspring of protein-restricted mothers. Brit. J. Nutr. 76:591-603.

Devillers, N., C. Farmer, J. Le Dividich, and A. Prunier. 2007. Variability of colostrum yield and colostrum intake in pigs. Animal 1:1033-1041.

Devillers, N., M. Jvan, A. Prunier, and J. Le Dividich. 2004. Estimation of colostrum intake in the neonatal pig. Animal 78(2):305-315.

Doty, S., J. Suárez, M. Latorre, J. Guada, and M. Fondevilla. 2014. Effect of dietary starch source on growth performanace digestibility and quality traits of growing pigs. Livest. Sci. 164:119-127.

Dunshea, F., D. Kerton, P. Cranwell, R. Campbell, B. Mullan, R. King, G. Power, and J. Pluske. 2003. Lifetime and post-weaning determinants of performance indices of pigs. Aust. J. Agric. Res. 54:363-370.

Dwyer, C. M., and N. C. Stickland. 1991. Sources of variation in myofiber number and within and between litters of pigs. Anim. Prod. 52:527-533.

Dwyer, C. M., J. M. Fletcher, and N. C. Stickland. 1993. Muscle cellularity and postnatal growth in the pig. J. Anim. Sci. 71:3339-3343.

E

Edwards, S. A. 2002. Perinatal mortality in the pig: environmental or physiological solutions? Livest. Sci. 78:3-12.

English, P. R., W. J. Smith, and A. MacLean. 1977. The sow: improving her efficiency. Farming Press Ltd, Ipswich, UK. pp 76-89.

Erkens, T., M. van Poucke, K. Goossens, A. van Zeveren, and L. J. Peelman. 2006. Development of a new set of reference genes for normalization of real-time RT-PCR data of porcine backfat and *longissimus dorsi* muscle, and evaluation with *PPARGCIA*. BMC Biotechnol. 6:41-47.

F

Ferguson, N. 2006. Basic concepts describing animal growth and feed intake. Mechanistic Modelling in Pig and Poultry Production. pp 22-53.

Ferguson, N. 2008. Application of an integrated management system to improve performance and profitability in finishing pigs. In: Advances in pork production. Proc., Banff Pork Seminar, Alberta, CA. pp 85-91.

Ferrer, R., J. Medrano, M. Diego, R. Calpena, L. Graells, M. Moltó, T. Pérez, F. Pérez, and G. Salido. 2000. Effect of exogenous insulin and glucagon on exocrine pancreatic secretion in rats in vivo. Int. J. Pancreatology 28:67-75.

Fix, J. S., J. Cassady, W. Herring, J. Holl, M. Culbertson, and M. See. 2010. Effect of piglet birth weight on body weight, growth, backfat, and Longissimus muscle area of commercial market swine. Livest. Sci. 127:51-59.

Flores, S., S. Rodriguez, J. Velasquez, M. Valencia, and S. Serrano 1996. Reanimacion del recien nacido. Reanimacion del recien nacido. In: Urgencias en Pediatria. Interamericana-McGraw-Hill, Mexico. pp 97-100.

Florini, J. R., D. Z. Ewton, and S. A. Coolican. 1991. Growth hormone and the insulin-like growth factor system in myogenesis. Endocr. Rev. 17:481-517.

Foxcroft, G., G. Bee, W. Dixon, M. Hahn, J. Harding, J. Patterson, T. Putman, S. Sarmento, M. Smit, W. Tse, and S. Town. 2007. Consequences of selection for litter size on piglet development. Editors J. Wiseman, M. Varley, S. McOrist and B. Kemp. In: Paradigms of Pig Science. Nottingham University Press, Nottingham, UK. pp 207-229.

Foxcroft G., W. T. Dixon, S. Novak, C. T. Putman, S. C. Town, and M. D. Vinsky. 2006. The biological basis for prenatal programming of postnatal performance in pigs. J. Anim. Sci. 84:E105-E112.

Foxcroft, G. R., and S. C. Town. 2004. Prenatal programming of postnatal performance - the unseen cause of variance. Advances in pork production : Proceedings of the Banff Pork Seminar 15. pp 269-279.

Fraser, D., and R. M. Jones. 1975. Teat order of suckling pigs. 1. Relation to birth weight and subsequent growth. J. Agric. Sci. 84:387-391.

Fritz, H., G. Hartwich, and E. Werle. 1966. On protease inhibitors. I. Isolation and characterization of trypsin inhibitors from dog pancreas tissue and pancrease secretion. Hoppe-Seyler's Zeitschrift für physiologische Chemie 12:345-350.

G

Gathercole, L. L., S. A. Morgan, and J. W. Tomlinson. 2012. Hormonal regulation of lipogenesis. Vitamins and hormones 91:1-27.

Genstat Committee. 2000. Reference Manual. Procedure Library PL12. VSN International. Oxford, UK.

Gerrard D. E., C. S. Okamura, M. A. Ranalletta, and A. L. Grant. 1998. Developmental expression and location of IGF-I and IGF-II mRNA and protein in skeletal muscle. J. Anim. Sci. 76:1004-1011.

Gieling, E. T., S. Y. Park, R. E. Nordquist, and F. J. van der Staay. 2011. Cognitive performance of low-and normal-birth-weight piglets in a spatial hole-board discrimination task. Pediatr. Res. 39:71-76.

Gondret, F., L. Lefaucheur, H. Juin, I. Louveau, and B. Lebret. 2006. Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the Longissimus muscle in pigs. J. Anim. Sci. 84:93-103.

Gondret, F., L. Lefaucheur, I. Louveau, B. Lebret, X. Pichodo, and Y. Le Cozler. 2005. Influence of piglet birth weight on postnatal growth performance, tissue lipogenic capacity and muscle histological traits at market weight. Livest. Prod. Sci. 93:137-146.

Greef, K. 1995. Prediction of growth and carcass parameters. Editors. P. Mogan, M. Verstegen and M. Visser. In: Modelling growth in the pig. Wageningen Press, Wageningen, the Netherlands. pp 151-163.

H

Hales, C. N., and D. J. Barker. 1992. Type 2 (non insulin dependent) diabetes mellitus – The thrifty phenotype hypothesis. Diabetologia 35:595-601.

Han, F., L. Hu, Y. Xuan, X. Ding, Y. Luo, S. Bai, S. He, K. Zhang, and L. Che. 2013. Effects of high nutrient intake on the growth performance, intestinal morphology and immune function of neonatal intrauterine growth retarded pigs. Brit. J. Nutr. 110:1819-1827.

Handel, S. E., and N. C. Stickland. 1987. Muscle cellularity and birth weight. Anim. Prod. 44:311-317.

Harada, E., M. Shizuyama, N. Ihara, and T. Takeuchi. 2003. Impaired pancreatic endocrine and exocrine responses in growth-retarded piglets. J. Vet. Med. A. 50:433-441.

Hayashi, M., D. Ingram, and M. Dauncey. 1987. Heat production and respiratory enzymes in normal and runt newborn piglets. Neonatology 51:324-331.

Herpin, P., M. Damon, and J. Le Dividich. 2002. Development of thermoregulation and neonatal survival in pigs. Livest. Prod. Sci. 78:25-45.

Hofman, P. L., W. Cutfield, E. Robinson, R. Bergman, R. Menon, M. Sperling, and P. Gluckman. 1997. Insulin resistance in short children with intrauterine growth retardation. J. Clin. Endocrynol. Metab. 82:402-406.

I

International Organization for Standardization ISO. 1997. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. Kjeldahl method. ISO 5983. International Organization for Standardization. Geneva, Switzerland.

International Organization for Standardization ISO 1998a. Animal feeding stuffs, animal products, and feces or urine. Determination of gross calorific value. ISO 9831. International Organization for Standardization. Geneva, Switzerland.

International Organization for Standardization ISO 1998b. Animal feeding stuffs. Determination of moisture and other volatile matter content. ISO 6496. International Organization for Standardization. Geneva, Switzerland.

International Organization for Standardization ISO 2002. Animal feeding stuffs. Determination of crude ash. ISO 5984. International Organization for Standardization. Geneva, Switzerland.

J

Jones, C. K. 2012. Characterizing the Fallback Pig. PhD Diss. Iowa State University, Iowa.

K

Kalbe C., M. Mau, and C. Rehfeldt. 2008. Developmental changes and the impact of isoflavones on mRNA expression of IGF-I receptor, EGF receptor and related growth factors in porcine skeletal muscle cell cultures. Growth Horm. IGF Res. 18:424-433.

Kalbe, C., J. Bérard, M. Porm, C. Rehfeldt, and G. Bee. 2013. Maternal L-arginine supplementation during early gestation affects foetal skeletal myogenesis in pigs. Livest. Sci. 157:322-329.

Katz, A., S. Nambi, K. Mather, A. Baron, D. Follmann, G. Sullivan, and M. Quon. 2000. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J. Clin. Endocrinol. Metab. 85:2402-2410.

Kaufman, D., M. Banerji, I. Shorman, E. Smith, J. Coplan, L. Rosenblum, and J. Kral. 2007. Early life stress and the development of obesity and insulin resistance in juvenile bonnet macaques. Diabetes 56:1382-1386.

v. Kempen, T. 2007. Sports supplements to facilitate parturition and reduce perinatal mortality. Editors P. Garsworth and J. Wijeman. In: Recent advances in animal nutrition. Nottingham Feed Conference, Nottingham, UK. pp 317-330.

v. Kempen, T., R. Prajwal, R. Regmi, J. Matte, and R. Zijlstra. 2010. In vitro starch digestion kinetics, corrected for estimated gastric emptying, predict portal glucose appearance in pigs. J. Nutr. 140:1227-1233.

Kerr, M. G. 2008. Veterinary laboratory medicine. 2nd edition. Blackwell Science Ltd, Oxford, UK.

Kersten, S. 2001. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO reports 2:282-286.

Kim, S. W., W. L. Hurley, I. K. Hant, and R. A. Easter. 2000. Growth of nursing pigs related to the characteristics of nursed mammary glands. J. Anim. Sci. 78:1313-1318.

Koishi K., M. Zhang, I. S. McLennan, and A. J. Harris. 1995. MyoD protein accumulates in satellite cells and is neurally regulated in regenerating myotubes and skeletal muscle fibers. Dev. Dyn. 202:244-254.

Koopmans, S., M. Jong, I. Que, V. Dahlmans, H. Pijl, J. Radder, M. Frölich, and L. Havekes. 2001. Hyperlipidaemia is associated with increased insulin mediated glucose metabolism, reduced fatty acid metabolism and normal blood pressure in transgenic mice overexpressing human apolipoprotein C1. Diabetologia 44:437-443.

Kraft, W., and U. Dürr. 2005. Klinische Labordiagnostik in der Tiermedizin. Schattauer GmbH., Stuttgart, Germany. pp 534.

Krueger, R. , M. Derno, S. Goers, B. Metzler, G. Nuernberg, K. Martens, and R. Constanze. 2013. Higher body fatness in intrauterine growth retarded juvenile pigs is associated with lower fat and higher carbohydrate oxidation during *ad libitum* and restricted feeding. European J. Nutr. 1:1-15.

Kuang, S., K. Kuroda, F. Le Grand, and M. A. Rudnicki. 2007. Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129:999-1010.

L

Labussière, E., J. van Milgen, C. F. de Lange, and J. Noblet. 2011. Maintenance energy requirements of growing pigs and calves are influenced by feeding level. J. Nutr. 141:1855-1861.

v. Laere, A., M. Nguyen, M. Braunschweig, C. Nezer, C. Collette, L. Moreau, A. L. Archibald, C. Haley, N. Buys, M. Tally, G. Anderson, M. Georges, and L. Anderson. 2003. A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425:832-836.

Layer, P., A. Zinsmeister, and E. DiMagno. 1986. Effects of decreasing intraluminal amylase activity on starch digestion and postprandial gastrointestinal function in humans. Gastroenterology 91:41-48.

Le Dividich, J., J. A. Rooke, and P. Herpin. 2005. Nutritional and immunological importance of colostrum for the newborn pig. J. Agric. Sci. 143:469-485.

Le Dividich, J., and J. Noblet. 1982. Growth rate and protein and fat gain in early weaned piglets housed below thermoneutrality. Livest. Prod. Sci. 9:731-742.

Le Dividich, J., P. Morméde, M. Catheline, and J. Caritez. 1991. Body composition and cold resistance of the neonatal pig from European (Large White) and Chinese (Meishan) breeds. Biol. Neonate 59:268-277.

Lee, C. Y., C. S. Chung, and F. A. Simmen. 1993. Ontogeny of the porcine insulin-like growth factor system. Mol. Cell. Endocrinol. 93:71-80.

Le Goff, G., and J. Noblet. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. J. Anim. Sci. 79(12):2418-2427.

Lossec, G., P. Herpin, and J. Le Dividich. 1998. Thermoregulatory responses of the newborn pig during experimentally induced hypothermia and rewarming. Exp. Physiol. 83:667-678.

Lowry, C., O. Rosebrough, A. Farr, and R. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 28:193-265.

M

v.d. Maagdenberg, K., A. Stinckens, E. Claeys, N. Buys, and S. De Smet. 2008a. Effect of the insulin-like growth factor-II and RYR1 genotype in pigs on carcass and meat quality traits. Meat Sci. 80:293-303.

v.d. Maagdenberg, K., A. Stinckens, L. Lefaucheur, N. Buys, and S. De Smet. 2008b. The effect of mutations in the insulin-like growth factor-II and ryanodine receptor-1 genes on biochemical and histochemical muscle fibre characteristics in pigs. Meat Sci. 79:757-766.

Magowan, E., M. E. Ball, K. J. McCracken, V. E. Beattie, R. Bradford, M. J. Robinson, M. Scott, F. J. Gordon, and C. S. Mayne. 2011. The performance response of pigs of different wean weights to `high' or `low' input dietary regimes between weaning and 20 weeks of age. Livest. Sci. 136:232-239.

Mahan, D. C., and A. J. Lepine. 1991. Effect of pig weaning weight and associated nursery feeding programs on subsequent performance to 105 kilograms body weight. J. Anim. Sci. 69:1370-1378.

Mallows, C. L. 1973. Some comments on Cp. Technometrics 15(4):661-675.

Martineau, G., and P. B. Badouard. 2009. Managing highly prolific sows. London Swine Conference – Tools of the Trade. pp 1-2.

Matthews, D., J. Hosker, A. Rudenski, B. Naylor, D. Treacher, and R. Turner. 1985. Homeostasis model assessment: insulin resistance and β cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412-419.

Mauro, A. 1961. Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol. 9:493-495.

McBride, G. 1963. The “teat order” and communication in young pigs. Animal Behaviour 11:53-56.

McCance, R., and E. M. Widdowson. 1974. Review lecture: the determinants of growth and form. Proceedings of the Royal Society of London. Series B. Biol. Sci. 185:1-17.

McMillen, I. C., M. B. Adams, J. T. Ross, C. L. Coulter, G. Simonetta, J. A. Owens, J. S. Robinson, and L. J. Edwards. 2001. Fetal growth restriction: adaptations and consequences. Reproduction 122:195-204.

v.d. Meulen, J., G. Bakker, B. Smits, and H. de Visser. 1997. Effect of source of starch on net protal flux of glucose, lactate, volatile fatty acids and amino acids in the pig. Brit. J. Nutr. 78:533-544.

Michiels, J., M. De Vos, J. Missotten, A. Ovyn, S. De Smet, and C. van Ginneken. 2012. Maturation of digestive function is retarded and plasma antioxidant capacity lowered in fully weaned low birth weight piglets. Br. J. Nutr. 109:65-75.

Milligan, B. N., D. Fraser, and D. L. Kramer. 2001. Birth weight variation in the domestic pig: effects on offspring survival, weight gain and suckling behaviour. Appl. Anim. Behav. Sci. 73:171-191.

Montgomery, D. C., and E. A. Peck. 1992. Introduction to linear regression analysis. 2nd. Edition. Wiley-Interscience. New York, United States. pp 527.

Morrison, J., J. Duffield, B. Muhlhausler, S. Gentili, and I. McMillen. 2010. Fetal growth restriction, catch-up growth and the early origins of insulin resistance and visceral obesity. Pediatr. Nephrol. 25:669-677.

Mosby, E. 2009. Mosby's medical dictionary. Elsevier St. Louis, MO.

Myer, R., J. Brendemuhl, and R. Bucklin. 2008. Effect of season on growth performance of finishing pigs fed low-protein, amino acid supplemented diets. J. Appl. Anim. Res. 34:1-8.

N

National Research Council. 1998. Nutrient Requirements of Swine: 10th Rev. Ed. National Academy Press, Washington DC, USA.

Nissen, P. M., P. F. Jorgensen, and N. Oksbjerg. 2004. Within-litter variation in muscle fiber characteristics, pig performance, and meat quality traits. J. Anim. Sci. 82:414-421.

Noblet, J., and K. E. Knudsen. 1997. Comparative digestibility of wheat, maize and sugar beet pulp non-starch polysaccharides in adult sows and growing pigs. In: Proc. VII Int. Cong. Digestive physiology in pigs. pp 115-148.

Novikoff, A. B., W. Shin, and J. Drucker. 1961. Mitochondrial localization of oxidative enzymes: staining results with two tetrazolium salts. J. Biophys. Biochem. Cytol. 9:47-61.

O

Oksbjerg, N., F. Gondret, and M. Vestergaard. 2004. Basic principles of muscle development and growth in meat-producing mammals as affected by the insulin-like growth factor (IGF) system. Domest. Anim. Endocrinol. 27:219-240.

Oliver J., T. 1965. Temperature regulation and heat production in the newborn. Pediatr. Clin. N. Amer. 12:765-779.

P

Paredes, S. P., A. J. M. Jansman, M. W. A. Verstegen, A. Awati, W. Buist, L. A. den Hartog, H. M. J. van Hees, N. Quiniou, W. H. Hendriks, and W. J. J. Gerrits. 2012. Analysis of factors to predict piglet body weight at the end of the nursery phase. J. Anim. Sci. 90:3243-3251.

Paredes, S. P., A. J. M. Jansman, M. W. A. Verstegen, L. A. den Hartog, H. M. J. van Hees, J. E. Bolhuis, and W. J. J. Gerrits. 2014. Identifying the limitations for growth in low performing piglets from birth until ten weeks of age. Animal 8(6):923-930.

Paredes, S. P., C. Kalbe, A. J. M. Jansman, M. W. A. Verstegen, H. J. M. van Hees, D. Lösel, W. J. J. Gerrits, and C. Rehfeldt. 2013. Predicted high-performing piglets exhibit more and larger skeletal muscle fibers. J. Anim. Sci. 12:5589-5598.

Patruno, M., F. Caliaro, L. Maccatrozzo, R. Sacchetto, T. Martinello, L. Toniolo, C. Regginani, and F. Mascarello. 2008a. Myostatin shows a specific expression pattern in pig skeletal and extraocular muscles during pre-and post-natal growth. Differentiation 76:168-181.

Patruno M., F. Caliaro, T. Martinello, and F. Mascarello. 2008b. Expression of the paired box domain Pax7 protein in myogenic cells isolated from the porcine semitendinosus muscle after birth. Tissue Cell 40:1-6.

Père, M. C., and M. Etienne. 2000. Uterine blood flow in sows: Effects of pregnancy stage and litter size. Reprod. Nutr. Dev. 40:369-382.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:45-47.

Pierzynowski, S., and W. Barej. 1984. The dependence of exocrine pancreatic secretion on insulin in sheep. Exp. Physiol. 69:35-39.

Pierzynowski, S., B. Westrom, J. Svendsen, and B. Karlsson. 1990. Development of exocrine pancreas function in chronically cannulated pigs during 1-13 weeks of postnatal life. J. Pediatric Gastroenterol. Nutr. 10:206-212.

Pluske, J., D. Kerton, P. Cranwell, R. Campbell, B. Mullan, R. King, G. Power, S. Pierzynowski, B. Westrom, and C. Rippe. 2003. Age, sex, and weight at weaning influence organ weight and gastrointestinal development of weanling pigs. Crop Pasture Sci. 54:515-527.

Pluske, J., J. Kim, D. McDonald, D. Pethick, and D. Hampson. 2001. Non-starch polysaccharides in the diets of young weaned piglets. The weaner pig. Nutrition and management. CABI Publishing, Wallingford, UK. pp 81-112.

Poore, K., and A. Fowden. 2002. The effect of birth weight on glucose tolerance in pigs at 3 and 12 months of age. Diabetologia 45:1247-1254.

Poore, K. R., and A. L. Fowden. 2004. Insulin sensitivity in juvenile and adult Large White pigs of low and high birth weight. Diabetologia 47:340-348.

Powell, S. E., and E. D. Aberle. 1980. Effects of birth weight on growth and carcass composition of swine. J. Anim. Sci. 52:748-756.

Q

Quesnel, H., L. Brossard, A. Valancogne, and N. Quiniou. 2008. Influence of some sow characteristics on within-litter variation on piglet birth weight. Animal 2:1842-1849.

Quinou, N., J. Dagorn, and D. Gaudré. 2002. Variation of piglets' birth weight and consequences on subsequent performance. Livest. Prod. Sci. 78:63-70.

Quiniou, N., and J. Noblet. 1999. Influence of high ambient temperatures on performance of multiparous lactating sows. J. Anim. Sci. 77(8):2124-2134.

R

Rehfeldt, C. 2007. Point: Counterpoint Comments: Satellite cell addition is / is not obligatory for skeletal muscle hypertrophy. J. Appl. Physiol. 103:1104-1106.

Rehfeldt, C., A. Tuchscherer, M. Hartung, and G. Kuhn. 2008. A second look at the influence of birth weight on carcass and meat quality in pigs. Meat Sci. 78:170-175.

Rehfeldt, C., B. Stabenow, R. Pfuhl, J. Block, G. Nürnberg, W. Otten, C. C. Metges, and C. Kalbe. 2012b. Effects of limited and excess protein intakes of pregnant gilts on carcass quality and cellular properties of skeletal muscle and subcutaneous adipose tissue in fattening pigs. J. Anim. Sci. 90:184-196.

Rehfeldt, C., and G. Kuhn. 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J. Anim. Sci. 84:E113-E123.

Rehfeldt, C., G. Kuhn, G. Nürnberg, E. Kanitz, F. Schneider, M. Beyer, K. Nürnberg, and K. Ender. 2001. Effects of exogenous somatotropin during early gestation on maternal performance, fetal growth and compositional traits in pigs. J. Anim. Sci. 79:1789-1799.

Rehfeldt, C., I. Fiedler, G. Dietl, and K. Ender. 2000. Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. Livest. Prod. Sci. 66:177-188.

Rehfeldt, C., I. Fiedler, and N. C. Stickland. 2004. Number and size of muscle fibres in relation to meat production. Editors M. F. W. te Pas, M. E. Everts and H. P. Haagsman. In: Muscle Development of Livestock Animals. CAB International, Oxfordshire, UK. pp 1-38.

Rehfeldt, C., L. Lefaucheur, J. Block, B. Stabenow, R. Pfuhl, W. Otten, C. C. Metges, and C. Kalbe. 2012a. Limited and excess protein intake of pregnant gilts differently affects body composition and cellularity of skeletal muscle and subcutaneous adipose tissue of newborn and weanling piglets. Eur. J. Nutr. 51:151-165.

Rijnen, M., M. Verstegen, M. Heetkamp, J. Haaksman, and J. Schrama. 2001. Effects of dietary fermentable carbohydrates on energy metabolism in group-housed sows. J. Anim. Sci. 79:148-154.

Ritacco, G., S. V. Radecki, and P. A. Schoknecht. 1997. Compensatory growth in runt pigs is not mediated by insulin-like growth factor I. J. Anim. Sci. 75:1237-1243.

Ropka-Molik, K., R. Eckert, and K. Piorkowska. 2011. The expression pattern of myogenic regulatory factors MyoD, Myf6 and Pax7 in postnatal porcine skeletal muscles. Gene Expr. Patterns 11:79-83.

Rueda, C., V. Dolinsky, J. Morton, S. Proctor, J. Dyck, and S. Davidge. 2011. Hypoxia induced intrauterine growth restriction increases the susceptibility of rats to high fat diet induced metabolic syndrome. Diabetes 60:507-516.

Rutherford, K., E. Baxter, R. D'Earth, S. Turner, G. Arnott, R. Roehe, B. Ask, P. Sandoe, V. Moustsen, and F. Thorup. 2013. The welfare implications of large litter size in the domestic pig I: biological factors. Anim. Welfare 22:199-218.

S

Sabbioni, A., V. Beretti, R. Manini, C. Cervi, and P. Superchi. 2010. Effect of sex and season of birth on Gompertz growth curve parameters in "Nero di Parma" pigs. Ital. J. Anim. Sci. 8:719-726.

Sacy, A., Y. Le Treut, P. Schmidely, and E. Chevaux. 2010. Caractérisation de l'immaturité des porcelets á la naissance. In: Proceedings of 42nd Journées Recherche Porcine, 2-3 February 2010, Paris, France, pp 259-260.

Safranski, T. J., A. M. Williams, and M. C. Lucy. 2010. Physiological and reproductive response to periparturient heat stress in sows. In Proc. Midwestern ASAS, Iowa, United States of America. pp 70-75.

Sandström, J., B. Weström, S. Pierzynowski, B. Ohlsson, and B. Karlsson. 1986. Immunoreactive trypsin in blood serum as a marker of pancreatic function and development in the youn pig. Proc. 9th Int. Pig Vet. Soc. Congress July 15-18. Barcelona, Spain. pp 468.

Sankaran, S., and P. M. Kyle. 2009. Aetiology and pathogenesis of IUGR. Best Pract. Res. Clin. Obstet. Gynaecol. 23:765-777.

SAS Institute Inc. 2011. SAS User's guide: Statistics. SAS for Windows, Version 9.3 SAS Institute, Inc., Cary, NC, USA.

Schinckel, A., B. Richert, D. Sholly, J. Radcliffe, A. Sutton, and M. Einstein. 2009. Impact of weaning weight and early postweaning growth of pigs to late finishing growth when fed either corn-and soybeanmeal based diets or low nutrient excretion nutrient-dense diets. The Professional Animal Scientist 25:175-188.

Schinckel, A. P., M. E. Einstein, S. Jungst, C. Booher, and S. Newman. 2010. Evaluation of the impact of pig birth weight on grow-finish performance, backfat depth, and loin depth. The Professional Animal Scientist 26:51-69.

Schoknecht, P. A., W. G. Pond, H. J. Mersmann, and R. R. Maurer. 1993. Protein restriction during pregnancy affects postnatal growth in swine progeny. J. Nutr. 123:1818-1825.

Schultes, B., A. Peters, M. Hallschmid, C. Benedict, V. Merl, K. Oltmanns, J. Born, H. Fehrn, and W. Kern. 2005. Modulation of food intake by glucose in patients with type 2 diabetes. Diabetes care 28(12):2884-2889.

Seale, P., L. A. Sabourin, A. Girgis-Gabardo, A. Mansouri, P. Gruss, and M. A. Rudnicki. 2000. Pax7 is required for the specification of myogenic satellite cells. Cell 102:777-7786.

Smit, M., J. Spencer, F. Almeida, J. Patterson, H. Chiarini, M. Dyck, and G. Foxcroft. 2013. Consequences of a low litter birth weight phenotype for postnatal lean growth performance and neonatal testicular morphology in the pig. Animal 7(10):1681-1689.

Smith, A. L., K. J. Stalder, T. V. Serenius, T. J. Baas, and J. W. Mabry. 2007. Effect of piglet birth weight on weights at weaning and 42 days post-weaning. J. Swine Health Prod. 15:213-218.

Stamataris, C., I. Kyriazakis, and G. Emmans. 1991. The performance and body composition of young pigs following a period of growth retardation by food restriction. Anim. Prod. 53:373-381.

Steyn, W. J., N. H. Casey, and C. Jansen. 2012. Effects of different penning conditions, feeding regimens and season on growth and carcass attributes of boars of a selected genetic line. S. Afr. J. Anim. Sci. 42:178-188.

Stinckens, A., K. van den Maagdenberg, T. Luyten, M. Georges, S. De Smet, and N. Buys. 2007. The RYR1 g.1843C>T mutation is associated with the effect of the IGF2 intron3-g.3072G>A mutation on muscle hypertrophy. Anim. Genet. 38:67-71.

Souza, C. 2013. Fermentation in the gut to prolong satiety. Exploring mechanisms by which dietary fibres affect satiety in pigs. PhD Diss. Wageningen University, the Netherlands.

Surek, D., L. Barrilli, E. Bueno, I. Krabbe, E. Alberton, and G. Maiorka. 2014. Growth of suckling piglets in litters standardized by weight. J. Anim. Sci. 92:177-181.

Svendsen, L., B. Weström, J. Svendsen, A. C. Olsson, and B. Karlsson. 1991. Blood serum characteristics of newborn pigs: comparison of unaffected pigs with pigs belonging to five mortality groups. Acta Vet. Scand. 32(3):287-290.

Swatland, H. J., and R. G. Cassens. 1972. Muscle growth: The problem of muscle fibers with an intrafascicular termination. J. Anim. Sci. 35:336-344.

te Pas, M. F., and A. Soumillion. 2001. Improvement of livestock breeding strategies using physiologic and functional genomic information of the muscle regulatory factors gene family for skeletal muscle development. Curr. Genomics 2:285-304.

te Pas, M. F., F. J. Verburg, C. L. Gerritsen, and K. H. de Greef. 2000. Messenger ribonucleic acid expression of the MyoD gene family in muscle tissue at slaughter in relation to selection for porcine growth rate. J. Anim. Sci. 78:69-77.

Thorn, S. R., P. J. Rozance, L. D. Brown, and W. W. Hay. 2011. The intrauterine growth restriction phenotype: fetal adaptations and potential implications for later life insulin resistance and diabetes. Semin. Reprod. Med. 29:225-236.

Thornbury, J. C., P. D. Sibbons, D. Vanvelzen, R. Trickey, and L. Spitz. 1993. Histological investigations into the relationship between low-birth-weight and spontaneous bowel damage in the neonatal piglet. Pediatr. Pathol. 13:59-69.

Tristan, F., O. Albors, G. Ramis, J. Vazquez, M. Martinez, J. Martinez, and F. Gil. 2009. Relationship of birth weight with the size, number and proportion of fibres in the pig Semitendinosus muscle. Anatom. Histol. Embryol. 38:275-278.

Tse, W. Y. 2005. Consequences of uterine crowding during early gestation on myogenesis in the pig. MSc Thesis, Univ. Alberta, Edmonton, Alberta, Canada.

V

de. Vries, S., A. Pustjens, M. Kabel, R. Kwakkel, and W. Gerrits. 2014. Effects of processing technologies and pectolytic enzymes on degradability of nonstarch polysaccharides from rapeseed meal in broilers. Poultry Sci. 93(3):589-598.

W

Wang, T., J. Yong, F. Shi, R. Jun, J. Reinhold, J. Hutz, and C. Berit. 2005. Effects of intrauterine growth retardation on development of the gastrointestinal tract in neonatal pigs. Biol. Neonate 88:66-72.

Wang, X. Q., W. Wu, G. Lin, G. Wu, and J. Wang. 2010. Temporal proteomic analysis reveals continuous impairment of intestinal development in neonatal piglets with Intrauterine Growth Restriction. J. Proteome Res. 9:924-935.

Wang, X. Q., W. J. Yang, Z. Yang, G. Shu, S. B. Wang, Q. Y. Jiang, L. Yuan, and T. S. Wu. 2012. The differential proliferative ability of satellite cells in Lantang and Landrace pigs. Plos One 7(3):325-337.

Wenk, C. 2001. The role of dietary fibre in the digestive physiology of the pig. Anim. Feed Sci. Tech. 90:21-33.

Widdowson, E. M. 1971. Intra-uterine growth retardation in the pig. Biol. Neonate 19:329-340.

Widdowson, E. 1971. Intra-uterine growth retardation in the pig. I. Organ size and cellular development at birth and after growth to maturity. Neonatology 19:329-340.

Widdowson, E., and R. McCance. 1960. Some effects of accelerating growth. 1. General somatic development. Proceedings of the Royal Society of London. Series B. Biol. Sci. 152:188-190.

Wientjes, J., N. Soede, B. Laurenssen, R. Koopmanschap, H. van den Brand, and B. Kemp. 2013. Insulin-stimulating diets during the weaning-to-estrus interval do not improve fetal and placental development and uniformity in high-prolific multiparous sows. Animal 7(8):1307-1316.

Winick, M., P. Rosso, and J. Waterlow. 1970. Cellular growth of cerebrum, cerebellum, and brain stem in normal and marasmic children. Experimental Neurology 26:393-400.

Wolf, J., E. Zaková, and E. Groeneveld. 2008. Within-litter variation of birth weight in hyperprolific Czech Large White sows and its relation to litter size traits, stillborn piglets and losses until weaning. Livest. Sci. 115:195-205.

Wolter, B., and M. Ellis. 2001. The effects of weaning weight and rate of growth immediately after weaning on subsequent pig growth performance and carcass characteristics. Can. J. Anim. Sci. 81:363-369.

Wolter, B., M. Ellis, B. P. Corrigan, and J. M. DeDecker. 2002. The effect of birth weight and feeding of supplemental milk replacer to piglets during lactation on preweaning and postweaning growth performance and carcass characteristics. J. Anim. Sci. 80:301-308.

Wu, G., F. W. Bazer, T. A. Cudd, C. J. Meininger, and T. E. Spencer. 2004. Maternal nutrition and fetal development. J. Nutr. 134:2169-2172.

Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Board invited review: Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84:2316-2337.

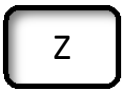
Wu, M., Z. Chen, V. Jarrell, and P. Dziuk. 1989. Effect of initial length of uterus per embryo on fetal survival and development in the pig. J. Anim. Sci. 67:1767-1772.

Wu, W., X. Wang, G. Wu, S. Kim, F. Cheng, and J. Wang. 2010. Differential composition of proteomes in sow colostrum and milk from anterior and posterior mammary glands. J. Anim. Sci. 88:2657-2664.

X

Xu, R. J., D. J. Mellor, M. J. Birtles, G. W. Reynolds, and H. V. Simpson. 1994. Impact of intrauterine growth-retardation on the gastrointestinal tract and the pancreas in newborn pigs. J. Pediatr. Gastroenterol. Nutr. 18:231-240.

Yablonka-Reuveni, Z., and A. J. Rivera. 1994. Temporal expression of regulatory and structural muscle proteins during myogenesis of satellite cells on isolated adult rat fibers. Dev. Biol. 164:588-603.



Zammit, P. S., and J. R. Beauchamp. 2001. The skeletal muscle satellite cell: stem cell or son of stem cell? Differentiation 68:193-204.

Zammit, P. S., F. Relaix, Y. Nagata, A. Ruiz, C. Collins, T. Partridge, and J. Beauchamp. 2006. Pax7 and myogenic progression in skeletal muscle satellite cells. J. Cell Sci. 119:1824-1832.

LIST OF ABBREVIATIONS

AA	amino acids
ADFI	average daily feed intake
ADG	average daily gain
AG	average gain
AID	apparent ileal digestibility
AL	average birth weight piglets with a lower than average gain during the first six weeks of age
ARC	Agricultural Research Council
AUC	area under the curve
BiW	birth weight
BW	body weight
$BW^{0.75}$	metabolic body weight
C	centigrade
CCK	Cholecystokinin
cm	centimeter(s)
CO	control diet
CP	crude protein
C_p	Mallows Cp
CV	coefficient of variation
CVB	Centraal Veevoeder Bureau
d	day(s)
DM	dry matter
DE	digestible energy
e.g.	*exempli gratia*
FC	feed consumption
FCR	feed conversion ratio
FCSA	fiber cross sectional area
FI	feed intake
g	gram(s)

g/d	grams per day
GE	gross energy
G:F	gain:feed
GGT	gamma glutamyl transpeptidase
GI	gastrointestinal tract
GOT	gamma oxaloacetate transaminase
GPT	glutamine pyruvate transaminase
h	hour(s)
HP	high performing piglets
i.e.	*id est*
IGF1	insulin growth factor 1
IGF2	insulin growth factor 2
IgG	inmunoglobulin G
IRCT	immuno-reactive trypsin
ISO	International Organization for Standardization
IUGR	intrauterine growth retarded
kg	kilogram(s)
LM	*Longissimus dorsi*
LP	low performing piglets
MCSA	muscle cross sectional area
min	minute(s)
MJ	mega joule
MRF	muscle regulatory factors
MYF5	myogenic factor 5
MYOD	myogenic differentiation factor
N	nitrogen
NE	net energy
n.i.	not included
NRC	National Research Council
OGTT	oral glucose tolerance test
OSTT	oral starch tolerance test

PAX7	paired box 7
PCNA	proliferating cell nuclear antigen
PW	post-weaning
Quicky	quantitative insulin sensitivity check index
rpm	revolutions per minute
RT	reverse transcription
s	second(s)
SD	standard deviation
SE	standard error
SI	small intestine
SID	standardized ileal digestibility
SO	suboptimal diet
STN	*Semitendinosus* muscle
TBP	TATA box binding protein
TFN	total fiber number
vs.	versus
wk	weeks
χ^2	chi square test
Y/N	yes or no

Summary

SUMMARY

The evolution of hyper-prolific sow breeds has led to a higher number of piglets born per sow per year. This increase in litter size has enlarged the number of light weight (or growth retarded) piglets, increased pre-weaning mortality and heterogeneity at the end of the nursery phase (ten weeks of age). These poorly performing piglets represent a challenge to the swine industry as their presence in the herd has economic and welfare implications, as it is associated with chilling, starvation, crushing and less vigour, mainly by the light piglets; leading to higher mortality. The major pre-weaning mortality occurs during the first 72 h of life and is mainly related to a low birth weight. Even if the piglets survive the critical 72 h there is still a risk that insufficient colostrum intake leads to higher susceptibility for disease and lower performance in further life stages. Reducing the heterogeneity at the end of the nursery phase is relevant, as it influences the efficiency of use of the grower and finisher facilities, and/or it reduces penalties for delivering underweight piglets to the slaughterhouse. The focus of this thesis was the end of the nursery phase, as this is the time point where piglets are transferred to the grower and finisher facilities.

The aim of this thesis was to identify and describe the causes of growth retardation in the nursery phase to provide a basis to look for alternative nutrition or management solutions.

Most literature which reports the effect of within-litter variation on performance assumes that all light piglets belong to the same group, without discriminating on time when the growth retardation has occurred (in-utero, postnatal). For this thesis, though, we assumed that light piglets are a heterogeneous population with a similar outcome: low body weight at the end of the nursery phase.

The database analysis described in **Chapter 2** defines the factors for predicting piglets' body weight (**BW**) at the end of the nursery phase based on the analysis of three datasets from swine research centres in the

Netherlands and France. The entire dataset contained information on 77,868 individual piglets born in the period between 2005 and 2010. The BW was determined at different moments in the pre- and post-weaning phase. In addition, sex, season of birth, litter information (litter size at day of birth and after cross-fostering, number of piglets born alive per litter, number of total born littermates, sow parity number), cross-fostered (yes or no), and pen group size over the post-weaning period were recorded. The factors that significantly contributed to the prediction of piglet BW at the end of the nursery phase corrected for age, based on the risk factor analysis were: season of birth, body weight at birth, at weaning and at six weeks of age. Remarkably, litter characteristics and sow parity were not included in the prediction factors. Focusing on the light birth weight piglets, it was determined that piglets with a birth weight below the mean minus 2.0 times the SD from the mean of the total population had 80% mortality. Piglets with a birth weight above the mean minus 2.0 times the SD from the mean of the total population have the potential to compensate during the subsequent phases of growth.

Based on the algorithm developed in **Chapter 2**, for predicting piglet's BW at the end of the nursery phase, we defined our target population (piglets with a predicted BW at the end of the nursery phase below the mean minus one time the SD from the mean of the total population; considered Low Performing piglets, **LP**) and compared them to their heavier littermates (piglets with a predicted BW at the end of the nursery phase above the mean plus one time the SD from the mean of the total population; considered High Performing piglets, **HP**) for the studies described in this thesis.

The study described in **Chapter 3** characterized differences between the LP and HP piglets in terms of performance, body morphology, behaviour, voluntary feed intake, BW gain, and apparent total tract and ileal nutrient digestibility from six to ten weeks of age. A total of 60 piglets (30 LP and 30 HP) were selected from a pool of 368 clinically healthy piglets at six weeks of age. Piglets were housed individually and were fed a highly digestible diet. Compared to the HP piglets, the LP piglets grew slower, ate less and

were lighter at ten weeks of age. The LP piglets tended to take more time to touch a novel object and spent more time eating. They also had a higher body length and head circumference relative to BW. Relative to BW, LP piglets had a heavier and longer small intestine. No differences were observed between groups in dry matter, nitrogen and gross energy digestibility. From this study it was concluded that the low performance of the LP piglets was due to their inability to engage compensatory gain or compensatory feed intake as efficiency of nutrient utilization and feed intake per kg $BW^{0.75}$ was unaffected. Low performing piglets tend to be more fearful towards novel objects. The morphological comparison showed that LP piglets have increased body length and head circumference relative to BW. This implies that the LP piglets have an increased priority for skeletal growth.

In **Chapter 4** it was hypothesised that the differences in performance between LP and HP piglets would be enlarged when fed a suboptimal diet (**SO**) compared to a control diet (**CO**) from six to ten weeks of age. We expected differences in apparent total tract and ileal digestibility as a consequence of a reduced feed intake of LP piglets or due to the high fibre content of the SO diet. A total of 60 piglets (30 LP and 30 HP) were selected from a pool of 470 clinically healthy piglets at six weeks of age. Piglets were housed individually. The LP and HP piglets were fed either a CO or a SO diet. The SO diet contained poorly digestible protein sources and had a high NSP content but both diets were formulated to have an equal ratio of SID amino acids to net energy. The LP piglets grew slower, ate less and were lighter at ten weeks of age. The SO fed piglets had a reduced gain:feed (**G:F**). Feed intake was not increased in the SO fed piglets, demonstrating that both LP and HP pigs are unable to compensate for the reduced energy content by increasing their feed intake. Starch and fat apparent total tract digestibility were reduced in the SO fed piglets. Apparent total tract and ileal protein digestibility were also reduced in the SO fed piglets. Digestibility of other macronutrients, with the exception of NSP, was unaffected by piglet group. Despite the reduced feed intake, apparent total tract digestibility of sugars in non-glucose polysaccharides, particularly xylose and uronic acid, was reduced in the LP piglets,

particularly in the SO fed piglets. In conclusion, macronutrient apparent total tract digestibility is equal for both piglet groups. Yet, the fermentative capacity of LP is reduced which places them in disadvantage for future performance.

Postnatal (muscle) growth potential depends on the total number and hypertrophy of myofibers in skeletal muscle tissue. In **Chapter 5**, we aimed to determine whether the differences in growth performance between LP and HP piglets could be the result of different skeletal muscle properties. A total of 40 piglets (20 LP and 20 HP) were selected from a pool of 368 clinically healthy piglets at six weeks of age (originating from the pool selected in **Chapter 3**). Piglets were housed individually and were fed a highly digestible diet. At ten weeks of age samples from the right *Semitendinosus* muscle were collected for histochemistry and gene expression analysis. The low performance of the LP piglets was associated with a lower plasma IGF1 concentration. The lower weight and muscle cross sectional area in LP piglets was related to a decrease in total fiber number and fiber cross-sectional area compared to the HP piglets. The mRNA expression of muscle regulatory factors (MYF5, MYOD, PAX7 and PCNA) did not differ between groups. From this study it was concluded that the LP piglets have a low muscularity in terms of lower total fiber number and fiber cross sectional area, which might be of disadvantage for lean mass accretion in further life and for meat quality.

In **Chapter 6** the target population was piglets with an average birth weight but which fell behind in growth in the lactation phase, the second major group of LP piglets (average birth weight, lower than average gain in the first six weeks of age, **AL**). The AL piglets were compared to piglets with an average birth weight but with a higher than average gain during the first six weeks of age (**AH**). We aimed to determine if growth retardation in early life affected insulin-mediated glucose tolerance in the nursery phase. A total of 16 piglets (8 AL and 8 AH) were selected from a pool of 435 clinically healthy piglets. Piglets were housed individually. At 8 and 9 weeks of age, insulin-mediated glucose tolerance was determined using oral glucose and starch tolerance tests, and hyperglycaemic clamp test at stable

glucose concentrations of 8 and 15 mmol/L. Subsequently, all piglets were sacrificed for determination of pancreatic enzyme activity. The oral starch tolerance test and the hyperglycaemic clamp at 8 mmol/L blood glucose concentration showed that the AL piglets exhibit insulin resistance. The oral glucose tolerance test and hyperglycaemic clamp at 15 mmol/L blood glucose concentration showed that at hyperglycaemia, the AL piglets' exhibit insulin and glucose resistance. Pancreatic protein content and amylase activity were lower in the AL piglets compared to the AH piglets. From this study it was concluded that piglets suffering from growth retardation in the first six weeks of age (originating in the lactation phase) expressed insulin resistance and had diminished pancreatic amylase activity, which may in part explain their permanent growth retardation during the nursery phase.

In **Chapter 7** a comparison of the main findings from **Chapter 2 to 6** with literature characterizing growth retarded piglets is presented. To determine the economic impact of light piglets entering the grower and finisher facilities, the modelling program (Watson 2.0, Nutreco Canada) was used to model the data of light and heavy piglets from 10 weeks until slaughter weight (110 kg BW). The modelled data outcome was that there is a 25 day increase in days to market for the light pigs when compared to the heavier pigs.

The studies reported in this thesis describe a novel method for selecting growth retarded piglets in the nursery phase and provide insight into possible mechanisms for growth retardation in the piglet phase.

SAMENVATTING

De genetische vooruitgang in de huidige zeugenlijnen heeft geleid tot een sterke toename van de toomgrootte en het totaal aantal geboren biggen per zeug per jaar. Een negatief gevolg van deze toename in toomgrootte is echter dat het aandeel lichte biggen, de uitval voor het spenen en de variatie in lichaamsgewicht aan het einde van de biggenopfokfase (op circa 10 weken) toeneemt.

De biggen met een achterblijvende gewichtsontwikkeling zijn een uitdaging voor de varkenshouderij omdat hun aanwezigheid implicaties heeft voor het welzijn en voor de economische rendabiliteit van een varkensbedrijf. Een laag lichaamsgewicht is immers geassocieerd met een verhoogde kans op verkleumen, verhongeren, dooddrukken, verminderde levensvatbaarheid en met een verhoogde kans op sterfte.

Het grootste aantal biggen dat sterft, overlijdt in de eerste 72 uur na geboorte. Het risico hierop is gerelateerd aan een laag geboortegewicht. Zelfs wanneer biggen deze kritische fase overleven, dan nog leidt hun lagere colostrumopname tot een verhoogde ziektegevoeligheid en lagere groeiprestaties in de latere levensfasen.

Het terugdringen van heterogeniteit aan het einde van de biggenopfokfase is van belang omdat hierdoor het efficient gebruik van de beschikbare vleesvarkensplaatsen verbetert. Daarnaast kunnen voedings of managemenstrategieën worden ontwikkeld om deze variatie te verminderen. Tenslotte is het waarschijnlijk dat het verminderen van heterogeniteit aan het einde van de opfokfase ook de variatie in slachtgewicht vermindert, en daarmee de kans verkleint op gewichtskortingen in het uitbetalingsschema van een slachterij.

Het doel van dit proefschrift was het identificeren van oorzaken voor groeivertraging in de biggenopfok. Met deze kennis kan aangepaste voeding en management ontwikkeld worden.

Het overgrootte deel van de bestaande literatuur over gewichtsvariatie bij jonge biggen gaat er van uit dat lichte biggen tot één groep behoren, zonder onderscheid te maken tussen het tijdstip waarop de groeivertraging optrad (*in utero* of post nataal). Onze benadering was dat we lichte biggen als een heterogene sub-populatie beschouwen met als grote gemene deler een laag lichaamsgewicht op 10 weken leeftijd.

In **hoofdstuk 2** is een data analyse beschreven waarmee een formule is ontwikkeld, die het gewicht van biggen aan het einde van de opfok kan voorspellen. Onze database bevatte gegevens van bijna 78000 biggen geboren tussen 2005 en 2010 en afkomstig van drie varkensonderzoeksbedrijven in Nederland en Frankrijk. Van elke big was het lichaamsgewicht bepaald op verschillende momenten van de opfok. Daarnaast waren hun geslacht, het geboorteseizoen, moeder- en toomgegevens, het feit of ze al dan niet waren overgelegd en het aantal hokgenoten na spenen bekend. Uit de analyse bleken de volgende factoren significant bij te dragen aan het voorspellen van het gewicht op het einde van de opfok (10 weeken): seizoen, lichaamsgewicht bij geboorte, spenen en op zes weken leeftijd. Opvallend genoeg kwamen toomeigenschappen en pariteit van de zeug niet naar voren als belangrijk invloedsfactoren

Daarnaast concludeerden we dat 80% van de biggen, die lichter waren dan tweemaal de standaardafwijking onder het gemiddelde van de hele populatie, stierven voor spenen. Biggen met een gewicht hoger dan tweemaal de standaardafwijking onder het gemiddelde gewicht hadden de potentie om een inhaalgroei te vertonen.

Op basis van het algoritme dat we ontwikkelden in **hoofstuk 2**, werden twee sub populaties gedefinieerd voor de vervolgstudies: biggen die een verwacht eindgewicht hadden dat meer dan 1 standaardafwijking onder het gemiddelde van de populatie lag. Deze biggen werden aangeduid als ‘low performing’ (**LP**). Deze subpopulatie werd vergeleken met een populatie goed groeiende biggen, dat wil zeggen biggen die een verwacht eindgewicht hadden dat boven het gemiddeld plus één standaardafwijking lag (‘High performing’, **HP**).

In de studie die is beschreven in **hoofdstuk 3** werden verschillende kenmerken van LP en HP biggen bepaald: lichaamsvorm, gedrag, vrijwillige voeropname, gewichtstoename en nutriënt verteerbaarheid (zowel op dunne darm als fecaal niveau). Hiertoe werden tweemaal dertig biggen geselecteerd uit een populatie van 368 klinisch gezonde biggen van zes weken leeftijd die nagenoeg gelijktijdig waren geboren. De biggen werden individueel gehuisvest en kregen een hoogwaardige voeding. Onder deze proefomstandigheden groeiden de LP biggen langzamer, namen minder voer op en waren lichter op tien weken leeftijd. Deze verschillen in voeropname en groei verdwenen als ze werden uitgedrukt relatief ten opzichte van het metabool gewicht (gewicht^{0,75}). De voederconversie was vergelijkbaar, maar er was een tendens tot een hogere efficientie van opgenomen voer boven de onderhoudsbehoefte in HP biggen. Opvallend genoeg vonden we geen verschillen in verteerbaarheid.

Bovendien bleek uit gedragsobservaties, dat LP biggen meer tijd namen om een onbekend voorwerp te benaderen. Deze observaties zouden kunnen wijzen op het angstiger zijn. Ondanks de lagere voeropname spendeerden LP biggen meer tijd aan de voerbak, hetgeen duidt op een lagere vreetsnelheid.

Ook constateerden we dat hun relatieve romplengte, kopomtrek, dunne darmlengte en – gewicht groter was. Alles bijeengenomen zouden onze bevindingen kunnen duiden op het feit dat LP biggen prioriteit geven aan de groei van hun skelet en minder aan de aanzet van spier- en vetweefsel.

In **hoofdstuk 4** werd de hypothese getoetst dat verschillen in groeiprestatie tussen LP en HP biggen verder zouden toenemen als ze een suboptimaal dieet (**SO**) zouden krijgen. De verwachting was dat het uitblijven van compensatoire voeropname in LP biggen veroorzaakt wordt door een lagere verteringscapaciteit. Dit effect zou meer uitgesproken moeten zijn als biggen een vezelrijker en minder goed verteerbaar voer verstrekt krijgen. Om deze hypothese te testen werden, op een vergelijkbare manier als in **hoofdstuk 3**, tweemaal dertig biggen (LP en HP) op zes weken leeftijd geselecteerd uit een totale populatie van 470 klinisch gezonde biggen. De

individueel gehuisveste biggen kregen een hoog kwalitatief controle voer of een SO voer. Dit SO voer bevatte lager verteerbare eiwitbronnen en een hoger niet-zetmeel koolhydraat (**NSP**) gehalte. De verhouding tussen netto energie en gestandaardiseerde ileaal verteerbare aminozuren was wel gelijk voor beide voeders. Zoals verwacht, aten de LP dieren minder, groeiden langzamer en waren als gevolg hiervan lichter op 10 weken leeftijd. Het SO dieet leidde tot een lagere voerefficientie, maar niet tot een hogere voeropname. De schijnbare dunne darm- en fecale eiwitverteerbaarheid waren lager voor het SO voer. Klaarblijkelijk waren biggen niet in staat om te compenseren voor de lagere concentratie aan nutriënten in het SO voer door hun opname te verhogen. Dit gold echter in gelijke mate voor LP en HP biggen.

Behalve voor NSP was er geen verschil in verteerbaarheid tussen LP en HP biggen. De verteerbaarheid van xylose en uronzuren was lager bij de LP biggen, hetgeen duidt op een licht verlaagde vezelverteerbaarheid. Dit was opvallend, gezien de lagere voeropname in deze biggen, die normaal gesproken correspondeert met een hogere vezelverteerbaarheid.

Concluderend konden we stellen dat de verteerbaarheid van de meeste macronutrienten niet verschillende was tussen de twee populaties biggen. We vonden echter aanwijzingen dat de fermentatie capaciteit van LP biggen lager is, waardoor ze een mogelijk nadeel ondervinden in hun streven naar het bereiken van hun groeipotentieel.

De post natale (spier)groei hangt af van het totale aantal en de grootte van de aanwezige spiervezels. In **hoofdstuk 5** wilden we daarom onderzoeken of het verschil in groeiprestaties tussen LP en HP biggen het resultaat is van verschillen in spiervezelsamenstelling. Hiertoe werden bloed monsters en spierbiopten van 20 LP en 20 HP biggen uit dezelfde populaties als beschreven in hoofdstuk 3 onderzocht Op tien weken leeftijd werd bloed afgenomen en werden spiermonsters van de rechter *Semitendinosus* spier histo-chemisch onderzocht. Daarnaast werd de expressie van een aantal genen betrokken bij de spierontwikkeling in dit weefsel bepaald.

LP biggen hadden een lagere plasmawaarden voor insulin-like growth factor 1. Het hogere gewicht van de *Semitendinosus* en spierdiameter in HP biggen werd veroorzaakt door een 20% groter aantal spiervezels en een groter oppervlak van de dwarsdoorsnede van deze vezels (34%) ten opzichte van LP leeftijdsgenoten. De mRNA-expressie van enkele regulatiegenen (MYF5, MYOD, PAX7 en PCNA) verschilde echter niet.

Op grond van deze bevindingen concludeerden we dat LP biggen in het nadeel zijn voor wat betreft de aanzet van spiereiwit en mogelijk ook vleeskwaliteit.

In **hoofdstuk 6** richtten we ons op een sub-populatie van groeivertraagde biggen, namelijk de dieren die met een gemiddeld gewicht werden geboren maar vervolgens in de zoogperiode een groeivertraging opliepen. Deze groep dieren (aangeduid met **AL**) vertegenwoordigd de op één na grootste groep binnen de totale LP populatie. We vergeleken de AL dieren met dieren die ook een gemiddeld geboortegewicht hadden maar vervolgens een meer dan gemiddelde groei vertoonden in de eerste zes weken van hun leven (**AH**). De hypothese was dat een groeivertraging opgelopen in de eerste zes weken, gerelateerd is aan een lage op de insuline-afhankelijke glucose-tolerantie. Hiertoe werden acht AL en acht AH dieren geselecteerd uit een populatie van 435 klinisch gezonde dieren. Deze 16 dieren werden individueel gehuisvest en voorzien van de benodigde bloedvatcatheters.

Vervolgens werden ze op 8 en 9 weken leeftijd blootgesteld aan een orale glucose- en zetmeeltolerantietest alsmede een hyperglycaecemische clamp test (op twee niveaus). Uit de zetmeeltolerantietest en het lage niveau van de hyperglycaemische clamp test) bleek dat AL biggen lijden aan een vorm van insulineresistentie. De overige twee testen gaven aan dat AL biggen ook een insuline intolerantie kunnen laten zien.

Aan het einde van de studie werden de biggen opgeofferd en werd hun pancreas (alvleesklier) verwijderd. Het eiwitgehalte in pancreasweefsel, en

de amylase activiteit bleken lager in LP biggen in vergelijking met HP biggen.

Uit dit experiment werd geconcludeerd dat biggen die een groeivertaging oplopen in de zoogperiode zowel een insuline resistentie laten zien. Samen met de verlaagde amylase-activiteit is dit een mogelijke oorzaak van de achterblijvende groei in de biggenopfok.

In de algemene discussie (**hoofdstuk 7**) werden de bevindingen uit de voorgaande hoofdstukken (hoofdstukken 2 tot en met 6) vergeleken met literatuurinformatie over groeivertraagde biggen.

Om de economische consequenties van lichte biggen voor de vleesvarkensfase in te schatten werd het groeisimulatiemodel Watson 2.0 (Nutreco Canada) gebruikt. De modelberekeningen gaven aan dat LP biggen 25 dagen meer nodig hebben dan HP biggen om hun slachtgewicht te bereiken.

De studies beschreven in dit proefschrift geven een nieuwe methode om groeivertraagde opfokbiggen te selecteren. Ze geven tevens inzicht in de mechanismen, die mogelijk ten grondslag liggen aan de achterblijvende groei en kunnen daarom een basis vormen voor het ontwikkelen van (voedings en management) strategieën om groeivertraagde biggen te helpen.

RESUMEN GENERAL

La evolución en las líneas de cerdas híper-prolíficas ha incrementado el número de lechones nacidos por cerda por año. Este incremento en el tamaño de la camada ha aumentado el número de lechones de bajo peso (lechones con crecimiento retrasado), aumentado la mortalidad pre-destete y la heterogeneidad de la camada al final de la fase de lechón (diez semanas de vida). Estos lechones con bajo rendimiento representan un peligro para la producción porcina ya que su presencia en el hato tiene repercusiones económicas y de bienestar animal. Los lechones de bajo peso son más propensos a padecer de escalofríos, aplastamientos y menos vigor, lo cual conduce al incremento en la mortalidad. La mortalidad pre-destete ocurre principalmente durante las primeras 72 horas de vida y está altamente relacionada al bajo peso al nacimiento. Si el lechón logra sobrevivir las críticas primeras 72 horas de vida, aún existe la posibilidad que no haya consumido suficiente calostro, lo cual lo vuelve vulnerable a enfermedades y bajo rendimiento en las siguientes etapas productivas. La reducción de la heterogeneidad al final de la fase de lechón es importante, ya que el peso alcanzado al finalizar esta etapa influye en la eficiencia de la utilización de instalaciones de cebo y/o en la reducción de las penalizaciones de entrega de cerdos con bajo peso al matadero.

El objetivo de esta tesis fue identificar y describir causas relacionadas con el retraso en el crecimiento en la fase de lechón para proveer las bases para la búsqueda de alternativas nutricionales o de manejo.

La mayoría de literatura que describe el efecto de la variación de desarrollo entre los lechones de una camada asume que todos los lechones de bajo peso pertenecen al mismo grupo, sin discriminar la fase en la que ha ocurrido el retraso (intrauterina, post-parto). En esta tesis, asumimos que los lechones de bajo peso son una población heterogénea con un resultado común: bajo peso al final de la fase de lechón.

El análisis de base de datos descrito en el **Capítulo 2** define los factores utilizados para predecir el peso del lechón a las diez semanas de vida. Estos

factores están basados en el análisis de tres bases de datos de centros de investigación porcino en los Países Bajos y Francia. La base de datos final contiene información individual de 77,868 lechones nacidos en el período de 2005 y 2010. Esta base de datos contiene información del peso de los lechones en diferentes etapas en el pre y post-destete. Además incluye el sexo del lechón, estación anual en que se produjo el nacimiento, información de la camada (tamaño de la camada al nacimiento, después de interadopciones, número de lechones nacidos vivos, número de lechones en la camada, número de parto de la madre), interadoptado (si o no), tamaño del grupo en el post-destete. Los factores que contribuyeron significativamente a la predicción del peso al final de la fase de lechón, en base al análisis de factores de riesgo fueron: época del año en que se produjo el nacimiento, peso al nacimiento, al destete y a las seis semanas de vida. Sorprendentemente, factores relacionados con la camada y el número de parto de la cerda no fueron identificados para la predicción. Al enfocarse la población de lechones de bajo peso, se determinó que la mortalidad de los lechones con un peso al nacimiento menor a -2 veces la desviación standard de la media de la población al nacimiento es del 80%. Los lechones con un peso al nacimiento superior a –2 veces la desviación standard de la media de la población al nacimiento tienen la capacidad de compensar su desarrollo en las siguientes fases de desarrollo.

En base al algoritmo desarrollado en el **Capítulo 2**, para predecir el peso de los lechones a las diez semanas, nuestra población de interés (lechones con un peso predicho menor a la media menos -1 desviación standard al final de la fase de lechón, considerados lechones de bajo rendimiento LP (por sus siglas en inglés)). Durante todos los estudios descritos en esta tesis, los lechones LP fueron comparados con los lechones de mayor peso corporal de la camada (lechones con un peso predicho mayor a la media +1 desviación standard al final de la fase de lechón, considerados lechones con alto rendimiento HP (por sus siglas en inglés)). Estas dos poblaciones de lechones fueron estudiadas en los estudios reportados en esta tesis.

El estudio descrito en el **Capítulo 3** se describe las diferencias en rendimiento, antropometría, comportamiento, ingestión voluntaria de

alimento, ganancia de peso, digestibilidad total aparente e ileal entre LP y HP de seis a diez semanas de edad. Un total de 60 lechones (30 LP y 30 HP) fueron seleccionados de un grupo de 368 lechones sanos a las seis semanas de vida. Los lechones fueron alojados individualmente y alimentados con una dieta altamente digestible. Al compararlos con los lechones HP, el crecimiento de los lechones LP fue más lento, el consumo de alimento fue menor y el peso corporal a las diez semanas de vida fue inferior. Al evaluar el comportamiento, los lechones LP tardaron más tiempo en acercarse a un objeto nuevo y ocuparon más tiempo en el día para consumir alimentos. Los lechones LP presentan mayor longitud corporal y perímetro cefálico en relación al peso corporal. También en relación al peso corporal, los lechones LP presentan un intestino delgado más largo y pesado. No se observaron diferencias en la digestibilidad total aparente e ileal de materia seca, nitrógeno o energía bruta entre ambos grupos. En base a estos resultados se concluyó que el bajo rendimiento de los lechones LP es debido a su inhabilidad de exhibir ganancia o consumo de alimentos compensatorio, ya que su eficiencia en la utilización de alimento consumido por kilo de peso $^{0.75}$ no se vio afectada. Los lechones LP tienen mayor dificultad de acoplarse al cambio. Las características morfométricas demuestran que los lechones LP tienen una mayor longitud corporal y perímetro cefálico, lo que nos lleva a plantear la hipótesis que las prioridades de los lechones LP están relacionadas a desarrollo óseo sobre el desarrollo muscular.

En el **Capítulo 4** nuestra hipótesis fue que las diferencias en el rendimiento entre lechones LP y HP se incrementarían al alimentar ambos grupos con una dieta de baja digestibilidad (**SO**). Para este efecto comparamos la dieta SO con una dieta control (**CO**) durante las seis y las diez semanas de vida. El resultado esperado era diferencias en digestibilidad total aparente e ileal como consecuencia de la reducción en el consumo de alimento de los lechones LP o debido a la alta cantidad de fibra presente en la dieta SO. Un total de 60 lechones (30 LP y 30 HP) fueron seleccionados de un grupo de 470 lechones sanos. Los lechones fueron alojados individualmente y alimentados con la dieta SO o CO respectivamente. La dieta SO fue formulada para con una menor digestibilidad proteica y alto contenido de

polisacáridos no almidonados (**NSP** por sus siglas en inglés). Ambas dietas fueron formuladas con un mismo ratio de amino ácidos de digestibilidad ileal estandarizada y energía neta. Los lechones LP crecieron más despacio, consumieron menos alimento y fueron más livianos a las diez semanas de vida comparados con los lechones HP. Los lechones alimentados con la dieta SO tuvieron una reducción en la eficiencia alimenticia (**G:F** por sus siglas en inglés). El consumo de alimento no fue incrementado en los lechones alimentados con la dieta SO, demostrando que ambos grupos de lechones (LP y HP) no son capaces de compensar la reducción de contenido energético incrementado el consumo de alimento. La digestibilidad total aparente de almidón y lípidos fueron reducidos en la dieta SO. La digestibilidad de otros macronutrientes no fue afectada por la categoría de lechón (LP o HP), con excepción de los NSP. A pesar de la reducción en el consumo de alimento la digestibilidad de azúcares y polisacáridos no relacionados con la glucosa, particularmente xilosa y ácido urónico, fueron reducidos en los lechones LP, especialmente en los alimentados con la dieta SO. En conclusión la digestibilidad total aparente de macronutrientes es similar para ambos grupos de lechones (LP y HP). Sin embargo, la capacidad fermentativa de los lechones LP es más reducida, lo cual los coloca en desventaja en rendimiento en etapas posteriores.

El potencial de crecimiento (muscular) post-destete depende del número total y capacidad de hipertrofia de las miofibras del tejido muscular esquelético. En el **Capítulo 5**, tratamos de determinar si las diferencias en el crecimiento de los lechones LP y HP estaban relacionadas con diferencias en las propiedades del músculo esquelético. Un total de 40 lechones (20 LP y 20 HP) fueron seleccionados de un grupo de 368 lechones sanos a las seis semanas de vida (originados del grupo descrito en el **Capítulo 3**). Los lechones fueron alojados individualmente y fueron alimentados con una dieta altamente digestible. A las diez semanas de edad, muestras del músculo Semitendinoso derecho fueron colectadas para análisis de histoquímica y expresión genética. Los lechones LP presentaron una menor concentración de IGF1 en plasma sanguíneo. El bajo peso y menor área transversal de tejido muscular fueron relacionadas con una disminución en el número total de fibras y área de fibras transversales, comparados con los

lechones HP. La expresión mRNA de los factores regulatorios (MYF5, MYOD, PAX7 y PCNA) no difirió entre grupos. En base a este estudio se concluyó que los lechones LP tienen una menor capacidad muscular, reflejada en menor número de fibras musculares totales y fibras del área transversal, lo cual los coloca en desventaja para la acumulación de masa magra y mejor calidad de canal en etapas posteriores de crecimiento.

En el **Capítulo 6**, la población de interés fue modificada ligeramente ya que se utilizaron lechones con un peso promedio al nacimiento, pero con un rendimiento menor al promedio en la fase de lactancia (**AL** por sus siglas en inglés), los cuales representan el segundo grupo de importancia de lechones LP. Los lechones AL fueron comparados con lechones con un peso promedio al nacimiento pero con un rendimiento mayor a la media durante las primeras seis semanas de vida (**AH** por sus siglas en inglés). El objetivo de este estudio fue determinar si el bajo rendimiento exhibido durante las primeras etapas está relacionado con insulinoresistencia en la fase de lechón. Un total de 16 lechones (8 AL y 8 AH) fueron seleccionados de un grupo de 435 lechones sanos y fueron alojados individualmente. A las 8 y 9 semanas de vida, la insulinoresistencia fue determinada utilizando una prueba de tolerancia oral a la glucosa y almidón y una prueba de clamp hiperglucémico a una concentración en plasma sanguíneo de 8 y 15 mmol/L de glucosa.

Al final de las 3 pruebas todos los lechones fueron eutanaciados para determinar la actividad de las enzimas pancreáticas. La prueba de tolerancia oral a la glucosa administrando almidón y el clamp hiperglucémico a una concentración en plasma sanguíneo de 8 mmol/L de glucosa demostraron que los lechones AL sufren de resistencia a la insulina. La prueba de tolerancia oral a la glucosa y el clamp hiperglucémico a una concentración en plasma sanguíneo de 15 mmol/L demostraron que en un estado de hiperglicémico los lechones AL sufren de resistencia a la insulina. El contenido de proteína pancreática y la actividad de amilasa pancreática fueron menores en los lechones AL comparados con los lechones AH. En base a este estudio concluimos que los lechones que sufren de retraso en el crecimiento durante las primeas seis semanas de vida (originado en la

fase de lactancia) sufren de resistencia a la insulina y una menor actividad pancreática de la amilasa, lo cual explica parcialmente el retraso en el crecimiento exhibido durante la fase de lechón.

En el **Capítulo 7**, se describe una comparación de los hallazgos descritos en los **Capítulos 2 a 6** con la literatura relacionada con lechones de bajo crecimiento. Para determinar el impacto económico del bajo peso de los lechones en la fase de desarrollo y cebo, el programa (Watson 2.0, Nutreco Canadá) fue utilizado para modelar el crecimiento y determinar este impacto y diferencias entre cerdos livianos a partir de las 10 semanas de vida hasta el peso al sacrificio (110 kg peso corporal). El resultado de este análisis fue que existe una diferencia de 25 días para alcanzar el peso al sacrificio entre los cerdos livianos y pesados.

Los estudios descritos en esta tesis describen un método novedoso para seleccionar lechones de bajo peso en la fase de lechón y provee información sobre los posibles mecanismos relacionados con el retraso en el crecimiento en esta fase.

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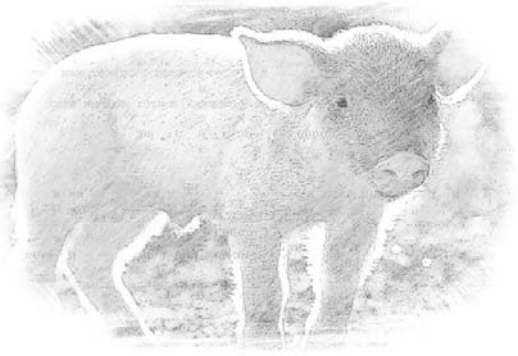
About the author



CURRICULUM VITAE

Sandra Paredes Escobar was born on July 2nd, 1981 in Guatemala City, Guatemala. In 2007 she obtained her BSc in Veterinary Medicine at the Universidad de San Carlos de Guatemala. After obtaining her degree she worked as a veterinarian in the National Pork Association (APOGUA). In September 2007 she got a position and Technical Sales Representative for Central America and Caribbean for Trouw Nutrition Guatemala. Also in 2007 she started an MSc in Farm Animal Reproduction in Universidad de San Carlos de Guatemala of which she graduated *Cum Laude*. Her master thesis focused on developing an economical assessment tool to determine the economic impact of artificial insemination in swine farms in Guatemala and a guide to reduce cost related to swine production in small and medium size farms. After receiving her MSc in 2009, Sandra went to the Netherlands to pursue a position as Project Manager Piglet Nutrition in the Swine Research Centre, Nutreco Nederland, B. V. (Boxmeer, the Netherlands) position she holds until now. In 2010 she started her PhD in the Animal Nutrition Group (ANU) of Wageningen University aimed to determine causes for growth retardation in piglets. This project was a collaboration between Wageningen UR Livestock Research Centre, ANU Group of Wageningen University and Nutreco Nederland, B. V.

LIST OF PUBLICATIONS

Peer reviewed scientific publications

Paredes, S. P., A. J. M. Jansman, M. W. A. Verstegen, A. Awati, W. Buist, L. A. den Hartog, H. M. J. van Hees, N. Quiniou, W. H. Hendriks, and W. J. J. Gerrits. 2012. Analysis of factors to predict piglet body weight at the end of the nursery phase. J. Anim. Sci. 90:3243-3251.

Paredes, S. P., A. J. M. Jansman, M. W. A. Verstegen, L. A. den Hartog, H. M. J. van Hees, J. E. Bolhuis, and W. J. J. Gerrits. 2014. Identifying the limitations for growth in low performing piglets from birth until ten weeks of age. Animal 8(6):923-930.

Paredes, S. P., C. Kalbe, A. J. M. Jansman, M. W. A. Verstegen, H. J. M. van Hees, D. Lösel, W. J. J. Gerrits, and C. Rehfeldt. 2013. Predicted high-performing piglets exhibit more and larger skeletal muscle fibers. J. Anim. Sci. 12:5589-5598.

Conference and symposia proceedings

Paredes, S. P., A. Awati, A. J. M. Jansman, H. M. J. van Hees, M. W. A. Verstegen, and W. J. J. Gerrits. 2012. Feed intake rather than digestion is the growth limiting factor in poor performing piglets, XII Internation Symposium Digestive Physiology of Pigs, Colorado, USA, May 29 to June 1. p. abstract # 3014.

Paredes, S. P. , L. A. den Hartog, A. J. M. Jansman, H. M. J. van Hees, M. W. A. Verstegen, and W. J. J. Gerrits. 2014 Unveiling causes of poor performance in piglets during the nursery phase. Advances in Animal Biosciences, BSAS, Nottingham, UK. p. abstract # 122.

Education certificate*

Completed Training and Supervision Plan

Description	Year
Basic package (3 ECTS†)	
WIAS Introduction course	2010
WSG course Ethics and Philosophy of Animal Science	2011
International conferences (5 ECTS)	
12th Darmendag, Ghent, Belgium	2010
43st Journées de lal Recherche Porcine, Paris, France	2011
Pre-conference symposium Gut Chemosensing, Colorado, USA	2012
12th International Symposium on Digestive Physiology in Pigs, Colorado, USA	2012
12th International Symposium on Insulin receptors and insulin action, Barcelona, Spain	2013
2nd International conference Nutrition and Growth, Barcelona, Spain	2014
BSAS Advances in Animal Biosciences, Nottingham, UK	2014
Seminars and workshops (1 ECTS)	
Nutreco Sow Nutrition Seminar, Boxmeer, the Netherlands	2010
WIAS seminar "Scientific Research in Animal Welfare: Do we make a difference", Wageningen, the Netherlands	2011
WIAS Science Day, Wageningen, the Netherlands	2011
Nutreco Fibres for monogastrics Seminar, Boxmeer, the Netherlands	2011
Presentations (5 ECTS)	
12th International Symposium on Digestive Physiology in Pigs, Colorado, USA (poster)	2012
Global Swine Meeting, The Hague, the Netherlands (poster and oral)	2012
Sunjin Co. Ltd, Swine Husbandry and Nutrition, Boxmeer, the Netherlands (oral)	2013

GRETECEG monthly meeting, Guatemala City, Guatemala (oral)	2013
BSAS Advances in Animal Biosciences, Nottingham, UK (oral)	2014
In-Depth Studies (6 ECTS)	
Statistical Course, Boxmeer, the Netherlands	2010
Orientation on Mathematical Modelling in Biology, Wageningen, the Netherlands	2011
Advances in Feed Evaluation Science, Wageningen, the Netherlands	2011
Advanced Statistics Course, Wageningen, the Netherlands	2012
Epigenesis and Epigenetic, Wageningen, the Netherlands	2014
Statistical course, Boxmeer, the Netherlands	2014
Statutory Courses (3 ECTS)	
Laboratory Animal Science, Utrecht University, the Netherlands	2009
Professional Skills Support Courses (4.3 ECTS)	
Workshop Scientific Publishing, Wageningen, the Netherlands	2011
Scientific Writing Course, Wageningen, the Netherlands	2011
Effective behaviour in your professional surroundings, Wageningen, the Netherlands	2012
Improve your writing, Wageningen, the Netherlands	2013
Data management course, Wageningen, the Netherlands	2013
Research Skills Training (6 ECTS)	
Preparing own PhD research proposal	2010
Didactic Skill Training (5 ECTS)	
Supervising 1 BSc student and 2 MSc Major	2010 - 2012
Education and Training Total	**38 ECTS**

* Completed in fulfilment of the requirements for the education certificate of the Graduate School WIAS (Wageningen Institute of Animal Sciences)

† One ECTS equals a study load of 28 h

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