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Strathe, Anja Varmløse

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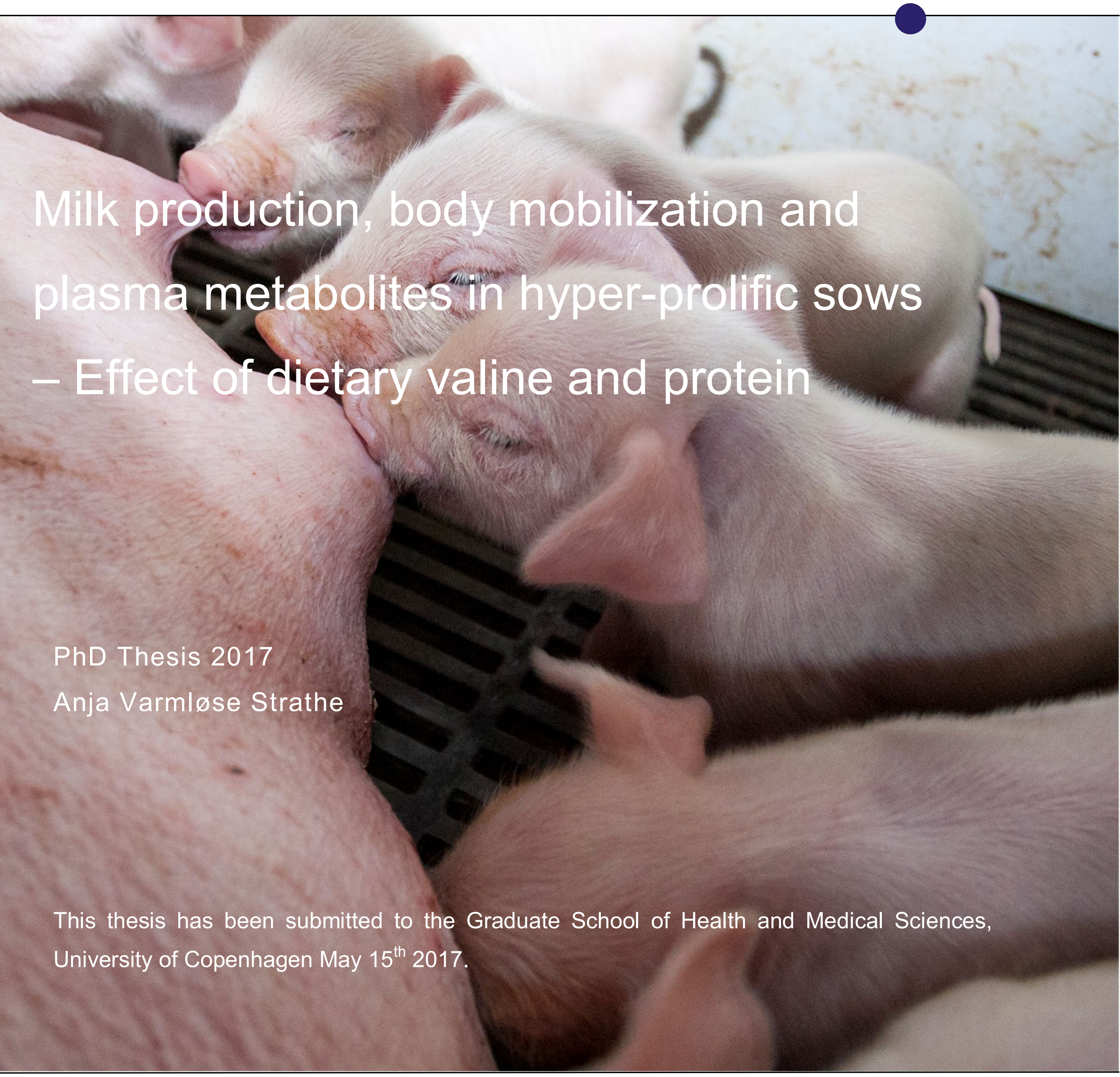

Milk production, body mobilization and plasma metabolites in hyper-prolific sows – Effect of dietary valine and protein

PhD Thesis 2017

Anja Varmløse Strathe

This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen May 15th 2017.

Supervisor:

Professor Christian Fink Hansen, Department of Veterinary and Animal Sciences, University of Copenhagen.

Co-supervisors:

Professor Anne-Helene Tauson, Department of Veterinary and Animal Sciences, University of Copenhagen.

Senior specialist Thomas Sønderby Bruun, SEGES Danish Pig Research Centre.

Assessment Committee:

Associate professor Anni Øyan Pedersen (Chairman)
Department of Veterinary and Animal Science
University of Copenhagen

Professor Nathalie L. Trottier
Department of Animal Science
Michigan State University, USA

Scientist Hélène Quesnel
Unit of Physiology, Environment, and Genetics for the Animal and Livestock Systems
Institut National de la Recherche Agronomique (INRA), France

Table of contents

1 PREFACE AND ACKNOWLEDGEMENT

This PhD thesis is intended to fulfill the requirement for the PhD degree at University of Copenhagen, Faculty of Health and Medical Sciences, Department of Animal and Veterinary Sciences, Denmark. The scholarship was awarded by the Faculty of Health and Medical Sciences at University of Copenhagen. The experiments were conducted in collaboration with SEGES Danish Pig Research Centre and AA and AA analyzes were sponsored by Evonik Nutrition & Care GmbH Tyskland Filial Denmark.

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Frederiksberg, May 2017
Anja Varmløse Strathe

2 ABBREVIATIONS

AA = Amino acid(s)
ADG = Average daily gain

Ala = Alanine

Asp = Aspartic acid

BCAA = Branched-chain amino acid(s)

BF = Back fat

BW = Body weight

CP = Crude protein

Cys = Cysteine

D_2O = Deuterium oxide

EAA = Essential amino acid(s)

Gly = Glycine

His = Histidine

Ile = Isoleucine

Leu = Leucine

LH = Lutheinizing hormone

Lys = Lysine

Met = Methionine

N = Nitrogen

NEAA = Non-essential amino acid(s)

NEFA = non-esterified fatty acid(s) / free fatty acid(s)

Phe = Phenylealanine

Pro = Proline

PUN = Plasma urea nitrogen

Ser = Serine

SID = Standardized ileal digestible

Thr = Threonine

Val = Valine

Val:Lys = Valine-to-lysine ratio

3 SUMMARY

The productivity of Danish sows has increased during the last decades resulting in sows giving birth to more piglets, and simultaneously the sow has become larger and leaner. This has increased the demand for nutrients for milk production, because hyper-prolific sows nurse more piglets. To improve the performance of hyper-prolific sows and their piglets a better understanding of amino acid and protein metabolism of the lactating sow is important in order to determine the nutritional requirements of hyper-prolific sows. The overall objective of this thesis was to determine the optimum dietary valine-to-lysine ratio (Val:Lys) and dietary concentration of balanced crude protein in diets to hyper-prolific sows to optimize sow and piglet performance during the lactation period. These data should form the basis for updating dietary recommendations to match the requirements of hyper-prolific sows.

This PhD project consisted of two experiments. First, an experiment testing six dietary total Val:Lys (0.84, 0.86, 0.88, 0.90, 0.95 and 0.99:1) during lactation including 558 sows was carried out. Second, the effect of increasing levels of standardized ileal digestible (SID) protein (104, 113, 121, 129, 139 and 150 g/kg) was investigated on a total of 544 sows. All litters were standardized to 14 piglets 24-48 hours *postpartum* and in both experiments diets based on wheat, barley and soybean meal were fed from the day after farrowing until weaning (day 24-26 *postpartum*). The body weight (BW) and back fat (BF) thickness of sows were measured and litter weight was recorded. On a subsample of sows milk, urine and blood samples were taken. In experiment 2 body composition was measured using the deuterium oxide (D_2O) dilution technique.

In experiment 1 (Val:Lys) litter gain, concentrations of blood metabolites, BW and BF loss of sows were similar in all groups. The milk contents of fat, protein and lactose were also unaffected by dietary treatment, but concentrations of branched-chain amino acids (BCAA) increased with increasing dietary Val:Lys. In experiment 2 (protein), increasing the dietary crude protein concentration increased litter gain, BF loss, milk protein, casein and fat content and plasma concentrations of urea nitrogen (N). While the BW loss and body protein loss of the sows was minimized.

The results of these experiments have contributed with new knowledge on milk production, body mobilization and concentrations of blood metabolites of hyper-prolific Danish sows. This knowledge has formed a qualified basis for new recommendations for dietary protein provision to

lactating hyper-prolific sows. Based on results of experiment 1 and 2 of the thesis total dietary Val:Lys do not need to exceed 0.84 and sow productivity and metabolism was maximized at a dietary SID protein concentration at 135 g/kg.

4 SAMMENDRAG (Danish summary)

Produktiviteten hos danske søer er steget gennem de sidste årtier, så søerne nu føder flere grise og i samme periode er søerne blevet større og mere magre. Dette har øget behovet for næringsstoffer til mælkeproduktion, fordi disse højproduktive søer skal passe flere pattegrise end tidligere. Mere viden om søernes aminosyre- og proteinomsætning er nødvendigt for at bestemme søernes næringsstofbehov under diegivningen og dermed optimere søernes produktivitet og pattegrisenes tilvækst. Det overordnede formål med denne afhandling var at bestemme den bedste valin:lysin ratio og koncentration af balanceret protein i foderet til højproduktive søer, for at opnå den maximale produktivitet hos søer og pattegrise. Resultaterne skulle danne grundlaget for en opdatering af aminosyre- og proteinnormer til diegivende søer.

PhD-projektet bestod af to forsøg. I det første forsøg blev seks total valin:lysin ratioer (0.84, 0.86, 0.88, 0.90, 0.95 and 0.99:1) i foderet testet gennem diegivning (558 søer). I det andet forsøg blev effekten af stigende koncentrationer af standard ileal fordøjeligt balanceret protein (104, 113, 121, 129, 139 and 150 g/kg) i foderet testet (544 søer). Alle kuld blev udjævnet til 14 grise og i begge forsøg blev søerene tildelt foder baseret på byg, hvede og sojaskrå fra dagen efter faring indtil fravænning (dag 24-26 efter faring). Alle søer fik målt kropsvægt og rygspæktykkelse og kuldet blev vejet. På nogle af søerne blev der desuden taget mælkeprøver og blodprøver, og i forsøg 2 blev søernes kropssammensætning målt ved hjælp af deuteriumoxid.

I forsøg 1 (valin:lysin ratio) var der ingen effekt af forsøgsfoderet på pattegrisenes tilvækst, søernes vægt og rygpæktykkelse og koncentrationer af metabolitter i plasma. Indholdet af fedt, lactose og protein i mælken var også end for alle grupper, men der var en stigning i koncentrationen af forgrenede aminosyre med stigende valin:lysin ratio. I forsøg 2 (protein) resulterede den stigende koncentration af protein i foderet i øget kuldtilvækst, rygspæktab, mælkeprotein, -kasein, -fedt og -laktose og plasma koncentrationer af urea nitrogen. Modsat blev der observeret et fald i søernes vægttab og mobilisering af kropsprotein.

Resultater fra disse forsøg har bidraget med ny viden om mælkeproduktion, kropsmobilisering og plasma metabolitter hos højtproducerende danske søer og har dannet grundlag for nye anbefalinger for indholdet af protein i søernes foder under diegivningen. Ud fra resultater i forsøg 1 og 2 blev det bestemt at foderets totale valin:lysin ratio ikke behøver at overstige 0.84 og at søernes produktivitet og stofskifte var optimalt ved 135 g SID protein/kg foder.

5 OUTLINE OF THE THESIS

The thesis consists of a synopsis and four papers. First, a short introduction and thorough background for the subject of the thesis is given leading to the formulated objectives and hypotheses. This is followed by a description of material and methods as well as the results of the experimental work. From the experimental work four papers are included in the thesis. In the general discussion the applied methods and obtained results of the experiments are discussed in relation to existing knowledge and scientific literature. In the end conclusions of the thesis are given together with the practical implications for commercial pig production as well as future scientific perspectives.

6 LIST OF INCLUDED PAPERS

The thesis is based on four papers. The first paper examined the effect of different dietary Val:Lys for lactating sows and the effect on litter growth, changes in BW and BF thickness of sows, milk composition and blood metabolites of sows. The second paper investigated what characterized hyper-prolific lactating sows regarding feed intake, BW loss and litter growth. The third paper concerns the effect of dietary protein levels of lactating sows on changes in sow BW and BF thickness, litter growth, milk composition and the following reproductive cycle. The fourth paper addresses the effect of dietary protein levels of lactating sows on changes in sow body composition, blood metabolites, urine pH and milk composition.

Paper I: The effect of increasing the dietary valine-to-lysine ratio on sow metabolism, milk production, and litter growth. 2016. A.V. Strathe, T.S. Bruun, J.-E. Zerrahn, A.-H. Tauson, C.F. Hansen. *Journal of Animal Science 94,155-164.*

Paper II: Sows with high milk production had both a high feed intake and high body mobilization. 2017. A.V. Strathe, T.S. Bruun, C.F. Hansen. *ANIMAL. doi: 10.1017/S1751731117000155*

Paper III: Increased dietary protein levels during lactation improved sow and litter performance. 2017. A.V. Strathe, T.S. Bruun, N. Geertsen, J.-E. Zerrahn, C.F. Hansen. Submitted to *Animal Feed Science and Technology*

Paper IV: Increased dietary protein for lactating sows affects body composition, blood metabolites and milk production. 2017. A.V. Strathe, T.S. Bruun, A.-H. Tauson, P.K. Theil, C.F. Hansen. Submitted to *ANIMAL*

1 INTRODUCTION

The modern hyper-prolific sow has undergone major changes during the last decades, which may have affected the metabolism of the sow. Sows have become larger and leaner due to genetic selection for high growth rate and feed efficiency. Simultaneously litter size has increased (Nielsen and Jultved, 2000, Jultved, 2006, Jessen, 2016; see **Figure 1a**) and as a result sows nurse large litters with 12 or more piglets (see **Figure 1b**). A high milk yield with an adequate nutrient composition that matches the energy and nutrient requirement of the newborn piglet is essential to ensure a high average daily gain (ADG) and survival of the piglets (Quesnel *et al.*, 2015).

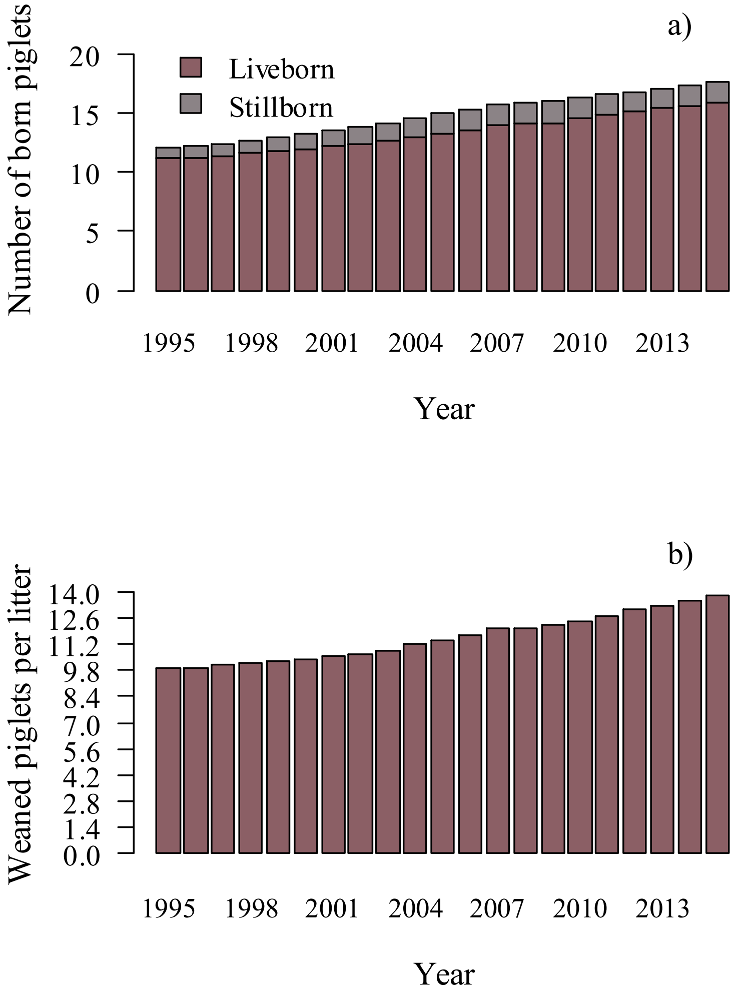

Figure 1. The development of **a)** live born and stillborn piglets and **b)** number of weaned piglets per litter in Danish sows from 1995 to 2015 (Nielsen and Jultved, 2000, Jultved, 2006, Jessen, 2016).

It is crucial for the lactating sow to have a high milk yield to ensure survival of the piglets and a high growth rate of the litter. Studies have shown that the milk production is limiting for growth of the piglets after the first week of lactation (Boyd *et al.*, 1995), which emphasizes the importance of determining nutrient requirements for the modern lactating sow to enable optimization of milk production and thereby piglet growth (Kim *et al.*, 2009). Milk production is prioritized above most other metabolic processes and therefore most sows turn catabolic during lactation. The requirement of nutrients for milk production often exceeds the dietary intake of the sow and therefore the sow mobilizes body fat and protein for milk synthesis (King *et al.*, 1993, Kim *et al.*, 2001a). Excessive mobilization from body reserves during lactation can affect the following reproductive cycle by increasing the weaning-to-estrus interval, decreasing embryo survival and number of piglets born in next litter and reducing the longevity of the sow (Koketsu *et al.*, 1996, Zak *et al.*, 1997). Very few studies, and none with hyper-prolific sows, have investigated the correlation between dietary intake and changes in sow body composition during lactation. It is vital to determine the extent of mobilization from body tissues to develop new dietary recommendations that both ensure a high productivity and longevity of sows (Trottier and Guan, 2000, Kim *et al.*, 2009).

However, despite these significant changes of the sow and her production level recommendations for dietary amino acids (AA) and crude protein (CP) have not been developed accordingly. It is important that firstly the AA profile of the dietary protein is correct and secondly that the amount of balanced CP should match the actual requirement of all essential amino acids (EAA) of the sow. The latest evaluation of the Danish dietary recommendations of the EAA and balanced CP for lactating sows was undertaken in 2002 (Tybirk *et al.*, 2016) based on experiments with a limited number of animals.

Recently the NRC updated their dietary recommendations for pigs (NRC, 2012) and their model predicting requirements of the lactating sow together with the model by Strathe *et al.* (2015) for prediction of AA and balanced CP requirements during lactation, clearly indicates that the modern sows nursing large litters have a higher requirement than stated in the former Danish recommendations (2013). However, these models are either based on data with no (NRC, 2012) or only limited (Strathe *et al.*, 2015) number of sows nursing 13-14 piglets and therefore the validity of predictions of requirements of hyper-prolific sows made from these models needs to be validated. In addition, several studies have recently demonstrated the need for updated AA recommendations of lactating sows because of the increased number of nursing piglets (Kim *et al.*, 2009, Manjarin *et al.*, 2014, Strathe *et al.*, 2015), but nevertheless these studies were not performed on sows as hyper-prolific as the current Danish DanAvl sows. The increased production level of the sow means that the overall balanced CP requirement has increased, because the increased milk production demands more AA (Guan *et al.*, 2004a). However, there has also been an increased focus on the requirement of the individual EAA and their ratio relative to lysine (Lys), and many studies have been conducted particularly on Lys, because it is the first limiting AA of pigs (NRC, 2012). Recently BCAA (Val, Leu and Ile) has also been given more attention, because the excretion of BCAA in milk is much lower that the uptake to the mammary gland and in addition, Val might be the second or third most limiting AA for lactating sows (Kim *et al.*, 2009). Crystalline Val has become commercially available and it is therefore possible more precisely to dose Val when formulating diets, but there are large discrepancies in the few studies determining the optimum valine-to-lysine ratio (Val:Lys) of lactating sows. Hence, the Danish recommendation was 0.76 (Christensen *et al.*, 2013) and the NRC recommends 0.85 (NRC, 2012).

Altogether this challenges the former Danish dietary recommendations (2013) for lactating sows and emphasizes the need of re-defining the dietary requirement of both Val:Lys and balanced CP of hyper-prolific sows. New recommendations for dietary Val:Lys and CP should accommodate both an optimization of milk production and thereby piglet growth and at the same time minimize mobilization from muscle protein to ensure good reproductive performance, health and longevity of hyper-prolific sows nursing 13-14 piglets.

2 BACKGROUND

2.1 The metabolism of modern, hyper-prolific lactating sow

It is important to know the general metabolism of modern hyper-prolific sows in order to determine requirements and develop feeding strategies that match their production level. This section will give a general description of milk production in sows and the metabolism of the mammary glands. In addition the interplay between feed intake, body mobilization and changes in body composition will be described.

2.1.1 Milk yield

Many factors affect the magnitude of the sow's milk yield. Amongst these are parity of the sow, breed, feed intake, dietary composition, ambient temperature and body condition of the sow, but the major determinants of milk yield are size and the number of nursing piglets (Hansen *et al.*, 2012b, Quesnel *et al.*, 2015). **Figure 2** shows the effects of litter size and ADG of the litter on milk yield.

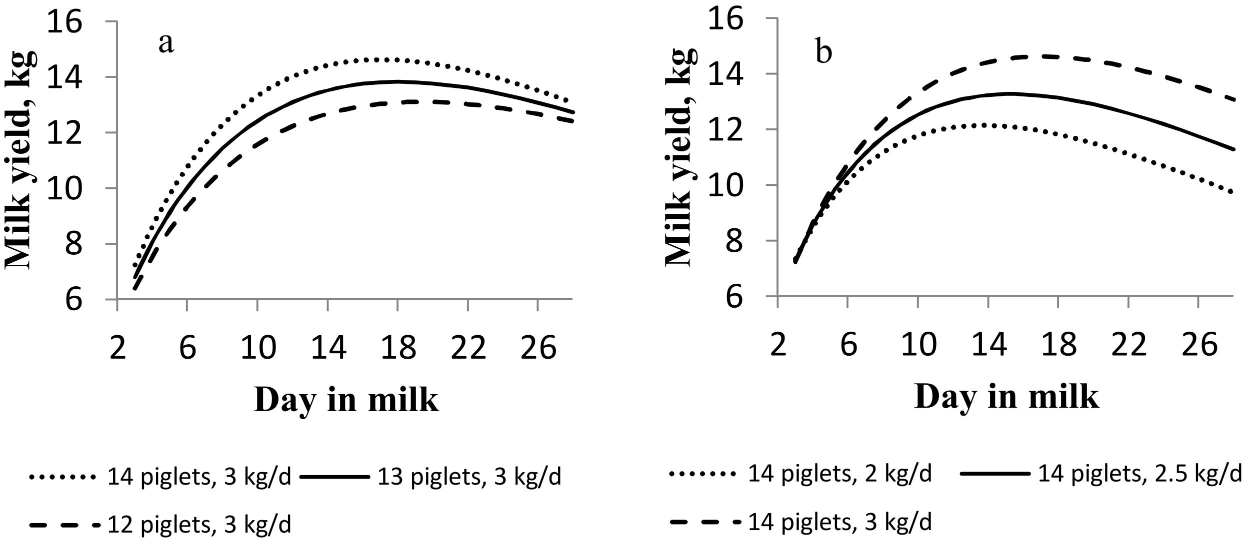

Figure 2. Lactation curves estimated from equations by Hansen *et al.* (2012b) to illustrate **a)** the effect of litter size on milk yield and **b)** the effect of litter gain on milk yield.

The number of suckling piglets has a great influence on milk yield and several studies have demonstrated a clear increase in milk yield when increasing litter size from six to 14 piglets (King *et al.*, 1989, Auldist *et al.*, 1998). Milk production is higher when sows nurse large litters (12-16 piglets) compared to nursing fewer piglets, because all mammary glands are regularly suckled. Unsuckled mammary glands regress and undergo involution, and it was reported by Theil *et al.* (2006) that mammary glands that were not suckled or transiently suckled from 12 hours *postpartum* to six days *postpartum* stopped producing milk and were involuted. Similarly, Kim *et al.* (2001b) also described that unsuckled mammary glands rapidly regressed. In sows, the stimulation of the udder and removal of milk by the piglets are essential for milk let-down and maintaining milk production (Quesnel *et al.*, 2015). Therefore, the size of the nursing piglets impact milk yield, because larger piglets are better at stimulating the udder. The average milk yield increased with 1-2 kg/day when sows were given 2-week old piglets two days *postpartum* compared to sows nursing newborn piglets (King *et al.*, 1997). These results demonstrate that sows have a high potential for milk production, but that it is very dependent on the stimulation by the litter.

Piglets have a high potential for growth and it seems that the milk production of the sow is the limiting factor for their ADG. Studies where piglets either were given extra milk or solely fed milk replacer compared with piglets only nursed by the sow showed that piglets increase their ADG when given extra milk (Zijlstra *et al.*, 1996, Cabrera *et al.*, 2010). This emphasizes the importance of improving the milk production of the sow through a better understanding of the nutritional requirements of the sow for exploiting the potential piglet growth rate during lactation.

When sows are undernourished it is possible to increase milk production by increasing the feed allowance or nutrient intake of the sow and this is referred to as a "push effect". For instance sows in the study by King *et al.* (1993) increased milk yield from 7.0 to 8.9 kg/day when increasing dietary CP concentration from 6.3 to 23.8 %. On the other hand if the sow is normal or well-nourished the interplay between the mammary gland and nutrients are regarded as a "pull effect", because it is not possible to push the mammary gland to produce more milk unless there is a demand for it (Theil *et al.*, 2012). This was demonstrated in a study by Eissen *et al.* (2003) testing the effects of increasing litter size from 7 to 14 piglets on feed intake of *ad libitum* fed (4.6 to 5.0 kg/day) sows and sows increased their intake until a litter size of 11 piglets. The sows most likely reached their upper physical limit for feed intake, because BW and BF loss continued to increase with higher litter sizes (Eissen *et al.*, 2003).

2.1.2 Milk synthesis and composition

The major components of sow milk are lactose, protein and fat (**Table 1**) and these nutrients are made available for milk synthesis through the diet or from body reserves. The milk composition changes from colostrum to milk continuous during the first 3-4 days *postpartum*. The greatest difference between colostrum and milk is the much higher protein content in colostrum, because of the high content of immunoglobulins in colostrum compared to milk (Theil *et al.*, 2014).

Table 1. Composition of milk from hyper-prolific Danish sows on day 3 and 17 *postpartum* (Theil *et al.*, 2014).

Composition, %	**Day 3 *postpartum***	**Day 17 *postpartum***
Lactose	4.8	5.1
Protein	6.1	4.7
Fat	9.8	8.2

The major part of AA utilized during lactation is taken up by the mammary gland as substrate for milk protein (Boyd and Kensinger, 1998). The AA are transported from the blood into the endothelial cells by several transport mechanisms (Shennan and Peaker, 2000), and the milk protein is synthesized in the rough endoplasmic reticulum. Glucose is transported via glucose transporters and lactose is synthesized from glucose and galactose in the Golgi apparatus. The main pathway by which AA and lactose crosses the alveolar epithelium and enter the milk is by exocytosis, where casein micelles, whey proteins, and lactose amongst others are collected in secretory vesicles in the Golgi apparatus. Triglycerides, which constitute the majority of milk lipids, are synthesized in the smooth endoplasmic reticulum from fatty acids and glycerol. The triglycerides are gathered in lipid droplets within the endothelial cell and gradually become enveloped in the apical membranes, and then finally the droplets are released into the lumen (Shennan and Peaker, 2000). Lactose is the major osmotic factor in milk and therefore accountable for drawing water into the secretory vesicles. These vesicles fuse with the apical membrane and the contents are released into the lumen of the alveolus (Shennan and Peaker, 2000). Approximately 50 % of the fatty acids in milk come from triglycerides and another 50 % is synthesized de novo from glucose in the mammary gland (Boyd and Kensinger, 1998).

Generally fat is regarded as the most variable component of milk, whereas lactose is the most stable component closely followed by the protein content. The fat content and fatty acid composition can be affected by dietary fat content and fatty acid composition (Lauridsen and Danielsen, 2004); however sow milk only contains small amounts of C4:0 to C12:0 fatty acids (Hurley, 2015). The fat content seems to be unaffected by body protein mobilization (Clowes *et al.*, 2003a), whereas mobilized body fat can be used for milk fat synthesis (Theil *et al.*, 2012).

Table 2. The AA composition of sow milk. Modified from the review by Hurley (2015). The range of the reported contents is given in brackets.

Amino acid	% of total protein (range)	Content as % of Lys
Lysine	7.3 (7.0-7.9)	-
Alanine	3.4 (2.8-3.9)	47
Arginine	5.2 (4.6-5.8)	71
Aspartic acid	8.1 (7.3-8.6)	111
Cysteine	1.5 (1.3-1.7)	21
Glutamic acid	22.0 (18.9-28.8)	301
Glycine	3.2 (2.3-3.6)	44
Histidine	2.9 (2.3-3.9)	40
Isoleucine	4.0 (2.9-4.4)	55
Leucine	8.8 (8.1-10.1)	121
Methionine	1.8 (1.4-2.0)	25
Phenylalanine	3.9 (3.6-4.2)	53
Proline	11.9 (10.9-12.3)	163
Serine	5.3 (4.5-5.8)	73
Threonine	4.1 (3.6-4.4)	56
Tryptophan	1.4 (1.3-1.6)	19
Tyrosine	4.2 (3.9-4.9)	58
Valine	4.9 (3.9-5.5)	67

Milk protein can be divided into two subgroups: caseins and whey proteins. The whey proteins include immunoglobulin G, immunoglobulin A, Immunoglobulin M, lactoferrin, albumin, α-lactalbumin, β-lactoglubulin, and other minor proteins, whereas each type of casein has its own AA composition. The AA composition of milk only varies a little (**Table 2**), but during lactation a decrease in EAA was observed, whereas the amount of non-essential AA (NEAA) stays the same or slightly increases during lactation (Csapó *et al.*, 1996). Glutamic acid is the most abundant protein bound AA in milk with 17-22 %, followed by proline, which accounts for 10-12 %. As a group the BCAA account for 18-19 % of the protein bound protein in milk (Hurley, 2015).

2.1.3 Feed intake, body mobilization and body composition of the lactating sow

Mobilization from body reserves and changes in body composition of the sow during lactation depend on, body condition at farrowing, feed intake, dietary composition and milk production.

High milk production puts a high demand on nutrient availability for milk nutrients. A sow nursing 14 piglets with a litter ADG of 3 kg/day will produce over 14 kg milk per day at peak lactation and excrete approximately 1000 g fat, 700 g lactose and 700 g protein per day in the milk (Hansen *et al.*, 2012b). Preferably these nutrients are provided through the dietary intake of the sow, but often the requirement for milk production exceeds the intake capacity of the sow and the sow becomes catabolic. Milk production has a high priority for the sow, which is the reason that sows can maintain a high milk yield despite an insufficient dietary intake and a high body mobilization. In a study by Dourmad *et al.* (1998) sows fed lower levels of CP (15.5 %) and Lys (0.66 %) had a higher BW loss than sows on diets higher in CP (17.1 %) and Lys (0.77 %), but all sows had similar milk yields during lactation, showing that sows are compensating for a lower nutrient intake by mobilizing from body reserves to maintain milk production.

Body composition can be measured either directly by slaughtering the sows or indirectly using D_2O dilution technique (Rozeboom *et al.*, 1994) or some type of body scan. Accordingly, equations have been developed to estimate the body composition from BW and BF thickness (Pettigrew *et al.*, 1992, Dourmad *et al.*, 1997) as BW and BF thickness of the sows are easy to obtain.

A study by Kim and Easter (2001) with litter sizes varying from 6 to 12 piglets investigated the differences in sow body composition at weaning, and found that protein loss was increased by 600 g when litter size increased by one piglet and body ash content was lower when nursing 12 piglets compared to 6-10 piglets (Kim and Easter, 2001). This underlines that the mammary gland is the main driver of metabolism during lactation and the increased demand for milk when nursing more piglets is increasing the flow of nutrients towards milk production. In the study by Kim and Easter (2001) the litter size did not affect the body fat content. Interestingly, Kim and Easter (2001) showed that the protein content of the reproductive tract, gastrointestinal tract and the liver was lower at weaning in sows nursing 12 piglets compared to smaller litter sizes, whereas the fat content of these tissues was unaffected by litter size (Kim and Easter, 2001) showing that body protein is not solely mobilized from muscle protein.

An energy deficit will mainly be covered by body fat mobilization, whereas sows only undersupplied with protein mainly will mobilize body protein. When comparing the studies measuring the change in body composition during lactation it is reported that sows mobilize both body fat and protein, but the ratio between mobilized fat and protein differs substantially (Dourmad *et al.*, 1998, Sauber *et al.*, 1998, Jones and Stahly, 1999, McNamara and Pettigrew, 2002, Gill, 2006), and this probably depends on if the sow is more in an energy or protein deficit. If body fat mobilization becomes limited, body protein may be mobilized and used as an energy source.

Mobilization from body reserves not only affects the performance during lactation, but can result in carry-over effects to the next reproductive cycle. A high BW loss can result in a prolonged weaning-to-estrus interval. For example, Zak *et al.* (1998) showed that sows fed restrictedly (2.8 kg/day) during lactation had a weaning-to-estrus interval that was 2 days longer and lost 38 kg of BW in comparison with sows with *ad libitum* access to feed (5.1 kg/day) and a BW loss at 16 kg. An increase in luteinizing hormone (LH) and a high frequency of LH pulses is a prerequisite for sows going into estrus. Hence, the prolonged weaning-to-estrus interval was most likely a result of the delay in the increase of LH after weaning and a lower frequency of LH pulses shown in the experiment (Zak *et al.*, 1998). In another study by Zak *et al.* (1997) the ovulation rate was also lower in restrictedly fed sows compared to sows with *ad libitum* access to feed. These restrictedly fed sows also had a higher BW and BF loss (Zak *et al.*, 1997), which eventually could have a negative effect on the number of total born piglets in next litter. In addition, Baidoo *et al.* (1992) found that sows with a lactational BW and BF loss of 39 kg and 6.4 mm had a lower embryo survival rate compared to sows losing 16 kg of BW and 2.3 mm BF, respectively. These studies collectively showed that there was a close connection between low feed intake, high body mobilization and reproductive problems, but mainly when excessive mobilization from the body occurred.

It is not fully elucidated where the limit between a normal level and excessive body mobilization is and if body mobilization should be measured as BW, BF, protein or fat loss. In this thesis the body mobilization was regarded as excessive when milk production or reproduction in the following cycle were compromised. Dourmad *et al.* (1996) showed that sows loosing up to 35 kg during lactation were able to regain the weight loss during gestation, but only when fed a high energy level (42.0 MJ ME/day), so the upper level for BW loss should probably be lower than 35 kg, because sows are normally fed restrictedly during gestation. Another study (Schenkel *et al.*, 2010) showed that when first parity sows mobilized more than 20 % of their body fat during lactation the number of total born piglets in next litter was reduced. It is a general claim, that excessive protein mobilization is more harmful and undesirable than excessive fat mobilization. One reason is that mobilization from body protein seems to have more negative effects on the following reproductive cycle than body fat loss. Sows with a high or low body protein loss (12 vs. 6 % of body protein), but with similar fat loss, during lactation, had a similar weaning-to-estrus interval, number of viable embryos and embryo survival (Mejia-Guadarrama *et al.*, 2002). However, sows with the highest protein loss during lactation had a lower ovulation rate and a lower ovarian weight (Mejia-Guadarrama *et al.*, 2002). The results of Mejia-Guadarrama *et al.* (2002) indicates that body protein loss had a negative effect on reproduction, but only when excessive protein mobilization (12 % of body protein) occurred. In addition the body protein mass at farrowing has a great impact on how affected the reproductive performance is, because sows with a higher body protein mass can mobilize more from the body protein pool than sows with a lower protein mass at farrowing. Thus, larger sows can mobilize more than smaller and younger sows.

The mobilized body reserves must be regained by the sow during the following gestation and in the same period young sows also needs to gain maternal weight to reach mature size. The capability of the sow to restore body reserves depends both on the degree of mobilization during lactation (Dourmad *et al.*, 1996, Huang *et al.*, 2013) and of the nutrient intake in the following gestation (Everts and Dekker, 1995). A study (Huang *et al.*, 2013) showed that first parity sows with similar BW at farrowing but fed low CP (17.5 %) and Lys (0.95 %) intakes during lactation had higher BW loss than sows fed higher levels of CP (19.0 %) and Lys (1.10 %). Sows fed low levels of CP and Lys only just reached the same BW after the second farrowing as at first farrowing indicating that these sows did not have any of the expected maternal gain during their second gestation. In contrast, sows with the lowest BW loss were able to reach a higher BW after the second farrowing compared to first farrowing showing that these sows were able to gain maternal weight during their second gestation (Huang *et al.*, 2013). Another study (Everts and Dekker, 1995) also showed that, feeding a gestation diet with low CP (12.0 %) and AA levels during the first gestation resulted in sows retaining less protein compared to sows on a high CP

(17.8 %) and AA diet. These sows were not able to fully compensate for this lower protein retention during the second and third reproductive cycle (Everts and Dekker, 1995). In line with this Dourmad *et al.* (1996) showed that sows with a high BW loss (35 kg) during lactation fed a low energy diet (29.6 MJ ME/day) during the following gestation was unable to regain the total BW loss and had a lower N retention during gestation (Dourmad *et al.*, 1996).

2.2 Amino acid and protein metabolism during lactation

This section will describe the importance of dietary AA and protein and there will be an in-depth description of BCAA metabolism in relation to lactation. In addition there will be a description of AA and protein metabolism of the mammary gland of the sow.

2.2.1 The importance of ideal amino acid composition of dietary protein

Utilization of dietary protein depends on protein digestibility and its content and balance of AA (Boisen, 2003, van Milgen and Dourmad, 2015). Protein digestibility is among others linked to the source of protein, dietary composition, age of the pig and feed intake, but the efficiency at which the pig uses dietary protein for different life processes also largely depends on the AA composition of the protein. The role of the dietary protein is to provide the EAA, but also to deliver adequate N as substrate for synthesis of NEAA.

Ideal protein composition describes a situation where all EAA are co-limiting for a given life process e.g. milk production, which means the dietary supply of AA exactly fits the AA requirement of the animal (van Milgen and Dourmad, 2015). The AA requirement is generally expressed on "standardized ileal digestible" (SID) basis. The ileal digestibility is measured in digesta sampled from the most distal end of the small intestine just before entering the caecum. The purpose of the digestibility value is to know how much of the dietary AA that are available for the pig. In the standardized digestibility a correction is made for the basal endogenous losses (Trottier *et al.*, 2015). The total tract digestibility is a less precise measurement of AA availability, because the microbes in the hindgut alter the AA composition of digesta both via degradation and production of AA and using this value can lead to over- or underestimation of the AA availability.

As stated above the protein and AA requirements will depend on the age and production level of the animal. In the case of the lactating sow the milk production will be the main determinant of the requirement, and the optimal AA composition of ideal protein is therefore largely determined by the AA composition of the milk. However, there are some limitations to this approach, because some AA as for instance BCAA are taken up by the mammary gland in excess of what is excreted in the milk (Trottier *et al.*, 1997, Jackson *et al.*, 2000). It is therefore important to include studies on arterio-venous differences for AA of the mammary gland to include the different efficiencies by which AA taken up by the mammary gland are excreted as AA in milk. The efficiency of utilization of SID CP for milk CP is approximately 0.75, whereas the efficiencies for the individual EAA vary slightly depending on the extent to which the AA is used for other things than synthesis of milk CP in the mammary gland (NRC, 2012).

The perception of the AA composition of ideal protein for lactating sow differs slightly between countries, and these discrepancies can mostly be ascribed to the differences in the data that lies behind the given AA composition, but also production level and genetics of the sows play a role. The largest difference between countries concern the Val:Lys, whereas the ratio of the other EAA to Lys are similar. **Table 3** gives an overview of the proposed ideal AA composition of dietary protein in Denmark (Tybirk *et al.*, 2016), INRA in France (Dourmad *et al.*, 2008) and NRC in North America (NRC, 2012).

Table 3. Ideal amino acid composition of dietary protein during lactation given as ratios to lysine according to Danish (Tybirk *et al.*, 2016), French (Dourmad *et al.*, 2008) and North American (NRC, 2012) recommendations.

Amino acid, % of Lysine	**Denmark**	**North America**[1]	**France**
Lysine	100	100	100
Methionine	32	27	30
Methionine + Cystine	60	53	60
Threonine	65	67	66
Tryptophan	20	19	19
Isoleucine	56	56	60
Leucine	115	112	115
Histidine	39	40	42
Phenylalanine	55	54	60
Phenylalanine + Tyrosine	113	112	115
Valine	76	86	85

[1] Calculated for sow: BW = 240 kg, ADG of litter: 3 kg/day, number of nursing piglets: 13.

For a growing pig it is rather simple to define the optimum production level using either the ADG or feed-conversion ratio, but lactation is physiologically and nutritionally distinctly different from the other stages of the pig's life. Therefore, the AA requirement of lactating sows not only needs to maximize milk production for litter growth, but also needs to consider the degree of body mobilization and feed intake since most sows turn catabolic during lactation (Trottier and Guan, 2000, Kim *et al.*, 2001a).

The AA requirement of ideal protein is often expressed relative to Lys, because Lys generally is the first-limiting AA in pig diets. However, because of the complex interplay between AA supply from diet and body protein for milk production it is complicated to determine both the ratio of other AA to Lys, and also the order of the limiting AA for lactating sows (Kim *et al.*, 2009).

2.2.2 Amino acid uptake by the mammary gland

Several factors affecting or limiting the uptake of AA in the mammary gland have been investigated and amongst them are suckling intensity, availability of AA in the blood, AA extraction rates, abundance of AA transporters and mammary blood flow.

The AA are transported to the mammary gland through the blood stream and therefore the mammary gland blood flow is a major determinant of the availability of AA for milk CP synthesis. Mammary blood flow can be slightly affected by postural changes of the sow and after a meal the blood flow slightly increases, but the number of suckling piglets is the major determinant of blood flow. The blood flow increased from 2000 L/day to more than 5000 L/day when increasing litter size from 3 to 13 piglets (Farmer *et al.*, 2008). Renaudeau *et al.* (2002) found that the mammary blood flow had decreased with 40 % already 8 hours after weaning of the piglets. In line with this, the effect of suckling intensity on AA uptake by the mammary gland was investigated by Nielsen *et al.* (2002) by measuring arterio-venous differences of the mammary gland of sows nursing 3 to 14 piglets at different stages of lactation. Milk yield and mammary gland plasma flow increased linearly with increased number of nursing piglets, but the extraction rate of the EAA and the blood flow did not change during lactation. The study by Nielsen *et al.* (2002) indicated that the mammary gland uptake of AA was affected by litter size mainly mediated by an increased blood flow. In addition the changes of AA uptake during lactation was a result of increased arterio-venous differences and higher extraction rates, because day of lactation did not affect the mammary gland blood flow (Nielsen *et al.*, 2002).

Several studies have investigated the effect of increasing AA intake or optimizing the AA balance of the sow on the abundance of AA transporters, but the availability of dietary AA do not seem to affect the expression of genes encoding for AA transporters (Manjarin *et al.*, 2012, Huber *et al.*, 2016). Optimization of the dietary AA composition increases the efficiency of utilizing dietary AA for milk AA, but the reason must be something different than an up regulation of the gene expression of AA transporters. It was suggested by Huber *et al.* (2016) and Manjarin *et al.* (2012) that the competition between structurally similar AA for the same transporters may be reduced when the dietary AA composition is optimized.

Uptake of AA by the mammary gland will increase with increasing plasma concentration of AA, because the K_m value of AA transporters in the mammary gland is higher than the normal concentrations of these AA in plasma (Hurley *et al.*, 2000, Manjarin *et al.*, 2011). Guan *et al.* (2004b) showed that increasing levels of dietary CP did not affect mammary gland plasma flow, but for most AA a linear increase in the uptake was recorded. In accordance with this Krogh (2017) reported a positive correlation between arterial EAA concentrations and the A-V difference of EAA in the mammary gland, and it was suggested that the availability of AA in the blood was limiting the milk synthesis (Krogh, 2017)

There are many indications of the effects and interplay between mammary gland blood flow, plasma AA concentration, dietary AA intake, abundance of transporters and AA extraction rates on AA uptake by the mammary gland, but many of the underlying mechanisms are not understood and needs further research. However, the availability of AA in the blood for milk production seems to be a limiting factor for milk production.

2.2.3 Branched chain amino acids and milk production

The BCAA (Val, Leu, Ile) and especially Val is an area of AA nutrition that recently has received increased attention for several reasons: 1) crystalline Val has become commercially available, 2) Val is regarded as the second or third limiting AA for lactating sows (Tokach *et al.*, 1993, Kim *et al.*, 2009), and 3) the mammary gland uptake of BCAA is much higher than the output in milk (Trottier *et al.*, 1997, Jackson *et al.*, 2000).

Valine deficient diets can negatively affect the feed intake, which has been shown in several studies with growing pigs (Theil *et al.*, 2004, Barea *et al.*, 2009) and it is hypothesized that it is a preventive action to make the pig stop ingesting a deficient diet, but the mechanism behind this response is not fully understood (Gloaguen *et al.*, 2012).

Most AA that enter the blood from the intestine are catabolized in the liver, but for BCAA the major sites of catabolism are extra-hepatic tissues as for instance muscle, adipose tissue and the mammary gland. The BCAA are not converted into any unique metabolites but Leu (ketogenic) forms acetoacetate and Coenzyme A (CoA), Ile (ketogenic and glucogenic) forms acetyl CoA and propionyl CoA, and Val (glucogenic) gives rise to succinyl CoA.

Jackson *et al.* (2000) investigated the effect of incubating sow mammary gland tissue obtained at day 21 of lactation in two different concentrations of Val on the transport mechanism for Val uptake in mammary cells. The uptake was dependent on the sodium gradient and the uptake of Val increased with increasing Val concentration. The kinetic parameters of the uptake indicated that the potential limit of the transport system is not exceeded in a lactating sow and is therefore not limiting for synthesis of milk protein. Jackson *et al.* (2000) also showed that the uptake of Val was inhibited by other AA (Met, Ala, glutamine, Leu and Lys), which indicates that these AA may share some transport systems of the mammary gland (Hurley *et al.*, 2000, Jackson *et al.*, 2000, Guan *et al.*, 2002). Besides sharing transport systems into the endothelial cells of the mammary gland several interactions have been detected between the BCAA. All BCAA share the same enzymes (branched-chain amino acid transaminase and branched-chain α-keto acid dehydrogenase) for the first two steps in BCAA catabolism. In addition, Leu given in excess might increase the catabolism of all three BCAA leading to a deficiency of Val and Ile (Wiltafsky *et al.*, 2010), because of a decrease in nitrogen utilization (Langer and Fuller, 2000).

The recommendation for dietary AA during lactation is often based on the AA composition of the milk, but for BCAA the uptake exceeds the excretion in milk and therefore this approach is not valid for determining the requirement of BCAA during lactation (Linzell *et al.*, 1969, Trottier *et al.*, 1997, Nielsen *et al.*, 2002). Trottier *et al.* (1997) showed that approximately 60 % of the BCAA taken up by the mammary gland was excreted in the milk, which indicates that the mammary gland catabolizes the remaining 40 % of the BCAA. The same study (Trottier *et al.*, 1997) showed that the uptake of glutamine was only 44 % of what was excreted in the milk, and Li *et al.* (2009) found in an *in vitro* study with porcine mammary gland tissue that around 40 % of the transaminated BCAA were converted to branched-chain α-keto acids and the rest underwent oxidative decarboxylation. The end products of the BCAA transamination were mostly glutamine and Asp, but Ala, glutamate and asparagine were also synthesized and accordingly, the mammary gland tissue expressed the enzymes branched-chain aminotransferase, branched-chain α-keto dehydrogenase, glutamine synthase, asparagine synthase, glutamate-oxaloacetate aminotransferase and glutamate-pyruvate aminotransferase, and especially branched-chain aminotransferase had a much higher activity in the mammary gland compared to skeletal muscle, liver and small intestine (Li *et al.*, 2009).

3 HYPOTHESES AND OBJECTIVES

Based on the introduction and background chapters the overall objective of this thesis was to determine the optimum dietary Val:Lys and dietary concentration of balanced CP of the hyper-prolific sow for optimizing sow and piglet performance during lactation. The results would provide the basis for updating the present dietary recommendations to match the requirements of modern hyper-prolific sows.

It was hypothesized that optimizing Val:Lys and CP nutrition of lactating sows would improve litter growth and milk production, while sow body mobilization was minimized. Moreover it was hypothesized that such dietary interventions would affect sow metabolism through improved N-balance. Based on discrepancies of results on current studies on Val:Lys and recommendations from Dourmad *et al.* (2008) and NRC (2012) for lactating sows it was hypothesized that the old recommendation of dietary SID Val:Lys at 0.76 should be increased. In addition, it was hypothesized that the increased production level of hyper-prolific sows has increased their requirement of balanced dietary CP.

To address the overall objective and test the hypothesis of the thesis two feeding experiments were carried out in a commercial Danish sow herd using practically applicable diets based on wheat, barley and soybean meal supplemented with commercially available crystalline AA. The following objectives and hypotheses were formulated for the different parts of the thesis:

- The objective was to determine the optimal dietary Val:Lys of hyper-prolific lactating sows (Paper I).
    - It was hypothesized that improving the AA composition of dietary CP by altering the Val:Lys would increase litter ADG through improved milk production, minimize BW and BF losses of the sow and affect blood metabolites related to protein metabolism.

- The objective was to determine correlations between feed intake, BW loss and litter gain of hyper-prolific lactating sows (Paper II).
    - It was hypothesized that ADG of the litter would be positively affected by high sow feed intake and also affected by the degree of sow body mobilization.

- The objective was to determine the level of balanced dietary SID CP where sow and litter performance was maximized (Paper III + IV).
    - It was hypothesized that increasing balanced dietary SID CP provision would increase litter ADG through improved milk production, and possibly an altered milk composition. It was also hypothesized that the SID CP provision would affect sow mobilization of body reserves, change concentrations of blood metabolites related to AA metabolism and affect the following reproductive cycle.

4 MATERIALS AND METHODS

The experimental design and methods used in the two experiments are designated in detail in **Paper I-IV**. This chapter will elaborate on the methods used for determination of body composition and milk production as well as a more detailed description of the dose-response approach for determining Val:Lys and CP requirements used in these trials and the use of breakpoint analysis for the interpretation of data. In addition, the analyzed plasma metabolites and their potential connection to protein and fat metabolism are described.

4.1 Animals and housing

The two experiments were carried out in a commercial Danish sow herd with 1,700 sows. The feeding system was a SpotMix feeding system (Schauer Agrotonic, Prambachkirchen, Austria) that enabled registration of the feed allowance of the individual sow and at the same time could mix and distribute six different experimental diets and thereby allow for performance of a dose-response trial. All sows were fed 2.3 kg from day 2 *postpartum* and the allowance was gradually increased to a maximum of 7.4 kg for first parity sows and 8.4 kg for multiparous sows at day 17 *postpartum* (**Paper I** and **Paper III**).

Sows were crossbred between Danish Landrace and Danish Yorkshire and were inseminated with semen from Duroc boars (DanAvl, Copenhagen, Denmark). The sows were loose housed in the gestation unit until approximately one week *prepartum* when they were moved into the farrowing unit with individual farrowing crates. Each crate had a covered area in the corner equipped with a heating lamp for the piglets and a farrowing rail for tethering the sow. Design of the farrowing pens are shown in **Figure 3**.

Figure 3. Design of farrowing pens used in experiments 1 and 2.

A new batch of sows was moved to the farrowing unit every week 4-7 days *prepartum*. In the trials 5 different sections in the farrowing unit were used, and the dietary treatments were rotated within each section for each batch of sows to avoid confounding between effects of diets, pens and sections. The experiments were carried out over 31 consecutive weeks.

4.2 Formulation of experimental diets

Diets in both experiments were formulated to be iso-energetic. In both experiments diets were based on soy bean meal as the main protein source, and wheat and barley as primary energy sources (starch). Crystalline AA (Lys, Met, Thr, Trp, and Val) were used to balance the AA composition of the diets. In both trials it was intended to use ingredients and formulate diets that would be practically applicable for Danish pig producers and therefore no so called "synthetic" diets were used. Synthetic diets are formulated with high amounts of crystalline AA and reduced contents of other protein sources as for instance soy bean meal and accordingly they have a lower CP content than standard diets (Manjarin *et al.*, 2012, Huber *et al.*, 2015).

4.2.1 Experiment 1: Val:Lys

The aim of experiment 1 was to evaluate increasing dietary Val:Lys and therefore the only difference between the six diets was an increasing concentration of Val resulting in six different Val:Lys (**Table 4**). It was chosen not to include a negative control in the trial because it was designed to test whether the Danish recommendation for Val:Lys (0.76) was too low (**Table 4**) compared to for instance NRC (2012) in North America and INRA in France (Dourmad *et al.*, 2008), and therefore a diet based on a high level of crystalline AA would not have any practical application for Danish pig producers. In **Paper I** a full table with ingredient and nutrient composition is given.

There was only a small increase in CP between the six diets caused by the addition of crystalline Val, because Lys concentration was maintained more or less the same in the six diets. The lowest Val:Lys was obtained by formulating a diet without adding crystalline Val and this resulted in a total dietary Val:Lys of 0.84. To avoid underestimating the optimal ratio between an EAA and Lys, Lys must be below the requirement and the requirement of other EAA must be fulfilled (Boisen, 2003, NRC, 2012). In this experiment the daily Lys supply of the sows would account for 70-75 % of the requirement according to estimations from a model by Strathe *et al.* (2015).

Table 4. Calculated and analysed composition of the experimental diets for experiment 1.

Composition	**Diet**					
Calculated	1	2	3	4	5	6
DM, %	86.3	86.3	86.3	86.3	86.3	86.3
Energy, MJ ME/kg	13.0	13.0	13.0	13.0	13.0	13.0
CP, %	14.2	14.2	14.2	14.2	14.2	14.2
SID Lys	7.1	7.1	7.1	7.1	7.1	7.1
SID Val	5.4	5.6	5.8	6.1	6.5	6.9
SID Val:Lys	75.8	79.0	82.0	85.0	91.0	97.0
Total Val:Lys	80.1	82.9	85.5	88.1	93.3	98.5
Analyzed						
CP, %	14.4	14.4	14.5	14.4	14.4	14.5
Total Lys, %	8.0	8.0	8.1	8.1	8.2	8.1
Total Val, %	6.7	6.9	7.1	7.3	7.8	8.0
Total Val:Lys	0.84	0.86	0.88	0.91	0.95	0.99

4.2.2 Experiment 2: Crude protein

The aim of experiment 2 was to test increasing dietary levels of balanced CP. The diets were formulated to fulfill the applicable Danish dietary recommendations for EAA composition of CP for lactating sows. In the formulation of the six diets the commercially available crystalline AA (Lys, Met, Tre, Val) were used in order to ensure the lowest possible CP concentration and that the dietary AA composition would be as close as possible to the desired ideal protein composition (**Table 5**).

It was not possible to match the ratio of these AA to Lys of these AA completely only by the use of crystalline AA, therefore soy bean meal was used as a source of the most limiting AA. Hence, the CP concentration of the diets was determined by the most limiting AA, typically Leu and His.

Table 5. The calculated SID CP and AA composition of the dietary treatments in experiment 2 compared to the Danish recommendation (in %). The diet of group 3 had an AA level complying with the Danish recommendation in 2014.

Amino Acid	**Recommended concentration, g/kg**	**Dietary group**					
		1	2	3	4	5	6
SID CP	117	84	92	98	105	114	124
SID Lys	7.0	83	92	100	108	118	129
SID Met	2.2	84	93	101	110	120	132
SID Met + Cys	4.2	89	97	103	110	118	128
SID Thr	4.6	83	92	100	107	118	129
SID Trp	1.4	93	102	110	118	128	139
SID Leu	8.1	83	92	100	108	118	129
SID Ile	3.9	92	103	112	121	135	148
SID His	2.8	84	93	101	109	120	130
SID Phe	3.8	119	132	142	153	168	182
SID Val	5.3	84	93	100	108	119	129

This resulted in six diets with the same AA composition of the dietary protein and with simultaneously increasing concentrations of CP and the individual AA (**Paper III** and **Paper IV**, **Table 5**).

4.3 Selection of sows for the experiments

When sows were moved into the farrowing unit BW and BF thickness of the sows were measured and sows were allocated to one of the six dietary treatments based on body condition and parity. In both experiments parity 1-4 sows were included. It was intended to have similar BW, BF thickness and parity number amongst the six dietary groups at day 2 *postpartum* (**Figure 4**). The BW and BF thickness of the sows were measured again at day 2 *postpartum* to ensure that sows allocated to the experimental diets from day *postpartum* still fulfilled the inclusion criteria. Each week a batch of 18 sows was included in the experiment with three sows per dietary treatment and it was ensured that each group of six sows (one per treatment) was of same parity.

4.4 Measurements

Sows were moved into the farrowing unit 4-7 days *prepartum*. The day after parturition (day 2) the litters were standardized to 14 medium or large piglets to ensure that the sows were pushed to maximize milk production. The litters were weaned at day 25-26 *postpartum*. Some measurements were made on all sows included in the experiments, whereas other measurements were only carried out on different subsamples of the total number of sows.

Experiment 1: Dietary Val:Lys

- Total sows in the experiment: 558 (93 per treatment)
- Subsample of sows: 72 (12 per treatment; blood, urine, milk)

Experiment 2: Dietary CP

Total sows in the experiment: 544 (91 per treatment)

- Subsample 1 of sows: 72 (12 per treatment; blood, urine, milk)
- Subsample 2 of sows: 60 (10 per treatment; body composition)

In **Figure 4** a timeline for measurements and collection of samples in the two experiments is given. **Figure 5** shows measurements of litter weight and sow BW and BF thickness.

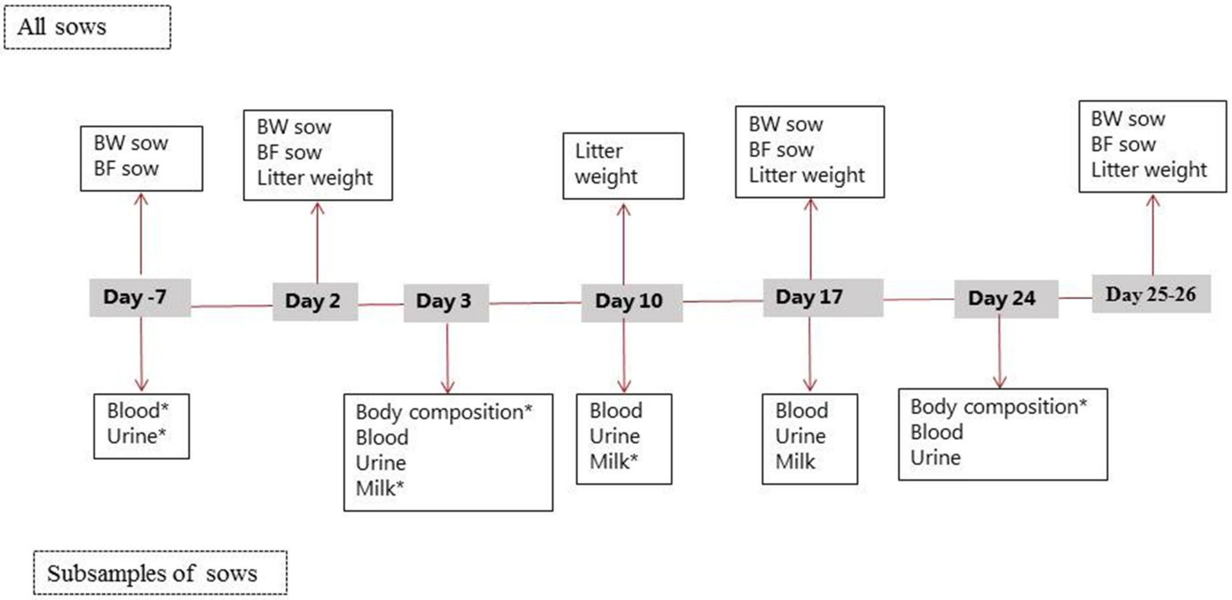

Figure 4. Timeline for measurements and collection of samples in the two experiments (1: Val:Lys and 2: CP). * = Measurements were only made in experiment 2. The measurements mentioned above the timeline were made on all sows in the experiments, whereas measurements listed below the time line were made on subsamples of sows.

Figure 5. Measurement of litter weight, sow BW and BF.

4.5 Body composition measured by the D_2O dilution technique

Body composition of the sows at litter standardization and at weaning was determined in experiment 2 (**Paper IV**) to investigate which body pools the sows were mobilizing from and the extent of this mobilization when increasing dietary protein intake. The advantage of using the D_2O dilution technique for determining body composition was that repeated measurements on the same animal were possible, and the sows did not have to be euthanized as in the slaughter technique. Deuterium oxide is a form of water with a larger amount of the hydrogen isotope deuterium, and D_2O is therefore also known as heavy water.

The principle of the D_2O dilution technique is that the sows are enriched with a known dose and concentration of D_2O and when equilibrium within the body water pool has been established the extent of the dilution can be determined.

The method was used to determine the D_2O-space of the sows at day 3 and 24 *postpartum.* The practical performance of the method is described below:

1. A blood sample was drawn from the jugular vein.

   This sample was taken to determine the background level of D_2O *in the blood of the sows. At the same time a sample of the infusate (10 % solution of* D_2O *in saline) was taken to determine the exact concentration of* D_2O*.*

2. 0.2 mL of the infusate per kg BW was injected intramuscularly in the neck of the sow.

   The syringe used for the injection was weighed before and after to know the exact weight of the injected infusate.

3. Five hours later another blood sample was collected.
   It takes approximately 5 hours for the D_2O to disperse equally within the body fluids, which means that an equilibrium of water inside and outside the cells has been established.
4. Analysis of D_2O.
   Plasma and infusate were analyzed for the fraction of hydrogen found as D_2O and the results were given as atomic fractions (AF, e.g. AF = 1 [100 % D_2O], AF =0.01 [1 % D_2O]).

From the determined AF of D_2O in plasma and infusate the D_2O space of the sows were calculated (Theil *et al.*, 2002):

$$D_2O\text{ space, mole} = \frac{Injected\ D_2O\ (g)}{Molecular\ weight\ injected\ D_2O\ (g/mole)} \times \frac{(AF_{infusate} - AF_{background})}{(AF_{plasma} - AF_{background})}$$

$$D_2O\text{ space, kg} = \frac{D2O\ space\ (mole) \times 18.015\ \left(\frac{g}{mole}\right)}{1000\ (g)}$$

The D_2O space of the sow was used in addition with BW and BF thickness to estimate the body pools of water, ash, fat and protein using equations by Rozeboom *et al.* (1994). The following equations developed for Yorkshire x Landrace gilts were used:

$$Water\ (kg) = 5.4 + 0.08 \times BW(kg) + 0.613 \times D_2O\ space(kg) - 0.11 \times BF(mm)$$
$$Fat(kg) = -7.7 + 0.649 \times BW(kg) - 0.61 \times D_2O\ space(kg) + 0.299 \times BF(mm)$$
$$Protein(kg) = 1.3 + 0.103 \times BW(kg) + 0.092 \times D_2O\ space(kg) - 0.108 \times BF(mm)$$
$$Ash(kg) = 0.04 - 0.01 \times BW(kg) + 0.054 \times D_2O\ space(kg) - 0.034 \times BF(mm)$$

The sum of the estimated values generally accounts for 90-95 % of the BW, because equations estimate the empty body content of water, fat, protein and ash. Hence, the empty body does not include contents of the intestines and bladder as the live BW does.

4.6 Milk yield and composition

In both experiments the individual milk yield of the sows was estimated and milk samples were obtained from sows and analyzed for nutrient composition. This was done to determine diet effects on both quantitative and qualitative aspects of milk production of the sows, because milk is the main product of the sow during lactation.

Milk samples were obtained in both experiments, and one and three samples were collected in experiment 1 and 2, respectively. Piglets were removed from the sow for 30-60 minutes and hereafter the sow was given an intramuscular injection of oxytocin to induce milk let-down (**Figure 6**). Milk was sampled from 4 to 5 teats to get at least 40 mL. The milk samples were analyzed for dry matter, fat, protein, lactose and casein using a MilcoScan FT2 (Foss Electric, Hillerød, Denmark). In addition, milk samples were analyzed for AA composition in experiment 1.

Milk yield of the sows was estimated from the ADG of the litter and the average litter size during lactation using equations by Hansen *et al.* (2012b). The milk yield was multiplied with the analyzed nutrient concentrations of the milk to calculate the total daily output of the nutrients.

Figure 6. Milk let-down was induced with oxytocin after removal of the piglets from the sow.

4.7 Calculation of efficiencies for utilization of amino acids

To determine the efficiency of utilization of dietary SID CP and Lys for milk protein quantitative information on intake, maintenance requirement, body protein mobilization and output in milk was needed. The efficiency was calculated as follows (NRC, 2012, Huber *et al.*, 2016):

$$\boxed{K = \frac{Output\ in\ milk - Mobilized\ from\ body\ protein \times 0.88}{SID\ intake - SID\ Maintenance\ requirement} \times 100}$$

where K is the efficiency of utilizing dietary SID CP or AA for milk CP synthesis and 0.88 (Hansen *et al.*, 2014) is the efficiency of utilizing mobilized CP or AA from body protein for milk CP synthesis. It was assumed that all mobilized body protein that was used for milk was directed towards milk CP synthesis and was therefore subtracted from the milk output of protein. It was assumed that the remaining CP in milk was derived from dietary protein. The requirement for maintenance was subtracted from the intake and the residual was the SID CP available for milk CP synthesis.

The information on body protein mobilization was only available for the entire lactation period; hence the balance was calculated for the whole lactation period and divided by days in milk. The total intake of SID Lys and CP was calculated based on the dietary concentration and the total feed intake during lactation. The requirement of SID CP and AA for maintenance were calculated using values from NRC (2012): 1320.5 and 46.3 mg/ kg $BW^{0.75}$ for CP and Lys, respectively. Amino acid composition of milk was not measured in experiment 2, and therefore the AA composition of milk measured in experiment 1 was used (**Paper I**). The diets in experiment 2 had the same dietary AA composition (**Paper III** and **IV**) and it was assumed that the AA composition of milk was similar for the six dietary groups. The AA available from body protein reserves were calculated using the estimated body protein mobilization and values for AA composition of maternal body protein were used according to NRC (2012). A linear regression model was made with output in milk from SID intake as response variable and SID intake for milk as input, where the slope represented the efficiency (K). The intercept was set to zero, because at zero intake the efficiency should also be zero to make sense biologically.

4.8 Blood metabolites

Blood samples were drawn by venipuncture from the jugular vein of the sows 4 hours after the morning feeding and the samples were centrifuged shortly after to harvest plasma (**Figure 7**). Plasma urea N (PUN) is a major marker of changes in dietary protein quality, intake and N-balance and it was decided to take the blood sample 4 hours after a feeding, because previously studies have shown that the best response on PUN was recorded at this time point (Eggum, 1970, Cai *et al.*, 1994).

This section will describe the measured blood metabolites and the use of them as potential markers for protein and fat metabolism of the sow to emphasize why plasma was analyzed specifically for these metabolites. The methods for chemical analysis of plasma and urine samples are described in **Paper I** and **IV**.

Figure 7. Blood samples were drawn from the jugular vein of the sow and centrifuged to harvest plasma.

4.8.1 Plasma urea nitrogen

Urea N is the end product of amino acid degradation in the urea cycle and is excreted via the urine. Catabolism of AA starts with a transamination of the AA and α-ketoglutarate forming the corresponding α-keto acid and glutamate. In the next step glutamate is converted to ammonium ion (NH_4^+) via oxidative deamination. Ammonium enters the urea cycle to form urea (Berg *et al.*, 2002).

The concentration of PUN reflects quality and quantity of dietary protein i.e. the AA composition, the protein/nitrogen intake and the time after feeding when the blood sample is drawn (Eggum, 1970). The concentration of PUN increases with increasing protein intake, so when using PUN as a measurement of protein quality the diets being compared must be isonitrogenous. Diets having the most ideal amino acid composition will have the highest protein quality, which results in the lowest PUN concentration, because urea excretion is decreased with a more efficient nitrogen utilization (Brown and Cline, 1974).

Plasma urea nitrogen can be used as a rapid response criterion to determine requirements of protein or amino acids by optimizing the nitrogen balance, because the nitrogen balance responds quickly to changes in dietary protein or amino acids (Coma *et al.*, 1996a). Coma *et al.* (1996a) investigated the response time of PUN to changes in dietary lysine in lactating sows, and found that there was a response already after 24 hours and that PUN concentration was stable 3-4 days after the feed change. For sows, the lowest concentration of PUN is obtained when the nitrogen balance is as close as possible to zero, which will be when minimal protein mobilization occurs. Eggum (1970) investigated how PUN changed over time in relation to feeding in growing pigs (68 kg). Plasma urea nitrogen was measured hourly in the portal vein and the concentration increased until 3-4 hours post feeding where a plateau was reached, and it was concluded that the most suitable time for blood sampling was 4-5 hours post feeding (Eggum, 1970). In another study (Cai *et al.*, 1994) it was tested if the diurnal pattern of PUN was affected by feeding strategy. The pigs (65 kg) had free access to feed in one period and were meal-fed in another period and blood samples were drawn from the jugular vein every second hour for 24 hours. The pigs with free access to feed had an almost constant concentration of PUN with only few fluctuations, whereas the meal-fed pigs had a higher diurnal variation. Plasma urea nitrogen of the meal-fed pigs peaked 3-4 hours post feeding.

Plasma urea N was measured in both experiment 1 and 2. In experiment 1 (**Paper I**) PUN was used as a measure of protein quality, because the aim was to determine the best dietary Val:Lys of the sows. In experiment 2 (**Paper IV**) PUN was used as a measure of the N-balance of the sows, because sows had different protein intakes.

4.8.2 Creatinine

A small amount of arginine in the urea cycle is diverted to synthesize creatine. Creatine is transported to the muscles where it is phosphorylated to creatine phosphate, which is used to generate ATP. Creatinine is formed continuously from the pool of creatine phosphate and creatinine passes from the blood to the kidneys, where it is excreted in the urine (Berg *et al.*, 2002). Creatinine is a measure of muscle mass, but can also be used as a marker for muscle catabolism (Rojkittikhun *et al.*, 1993); hence during periods where muscle protein is mobilized an increase in plasma or urine creatinine can be expected.

4.8.3 Triglycerides and NEFA

Triglycerides in the blood are mainly derived from dietary fat, whereas NEFA can be of both dietary fat and body fat origin. Non-esterified fatty acids in plasma shortly after a meal will also mainly be of dietary origin, but the concentrations of NEFA in plasma after feeding is much lower as compared to the concentrations in fasting sows. In addition, the differences between plasma NEFA concentration in the fed and fasting states are small during the first week of lactation and then becomes larger throughout lactation (Valros *et al.*, 2003, Mosnier *et al.*, 2010). The NEFA concentration in plasma can therefore be used as a marker for fat mobilization, but the time of sampling post feeding and *postpartum* must be considered carefully.

4.8.4 Albumin, total protein, AA

Albumin is synthesized in the liver and is the most abundant protein in the blood. The function of albumin is to serve as carrier for several molecules (e.g. water, fatty acids, hormones and cations) and to maintain the pressure of the blood. During periods where protein intake and muscle protein mobilization is insufficient to cover the requirements, albumin may be used as a protein reserve and the plasma concentration will therefore decrease (Wykes *et al.*, 1996).

Total protein of plasma includes all proteins of the body (e.g. structural protein, muscle protein, enzymes, transport proteins, hormones, immune proteins and dietary proteins). A low total plasma protein concentration can be a result of low protein synthesis caused by low dietary CP concentration or malabsorption of protein (Thorup *et al.*, 2012).

Amino acids in the blood can either be of dietary or muscle protein origin, so an elevated concentration of blood AA can be observed when intake of either protein or single AA is increased. During muscle protein mobilization AA are released to the blood and transported to other tissues as for instance the mammary gland for milk protein synthesis. Blood was only analyzed for AA in experiment 1 (**Paper I**), because here the different dietary AA profiles could result in a diverse AA composition of the blood as compared to experiment 2 (**Paper III** and **IV**), where the dietary AA profile was similar in all diets.

4.8.5 Enzymes related to amino acid metabolism

Generally, a higher concentration of an enzyme indicates an elevated activity, whereas a lower concentration would imply at lower activity or a faster use of the enzyme. Gamma-glutamyl transferase promotes the uptake of AA by the mammary gland (Viña et al., 1981b, Viña et al., 1981a). Both alanine amino transferase and aspartate amino transferase are involved in the transamination of AA in the cytosol of the liver cell, where α-keto glutarate is converted to glutamate. Glutamate is transported into the mitochondrion and converted to ammonium ions, which enters the urea cycle. Aspartate amino transferase is part of the transamination route where aspartate is converted to glutamate. Therefore it is expected that a higher protein intake would increase the activity of these enzymes (Das and Waterlow, 1974).

4.9 Determination of requirements using breakpoint analysis

In the two performed dose-response trials each experiment included six dietary treatments with either increasing Val:Lys or CP. Several feed analyses were performed throughout the experiments and together with the individual feed intakes of the sows a dietary Val:Lys or CP concentration was calculated for each sow. These individual concentrations were used as explanatory variables of the diet in the breakpoint analysis and therefore adds more variation to the dose-response curve.

It is common to analyze dose-response data using breakpoint analysis, because the breakpoint estimate and the belonging standard error can be interpreted as the requirement and above this point no significant changes are reported (Robbins *et al.*, 2006, NRC, 2012).

It was chosen to fit data to linear broken-line and quadratic broken-line models using nonlinear mixed effect models. The random effect of block was incorporated into plateau parameters of the models, while the fixed effect of parity was incorporated both in the requirement parameter and the plateau parameter of the models. It was tested if there was an effect of parity, and when significant it was included in the model. If the effect of parity was significant it was also tested if primi- and multiparous sows had different breakpoints. Most response variables were also fitted with a simple linear regression model for the comparison with the two breakpoint models. Three criteria were used to evaluate the output of the models: 1) comparing Akaike Information Criterion (AIC) and -2 log likelihood fit statistics for nested models, where smaller values indicate a better fit to data. For -2 log likelihood fit statistics there should be a drop by 3.84 units to conclude that one model had a better fit (Kanninen and Khawaja, 1999), 2) when the breakpoint of the model was outside the range of the tested dietary SID CP concentrations the model was excluded, because it was taken to be unreliable to describe data and 3) visually best fit to data (Parr et al., 2003).

The breakpoint analysis was only applied on data from experiment 2 (**Paper III** and **IV**), because no dietary effects were detected in experiment 1 (**Paper I**). A detailed description of the statistical analysis performed beside the breakpoint analysis is given in the individual papers (**I – IV**). All statistical analyses were performed in the statistical software R (R Core Team, Vienna, Austria).

5 RESULTS

In this chapter the main results of the two experiments are summarized. Results that were not shown in the papers are also presented in this chapter. The results are described in more detail in the **Paper I-IV**.

5.1 Experiment 1 (Paper I and II)

The objective of experiment 1 was to determine the optimal dietary Val:Lys during lactation, but since no diet effects on sow and litter performance recorded (**Paper I**) a second objective was formulated to determine correlations between feed intake, BW loss and litter ADG (**Paper II**).

5.1.1 Milk production and litter performance

The number of weaned pigs was unaffected by dietary treatments, accordingly each sow weaned 13.0±1.1 on average (**Paper I**; P=0.23) piglets. Increasing the total dietary Val:Lys above 0.84 did not affect the milk yield (P=0.49) or the ADG of the litter (P=0.84; **Paper I**). The litter gain was higher in mid lactation than in early and late lactation (**Paper II**; P<0.05). The ADG of the litter was positively correlated to the feed intake (r=0.45, P<0.001) of the sow and the BW (r=0.48, P<0.001) and BF loss during lactation (r=0.42, P<0.001; **Paper II**). Parity 1 sows had a lower ADG of the litter and milk yield compared to multiparous sows (P>0.001; **Paper II**).

Milk content of fat (7.2±2.1 %; P=0.37), CP (4.7±0.7 %; P=0.90), urea (1076±256 mg/dL; P=0.35) and DM (17.8±2.3 %; P=0.33) on day 17 of lactation was unaffected by dietary Val:Lys, whereas there was a tendency for a diet effect on lactose content (P=0.05). Nor was the total output of these nutrients in the milk affected by the treatment (P>0.05). There was an linear increase of concentrations of Val, Ile and Ala and a decrease in Arg (P<0.05) in the milk with increasing dietary Val:Lys (**Paper I**). There was a tendency for increased Leu (P=0.06) concentration in the milk (**Paper I**). In connection with the AA analyses of the milk the CP content was analyzed and the average concentration was 4.5±0.2 %. The concentration of the other AA in the milk was not affected by dietary treatment and the average concentrations of each AA are given in **Table 6** (Results not shown in **Paper I** and **II**). Of the EAA Leu was the most abundant AA (**Paper I**), whereas glutamic acid was the NEAA with the highest concentration (**Table 6**).

Table 6. Average AA composition of milk CP on day 17 of lactation for AA that were not affected by dietary treatment (n=17 sows).

Amino acid	**g/100 g CP**	**% of Lysine**
Lysine	6.90±0.27	-
Methionine	1.88±0.09	27.4±0.67
Methionine + Cysteine	3.25±0.14	47.3±1.70
Threonine	3.77±0.19	54.7±2.18
Arginine	4.38±0.47	63.4±6.78
Histidine	2.47±0.11	35.6±1.18
Phenylalanine	3.85±0.17	56.1±2.17
Glycine	3.33±0.17	49.0±4.08
Serine	4.77±0.30	69.4±3.71
Alanine	3.39±0.19	49.6±2.63
Aspartic acid	7.87±0.26	114±3.58
Proline	11.0±0.36	161±07.45
Glutamic acid	35.9±5.85	286±18.1

The concentration of glutamic acid in the blood was negatively correlated to the glutamic acid concentration in milk protein (r= -0.74; P<0.01) and there was also a tendency for a negative correlation between BCAA concentration in blood and glutamic acid in the milk (r= -0.53; P=0.08). There was no correlations between blood concentrations of total BCAA or single BCAA and the BCAA concentrations in milk protein (P>0.05).

5.1.2 Body mobilization of the sow

Increasing the dietary Val:Lys did not affect the BW (P=0.21) and BF (P=0.11) loss of sows during lactation (**Paper I**). Sows had a higher BW and BF loss from day 2 to 17 compared to day 17 to 26 (**Paper II**; P<0.001). The average feed intake of the sows was negatively correlated to both BW loss (r = -0.27; P<0.001) and BF loss (r = -0.19; P<0.001) during lactation. Sows with a high milk yield also had a higher loss of BW (r = 0.49; P<0.001) and BF (r=0.49; P<0.001; **Paper II**).

5.1.3 Sow metabolism: blood and urine metabolites

Serine concentration in the blood on day 17 was the only AA affected by dietary treatment ($P<0.05$; **Paper I**) and plasma concentrations of glucose, lactate, NEFA, creatinine and PUN as wells as urinary creatinine were unaffected by dietary Val:Lys ($P>0.05$; **Paper I**). Plasma concentrations of glucose and lactate were similar at all sampling days ($P>0.05$). A decrease from early to late lactation was found in concentrations of plasma creatinine and NEFA and urinary creatinine ($P<0.01$), whereas PUN increased during lactation ($P<0.001$; **Paper II**).

5.1.4 Sow performance in following reproductive cycle

The dietary treatments did not affect the reproductive performance of the sows in the following cycle ($P>0.05$). First parity sows had a longer weaning-to-estrus interval than multiparous sows ($P<0.001$) and the number of total born piglets in next litter was lowest in parity 1 sows, intermediary in second parity sows and highest in parity 3-4 sows ($P<0.001$; **Paper II**). Total born piglets in next litter was correlated to both feed intake of the sow ($r=0.24$; $P<0.001$) and BW loss during lactation ($r=-11$; $P<0.01$; **Paper II**).

5.2 Experiment 2 (Paper III and IV)

The objective of experiment 2 was to determine the level of balanced dietary SID CP where sows and litter performance was maximized.

5.2.1 Milk production and litter performance

The litter size at weaning was not affected by dietary protein concentration and sows in average weaned 12.8±1.2 piglets (**Paper III**; $P=0.30$). The ADG of the litter increased with increasing dietary SID CP until a breakpoint at 135 g SID CP/kg (**Table 8**; **Paper III**; $P<0.001$). The dietary treatment did not affect the incidence of litters treated for diarrhea ($P=0.97$), but first parity sows had a higher incidence than multiparous sows (**Paper III**; $P<0.001$).

Estimated milk yield increased with increasing dietary SID CP (**Paper III**; $P<0.001$). Milk composition was not affected by dietary treatment at day 3 and 10 of lactation (**Paper III** and **IV**; $P>0.05$). Lactose content of the milk was similar at all sampling days ($P=0.15$), but at day 17 the lactose content decreased until a breakpoint at 120 g SID CP/kg ($P<0.001$). Milk CP and casein (data for casein not shown in **Paper III** and **IV**) content was higher at day 3 compared to days 10 and 17 ($P<0.001$), the fat content of the milk decreased from day 3 to 17 (**Paper III**; $P<0.05$), and the urea concentration increased throughout lactation ($P<0.01$). The CP content of milk increased until a breakpoint at 136 g SID CP/kg (P<0.001) and fat content increased linearly (P<0.001). The concentration of casein was unaffected by dietary treatment at day 3 (4.42±0.60 %; P=0.97) and 10 (3.90±0.49 %; P=0.48), but at day 17 the casein content increased linearly with increasing dietary SID CP concentration (**Figure 8**; P<0.01). The casein content amounted to 88 %, 79 % and 77% of the protein content at day 3, 10 and 17, respectively. The urea concentrations of the milk was unaffected by dietary treatment (P=0.45), but the concentration increased during lactation with concentrations of 834, 1110, and 1223 mg/dL on day 3, 10 and 17, respectively (P<0.001). There was a positive correlation between milk urea and PUN (r=0.32; P<0.001) on all sampling days.

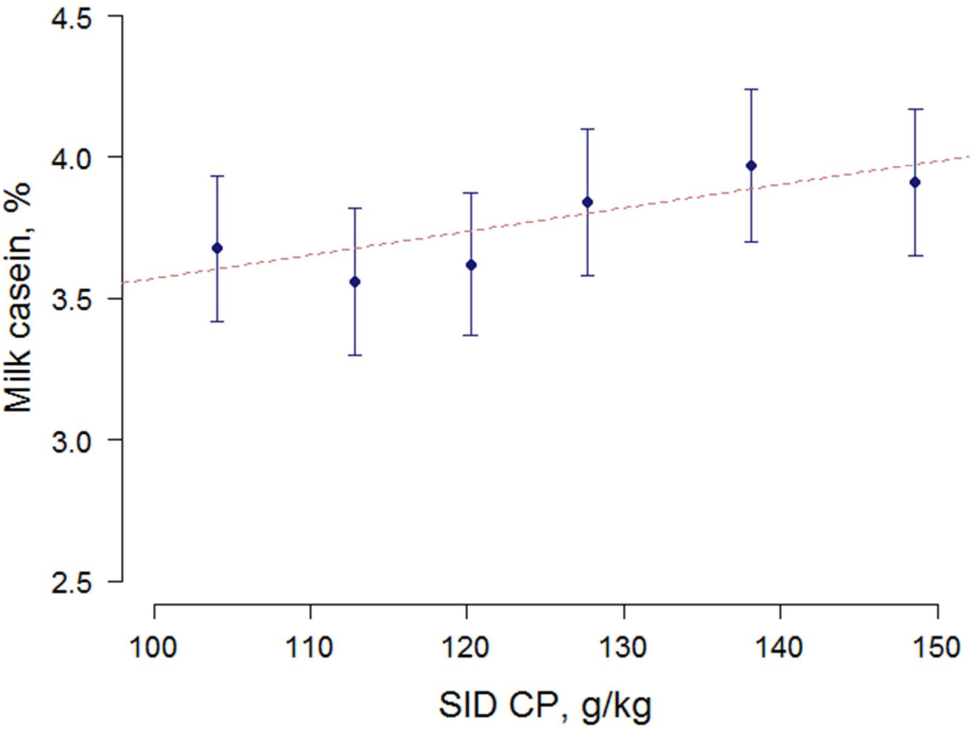

Figure 8. The content of milk casein increased linearly at day 17 (P<0.01) of lactation with increasing dietary SID CP concentration (Y = 3.4 + 0.005 x SID CP (g/kg)).

5.2.2 Body composition and body mobilization in the sow

The BW loss decreased and BF loss increased until breakpoints at 143 and 127 g SID CP/kg, respectively (**Paper III**; **Table 8**; $P<0.001$).

The loss of body water and CP decreased with increasing dietary SID CP concentration until breakpoints (**Table 8**; $P<0.001$), the body ash loss decreased linearly (**Table 8**; $P<0.001$) and fat loss was unaffected by dietary treatment (**Paper IV**; $P=0.41$). When adding up the estimated body pools of fat, CP, ash and water at day 3 and 24 it accounted for 92.8±0.8 % and 93.9±1.3 % of the live BW of the sows, respectively.

5.2.3 Sow metabolism: blood and urine metabolites

Only four of the measured plasma metabolites were affected by dietary SID CP level (**Paper IV**). Plasma concentration of albumin at day 17+ 24 ($P<0.05$) and gamma-glutamyl tranferase at all sampling days ($P<0.05$) increased linearly with increasing dietary CP concentration, whereas the concentration of alanine amino transferase at day 17 and 24 ($P<0.05$) decreased linearly. Plasma urea N concentration was stable until breakpoints at 139 and 133 g SID CP/kg in early and late lactation, respectively (**Paper IV**; $P<0.001$). Urinary pH was similar at all sampling days and increased quadratic until 141 g SID CP/kg. Effect of day *postpartum* on concentration of blood metabolites are shown in **Table 7**.

Table 7. Concentrations of plasma metabolites throughout lactation, where there was no effect of dietary treatments (P>0.05).

	Day *postpartum*						***P*-value**
	-4	3	10	17	24	SE	Day
Glucose, mmol/L	4.46^{a}	5.32^{b}	5.51^{bc}	4.97^{cd}	4.76^{ad}	0.14	<0.001
NEFA mmol/L[2,3]	0.26^{a} [0.22; 0.33]	0.37^{b} [0.30; 0.46]	0.32^{ab} [0.26; 0.40]	0.18^{c} [0.15; 0.22]	0.16^{c} [0.13; 0.20]	-	<0.001
Triglycerides, mmol/L	0.54^{a}	0.23^{b}	0.21^{b}	0.22^{b}	0.20^{b}	0.03	<0.001
Cholesterol, mmol/L	1.27^{a}	1.38^{a}	1.39^{a}	1.64^{b}	1.73^{b}	0.06	<0.001
Albumin,	34.5^{a}	40.5^{b}	38.0^{c}	[4]	[4]	1.09	<0.001
Creatinine, µmol/L	190^{a}	177^{b}	158^{c}	144^{d}	138^{d}	4.67	<0.001
Total protein, g/L	62.3^{a}	74.4^{b}	72.3^{b}	67.2^{c}	66.0^{ac}	1.91	<0.001
Aspartate amino transferase, U/L	22.8^{a}	39.9^{b}	36.1^{b}	37.6^{b}	36.7^{b}	1.53	<0.001
Alanine amino transferase,	37.6^{a}	38.5^{a}	38.2^{ab}	[4]	[4]	2.05	<0.001

[1] There was no effect of diet and parity (*P*>0.05). The interactions Diet x Parity and Diet x Day of lactation were not significant (*P*>0.05).

[2] Results were log transformed and are therefore given with a confidence interval instead of SE.

[3] NEFA = non-esterified fatty acids

[4] Diet effects on these days are shown in Paper IV.

[abcd] Values within a row with different superscripts differ significantly at *P*<0.05.

5.2.4 Sow performance in following reproductive cycle

The weaning-to-estrus interval was unaffected by dietary treatment (5.2±3.6 days; P=0.83) and there was a tendency for a lower number of total born piglets in next litter in groups fed 104 and 113 g SID CP/kg compared with the other four groups (**Paper III**; P<0.10).

5.2.5 Overview of breakpoints obtained in breakpoint analyses

The main results of the breakpoint analyses are shown in **Table 8** and the remaining results reported in **Paper III** and **IV**.

Table 8. Results of the breakpoint analyses (**Paper III** and **IV**).

Response variable	**Day/period**	**SID CP (g/kg) at breakpoint [CI]**[1]	**Response at breakpoint**
ADG litter	Day 2-25	135 [124; 145]	2.53 kg/day (parity 1) 3.07 kg/day (parity 2-4)
BW loss	Day 2-25	143 [120; 165]	0.6 kg/day
BF loss	Day 2-25	127 [112; 142]	3.0 mm
Milk Lactose	Day 17	120 [110; 130]	5.3 %
Milk CP	Day 17	136 [115; 158]	5.0 %
Body CP loss	Day 3-24	128 [111; 146]	1.5 % of body CP at day 3
Body water loss	Day 3-24	130 [117; 142]	2.5 % of body water at day 3
PUN	Day 3 + 10	139	3.8 mmol/L
PUN	Day 17 + 24	133	4.5 mmol/L

[1] CI = 95 % confidence interval.

5.2.6 Efficiencies of utilization of amino acids for milk production

The efficiency of utilizing dietary SID CP for milk CP was 0.75 when including all sows. Sows in the groups with the two highest dietary SID CP concentrations (139 and 150 g SID CP/kg) where most likely oversupplied with CP and when excluding these sows from the calculations the efficiency was 0.78 (**Figure 9**). The efficiency of utilizing dietary SID Lys for milk Lys was 0.86 when including all sows and when only sows in group 1 to 4 were included the efficiency was 0.91 (**Figure 10**). These results were not shown in **Paper III** and **IV**.

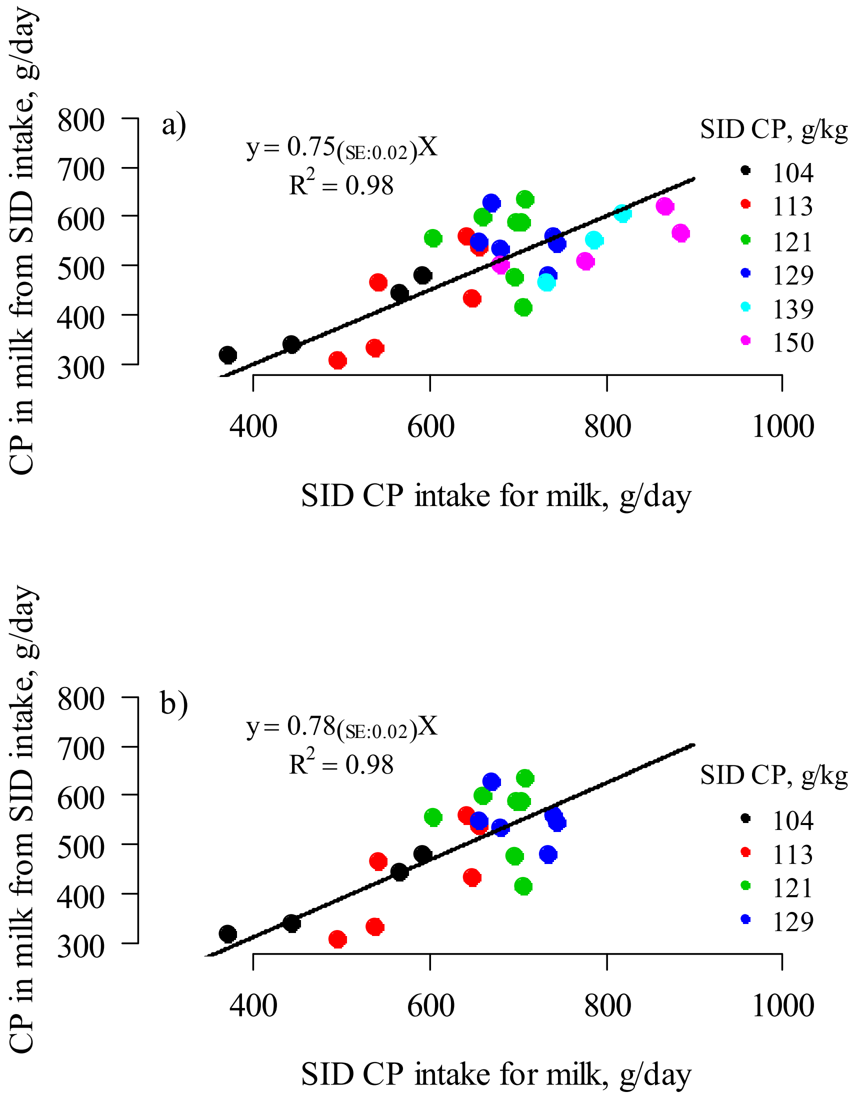

Figure 9. Efficiency of utilizing dietary SID CP for milk protein in experiment 2. Group 1 to 6 refers to the six dietary concentrations of SID CP: 104, 113, 121, 129, 139 and 150 g SID CP/kg, respectively. The slope represents the efficiency. **a)** Efficiency for all sows in group 1 to 6 at 0.75., and **b)** Efficiency for sows in group 1 to 4 at 0.78.

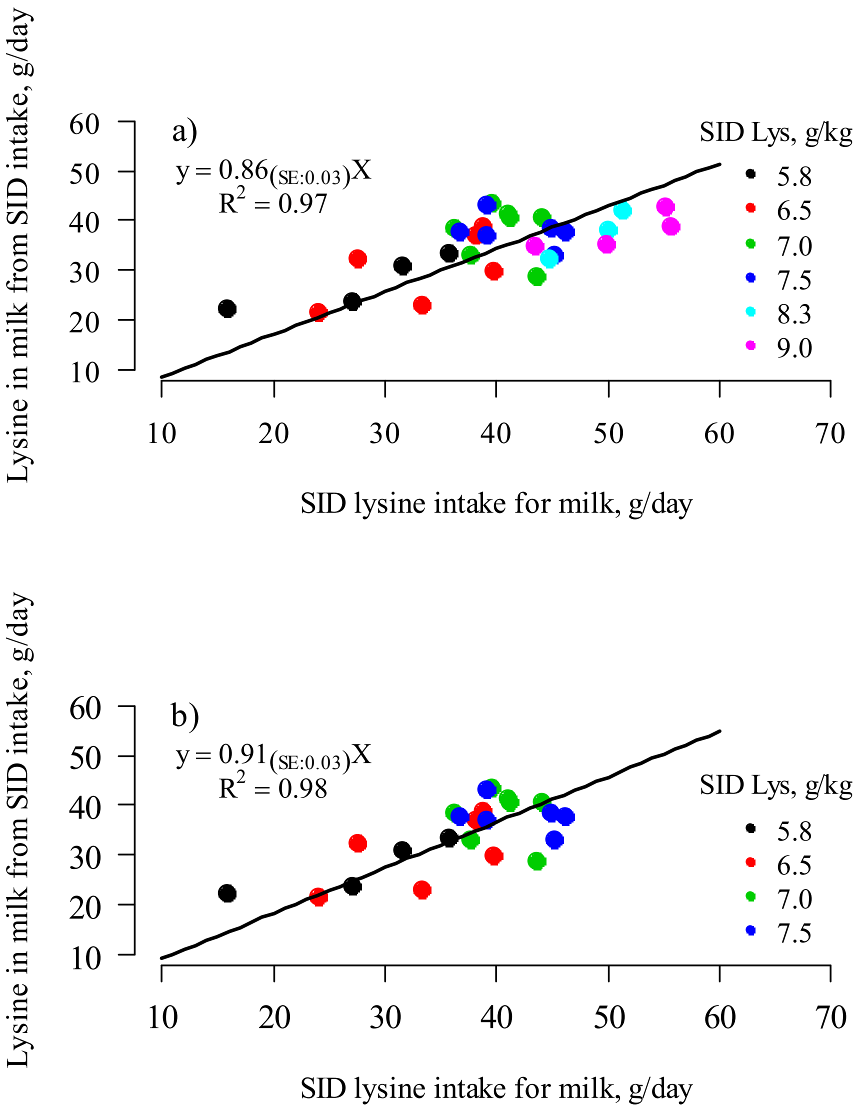

Figure 10. Efficiency of utilizing dietary SID lysine for milk lysine in experiment 2. Group 1 to 6 refers to the six dietary concentrations of SID lysine: 5.8, 6.5, 7.0, 7.5, 8.3 and 9.0 g SID Lys/kg, respectively. The slope represents the efficiency. **a)** Efficiency for all sows in group 1 to 6 at 0.86, and **b)** Efficiency for sows in group 1 to 4 at 0.91.

6 INCLUDED PAPERS

6.1 Paper I

The effect of increasing the dietary valine-to-lysine ratio on sow metabolism, milk production, and litter growth.

A.V. Strathe, T. S. Bruun, J.-E. Zerrahn, A.-H. Tauson and C.F. Hansen

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The effect of increasing the dietary valine-to-lysine ratio on sow metabolism, milk production, and litter growth[1]

A. V. Strathe,* T. S. Bruun,† J.-E. Zerrahn,‡ A.-H. Tauson,§ and C. F. Hansen*[2]

*Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Fredriksberg C, Denmark, †SEGES Pig Research Centre, Axeltorv 3, 1609 Copenhagen, Denmark; ‡Evonik Degusa International AG, Havneparken 2, 7100 Vejle, Denmark; and §Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark

ABSTRACT: A study was conducted to investigate the effect of increasing the dietary valine-to-lysine ratio (Val:Lys) for lactating sows weaning more than 12 piglets. Five hundred fifty-eight sows (parity 1 to 4) were allotted to 6 dietary treatments from 2 d postpartum, when litters were standardized to 14 piglets. Diets were analyzed to have a total dietary Val:Lys of 0.84, 0.86, 0.88, 0.90, 0.95, or 0.99:1. On all 558 sows, BW, back fat thickness (BF), and litter weight were registered at d 108 of gestation and d 2 and 25 (weaning) postpartum. On a subsample of 72 sows, additional measurements were made: sow BW and BF were measured on d 17 and litter weight was measured on d 10 and 17, and blood and urine samples were collected weekly. The litter size at weaning was not affected by the dietary Val:Lys ($P = 0.23$) and, on average, the sows weaned 13.0 ± 1.1 piglets. Average daily gain of the litter (2.93 ± 0.53 kg/d; $P = 0.84$), litter weight at weaning ($P = 0.67$), the average milk yield (11.3 ± 1.4 kg/d; $P = 0.49$), and milk contents of fat ($P = 0.57$), protein ($P = 0.18$), and lactose ($P = 0.20$) were not affected by the dietary Val:Lys. Increasing the dietary Val:Lys increased the milk concentration of Val ($P < 0.05$) and Ile ($P < 0.01$). The change in sow BW and BF were similar for all sows from d 2 to 17, d 17 to 25, and d 2 to 25 ($P > 0.05$). During lactation, sows, on average, had a BW and back fat loss of 22.1 ± 12.7 kg and 2.9 ± 1.7 mm, respectively. Plasma concentrations of glucose ($P = 0.26$), lactate ($P = 0.95$), urea N ($P = 0.84$), NEFA ($P = 0.24$), and creatinine ($P = 0.42$); urine concentration of creatinine ($P = 0.57$); and concentrations of AA in whole blood ($P > 0.05$) were not affected by the dietary Val:Lys. In conclusion, there was no effect of increasing the total dietary Val:Lys above 0.84:1 on sow metabolism and litter performance during lactation.

Key words: blood metabolites, dietary valine-to-lysine ratio, lactation, litter growth, milk composition, sows

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INTRODUCTION

During the last decades, litter size and milk production of modern genotype sows have drastically increased and nutritional requirements, therefore, need to be reevaluated. Research with lactating sows has indicated that Val is catabolized at a high rate in the mammary tissue (Li et al., 2009) and AA composition of milk, therefore, can not be used to predict the ideal dietary AA composition (Guan et al., 2004). Valine is regarded as the third most limiting AA for lactating sows after Lys and Thr (Kim et al., 2001, 2009). However, the few studies made on the correct dietary valine-to-lysine ratio (Val:Lys) for lactating sows were conducted with sows weaning 10 to 11 piglets or less, which is below the industry average (Vinther, 2014) and emphasizes the importance of a study on high-producing sows.

In addition, the empirical studies evaluating the Val:Lys for lactating sows show contradicting results. Richert et al. (1996) increased suckling piglet growth

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[2] Corresponding author: cfh@sund.ku.dk

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when increasing the total dietary Val:Lys from 0.83:1 to 1.11:1 and, in a subsequent study, Richert et al. (1997) concluded that the best total Val:Lys for lactating sows was 1.20:1 for optimizing piglet growth. To the contrary, Paulicks et al. (2003) reported an effect on only piglet growth, milk yield, and sow BW by increasing the total Val:Lys from 0.45:1 to 0.55:1, but no effect was recorded when further increasing the ratio from 0.64:1 to 1.44:1. Similarly, another study by Gaines et al. (2006) concluded that a total Val:Lys above 0.86:1 did not prevent excessive mobilization of body tissues or improved piglet gain; however, litter growth was compromised for a Val:Lys at 0.73:1.

The current NRC (2012) recommendation for standard ileal digestible (**SID**) Val:Lys is 0.85:1, whereas the Danish recommendation of SID Val:Lys is 0.76:1 (total Val:Lys of 0.80:1; Tybirk et al., 2014). Consequently, the aim of the current study was to investigate a total Val:Lys above 0.84:1 for high-producing sows weaning more than 12 piglets. It was hypothesized that optimization of the dietary Val:Lys would improve the metabolic status of the sow and litter growth.

MATERIAL AND METHODS

This study was conducted with the approval of the Danish Animal Experimentation Inspectorate (authorization number 2013-15-2934-00961).

Experimental Design, Animals, and Housing

The study was conducted in a commercial Danish herd from December 2013 to July 2014 using a total of 558 parity 1 to 4 sows (Danish Landrace × Danish Yorkshire; DanAvl, Copenhagen, Denmark) inseminated with Duroc semen (Hatting KS, Horsens, Denmark). The sows were randomly allocated based on body condition and parity (18, 36, 28, and 18% sows of parity 1, 2, 3, and 4, respectively) to 1 of 6 dietary treatments (n = 93) with 6 dietary ratios of (analyzed) total Val to Lys of 0.84:1, 0.86:1, 0.88:1, 0.90:1, 0.95:1, or 0.99:1 in a complete block design. Very fat or thin sows were not included in the experiment and the sow should have at least 14 functional teats. The animals were studied from 1 wk prepartum to d 25 postpartum, when the piglets were weaned. The herd had 5 farrowing sections with individual farrowing crates. Treatments were equally represented within each section, but treatments were rotated within each section to ensure that sows receiving the same treatments were not placed in the same pens in every block of sows. The temperature in the farrowing unit was set to 20°C and the farrowing unit was ventilated, using negative pressure, by wall inlets. In the farrowing rooms, artificial light was on from 0700 to 1600 h. Each pen had a covered area in the corner equipped with a heating lamp for the piglets. The heating lamp was regulated by infrared sensors (VengSystem A/S, Roslev, Denmark) and the temperature was gradually decreased from 34°C at farrowing to 22°C 15 d postpartum. Each week, a batch of 18 sows was selected for the experiment, moved to the farrowing unit 7 d before expected parturition, and placed in individual farrowing crates. Twenty-four hours postpartum (d 2), the litters of the experimental sows were standardized to 14 piglets (1.78 ± 0.06 kg average BW). All piglets were given iron injections and were tail docked, and males were surgically castrated on d 3 or 4 postpartum. Besides the registrations made in the experiment, all animal were managed according to the general routines of the herd. The health of the animals was monitored by the farm staff and normal practices for management, treatments, and vaccinations of the herd were followed. When dead or very weak piglets were removed, the date and weight of the excluded piglet were registered. Piglets were weaned at approximately 25 d postpartum, when the experiment ceased.

Feeding System and Diets

Sows were fed using a SpotMix feeding system (Schauer Agrotronic, Prambachkirchen, Germany) that enabled mixing of batches for small groups of sows and usage of individual feeding curves for each sow. Meals for each sow were weighed and registered by the SpotMix feeding system and fed by air-assisted transport, thereby avoiding mixing of the different diets. Feed residuals were not recorded. Sows generally ate their daily ration, but sows with many drops in feed intake were subsequently excluded from the data set, which made it valid to interpret the recorded feed allowances as the actually intake of the sows. From 7 d prepartum to 2 d postpartum, all sows were fed the same commercial formulated lactation diet based on wheat, soybean meal, and sugar beet pulp complying with Danish recommendations (Christensen et al., 2013; Tybirk et al., 2014). From d 2 to 10 of lactation, sows were fed twice daily, and from d 10 onward, sows were fed 3 times per day. Gilts were fed 2.3 kg from d 2 postpartum and the feed allowance was increased to a maximum of 7.4 kg at d 17. Multiparous sows were fed 2.3 kg from d 2 postpartum and feed allowance was gradually increased to a maximum of 8.4 kg at d 17. Sows had drinking nipples in the trough and free access to water. The feed allowance was adjusted daily for the individual sow. From d 2 postpartum, sows were allotted to 6 dietary treatments varying in Val:Lys (Table 1). The diets were formulated

Table 1. Ingredients and nutrient composition of the 6 dietary treatments (1–6; as-fed basis)[1]

	Diet					
Item	1	2	3	4	5	6
Ingredient, %						
Barley	40.0	40.0	40.0	40.0	40.0	40.0
Wheat	39.7	39.7	39.7	39.7	39.7	39.7
Soy bean meal	14.3	14.2	14.2	14.1	14.0	13.8
Palm oil	1.92	1.92	1.92	1.92	1.92	1.92
Micro grits	0.05	0.05	0.05	0.05	0.05	0.05
Monocalcium phosphate	1.06	1.06	1.06	1.06	1.06	1.06
Limestone	1.48	1.48	1.48	1.48	1.48	1.48
Salt	0.53	0.53	0.53	0.53	0.53	0.53
L-Lys	0.46	0.47	0.47	0.48	0.49	0.50
L-Thr	0.05	0.05	0.06	0.06	0.06	0.06
DL-Met	0.04	0.04	0.04	0.04	0.04	0.04
L-Val	–	0.06	0.13	0.19	0.31	0.43
Vitamin and mineral premix[2]	0.42	0.42	0.42	0.42	0.42	0.42
Phytase[3]	0.01	0.01	0.01	0.01	0.01	0.01
Composition (calculated)						
DM, %	86.3	86.3	86.3	86.3	86.3	86.3
CP, %	14.2	14.2	14.2	14.2	14.2	14.2
SID[4] Lys, g/kg	7.1	7.1	7.1	7.1	7.1	7.1
SID Val, g/kg	5.4	5.6	5.8	6.1	6.5	6.9
SID Met, g/kg	2.3	2.3	2.3	2.3	2.3	2.3
SID Cys, g/kg	2.2	2.2	2.2	2.2	2.2	2.2
SID Thr, g/kg	4.6	4.6	4.6	4.6	4.6	4.6
SID Trp, g/kg	1.6	1.6	1.6	1.6	1.6	1.6
SID Ile, g/kg	8.4	8.4	8.4	8.4	8.4	8.4
SID Leu, g/kg	4.6	4.6	4.6	4.6	4.6	4.6
SID His, g/kg	2.9	2.9	2.9	2.9	2.9	2.9
SID Phe + Tyr, g/kg	9.5	9.5	9.5	9.5	9.5	9.5
SID Val:Lys, %	75.8	79.0	82.0	85.0	91.0	97.0
Total Val:Lys	0.801	0.829	0.855	0.881	0.933	0.985
Energy, MJ ME/kg	13.0	13.0	13.0	13.0	13.0	13.0
Composition (analyzed)[5]						
Total Val:Lys, %	0.839	0.864	0.880	0.905	0.953	0.991
CP, %	14.4	14.4	14.5	14.4	14.4	14.5
Lys, g/kg	8.0	8.0	8.1	8.1	8.2	8.1
Val, g/kg	6.7	6.9	7.1	7.3	7.8	8.0
Met, g/kg	2.5	2.5	2.5	2.5	2.5	2.5
Cys, g/kg	2.7	2.7	2.7	2.7	2.7	2.7
Thr, g/kg	5.6	5.6	5.7	5.7	5.7	5.6
Trp, g/kg	2.0	2.0	2.0	2.0	2.0	2.0
Ile, g/kg	5.6	5.5	5.6	5.6	5.6	5.5
Leu, g/kg	10.3	10.4	10.4	10.3	10.5	10.2
His, g/kg	3.5	3.5	3.5	3.5	3.5	3.5
Phe, g/kg	6.9	7.0	7.0	7.0	7.0	6.8
Phe + Tyr, g/kg	11.6	11.9	11.9	11.9	11.7	11.3

[1] Diets were fed to sows from d 2 postpartum to d 25 (weaning) postpartum.

[2] Provided per kilogram of the diet: 9,070 IU vitamin A, 910 IU vitamin D_3, 170.1 mg DL-**α**-tocopherol, 4.32 mg vitamin K_3, 2.27 mg vitamin B_1, 5.67 mg vitamin B_2, 3.40 mg vitamin B_6, 0.02 mg vitamin B_{12}, 17.01 mg d-pantothenic acid, 22.68 mg niacin, 1.70 mg folic acid, 90.72 mg iron (FeSo4), 13.00 mg copper (CuSO4), 45.36 mg manganese (MnO), 0.23 mg iodine (Ca(IO3)2), and 0.38 mg selenium (Na2SeO3).

[3] Phyzyme XP (Danisco Animal Nutrition, Marlborough, United Kingdom) provided 500 units of phytase activity per kilogram of diet.

[4] SID = standard ileal digestible; SID values used in diet formulation were from Pedersen and Boisen (2002).

[5] Feed samples were sampled approximately every third week and 20 feed samples were analyzed per dietary treatment.

using standard ileal digestibility values of individual ingredients (Pedersen and Boisen, 2002) to be isoenergetic and isonitrogenous. The diets were formulated to ensure that Lys levels were deficient and that the requirement of other essential AA except Val was fulfilled. The Lys requirement of a high-producing sow (nursing 14 piglets with an ADG of 3 kg/d) was estimated using the model by Strathe et al. (2015). In the study, 6 diets analyzed to have dietary total Val:Lys varying from 0.84:1 to 0.99:1 were used.

Records, Sampling, and Chemical Analyses

The 6 experimental diets were sampled approximately every third week during the experiment by collecting 10 kg of each diet from the SpotMix feeding system and subsequently splitting the sample using a riffle sample divider. In total, 20 feed samples per treatment were analyzed for DM, crude fat, CP, ash, energy, minerals, and AA (Biochrom 20 plus; Biochrom Ltd., Cambridge, UK; [EC] 152/2009; European Commission 2009). Body weight and back fat thickness (**BF**) of all sows was measured at d 7 prepartum, d 2 postpartum, and weaning. Litter weight was recorded at litter standardization and at weaning. On a subsample of 72 second-parity sows (12 per dietary treatment), supplementary measurements were made. Additional measurements of sow BW and BF were made on d 17 and of litter weight at d 10 and 17 postpartum. Blood samples were collected from the jugular vein of these sows at d 2, 10, 17, and 25 postpartum. Blood samples were collected in 10-mL EDTA tubes 4 h after the morning feeding and placed on ice. The samples were centrifuged for 10 min (1,560 × *g*) at room temperature and plasma was harvested and stored at –20°C until analysis. Plasma was analyzed for concentrations of plasma urea nitrogen (**PUN**), creatinine, glucose, lactate, and NEFA (Advia 1800 Chemistry System; Siemens, Ballerup, Denmark). Blood for AA analyses was taken in 10-mL noncoated tubes and stored at –20°C until freeze-dried. Blood sampled at d 2 and 17 was analyzed for AA ([EC] 152/2009; European Commission 2009; Biochrom 20 plus; Biochrom Ltd.). Spot samples of urine were collected at the same days as blood samples and stored at –20°C until analysis. Urine was analyzed for creatinine (Advia 1800 Chemistry System; Siemens). Milk samples were obtained at d 17 postpartum. Piglets were removed from the dam for at least 30 min, and 2 mL oxytocin was given intramuscularly to induce milk letdown. Milk was sampled from 4 to 5 teats and stored at –20°C until analysis. Milk was analyzed for DM, lactose, fat, protein, urea (MilkoScan FT2; Foss Electric, Hillerød, Denmark), and AA ([EC] 152/2009; European Commission, 2009; Biochrom 20 plus; Biochrom Ltd.).

Calculations and Statistical Analyses

All calculations and statistical analyses were performed using the statistical software R (R Core Team, Vienna, Austria) with the individual sow as the experimental unit. The total nutrient output in the sow milk was calculated based on the analyzed nutrient concentrations in milk and the milk yield that was estimated from equations by Hansen et al. (2012).

Feed composition (CP, Val, Lys, and Val:Lys) was analyzed using the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij},$$

in which Y_{ij} is the response variable, μ is the overall mean, α_i is the effect of diet ($i = 1, \ldots, 6$), β_j is the effect of sample time ($j = 1, 2, \ldots, 8$), and ε_{ijk} is the random error component, which was assumed to be $N(0, \sigma^2)$.

Average daily gain of the litter, litter weight, milk yield, sow BW and BF, changes in sow BW and BF, and feed intake were analyzed using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk},$$

in which Y_{ijk} is the response variable, μ is the overall mean, α_i is the effect of diet ($i = 1, \ldots, 6$), β_j is the effect of parity ($j = 1, 2, \ldots, 4$), γ_k is the random effect of block ($k = 1, 2, \ldots, 11$), and ε_{ijk} is the random error component, which was assumed to be $N(0, \sigma^2)$. For analysis of ADG of litter and litter weight at weaning, the litter weight at standardization was used as covariates. In the analysis of change in sow BW and BF and BW and BF at weaning, BW and BF at standardization, respectively, were used as covariates. The interaction between diet and parity was tested, but it was not significant ($P > 0.05$) and was removed from the models.

Whole blood concentrations of AA, milk composition, daily nutrient output, and milk yield were analyzed using the following model:

$$Y_{ij} = \mu + \alpha_i + \gamma_j + \varepsilon_{ij},$$

in which Y_{ij} is the response variable, μ is the overall mean, α_i is the effect of diet ($i = 1, \ldots, 6$), γ_j is the random effect of block ($j = 1, 2, \ldots, 11$), and ε_{ij} is the random error component, which was assumed to be $N(0, \sigma^2)$.

Only 16 milk samples were available for AA analysis, and because of the very few observations per diet group, the data were analyzed using simple linear regression, testing the effect of the average dietary Val:Lys of the individual sow.

Table 2. Effect of dietary valine-to-lysine ratio (Val:Lys) on sow and litter performance

	Total Val:Lys[1]							
	0.84	0.86	0.88	0.90	0.95	0.99		*P*-value
	No.[2]							
Item	93	93	93	93	93	93	SE	Diet
Parity	2.5	2.4	2.5	2.5	2.5	2.5	0.11	0.99
Feed intake, kg/d	6.22	6.16	6.14	6.28	6.14	6.08	0.13	0.23
Val intake, g/d	41.8	41.8	42.9	44.5	44.2	45.6	1.81	0.35
Weaning day, d	25.0	25.0	25.5	25.3	25.3	25.3	0.32	0.06
Litter size, d 25	13.4	13.6	13.4	13.3	13.3	13.4	0.25	0.23
ADG (d 2–25), kg/d	2.85	2.93	2.93	2.89	2.88	2.92	0.06	0.84
ADG of piglets (d 2–25), g/d	214	218	219	217	217	219	4.2	0.89
Litter weight (d 2), kg	25.4	25.3	24.6	24.7	24.7	24.7	0.36	0.30
Litter weight (d 25), kg	90.6	93.2	94.0	92.5	91.9	93.7	1.65	0.67
Milk yield, kg/d	11.2	11.5	11.3	11.2	11.2	11.3	0.16	0.49
Sow BW								
d 108, kg	274	274	274	272	274	275	2.55	0.77
d 2, kg	241	242	242	240	241	242	2.23	0.73
d 25, kg	222	222	219	223	222	220	1.57	0.61
Loss (d –7 to 2), kg	32.7	32.1	32.1	32.6	32.8	32.4	1.22	0.30
Loss (d 2–25), kg	22.0	22.8	23.2	20.6	21.4	23.5	1.36	0.21
Sow back fat thickness								
d 108, mm	15.0	15.8	15.8	15.3	15.0	15.8	0.32	0.11
d 2, mm	14.3	14.9	14.9	14.5	14.5	14.9	0.30	0.19
d 25, mm	12.0	11.8	11.8	11.9	12.0	11.7	0.18	0.53
Loss d –7 to 2, mm	0.8	0.8	0.9	0.9	0.7	0.9	0.12	0.77
Loss d 2–25, mm	2.8	3.0	3.0	2.8	2.6	3.1	0.18	0.11

[1] Dietary treatments were fed to sows from d 2 postpartum to d 25 (weaning) postpartum.

[2] The measurements were made on 558 sows (parity 1 to 4).

Concentrations of blood metabolites were analyzed using the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_k + \nu_l + \varepsilon_{ijkl},$$

in which Y_{ijkl} is the response variable, μ is the overall mean, α_i is the effect of diet (i = 1, …, 6), β_j is the effect of day postpartum (j = 2, 10, 17, and 25), $\alpha\beta_{ij}$ is the interaction term between diet and day, γ_k is the random effect of block (k = 1, 2,…., 11), ν_l is the random effect of sow (l = 1, 2,……, 72), and ε_{ijkl} is the random error component, which was assumed to be N(0, σ^2). Plasma lactate and NEFA and urine creatinine concentrations were log transformed, because of variance heterogeneity. The interaction between diet and time was not significant for any of the response variables ($P > 0.05$).

Results are reported as least squares means and SE except for log-transformed response variables, where the back-transformed values and the 95% confidence interval are given. Statistical significance was declared at $P < 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$. Multiple comparisons were made when the ANOVA indicated that there were significant differences. Tukey's test was used in multiple comparisons of means to adjust the *P*-values. The multiple comparisons were done using the multcomp package in R.

RESULTS

Dietary Composition and Feed Intake

The total Val:Lys was higher than calculated in all treatment groups, which could be ascribed to higher or lower than expected concentrations of Val and Lys in barley, wheat, or soybean meal. As planned, the 6 experimental diets had similar concentrations of Lys (P = 0.79) and CP (P = 0.22) but increasing concentrations of Val ($P < 0.001$) and Val:Lys ($P < 0.001$). Average daily feed intake (6.1 ± 0.7 kg/d; P = 0.23) of the sows did not differ between the dietary treatments (Table 2). The daily Val intake varied from 41.8 ± 1.70 g/d in group 1 to 45.6 ± 1.81 g/d in group 6 (Table 2).

Sow and Litter Performance

Litter size at weaning (13.0 ± 1.1; P = 0.23) and day of weaning (P = 0.06) did not differ between the dietary treatments (Table 2). The total ADG (2.93 ± 0.53 kg/d; P = 0.84) and ADG in mid (P = 0.20) and late (P = 0.22)

Table 3. Effect of dietary valine-to-lysine ratio (Val:Lys) on ADG of the litter, litter weight on d 10 and 17 postpartum, and changes in sow BW and back fat thickness from d 2 to 17 and d 17 to 25

	Total Val:Lys[1]							
	0.84	0.86	0.88	0.90	0.95	0.99		*P*-value
	No.[2]							
Item	12	12	12	12	13	11	SE	Diet
Litter weight								
d 10, kg	47.7	47.8	49.9	48.3	45.7	49.1	1.86	0.29
d 17, kg	71.8	70.3	74.4	69.4	65.2	72.8	2.82	0.06
ADG of litter								
d 2–10, kg/d	2.80^{ab}	2.93^{ab}	3.14^{a}	3.01^{ab}	2.60^{b}	2.91^{ab}	0.13	0.03
d 10–17, kg/d	3.35	3.11	3.28	3.07	2.95	3.23	0.16	0.20
d 17–25, kg/d	2.89	2.69	3.13	2.94	2.73	2.96	0.24	0.22
BW change								
d 2–17, kg	–18.6	–18.3	–18.8	–16.6	–15.9	–20.4	3.46	0.39
d 17–25, kg	–8.9	–4.8	–9.2	–8.7	–6.0	–10.1	2.44	0.50
Back fat thickness change								
d 2–17, mm	–2.6	–2.0	–2.8	–2.6	–2.3	–3.3	0.46	0.48
d 17–25, mm	0.6	–0.8	–0.9	–1.4	0.2	–0.8	0.45	0.17

^{a,b}Within a row, values with common superscripts differ ($P < 0.05$).

[1] Dietary treatments were fed to sows from d 2 postpartum to d 25 (weaning) postpartum.

[2] The measurements were made on 72 second-parity sows (subsample of the 558 sows).

lactation were not affected by dietary treatment (Tables 2 and 3). In early lactation, ADG was higher at a Val:Lys of 0.88 than a Val:Lys of 0.95 ($P < 0.05$; Table 3). Litter weight at standardization ($P = 0.30$), d 10 ($P = 0.29$), d 17 ($P = 0.06$), and weaning ($P = 0.73$) was similar among all dietary treatments (Tables 2 and 3). The loss of BW and BF from d 108 to 2 (32.7 ± 10.9 kg and 0.9 ± 1.1 mm, respectively), from d 2 to weaning (22.1 ± 12.7 kg and 2.9 ± 1.7 mm, respectively), from d 2 to 17 (17.9 ± 11.7 kg and 2.6 ± 1.6 mm, respectively), and from d 17 to weaning (8.0 ± 7.9 kg and 0.7 ± 1.5 mm, respectively) were not affected by the dietary Val:Lys ($P > 0.05$; Tables 2 and 3). Body weight and BF of sows were similar at d 108, d 2, and weaning (Table 2).

Milk, Blood, and Urine Samples

The milk yield (11.3 ± 1.4 kg/d; $P = 0.49$), DM ($P = 0.33$), protein ($P = 0.90$), fat ($P = 0.37$), and urea ($P = 0.35$) concentrations of milk were not affected by dietary treatments, but there was a tendency toward a dietary effect on lactose ($P = 0.05$). Neither did the dietary Val:Lys affect the average daily secretion of CP ($P = 0.31$), fat ($P = 0.31$), lactose ($P = 0.36$), and urea ($P = 0.69$) in milk (Table 4). Increasing the dietary Val:Lys increased the Val, Ile, and Ala and decreased the Arg concentration in the milk ($P < 0.05$), and there was a tendency toward increased Leu ($P = 0.06$) and Met ($P = 0.07$) concentrations (Fig. 1). The concentration of the other AA in the milk was not affected by the dietary Val:Lys ($P > 0.05$; results not shown).

Increasing the dietary Val:Lys did not have any effect on concentrations of glucose, lactate, NEFA, PUN, and creatinine in plasma and creatinine in urine ($P > 0.05$). The plasma concentration of glucose ($P = 0.19$) and lactate ($P = 0.11$) was similar on all 4 d of measurements. The plasma concentration of urea N ($P < 0.001$) increased during lactation and the creatinine ($P < 0.001$) and NEFA ($P < 0.001$) concentrations in plasma and urine concentration of creatinine ($P < 0.01$) decreased throughout lactation. There was effect of dietary Val:Lys on blood concentration of Ser at d 17 ($P < 0.05$) and tendencies toward effects on blood concentrations of CP ($P = 0.10$), Met ($P = 0.06$), Thr ($P = 0.06$), and Val ($P = 0.08$) at d 17 (Table 5).

DISCUSSION

In the current experiment, it was decided to test only a total Val:Lys above 0.84:1 and, therefore, no negative control group was included. The reason was that it is difficult to obtain a total Val:Lys below 0.84:1 when formulating a standard European diet for lactating sows based on soybean meal, wheat, and barley as the main ingredients. A semisynthetic diet could have been formulated as a negative control using high amounts of crystalline AA, but it was considered that this kind of diet would not have any practical application.

When establishing the best possible ratio between Lys and other AA, Lys must be marginally deficient whereas the requirement of other essential AA should be fulfilled to avoid underestimation of the determined

Table 4. Effect of dietary valine-to-lysine ratio (Val:Lys) on milk composition and nutrient output in milk for sows. Milk samples were taken on d 17 of lactation

	Total Val:Lys[1]							
	0.84	0.86	0.88	0.90	0.95	0.99		*P*-value
	No.[2]							
Item	8	9	10	8	9	6	SE	Diet
Concentration								
DM, %	17.4	17.0	17.6	18.1	17.5	16.2	0.64	0.33
Lactose, %	5.8	5.6	5.7	5.4	5.4	5.9	0.20	0.05
Protein, %	4.7	4.8	4.6	4.8	4.8	4.7	0.18	0.90
Fat, %	7.3	6.8	7.4	7.7	7.2	6.2	0.57	0.37
Urea, mg/dL	1,130	1,250	1,101	1,126	1,092	1,238	88.7	0.35
Output[3]								
Lactose, g/d	680	641	700	650	564	632	41.1	0.36
Protein, g/d	571	525	569	537	499	533	29.2	0.31
Fat, g/d	848	769	892	826	704	812	65.4	0.31
Urea, g/d	127	133	132	127	117	110	10.1	0.69

[1] Dietary treatments were fed to sows from d 2 postpartum to d 25 (weaning) postpartum.

[2] The milk samples were taken from 50 second-parity sows (subsample of the 558 sows).

[3] Output of milk nutrients were calculated from the estimated milk yield and analyzed nutrient concentrations of milk. The milk yield was estimated from the litter size and ADG (kg/d) of the litter using equations by Hansen et al. (2012).

ratio (Boisen, 2003). In the current study, sows, on average, consumed 50 g Lys/d, which would cover 70 to 75% of their daily Lys requirement for maintenance and milk production (Strathe et al., 2015), meaning that the remaining had to be covered by body mobilization. It could be argued that a reason for the lack of response to the dietary treatments could be an effect of the diet being too deficient in Lys, but, on the other hand, the milk production of the sows was similar in all dietary groups. Sows, on average, weaned over 13 piglets and produced 11.3 kg of milk per day. A major difference between the current study and the other studies that have investigated dietary Val:Lys besides the high production level of the sows was the substantial loss of BW and BF, but this is similar to results from a survey on random sows in 8 Danish herds (Christensen and Sørensen, 2013). There were, therefore, no indications that the mobilization had been excessive or unusual for a high-producing Danish sow (DanAvl hybrid) and it is reasonable to assume that the optimal Val:Lys has been determined under appropriate conditions for sows in commercial production systems. The mobilization of body protein can make a considerable contribution of AA for milk production, but currently it is not advisable to rely on body mobilization to cover part of this requirement. More knowledge is warranted to determine when mobilization of especially body protein compromises reproduction and health of the sow.

Sow and Litter Performance

This study showed no effect of increasing the total Val:Lys above 0.84 on litter growth and sow body condition during lactation, which corresponds with results of an experiment with sows nursing 10 piglets by Gaines et al. (2006), who found no effect of Val:Lys on piglet growth and changes in sow body condition when the total dietary Val:Lys was 0.73:1, 0.86:1, and 1.25:1. In another study, the total dietary Val:Lys ranged from 0.45:1 to 1.45:1 and the diets with very low Val:Lys were formulated using high amounts of crystalline AA; piglet gain was similar for groups fed diets with Val:Lys from 0.65:1 to 1.45:1, but it decreased when Val:Lys was lowered from 0.65:1 to 0.55:1 (195 vs. 171 g/d) and further from 0.55:1 to 0.45:1 (171 vs. 146 g/d). Only when the total Val:Lys was decreased from 0.55:1 to 0.45:1 was sow BW loss increased and milk production significantly decreased (Paulicks et al., 2003). In accordance with the current study, Carter et al. (2000) did not report any effect on litter gain, litter size at weaning, or changes in sow BW when providing lactating sows nursing 10 to 12 piglets with total dietary Val:Lys of 0.77:1, 0.92:1, 1.03:1, or 1.22:1 from d 2 postpartum until weaning, and it was concluded that a total Val:Lys of 0.77:1 was sufficient to meet the requirement for litter growth. In contrast, Richert et al. (1996) found an effect on litter weight at d 21 and 26 and on total litter gain from d 0 to 21 and from d 0 to 26 by increasing the total dietary Val:Lys above 0.83:1. No effect of dietary treatment on sow BW and BF was reported and the change in BW and BF was close to 0, whereas in the current study, sows, on average, lost 22.1 kg BW and 2.9 mm BF, which could be explained by lower litter size and high average feed intake or the use of another breed in the study by Richert et al. (1996). In another study by Richert et al. (1997), it

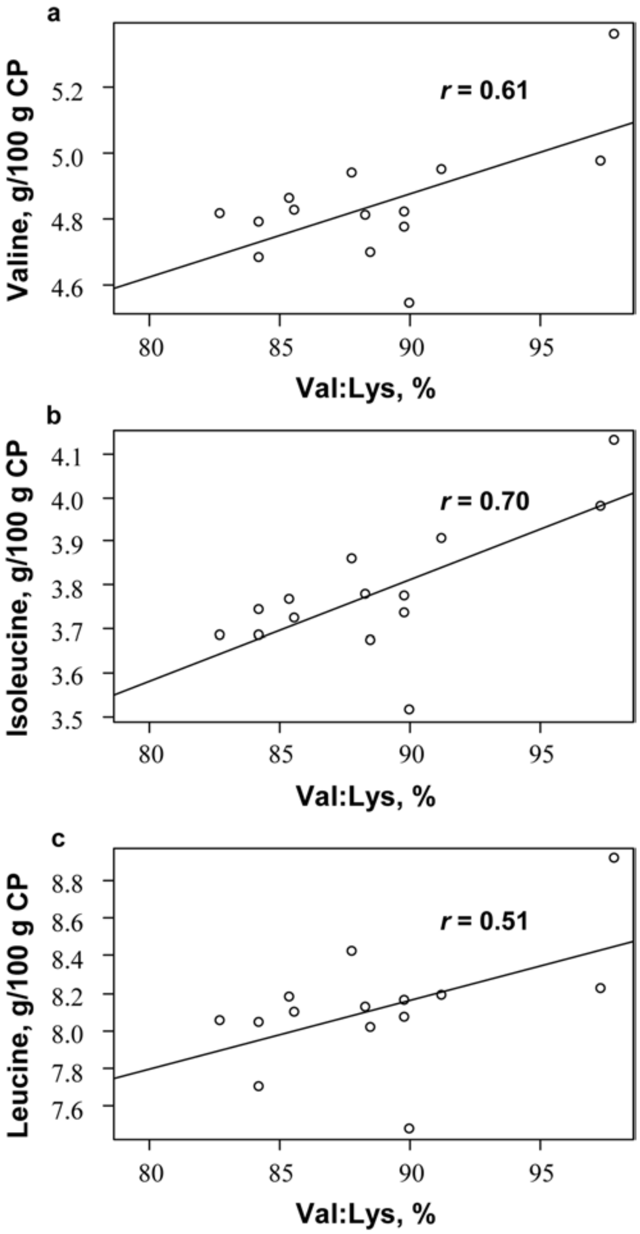

Figure 1. A milk sample was obtained on d 17 of lactation and analyzed for AA. The total dietary Val:Lys on the day of milk sampling had a linear effect on a) Val concentration ($y = 0.03x + 2.11$; $P < 0.05$), b) isoleucine concentration ($y = 0.03x + 1.32$; $P < 0.01$) and c) leucine concentration ($y = 0.05x + 3.93$; $P = 0.06$) in the milk.

was concluded that sows nursing more than 10 piglets required a total dietary Val:Lys of 1.20:1 compared with 1.00:1 and 0.80:1 to maximize litter growth, whereas Moser et al. (2000) showed an increased litter gain and BF loss of sows when the total Val:Lys was increased from 0.89:1 to 1.33:1.

Some of the mentioned studies indicated that the ADG of the litter will be compromised at very low Val:Lys, which were below the Val:Lys tested in current study, and also that body mobilization was not necessarily prevented by using a high Val:Lys. The NRC (2012) recommends a SID Val:Lys of 0.85:1 based on only 2 studies (Rousselow and Speer, 1980; Paulicks et al., 2003), and the current study suggest that a SID Val:Lys of 0.80:1 is sufficient. The number of sows used in the present experiment was rather high compared with other studies on dietary Val:Lys, which strongly supports the studies reporting results in agreement with the current findings.

Milk, Plasma, Whole Blood, and Urine Parameters

In the current study, the average milk yield of sows and the increasing dietary Val:Lys did not have any effect on concentration and average daily output of DM, CP, fat, lactose, and urea in milk. This is in accordance with Paulicks et al. (2003) and Roth-Maier et al. (2004) investigating total dietary Val:Lys from 0.64:1 to 0.84:1, but protein content of milk and milk yield decreased and fat percentage increased when the total Val:Lys was decreased below 0.45:1. In accordance with the findings of this study, Roth-Maier et al. (2004) did not find any differences in the urea concentration of milk when increasing the total dietary Val:Lys. Rousselow and Speer (1980) reported that milk yield of sows on d 14 and 20 was highest at a total dietary Val:Lys of 1.17:1. Protein content and total protein output (g/d) of the milk increased when the total dietary Val:Lys was increased from 0.40:1 to 1.17:1 but no effect was seen when the Val:Lys was further increased to 1.43:1, and it was concluded that a total Val:Lys of 1.17:1 was optimal (Rousselow and Speer, 1980). The studies (Paulicks et al., 2003; Roth-Maier et al., 2004) indicate that milk production will be compromised at very low Val:Lys, but these ratios are well below those included in the current study.

Concentrations of Val, Leu, Ile, Ala, and Met increased and Arg decreased in milk with increasing dietary Val:Lys, in agreement with the results by Dunshea et al. (2005), who investigated the effect of supplementing the lactation diet with protein and branched-chain AA (**BCAA**) and also found an increase in the milk concentrations of Val, Leu, Ile, Met, and Ala. In contrast, no significant increases in milk AA concentrations were found when the total dietary Val:Lys was increased above 0.64:1, but AA concentrations decreased when the total Val:Lys was lowered to 0.54:1 or 0.45:1(Roth-Maier et al., 2004). A likely explanation for increased Ala in milk is that oxidation of BCAA is the major donor of amino groups for Ala and Glu synthesis. An in vitro study (Li et al., 2009) found an increasing transport of BCAA into porcine mammary cells and catabolism of BCAA in the cells when increasing the extracellular concentration of BCAA. An increased oxidation of BCAA would also increase the use of Arg in the urea cycle to form ornithine and urea.

Plasma concentrations of glucose, lactate, NEFA, PUN and creatinine and the urinary concentration of

Table 5. Effect of dietary valine-to lysine ratio (Val:Lys) on blood AA concentrations (% of whole blood) at d 2 and 17 postpartum. Results are given as least squares means

	Total Val:Lys[1]							
	0.84	0.86	0.88	0.90	0.95	0.99		*P*-value
	No.[2]							
Item	6	6	6	6	6	6	SE	Diet
CP (d 2), %	18.0	18.1	17.7	17.7	18.7	17.9	0.52	0.80
CP (d 17), %	16.4	16.9	17.0	16.5	17.1	16.0	0.32	0.10
Lys (d 2), %	1.56	1.56	1.53	1.53	1.61	1.56	0.052	0.90
Lys (d 17), %	1.40	1.44	1.46	1.42	1.47	1.37	0.031	0.19
Met (d 2), %	0.13	0.13	0.13	0.13	0.13	0.13	0.004	0.94
Met, (d 17), %	0.12	0.12	0.12	0.12	0.12	0.11	0.003	0.06
Cys (d 2), %	0.23	0.24	0.23	0.24	0.24	0.23	0.007	0.64
Cys (d 17), %	0.22	0.23	0.22	0.23	0.22	0.22	0.006	0.87
Thr (d 2), %	0.66	0.68	0.65	0.67	0.69	0.66	0.015	0.50
Thr (d 17), %	0.61	0.63	0.63	0.62	0.64	0.60	0.011	0.06
Arg (d 2), %	0.80	0.81	0.79	0.80	0.83	0.81	0.022	0.81
Arg (d 17), %	0.74	0.76	0.76	0.74	0.77	0.72	0.013	0.09
Ile (d 2), %	0.23	0.25	0.22	0.24	0.23	0.23	0.006	0.15
Ile (d 17), %	0.22	0.22	0.21	0.22	0.22	0.21	0.007	0.92
Leu (d 2), %	2.23	2.22	2.20	2.19	2.32	2.22	0.078	0.87
Leu (d 17), %	2.01	2.07	2.11	2.02	2.11	1.95	0.050	0.15
Val (d 2), %	1.48	1.48	1.46	1.46	1.55	1.48	0.050	0.84
Val (d 17), %	1.35	1.39	1.42	1.36	1.42	1.30	0.031	0.08
His (d 2), %	1.16	1.14	1.15	1.13	1.21	1.16	0.046	0.85
His (d 17), %	1.04	1.07	1.10	1.04	1.10	1.01	0.030	0.15
Phe (d 2), %	1.16	1.17	1.15	1.16	1.22	1.17	0.038	0.85
Phe (d 17), %	1.06	1.09	1.11	1.07	1.11	1.03	0.024	0.13
Gly (d 2), %	0.80	0.80	0.79	0.79	0.84	0.80	0.027	0.83
Gly (d 17), %	0.73	0.75	0.77	0.74	0.77	0.71	0.017	0.71
Ser (d 2), %	0.83	0.83	0.82	0.83	0.87	0.82	0.022	0.68
Ser (d 17), %	0.76^{ab}	0.78^{ab}	0.79^{a}	0.77^{ab}	0.80^{a}	0.74^{b}	0.014	0.04
Pro (d 2), %	0.72	0.72	0.70	0.72	0.74	0.70	0.017	0.46
Pro (d 17), %	0.67	0.68	0.68	0.68	0.71	0.65	0.014	0.15
Ala (d 2), %	1.32	1.31	1.31	1.30	1.38	1.32	0.047	0.88
Ala (d 17), %	1.20	1.23	1.26	1.21	1.26	1.16	0.030	0.16
Asp (d 2), %	2.01	2.00	1.99	1.97	2.09	2.00	0.067	0.85
Asp (d 17), %	1.82	1.87	1.91	1.83	1.91	1.76	0.043	0.11
Glu (d 2), %	1.67	1.71	1.65	1.68	1.74	1.68	0.044	0.79
Glu (d 17), %	1.55	1.59	1.59	1.57	1.61	1.52	0.027	0.22

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

[1] Dietary treatments were fed to sows from d 2 postpartum to d 25 (weaning) postpartum.

[2] The blood was drawn from 36 second-parity sows (subsample of the 558 sows).

creatinine were not affected by the dietary Val:Lys, so there were no indications that the dietary Val:Lys had any effects on fat and protein mobilization and N balance of the sow. Rousselow and Speer (1980) investigated the effect of 5 levels of dietary Val:Lys on the N balance of sows and reported that the N balance was unaffected by diet but that the PUN concentration was lowest at a total dietary Val:Lys of 0.66:1. Richert et al. (1996) found increased serum urea N when increasing the total dietary Val:Lys from 0.83:1 to 1.28:1, but serum creatinine concentrations remained stable. In contrast, Roth-Maier et al. (2004) did not find any effect on plasma urea N with increasing Val:Lys.

Amino acids concentrations in blood were not affected by the dietary Val:Lys in this study, in accordance with the findings in other studies (Richert et al., 1996; Roth-Maier et al., 2004), with the exception being Val, which increased with increased Val:Lys and Val intake. In the current study, the average daily Val intake of the 6 dietary groups ranged from 41 to 47 g/d, which is a much smaller variation than seen between groups in the studies by Roth-Maier et al. (2004) and Richert et al. (1996), where the Val intake ranged from 20 to 64 g/d and from 47 to 71 g/d, respectively, and this could explain the lack of dietary response on blood Val concentration as well as sow and litter performance.

Conclusion

In conclusion, the results of the current study showed no effect of increasing the total dietary Val:Lys above 0.84:1 (SID Val:Lys of 0.80:1) on litter growth and sow metabolism. The dietary Val:Lys did affect milk concentrations of BCAA, but this did not affect litter growth. Concentrations of urea in plasma and milk were similar in all 6 groups, which emphasized that increasing the total dietary Val:Lys above 0.84:1 did not give a more ideal AA composition of the dietary protein for the lactating sow.

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6.2 Paper II

Sows with high milk production had both a high feed intake and high body mobilization

A.V. Strathe, T. S. Bruun and C.F. Hansen

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Sows with high milk production had both a high feed intake and high body mobilization

A. V. Strathe[1], T. S. Bruun[2] and C. F. Hansen[1]†

[1]*Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegaardsvej 2, 1870 Frederiksberg C, Denmark;*
[2]*SEGES Danish Pig Research Centre, Axeltorv 3, 1609 Copenhagen, Denmark*

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Selection for increased litter size have generated hyper-prolific sows that nurses large litters, however limited knowledge is available regarding the connection between milk production, feed intake and body mobilization of these modern sows. The aim of the current study was to determine what characterized sows with high milk production and nursing large litters, differences between sows of different parities and effects of lactational performance on next reproductive cycle. In total 565 sows (parity 1 to 4) were studied from 7 days before farrowing until weaning. On day 2 postpartum *litters were standardized to 14 piglets. Weight and back fat thickness of sows were measured at day 7* prepartum*, day 2* postpartum *and at weaning. Litters were weighed at day 2 and at weaning. Pearson correlation coefficients between variables were calculated and regression models were developed. The average daily feed intake (ADFI) of the sows was 6.1 ± 1.1 kg/day, average daily gain (ADG) of the litter was 2.92 ± 0.53 kg/day and sows weaned 13.0 ± 1.1 piglets. First parity sows generally had a lower ADFI and milk production and a decrease in total born piglets in next litter compared with parity 2 to 4 sows, which could be explained by a relatively higher proportion of their body reserves being mobilized compared with multiparous sows. The ADG of the litter was positively related by ADFI of the sows, litter size and BW loss and increasing the ADFI with 1 kg/day throughout lactation likely increased the ADG of the litter with 220 to 440 g/day in parity 1 to 4, respectively. Increasing the ADFI by 1 kg/day reduced the BW loss with 6.6 to 13.9 kg of parity 1 to 4 sows, respectively, during lactation, whereas increasing the average milk yield with 1 kg/day raised the BW loss with 4.3 to 21.0 kg of the four parities during lactation. The number of total born piglets in the next litter was positively related to the number of piglets born in the previous litter. In conclusion, both a high feed intake and a high mobilization of body reserves was a prerequisite for a high milk production. The sows might be very close to the physical limit of what they can ingest and future research should therefore, focus on optimizing the dietary energy and nutrient concentrations of diets for lactating hyper-prolific sows and herein distinguish between primiparous and multiparous sows.*

Keywords: body mobilization, feed intake, milk production, reproduction, sow

Implications

To obtain a high litter weight at weaning the sow must consume high amounts of feed, but a high milk yield often causes a high BW loss. Counterproductive, body mobilization is positive for the offspring, but might be harmful to the sow. This study showed that sows with the highest milk production and litter gain had both a high feed intake and large mobilization. The sows might have reached their upper physical limit for how much feed they could ingest and therefore future research should focus on correct nutrient concentrations of lactation diets of hyper-prolific sows.

Introduction

Milk production in sows is affected positively by feed intake during lactation (Koketsu *et al*., 1997; Vadmand *et al*., 2015). Many sows turn catabolic during the lactation period to maintain their milk production, because they are unable to increase the feed intake at the same rate as milk production is increasing (van den Brand *et al*., 2000; Hansen *et al*., 2012a). However, possibly due to differences in the number of nursing piglets, studies on sows show contradicting results when it comes to linking body mobilization and milk production, and whether a high feed intake (5.0 to 7.6 kg/day) can prevent excessive BW loss (Eissen *et al*., 2003; Mosnier *et al*., 2010). A low sow feed intake (2.8 to 3.0 kg/day) and great body mobilization (38 to 40 kg) during

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† E-mail: cfh@sund.ku.dk

lactation can have a negative effect on the following reproductive cycle by increasing weaning-to-estrus interval (WEI) and decreasing the number of total born piglets (Baidoo *et al*., 1992; Koketsu *et al*., 1996; Zak *et al*., 1998). In addition, parity of the sow also influences milk production and reproductive performance. First parity sows give birth to fewer piglets and have a lower feed intake compared with multiparous sows (Eissen *et al*., 2000). First parity sows might therefore have a different interplay between feed intake, body mobilization, milk yield and reproduction than older sows according to Dourmad *et al*. (1996). Hyper-prolific sows are pushed to the limit and beyond during lactation to increase milk yield, however, more knowledge is needed to clarify the connection between milk production, feed intake, body mobilization and subsequent reproduction in hyper-prolific sows nursing large litters of 12 to 14 piglets. The aim of this study was, therefore, to characterize hyper-prolific sows with high milk production. We hypothesized that milk yield would be influenced by number of nursing piglets, parity, feed intake and body mobilization.

Table 1 *Ingredients and dietary composition (as-fed) of the lactation diets*[1]

Ingredients (%)	Diet 1	Diet 2
Barley	80.8	40.0
Wheat	–	39.9
Soybean meal	10.2	14.3
Sugar beet pellet	6.0	–
Palm oil	–	1.62
Soy oil	0.01	0.30
Monocalcium phosphate	0.82	1.08
Limestone	1.29	1.68
Salt	0.48	0.54
L-lysine	0.18	0.32
L-threonine	0.03	0.07
DL-methionine	0.02	0.05
Vitamin and mineral premix 1[2]	0.15	–
Vitamin and mineral premix 2[3]	–	0.09
Phytase[4]	0.02	0.01
Composition (calculated)		
DM (%)	86.3	86.3
CP (%)	13.1	14.2
SID Lysine (g/kg)	6.1	7.1
Energy (MJ ME/kg)	12.4	13.0

SID = standardized ileal digestible; DM = dry matter; ME = metabolizable energy. Diet 1 was fed from day 80 of gestation until the day after farrowing and diet 2 was fed from day 2 *postpartum* and until weaning of the litter.

[1] In the original six dietary treatments (Strathe *et al*., 2016) L-valine was included in diet 2 from 0.00% to 0.43%, respectively.

[2] Provided per kg of the diet: 8130 IU vitamin A; 810 IU vitamin D_3; 101.6 mg DL-alfa tocoferol, 4.06 mg vitamin K_3; 2.03 mg vitamin B_1; 5.08 mg vitamin B_2; 3.05 mg vitamin B_6; 0.02 mg vitamin B_{12}; 15.24 mg D-pantothenic acid; 20.32 mg niacin; 1.52 mg folic acid; 81.30 mg iron ($FeSo_4$); 13.00 mg copper ($CuSO_4$); 40.65 mg manganese (MnO); 0.20 mg iodine ($Ca(IO_3)_2$); 0.36 mg selenium (Na_2SeO_3).

[3] Provided per kg of the diet: 9070 IU vitamin A; 910 IU vitamin D_3; 170.1 mg DL-alfa tocoferol, 4.32 mg vitamin K_3; 2.27 mg vitamin B_1; 5.67 mg vitamin B_2; 3.40 mg vitamin B_6; 0.02 mg vitamin B_{12}; 17.01 mg D-pantothenic acid; 22.68 mg niacin; 1.70 mg folic acid; 90.72 mg iron ($FeSo_4$); 13.00 mg copper ($CuSO_4$); 45.36 mg manganese (MnO); 0.23 mg iodine ($Ca(IO_3)_2$); 0.38 mg selenium (Na_2SeO_3).

[4] Phyzyme XP provided 500 phytase activity (FTU) per kg of diet (Danisco Animal Nutrition, Marlborough, UK)

Material and methods

This study was conducted with the approval of the Danish Animal Experimentation Inspectorate (Authorization No. 2013-15-2934-00961).

Animals and diets

The study was conducted in a commercial Danish herd using 565 parity 1 to 4 sows (Landrace × Yorkshire) mated with Duroc semen (Hatting KS, Horsens, Denmark). Originally the study was conducted to test the effect of six dietary valine-to-lysine ratios for lactating sows (see Strathe *et al*., 2016), but no treatment effects were seen on any of the measured parameters and therefore data was pooled for this assessment. Each week, a block of 18 sows were selected for the trial based on parity and in total 32 blocks of sows were included. The animals were studied from 1 week *prepartum* until weaning (day 26 *postpartum)*. Twenty four hours *postpartum* (day 2), the litters were standardized to 14 medium or large piglets (1.78 ± 0.24 kg). Sows were fed using a SpotMix feeding system (Schauer Agrotronic, Prambachkirchen, Austria) that enabled individual feeding curves for each sow. From 7 days *prepartum* to 2 days *postpartum* all sows were fed the same diet (Table 1). From day 2 *postpartum*, sows were allotted to six dietary treatments only varying in valine concentration (Table 1). Gilts were fed 2.3 kg from day 2 *postpartum* and feed allowance was increased to a maximum of 7.6 kg at day 17. Multiparous sows were fed 2.3 kg from day 2 *postpartum* and feed allowance was gradually increased to a maximum of 8.6 kg at day 17. These feeding curves were used as a guideline for the maximum daily allowance of the sows; and were the curves normally used in the herd, as it was the experience that they were close to the maximum intake capacity of the sows. The feed allowance was adjusted for the individual sows daily and when feed residuals were present in the trough the allowance was downgraded with 0.25 kg. Feed residuals were not recorded.

Records and sampling

Sows were weighed and back fat (BF) thickness of sows was measured by ultrasound at the P2 site (Sono-Grader II; Renco Corporation, Minneapolis, MN, USA) at day 7 *prepartum*, day 2 *postpartum* and at weaning. Litter weight was recorded at litter standardization and at weaning. On a subsample of 72 second parity sows, additional measurements of BW and BF were made on day 17 and litter weight at days 10 and 17 *postpartum*. Blood samples were collected from the jugular vein of these sows at days 2, 10, 17 and 26 *postpartum*. Spot samples of urine were collected on the same days as blood samples, and milk samples were obtained at day 17 *postpartum*. Detailed description of sampling and chemical analysis of feed, blood, urine and milk are given in Strathe *et al*. (2016).

Calculations and statistical analysis

All calculations and statistical analyses were carried out in R (R Core Team, Vienna, Austria) with the individual sow as the experimental unit. Milk yield was estimated from equations by Hansen *et al.* (2012b). Changes in body pools of fat and protein were estimated using equations by Dourmad *et al.* (2008).The descriptive statistics of data was reported as a mean and SD and results of analysis were reported as least squares mean and SE.

The effect of parity was analyzed using the following model:

$$Y_{ij} = \mu + \alpha_i + \gamma_j + \varepsilon_{ij}$$

where Y_{ij} is the response variable, μ the overall mean, α_i the effect of parity (i = 1, 2, 3, 4), γ_j the random effect of block (j = 1, 2, … , 32) and ε_{ij} the random error component which was assumed to be N $(0,\sigma^2)$. When analyzing the effect on sow BW and BF thickness, the interaction between parity and stage of lactation was also included in the model. Multiple comparisons were made when the ANOVA indicated that there were significant differences. Tukey's test was used in multiple comparisons of means to adjust the *P*-values. The multiple comparisons were done using the multcomp package in R. Statistical significance was declared at $P<0.05$. Relationships between response variables (average daily gain (ADG) of litter, BW and BF loss of sow during lactation, total born piglets in next litter and WEI) and explanatory variables were evaluated by Pearson correlation coefficients to determine which variables should be used in the multiple regression analysis. All explanatory variables that were significant at the 5% level were offered in multiple regression models. Multiple regression models were developed for the ADG of the litter, BW loss of the sow during lactation and total born piglets in next litter for parity 1 to 4, respectively, using a backward elimination approach for reduction of the model. Multiple coefficient of determination (adjusted R^2) and parameters of the explanatory variables and their SE were estimated.

Results

Descriptive analysis of data and time effects

The sows had an average daily feed intake (ADFI) at 6.1 kg/day during lactation (Figure 1). In average sows weaned 13.0 ± 1.1 piglets and the ADG of the litter was 2.92 ± 0.53 kg/day. Sows in average lost 22.1 ± 12.7 kg of BW and 2.9 ± 1.7 mm of BF from day 2 *postpartum* until weaning, respectively. Milk contents of lactose, fat and protein were 5.58 ± 0.89%, 7.17 ± 2.07% and 4.71 ± 0.71%, and the daily outputs of these nutrients were 648 ± 115, 832 ± 184 and 544 ± 74.3 g/day, respectively. Sow plasma concentrations of glucose (5.27 ± 0.64 mmol/l; P = 0.19) and lactate (1.76 ± 0.75 mmol/l; P = 0.11) were similar on all sampling days, whereas concentrations of non-esterified fatty acid (NEFA) decreased, plasma urea nitrogen increased and plasma and urine creatinine decreased during lactation ($P<0.001$, Figure 2).

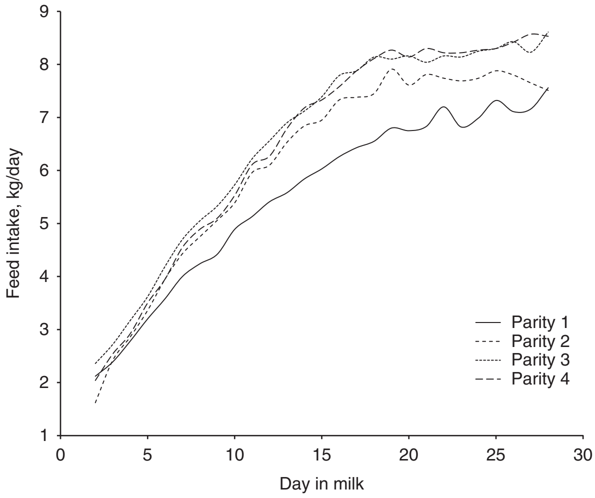

Figure 1 Feed allowance was registered daily for individual sows and feed allowances (kg/day) for parity 1 to 4 sows are given from litter standardization to weaning.

The ADG of the litter was highest in mid-lactation (3.15 ± 0.55 kg/day; days 10 to 17) compared with early (2.87 ± 0.52 kg/day; days 2 to 10) and late (2.89 ± 0.89 kg/day; days 17 to 26) lactation (Figure 3; $P<0.05$). The loss of BW and BF in sows was highest from days 2 to 17 (17.9 ± 11.7 kg and 2.6 ± 1.6 mm) compared with days 17 to 26 (8.0 ± 7.9 kg and 0.7 ± 1.5 mm; Figure 3; $P<0.001$).

Sow parities

The highest ADFI was recorded for the parity 3 and 4 sows, whereas parity 1 sows had the lowest ADFI ($P<0.001$; Figure 1). All parities weaned the same number of piglets (P = 0.15), but parity 1 sows had a lower ADG of the litter compared with multiparous sows (2.55 *v.* 2.97 to 3.04 kg/day, $P<0.001$). The total BW loss (kg) during lactation was lowest in parity 1 and 3 compared with 2 and 4 ($P<0.01$), but the total BF loss (mm) was similar among all parities (P = 0.45). On average, first parity sows lost 10% of their BW and 7.7% of body protein during lactation, which was the same as second parity sows, but first parity sows lost 26% of the body fat compared with 20% in second parity and 16% in parity 3 or 4 ($P<0.001$). In the subsequent reproductive cycle, parity 1 sows had a longer WEI ($P<0.001$) and a drop from 16.2 to 15.2 piglets in the next litter compared with the multiparous sows which had the same or increased litter size ($P<0.001$; Table 2).

Correlations

The ADG of the litter was positively correlated with BW and BF loss during lactation, respectively ($P<0.001$, Table 3). The ADFI during the entire lactation period and the weekly ADFI (ADFI 1 to 4) most likely had a positive effect on ADG of the litter ($P<0.001$; Table 3). Increased outputs of the individual nutrients in milk (lactose (r = 0.72), fat (r = 0.58) and protein (r = 0.64)) was positively related to ADG ($P<0.001$) of the litter. A high plasma creatinine (r = 0.28 to 0.49) throughout lactation seemed to have a positive

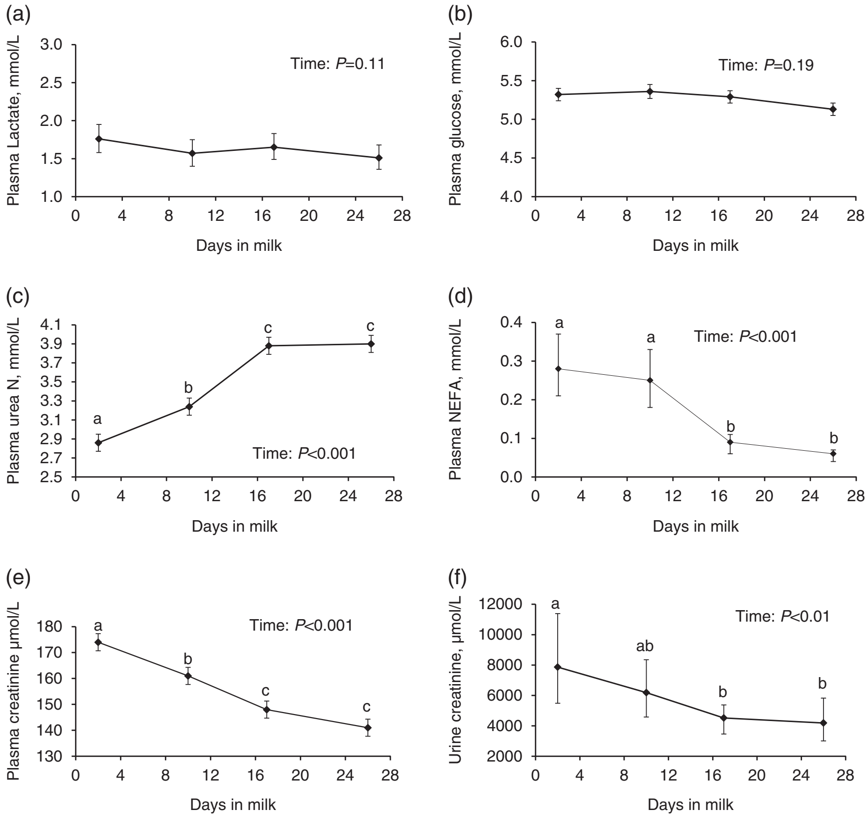

Figure 2 Plasma and urine samples were collected 4 h after the morning feeding on days 3, 10, 17 and 26 *postpartum*. Plasma was analyzed for (a) lactate, (b) glucose, (c) plasma urea N, (d) non-esterified fatty acids (NEFA) and (e) creatinine, and urine was analyzed for (f) creatinine. Results for plasma urea N, glucose and creatinine was given as means and SD and results for plasma NEFA and lactate and urine creatinine was given as means and 95% confidence intervals. ^{a,b,c}Means without a common superscript differ ($P<0.005$).

effect on ADG ($P<0.05$), whereas plasma NEFA had a positive effect in the last week ($r=0.35$; $P<0.01$), and plasma urea N had a negative effect in the 1st week ($r=-0.38$; $P<0.01$). A higher litter size and ADG of the litter was related to increased body mobilization during lactation ($P<0.001$, Table 3). The ADFI was negatively correlated to BW loss or BF loss during lactation ($P<0.001$, Table 3). Total daily output of fat in milk was positively correlated to the BF loss ($r=0.40$; $P<0.01$). High concentrations of NEFA ($r=0.30$ and 0.28; $P<0.05$) or creatinine ($r=0.36$ and 0.37; $P<0.01$) at days 10 and 17 was correlated with increased BW loss, whereas a high plasma urea N at day 2 was linked to a decreased BW ($r=-0.36$) and BF ($r=-0.32$) loss ($P<0.01$). A shorter WEI was associated with a high ADFI during the entire lactation period, and WEI was also positively correlated to a high number of born piglets in the next litter ($P<0.001$, Table 3). In contrast, WEI was correlated with a higher proportional loss of BW and BF during lactation ($P<0.05$; Table 3), a high number of weaned piglets ($P<0.01$, Table 3) or higher daily outputs of protein ($r=0.31$; $P<0.05$) or lactose ($r=0.29$; $P<0.05$) in milk. Weaning-to-estrus interval was likely negatively affected by plasma NEFA at day 17 ($r=-0.27$; $P<0.05$), plasma creatinine at day 10 ($r=-0.36$; $P<0.01$) and urine creatinine at day 10 ($r=-0.50$; $P<0.01$) and day 17 ($r=-0.29$; $P<0.05$). The ADFI of weeks 2 to 4 was positively correlated to number of total born piglets in next litter ($P<0.001$; Table 3).

Regression analysis

The results of the multivariate regression analysis are shown in Table 4. The variables included in the regression models of parity 1 to 4 sows explained 55%, 63%, 68% and 73% of the variation in ADG of the litter, respectively. Average daily feed intake and BW loss of the sow during lactation was incorporated in the regression models for all four parities. The parameters for ADFI were highest among the variables in the regression models and varied from 0.22 to 0.44 kg/day meaning that increasing the ADFI with 1 kg/day would increase the ADG of the litter with 220 to 440 g/day provided that other variables were held fixed. An increase in BW loss of 1 kg only increased ADG with 20 to 30 g/day, and an extra piglet in the litter increased ADG by 70 to 150 g/day. In total 70%, 69%, 65% and 73% of the variation in BW loss of the sows during lactation was explained by the models for parity 1 to 4, respectively. Average daily feed intake, BW at standardization, BF loss during lactation and milk yield were included in models for all four parities. Increasing the ADFI by 1 kg/day decreased the BW loss by 6.6 to 13.9 kg during lactation, whereas increasing the average milk yield with 1 kg/day raised the BW loss by 4.3 to 21.0 kg during lactation. In models for the number of total born piglets in next

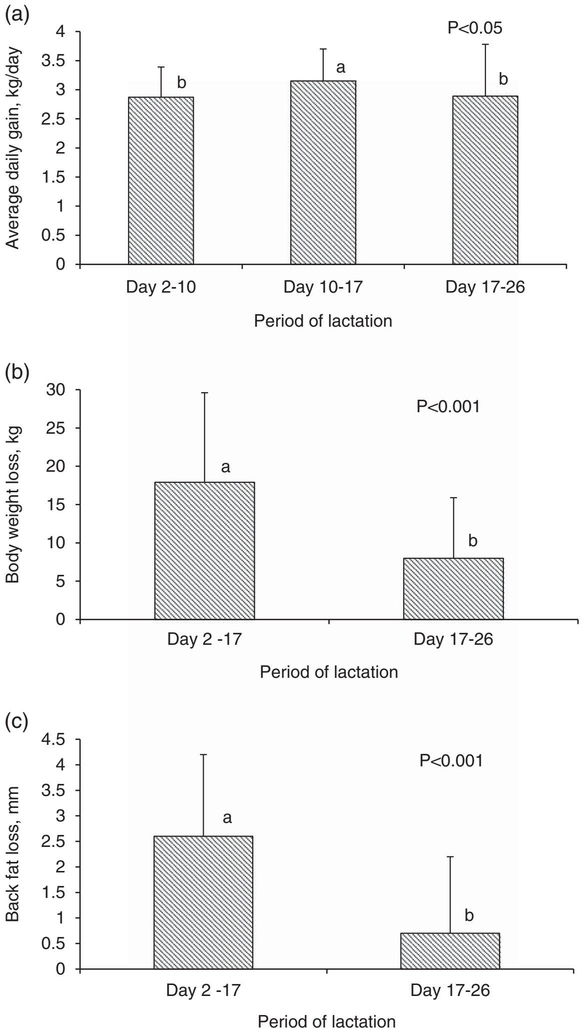

Figure 3 Changes in (a) average daily gain of the litter, (b) BW loss of the sow and (c) back fat loss of sow during different periods of lactation. Results are gives as mean and SD. ^{a,b}Means without a common superscript differ ($P < 0.005$).

litter no variables were included in all four models which only explained 8%, 6%, 4% and 14% of the variation in the number of total born piglets in next litter for parity 1 to 4, respectively. For parity 2 to 4 one extra pig born would increase litter size in the subsequent reproductive cycle with 0.18 to 0.26 piglets.

Discussion

Average daily gain of the litter

This study clearly showed that the ADG of the litter was positively affected by the ADFI of the sow, litter size and the BW loss of the sow during lactation. Average daily feed intake seemed to have the highest impact on ADG compared with litter size and BW loss. It might be difficult to increase the ADFI by 1 kg/day, but an increase in ADFI by 100 g/day could potentially increase the litter weaning weight by 0.5 to 1.0 kg. Milk production has the highest priority for the sow during lactation, which means that almost all dietary nutrients are directed toward the mammary glands. When the sow increases feed intake, more energy and nutrients become available for the synthesis of milk, which is converted into piglet growth (Koketsu *et al.*, 1997; Eissen *et al.*, 2003). The ADFI in individual weeks of lactation also had a positive correlation to the ADG of the litter, especially in weeks 3 and 4 even though the effect of the total ADFI for the entire lactation period was more pronounced. This makes sense because around days 16 to 18 *postpartum* the sows reach their maximum milk production and therefore has the highest energy and nutrient requirement (Strathe *et al.*, 2015); Koketsu *et al.*, (1997) also investigated the effect of ADFI in individual weeks of lactation on litter weight at weaning; and similarly found that ADFI of weeks 2 and 3 had a higher impact than ADFI in week 1. It is nevertheless important to mention that the sows in the current study did not have *ad libitum* access to feed, but an upper limit was set on the feed allowance. Consequently, it is not possible to determine for how long the positive effect of feed intake on ADG will continue to increase linearly before an upper limit will be reached. However, because both the ADFI and the body mobilization of the sows was high in litters with the highest ADG, it could be speculated that the feed allowance might have been very close to the physical limit for how much the sows could ingest. If this is the case, then it emphasizes the importance of investigating optimal daily requirements of energy and nutrients instead of feed allowances to ensure high productivity of hyper-prolific sows.

Sow body mobilization during lactation

The BW loss of all sows during lactation decreased with increasing feed intake, whereas an increased milk yield enlarged the BW loss. Again, it might be unrealistic to increase the ADFI with 1 kg/day, but a lower increase at for instance 200 g/day could potentially decrease the BW loss with 1.3 to 2.8 kg. A high milk yield is needed to ensure a high litter gain and it does, therefore, not from a production point of view make sense to decrease milk yield. Several studies have shown that increased litter size and ADG of the litter increased the BW and BF loss of the sows during lactation (Auldist *et al.*, 1994; Kim and Easter, 2001; Eissen *et al.*, 2003), because the sow compensates for the inadequate feed intake to maintain a high milk production. It is expensive for the sow to mobilize excessively from body reserves, because the body reserves must be reconstituted during the following gestation resulting in an increased feed cost. According to Dourmad *et al.* (1996), sows were capable of regaining BW and BF lost during lactation through the following gestation when sows lost between 12 or 35 kg BW and 2.5 and 4 mm BF, but only if fed a high-energy level (10.0 Mcal ME/day *v.* 7.1 and 8.5 Mcal ME/day). Several older studies showed that increasing the feed allowance of the sow can minimize the body mobilization (Clowes *et al.*, 1998; Pluske *et al.*, 1998; Eissen *et al.*, 2003), but if the physical upper limit for feed intake of the sow is already reached focus should be shifted toward optimizing nutrient concentrations of the feed instead and in this way try to limit the mobilization of body reserves.

Table 2 *Effects of parity on sow and piglet performance*

	Parity					*P*-value
	1	2	3	4	SE	Parity
n	100	206	156	103		
Feed intake						
ADFI (kg/day)	5.4^{c}	6.1^{b}	6.4^{a}	6.4^{a}	0.07	***
ADFI day 2 to 6 (kg/day)	2.8^{c}	3.0^{b}	3.3^{a}	3.2^{a}	0.08	***
ADFI day 7 to 13 (kg/day)	4.8^{a}	5.4^{b}	5.8^{a}	5.6^{a}	0.08	***
ADFI day 14 to 20 (kg/day)	6.4^{c}	7.3^{b}	7.8^{a}	7.8^{a}	0.12	***
ADFI day 21 to weaning (kg/day)	7.0^{c}	7.7^{b}	8.8^{a}	8.3^{a}	0.13	***
Piglets						
Total born	16.3^{b}	18.2^{a}	18.5^{a}	19.2^{a}	0.40	***
Litter size at weaning	13.2	13.1	12.9	12.9	0.17	NS
ADG piglet (g/day)	188^{b}	223^{a}	229^{a}	229^{a}	4.09	***
ADG litter (kg/day)	2.55^{b}	2.97^{a}	3.04^{a}	3.02^{a}	0.06	***
Body condition sows						
BW change day −7 to 2 (kg)	27.2^{c}	32.1^{b}	34.7^{ab}	35.3^{a}	1.18	***
BW day 2 (kg)	191^{d}	240^{c}	260^{b}	274^{a}	2.17	***
BW loss day 2 to 26 (kg)	19.6^{b}	24.5^{a}	20.4^{b}	21.9^{ab}	1.46	**
BW loss day 2 to 26 (% of BW day 2)	10.1^{a}	10.3^{a}	7.7^{b}	7.9^{b}	0.57	***
BF day 2 (mm)	13.7^{a}	16.0^{b}	16.0^{b}	16.0^{b}	0.30	***
BF loss day −7 to 2 (mm)	0.66	0.88	0.84	0.96	0.12	NS
BF loss day 2 to 26 (mm)	2.87	3.02	2.75	2.81	0.18	NS
BF loss day 2 to 26 (% of BF day 2)	21.9^{a}	19.8^{ab}	17.8^{b}	18.7^{ab}	1.15	*
Fat loss (kg)	8.17^{ab}	9.33^{a}	8.11^{b}	8.61^{ab}	0.46	*
Fat loss (% of fat day 2)	25.8^{c}	20.0^{a}	16.1^{b}	16.1^{b}	1.42	***
Protein loss (kg)	2.38^{b}	3.19^{a}	2.57^{b}	2.80^{ab}	0.22	**
Protein loss (% of protein day 2)	7.7^{ac}	8.3^{a}	6.0^{b}	6.2^{bc}	0.67	***
Milk production^{1}						
Average milk yield (kg/day)	10.7^{b}	11.4^{a}	11.5^{a}	11.5^{a}	0.16	***
Peak milk yield (kg)	12.9^{b}	14.0^{a}	14.1^{a}	14.1^{a}	0.23	***
Day of peak milk yield (day)	16.2^{b}	17.0^{a}	18.4^{a}	18.3^{a}	0.19	***
Reproduction						
Weaning-to-estrus interval (days)	5.6^{a}	4.2^{b}	4.0^{b}	4.1^{b}	0.18	***
Length of gestation (days)	117.2^{b}	117.4^{ab}	117.4^{ab}	117.8^{a}	0.17	***
Total born next litter	15.2^{c}	18.0^{b}	19.3^{a}	19.6^{a}	0.35	***

NS = not significant; ADFI = average daily feed intake; ADG = average daily gain; BF = back fat.

^{a,b,c}Means within a row with different superscript letters are different.

[1] Milk yield was estimated from litter gain (g/day) and litter size using equations by Hansen *et al.* (2012b).

$^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$, $^{***}P \leqslant 0.001$.

Sow productivity and metabolism

Differences were seen between sows of different parities regarding milk production and body mobilization. First parity sows might be more sensitive to nursing large litters and mobilizing high proportions of body fat reserves could have caused the second litter drop seen in the current study. First parity sows mobilized 26% of their body fat and Schenkel *et al.* (2010) showed that first litter sows dropped in second litter, when mobilizing >20% of their body fat, whereas mobilization of body protein did not affect litter size. In addition, first parity sows had a prolonged WEI compared with multiparous sows. This indicates that the metabolism of primiparous sows might differ from multiparous sows during lactation because they are not fully grown. Therefore, other feeding strategies or dietary compositions than used for older sows could help prevent negative effects of excessive mobilization on the subsequent reproductive cycle of these first parity sows.

Urea N in plasma increased during the first 3 weeks of lactation as a reflection of increased catabolism of amino acids caused by the higher protein intake (Le Cozler *et al.*, 1998; Mosnier *et al.*, 2010), which was in accordance with Mosnier *et al.* (2010) and Quesnel *et al.* (2009). The decrease in plasma creatinine throughout lactation matches the higher BW loss during the 1st weeks of lactation compared with the last week before weaning. In contrast, Mosnier *et al.* (2010) reported a constant concentration of creatinine in plasma, but in that study the sows did not lose weight during lactation. A higher mobilization of body protein increases plasma creatinine and the mobilized protein is to a great part redirected toward milk protein. In the current study, a higher daily protein output in milk resulted in a higher litter gain. A large protein mobilization during lactation is not desirable, because it is expensive for the sow to restore muscle protein in the following gestation (Dourmad *et al.*, 1996), and this

Table 3 *Pearson correlation coefficients between variables (*n *= 565)*

	ADG (kg/day)		BW loss (kg)		BF loss (mm)		WEI		Total born in next litter	
Items	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value
Feed intake										
ADFI (kg/day)	0.45	***	−0.27	***	−0.19	***	−0.17	***	0.24	***
ADFI day 2 to 6 (kg/day)	0.17	***	−0.20	***	−0.09	*	−0.07	†	0.08	†
ADFI day 7 to 13 (kg/day)	0.33	***	−0.17	***	−0.16	***	−0.15	***	0.21	***
ADFI day 14 to 20 (kg/day)	0.43	***	−0.19	***	−0.20	***	−0.15	***	0.21	***
ADFI day 21 to weaning (kg/day)	0.40	***	−0.20	***	−0.11	**	−0.14	**	0.23	***
Piglets										
Total born piglets	0.01	NS	−0.10	*	−0.10	*	−0.16	***	0.26	***
Litter weight day 2 (kg)	0.19	***	0.18	***	0.18	***	−0.05	NS	−0.07	NS
Litter size at weaning	0.50	***	0.33	***	0.30	***	0.13	**	−0.01	NS
ADG litter (kg/day)			0.48	***	0.42	***	−0.05	NS	0.14	**
Body condition sows										
BW loss day −7 to 2 (kg)	0.04	NS	−0.13	**	−0.03	NS	−0.03	NS	0.48	***
BW day 2 (kg)	0.36	***	0.26	***	0.05	NS	−0.30	***	0.35	***
BW loss day 2 to 26 (kg)	0.48	***			0.55	***	0.01	NS	−0.01	NS
BW loss day 2 to 26 (% of BW day 2)	0.39	***	0.96	***	0.56	***	0.09	*	−0.11	**
BF loss day −7 to 2 (mm)	−0.05	NS	−0.08	†	−0.19	***	−0.03	NS	0.15	***
BF day 2 (mm)	0.21	***	0.29	***	0.37	***	−0.16	***	0.04	NS
BF loss day 2 to 26 (mm)	0.42	***	0.55	***			0.02	NS	−0.03	NS
BF loss day 2 to 26 (% of BF day 2)	0.38	***	0.49	***	0.93	***	0.09	*	−0.05	NS
Fat loss day 2 to 26 (kg)[1]	0.47	***	0.89	***	0.84	***	−0.01	NS	0.03	NS
Protein loss day 2 to 26 (kg)[1]	0.42	***	0.97	***	0.33	***	−0.01	NS	0.01	NS
Milk production[2]										
Days in milk (days)	0.01	NS	0.05	NS	0.08	†	−0.01	NS	0.08	†
Milk yield (kg/day)	0.89	***	0.49	***	0.43	***	0.03	NS	0.09	*
Peak milk yield (kg)	0.93	***	0.49	***	0.43	***	0.01	NS	0.10	*
Day of peak milk yield (day)	0.82	***	0.34	***	0.29	***	−0.14	**	0.16	***
Reproduction										
WEI (days)	–		–		–				−0.11	*
Length of gestation (days)	–		–		–		–		−0.01	NS

NS = not significant; WEI = weaning-to-estrus interval; ADFI = average daily feed intake; ADG = average daily gain; BF = back fat.
ADG of the litter, BW and BF loss of the sow, WEI and total born in next litter were used as response variables.
[1] Estimated with equations by Dourmad *et al.* (2008).
[2] Estimated with equations by Hansen *et al.* (2012b).
†$P \leqslant 0.10$, *$P \leqslant 0.05$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$.

study indicates that milk production has a great impact on protein metabolism of the sow, and therefore an important focus of future research should be optimization of protein and amino acid requirements during lactation. The plasma concentration of NEFA, which is likely an indirect measurement of body fat mobilization (Le Cozler *et al.*, 1998; Mosnier *et al.*, 2010) was highest during the first 2 weeks and then decreased to almost zero in the last 2 weeks of lactation, which is in accordance with observations made by Trottier and Easter (1995). This conforms with the higher BF loss during the 1st week compared with the last week of lactation as reported in the current study.

Sow reproduction in the following cycle

A higher feed intake during lactation was associated with a shorter WEI, which is in accordance with findings in previous studies (Koketsu *et al.*, 1997; Zak *et al.*, 1997) that reported a higher frequency of LH pulses after weaning in sows with a high feed intake during lactation. Luteinizing hormone pulses are a prerequisite for the sow to return to estrus after weaning. The regression models with total born piglets in next litter as response variable only explained very little of the variation (4% to 14%) in our data. The reason could be that many factors besides the ones measured in the current study in-between mating and farrowing have a great influence on litter size. Sows giving birth to many piglets in the first litter are prone to give birth to a high number of piglets in the subsequent litter (Lucia *et al.*, 2000; Hughes *et al.*, 2010), and in the regression models for parity 2 to 4 a positive effect of number of born piglets in the preceding litter was seen. A positive Pearson correlation coefficient was found between ADG of the litter, and the litter size in the subsequent litter and sows nursing large litters similarly had the highest ADFI during lactation. Studies have shown that feed intake especially during the last week before weaning, can have a positive effect on embryo survival (Baidoo *et al.*, 1992; Zak *et al.*, 1997).

Table 4 *Regression models for average daily gain (ADG) of the litter, BW loss of sows during lactation and total born piglets in next litter for parity 1 to 4* (n = 565)[1]

Responses	Regression model	R^2
$\mathrm{ADG}_{\mathrm{parity\ 1}}$	$-1.07_{(SE = 0.44)} + 0.22_{(SE = 0.08)} \times \mathrm{ADFI} + 0.02_{(SE = 0.004)} \times \mathrm{BWL} + 0.07_{(SE = 0.03)} \times \mathrm{LS} + 0.10_{(SE = 0.02)} \times \mathrm{BFL} + 0.14_{(SE = 0.05)} \times \mathrm{ADFI3}$	0.55
$\mathrm{ADG}_{\mathrm{parity\ 2}}$	$-1.32_{(SE = 0.30)} + 0.44_{(SE = 0.04)} \times \mathrm{ADFI} + 0.02_{(SE = 0.002)} \times \mathrm{BWL} + 0.08_{(SE = 0.02)} \times \mathrm{LS} + 0.06_{(SE = 0.01)} \times \mathrm{BFL}$	0.63
$\mathrm{ADG}_{\mathrm{parity\ 3}}$	$-1.70_{(SE = 0.38)} + 0.28_{(SE = 0.07)} \times \mathrm{ADFI} + 0.02_{(SE = 0.003)} \times \mathrm{BWL} + 0.10_{(SE = 0.02)} \times \mathrm{LS} + 0.09_{(SE = 0.02)} \times \mathrm{BFL} + 0.13_{(SE = 0.04)} \times \mathrm{ADFI4}$	0.68
$\mathrm{ADG}_{\mathrm{parity\ 4}}$	$-1.92_{(SE = 0.41)} + 0.39_{(SE = 0.07)} \times \mathrm{ADFI} + 0.03_{(SE = 0.002)} \times \mathrm{BWL} + 0.15_{(SE = 0.03)} \times \mathrm{LS}$	0.73
$\mathrm{BWL}_{\mathrm{parity\ 1}}$	$-20.9_{(SE = 11.2)} -6.65_{(SE = 1.78)} \times \mathrm{ADFI} + 0.25_{(SE = 0.05)} \times \mathrm{BWS} + 4.29_{(SE = 0.73)} \times \mathrm{MY} + 1.24_{(SE = 0.51)} \times \mathrm{BFL} + 0.67_{(SE = 0.22)} \times \mathrm{LWS} -2.60_{(SE = 1.15)} \times \mathrm{ADFI3} -0.30_{(SE = 0.07)} \times \mathrm{BWF} -0.99_{(SE = 0.34)} \times \mathrm{BFS}$	0.70
$\mathrm{BWL}_{\mathrm{parity\ 2}}$	$-43.9_{(SE = 12.5)} -12.01_{(SE = 0.93)} \times \mathrm{ADFI} + 0.17_{(SE = 0.03)} \times \mathrm{BWS} + 12.9_{(SE = 2.89)} \times \mathrm{MY} + 0.80_{(SE = 0.38)} \times \mathrm{BFL} + 0.32_{(SE = 0.16)} \times \mathrm{LWS} + 1.78_{(SE = 0.49)} \times \mathrm{TPM} -0.19_{(SE = 0.05)} \times \mathrm{BWF} -5.85_{(SE = 2.12)} \times \mathrm{PMY}$	0.69
$\mathrm{BWL}_{\mathrm{parity\ 3}}$	$-83.5_{(SE = 18.6)} -13.9_{(SE = 1.64)} \times \mathrm{ADFI} + 0.10_{(SE = 0.04)} \times \mathrm{BWS} + 21.0_{(SE = 4.60)} \times \mathrm{MY} + 5.50_{(SE = 1.85)} \times \mathrm{ADFI2} + 0.51_{(SE = 0.20)} \times \mathrm{LWS} + 3.10_{(SE = 0.59)} \times \mathrm{TPM} -0.23_{(SE = 0.06)} \times \mathrm{BWF} -11.9_{(SE = 3.38)} \times \mathrm{PMY}$	0.65
$\mathrm{BWL}_{\mathrm{parity\ 4}}$	$-39.1_{(SE = 14.1)} -11.2_{(SE = 2.24)} \times \mathrm{ADFI} + 0.16_{(SE = 0.04)} \times \mathrm{BWS} + 4.44_{(SE = 0.69)} \times \mathrm{MY} + 1.80_{(SE = 0.54)} \times \mathrm{BFL} -2.94_{(SE = 1.17)} \times \mathrm{ADFI1} + 1.84_{(SE = 0.50)} \times \mathrm{TPM} + 3.30_{(SE = 1.53)} \times \mathrm{ADFI2} -0.79_{(SE = 0.28)} \times \mathrm{BFS}$	0.73
$\mathrm{TBNL}_{\mathrm{parity\ 1}}$	$22.6_{(4.23)} + 0.95_{(SE = 0.38)} \times \mathrm{BFF} -0.50_{(SE = 0.26)} \times \mathrm{TPM}$	0.08
$\mathrm{TBNL}_{\mathrm{parity\ 2}}$	$13.5_{(SE = 2.24)} + 0.21_{(SE = 0.06)} \times \mathrm{TBP} + 1.00_{(SE = 0.58)} \times \mathrm{ADFI} -0.74_{(SE = 0.44)} \times \mathrm{ADFI3}$	0.06
$\mathrm{TBNL}_{\mathrm{parity\ 3}}$	$36.3_{(SE = 9.17)} + 0.18_{(SE = 0.08)} \times \mathrm{TBP} + 10.04_{(SE = 4.87)} \times \mathrm{ADG} -1.23_{(SE = 0.58)} \times \mathrm{TPM} -1.98_{(SE = 0.94)} \times \mathrm{PMY}$	0.04
$\mathrm{TBNL}_{\mathrm{parity\ 4}}$	$18.4_{(SE = 4.92)} + 0.29_{(SE = 0.10)} \times \mathrm{TBP} + 3.58_{(SE = 1.14)} \times \mathrm{ADG} -0.82_{(SE = 0.39)} \times \mathrm{TPM}$	0.14

The adjusted R^2 is given for each model.

[1] ADG = average daily gain of the litter (kg/day); ADFI = average daily feed intake (kg/day); ADFI1 = ADFI from standardization to day 6 (kg/day); ADFI2 = ADFI from day 7 to 13 (kg/day); ADFI3 = ADFI from day 14 to 20 (kg/day); ADFI4 = ADFI from day 21 to weaning (kg/day); BWL = BW loss during lactation (kg); BFL = back fat loss during lactation (mm); BFL% = back fat loss (% of BF at day 2); LS = litter size at weaning (*n* piglets); TBP = total born piglets (*n* piglets); BWS = BW sow at standardization (kg); BFS = back fat at standardization (mm); BFF = back fat loss day −7 to 2 (mm); WEI = weaning-to-estrus interval (days); TBNL = total born piglets in next litter (*n* piglets); TPM = time of peak milk yield (day *postpartum*); MY = milk yield (kg); PMY = peak milk yield (kg); LWS = litter weight at standardization (kg).

In conclusion, primiparous sows had a lower feed intake, ADG of the litter and a lower litter size in the next reproductive cycle, which was likely caused by a higher degree of body mobilization compared with multiparous sows. Both a high feed intake and a high mobilization of body reserves were a prerequisite for a high milk production. A low feed intake increased body mobilization and had negative effects on the subsequent reproduction, although a large increase in daily feed intake is perhaps not realistic. Instead, future research should focus on optimizing the dietary energy and nutrient concentrations of diets for lactating hyper-prolific sows and herein distinguish between primiparous and multiparous sows.

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6.3 Paper III

Increased dietary protein levels during lactation improved sow and litter performance.

A.V. Strathe, T.S. Bruun, N. Geertsen, J.-E. Zerrahn and C.F. Hansen

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Increased dietary protein levels during lactation improved sow and litter performance

Anja V. Strathe[a]*, Thomas S. Bruun[b], Niels Geertsen[a], Jens-Erik Zerrahn[c] and Christian F. Hansen[a]

[a]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 2, 1870 Frederiksberg, Denmark,

†SEGES Danish Pig Research Centre, Axeltorv 3, 1609 Copenhagen, Denmark.

‡Evonik Nutrition & Care GmbH Tyskland Filial Denmark, Damhaven 3, 7100 Vejle, Denmark.

Abbreviations: AA, Amino acid(s); ADG, Average daily gain; AIC, Akaikes information criterion; BF, Back fat thickness; BW, Body weight; CP, Crude protein; d, Day; LSMeans, Least squares means; NE, Net energy; SID, Standardized ileal digestible; WEI, Weaning-to-estrus interval.

*Corresponding author.

Email-address: avst@sund.ku.dk (A.V. Strathe)

ABSTRACT

The study was conducted to investigate the effect of increasing balanced dietary protein for hyper-prolific lactating sows. In total 544 sows (parity 1 to 4) was allotted to one of six diets from d 2 post-partum until weaning. The diets were analyzed to have a standardized ileal digestible (SID) crude protein (CP) level of 104.3, 113.3, 120.9, 128.5, 139.2 or 150.0 g/kg. At d 2 post-partum litters were standardized to 14 piglets and body weight (BW), back fat (BF) thickness of sows and litter weight were recorded. Body weight, back fat thickness and litter weight was also recorded at weaning. On a subsample of 70 sows (parity 2 and 3) milk samples were obtained at d 3, 10 and 17 post-partum and analyzed for fat, CP and lactose. In the analysis of the dose-response data the dietary SID CP concentration of the individual sow was used as explanatory variable. The abovementioned response variables were fitted with linear broken-line, quadratic broken-line and linear regression models. Sow BW and BF loss reached a break point at 143 g SID CP/kg and 127 g SID CP/kg, where sows lost 0.58 kg/d and 3 mm, respectively ($P < 0.001$). Multiparous sows had a higher average daily gain of the litter than first parity sows (3.07 vs. 2.53 kg/d) at the break point at 135 g SID CP/kg ($P < 0.001$), but litter size (13.0 ± 1.2 piglets) at weaning was unaffected by dietary treatment ($P = 0.30$). Milk CP increased to 5.0 g/100 mL until a breakpoint at 136 g SID CP/kg, milk lactose decreased until a breakpoint at 120 g SID CP/kg to 5.3 g/100 mL ($P < 0.001$) and milk fat increased linearly ($P < 0.05$). The daily output of milk protein was increased at d 17 until a breakpoint at 130 g SID CP/kg (663 - 670 g/d; $P < 0.001$).The content of milk fat increased linearly with increasing dietary SID CP ($P < 0.05$). There was a tendency towards an increased number of total born piglets in next litter with increased dietary SID CP ($P = 0.06$), whereas the weaning-to-estrus interval was unaffected by treatment ($P = 0.83$). In conclusion, increasing dietary SID CP up till 135 g/kg or 850 g SID CP/d increased ADG of the litter, and this increase was caused by increased milk yield and increased daily protein output in milk.

Key words: dietary protein, lactation, litter weight gain, milk composition, reproduction, sow performance

1. Introduction

The modern sow has undergone major changes during the last decades and has become larger and leaner and gives birth to more piglets, but only few studies have recently investigated the effect of increasing dietary protein with a balanced AA composition of lactating sows (Laspiur et al., 2009; Manjarin et al., 2012; Huang et al., 2013). The high-producing sow nurses and weans more than 12 piglets (Strathe et al., 2016; Strathe et al., 2017), placing an increased demand on the nutrient availability for milk production. Increasing milk yield and nutrient contents is essential to meet the energy and nutrient demand of the large litters. Several studies find a higher litter ADG when increasing dietary protein (King et al., 1993; Yang et al., 2000a; Manjarin et al., 2012), but only few have found an increased milk yield (King et al., 1993; Sauber et al., 1998). However, the increased ADG could be a result of increased nutrient concentrations of the milk and several studies have shown increased milk protein as response to dietary protein (Guan et al., 2004; Laspiur et al., 2009). During lactation many sows turn catabolic to meet the milk demand of the litter (Dourmad et al., 1998; Strathe et al., 2015; Strathe et al., 2017). High mobilization of protein can have a negative effect on milk production and reproduction in the following cycle (Zak et al., 1997; Clowes et al., 2003b). In addition the process of compensating for a high protein loss during lactation by regaining the mobilized muscle protein in the following gestation can be incomplete for many sows (Everts and Dekker, 1995; Dourmad et al., 1996). Consequently, the objective was to test the effect of increasing dietary SID CP concentrations with similar AA composition on sow and litter performance and it was hypothesized that increasing the level of balanced dietary CP would have a positive effect on milk production and litter growth and prevent excessive body mobilization.

2. Materials and methods

This study was conducted with the approval of the Danish Animal Experimentation Inspectorate (Authorization No. 2013-15-2934-00961).

2.1 Experimental design, animals, and housing

The study was designed as a dose-response experiment to determine the minimum SID CP level (104 to 150 g SID CP/kg) to maximize sow and litter performance. The study was conducted in a commercial Danish piggery using a total of 544 parity 1 to 4 sows (Landrace × Yorkshire, DanAvl, Copenhagen, Denmark) mated with Duroc semen (Ornestation Mors, Redsted, Denmark). The sows were randomly allocated based on parity to 1 of 6 dietary treatments (n = 91) with 6 dietary levels of SID CP (Table 1) in a complete block design. The distribution of parities was similar in all six treatment groups. The animals were studied from 4 days pre-partum (day 4.2 ± 1.6) to approximately 4 weeks post-partum when the piglets were weaned at day 24 (day 24.4 ± 1.2). The herd had 5 farrowing sections with individual farrowing crates (1.7 x 2.6 m and 1.6 x 2.5 m) that were used in the study. Treatments were randomly rotated within each section to ensure that sows receiving the same dietary treatments were not placed in the same pens in every block of sows. Each week a batch of 18 sows was included in the study, moved to the farrowing unit 4 days pre-partum and placed in individual farrowing crates. The study was performed in 31 consecutive weeks. The temperature in the farrowing unit was set to 20°C and the farrowing unit was ventilated using negative pressure by wall inlets. In the farrowing rooms artificial light was on from 0700 to 1600 h. Each pen was equipped with a covered creep area with a heating lamp for the piglets. The heating lamp was regulated by infrared sensors (VengSystem A/S, Roslev, Denmark) and the temperature was gradually decreased from 34°C at farrowing to 22°C 15 d post-partum. Twenty four to 48 h (day 1.3 ± 0.7) post-partum (day 0) the litters of the experimental sows were standardized to 14 piglets (average BW: 1.76 ± 0.24 kg). All piglets had iron injections (0.5 mL; Solofer Vet., Pharmacosmos A/S, Holbæk, Denmark), were tail docked, and males were surgically castrated on day 3 or 4 post-partum with postoperative analgesia (0.1 mL; Melovem, Dopharma B.V., aamsdonksveer, The Netherlands). Besides the recordings made in the experiment all animals were managed according to the general routines of the herd. Health of the animals was monitored by the stock personnel and normal practices for management, treatments and vaccinations of the herd were followed. It was recorded if the litter were treated for diarrhea during the experimental period. When dead or very weak piglets were removed from the litter, date and weight of the excluded piglet were logged. At weaning litter weight and number of piglet below 5 kg was noted.

2.2 Diets and feeding system

From day 4 pre-partum to day 2 post-partum all sows were fed the same commercial formulated lactation diet based on wheat, soybean meal and sugar beet pulp complying with Danish recommendations (Tybirk et al., 2013). From day 2 post-partum and until weaning sows were allotted to 6 dietary treatments varying in balanced SID CP concentration (Table 1), but formulated to be isoenergetic based on NE. The diets were formulated to fulfill the Danish dietary recommendations for essential AA composition of CP for lactating sows (Tybirk et al., 2013) and based on barley, wheat and soy bean meal

(Table 1). It was intended to formulate diets based on normally used ingredients that would be practical applicable for the pig producer and therefore no so called "synthetic" diets were used. In the formulation of the six diets the commercially available synthetic AA (Lys, Met, Tre, Val) were used in order to ensure the lowest possible CP concentration and that the dietary AA composition would be as close as possible to the recommendation (Tybirk et al., 2013).

From farrowing to day 10 of lactation sows were fed twice daily and from day 10 onwards sows were fed three times per day. All sows were fed 2.3 kg from farrowing and feed allowance was gradually increased to a maximum of 7.4 kg for primiparous sows and 8.4 kg/d for multiparous sows at day 17. Sows were fed using a SpotMix feeding system (Schauer Agrotronic, Prambachkirchen, Austria) that enabled mixing of batches for small groups of sows and usage of individual feeding curves for each sow. Individual meals for the sows were weighed and logged by the SpotMix feeding system and fed by air-assisted transport thereby avoiding mixing of the different diets. Feed residuals were not recorded, but in general sows were eating their daily ration. Sows had drinking nipples in the trough and free access to water. The feed allowance was adjusted for the individual sows daily and the feeding curves were used as maximum allowance.

2.3 Records, sampling and chemical analysis

Feed samples were taken 12 times during the experimental period and analyzed for dry matter, CP, crude fat, ash, AA (except Trp), calcium, phosphorus and energy following the directives of the European Commission ([EC] 152/2009). On day 4 pre-partum, day 3 (day 2.9 ± 1.5) post-partum, and at weaning BW of the sows was measured using a walk-in scale (Bjerringbro Vægte ApS, Bjerringbro, Denmark) and BF of sows was measured by ultrasound at the P2 site using a Sono-Grader II (Renco Corporation, Minneapolis, US). In the following reproductive cycle WEI and total born piglets in next litter was registered. Litter weight was recorded at litter standardization and at weaning using a weight trolley (Bjerringbro Vægte ApS, Bjerringbro, Denmark). On a subsample of 70 parity 2 to 3 sows (10-12 per dietary treatment) supplementary measurements were made. Additional measurements of BW and BF were made on day 17 (day 17.1 ± 1.5) and litter weight at day 10 (day 10.0 ± 1.5) and day 17 post-partum. Milk samples were obtained at day 3, 10 and 17 post-partum. Piglets were removed from the dam for at least 30 min and 2 mL oxytocin (10 IU/mL; Løvens Kemiske Fabrik, Ballerup, Denmark) were given i.m. to induce milk letdown. Milk was sampled in 50 mL sterile tubes (Sarstedt, Nümbrecht, Germany) and stored at -20°C until analysis. Milk was analyzed for dry matter, lactose, fat and protein (MilkoScan FT2, Foss Electric, Denmark).

2.4 Calculations and statistical analyzes

Average daily gain of the litter was calculated from litter size and weight at weaning, and weight of piglets removed from the litter was added. Milk yield was estimated based on ADG of litter and litter size using Eq. by Hansen et al. (2012b). Total output of milk nutrients at day 2, 10 and 17 was calculated from the measured milk composition and the calculated milk yield at the given day. In the analysis of the dose-response data the dietary SID CP concentration of the diets were used as explanatory variable. Based on the recordings of individual daily feed intakes of the sows, chemical analysis of dietary composition and digestibility coefficients of CP of the six diets an average dietary SID CP concentration was calculated for the individual sow.

Data were subjected to ANOVA using the nlme package in R (R Core Team, Vienna, Austria). The statistical model included diet and parity as fixed effects and block as random effect with sow as the experimental unit. There was no difference between sows of parity 2 to 4, and therefore the variable parity was divided into two groups (primi- vs. multiparous sows) for the 544 sows. For analysis of ADG of litter and litter weight at weaning, the litter weight at standardization was used as a covariate. In the analysis of change in sow BW and BF, and BW and BF at weaning, BW and BF at standardization were used as covariates, respectively. The interaction between diet and parity was tested, but was not significant ($P > 0.05$) and therefore removed from the models. Milk composition and litter weights, sow BW and BF of the subsample of sows were analyzed as repeated measurements and the model also included time and the interaction between time and diet as fixed effects and sow as random effect nested within block. The results of neonatal diarrhea treatments were analyzed using logistic regression. Results are given as LSMeans and SE or CI. When estimating LSMeans the skewed distribution between the two parity groups (primi- vs. multiparous sows) was taken into account by weighing by the proportion of sows in each group (0.10 vs. 0.90). Statistical significance was declared at $P < 0.05$ and tendencies at $P < 0.10$.

Linear broken-line and quadratic broken-line models were fitted to data using nonlinear mixed effect models. The random effect of block was incorporated into plateau parameters of the models, while the fixed effect of parity was incorporated both in the requirement parameter and the plateau parameter of the models. It was tested if there was an effect of parity, and when significant it was included in the model. If the effect of parity was significant it was also tested if primi- and multiparous had different breakpoints. The output of the models was evaluated using 3 criteria: 1) comparing AIC and -2 log likelihood fit statistics for nested models, where smaller values indicate a better fit to data. For -2 log likelihood fit statistics there should be a drop by 3.84 units to conclude that one model had a better fit (Kanninen and Khawaja, 1999), 2) when the breakpoint of the model was outside the range of the tested dietary SID CP concentrations the model were excluded, because it was taken to be unreliable to describe data and 3) visually best fit to data (Parr et al., 2003).

3. Results

The protein concentration was slightly higher in the 6 diets than calculated, because the analysis of wheat and barley revealed a higher content of protein than values used in the feed formulation (Table 1).

Results from ANOVA are shown in Table 2 to 4 and results of the dose-response analysis are seen in Table 5. For the BW loss, BF loss and ADG of the litter for the entire lactation period AIC or -2 log likelihood fit statistics indicated that the linear broken line model was the best fit to data, which was stressed by a better visual fit than the linear model because of the clear appearance of a break point. The quadratic model was regarded as inappropriate to describe these data because the break points were above the tested dietary SID AA concentrations. The BW loss in early to mid and late lactation was clearly best described by linear broken-line and BF loss in late lactation by linear model according to AIC and -2 log likelihood fit statistics. The linear model was chosen to describe ADG in mid and late lactation, because the linear and quadratic broken-line models did not converge. The quadratic broken line models were disregarded for milk CP, because of the high breakpoints. The linear model was the best fit to the milk fat data, whereas the milk CP data showed a clear plateau and hence the linear broken-line model was chosen here. For milk lactose a broken line model was selected because data plateaued and a linear broken line model was chosen over the quadratic broken line model as a result of the slightly lower AIC and -2 log likelihood fit statistics. For total milk protein output the linear and quadratic broken line model had the same breakpoint.

3.1 Litter characteristics

Litter size (13.0 ± 1.2 piglets) at weaning was not affected by dietary treatment (*P* = 0.30, Table 2), but increasing the dietary SID CP decreased number of piglets below 5 kg at weaning (*P* < 0.01; Table 2). Increasing the dietary SID CP level increased the ADG of the litter (*P* < 0.001; Table 5) until a breakpoint at 135 g SID CP/kg. The ADG was also affected by parity, and primi- and multiparous sows reached maximum at an ADG of 2.53 and 3.07 kg/d, respectively (Fig. 1, Table 5). The ADG of the litter in early lactation (day 2 - 10) was not affected by dietary treatment (*P* = 0.80; Table 2), but in mid (day 10 - 17; *P* < 0.10; Table 5) and late (day 17 - 26; *P* < 0.05; Table 5) ADG of the litter responded linearly to increased dietary SID CP levels (Table 5). Number of litters treated for diarrhea was not affected by dietary treatment (*P* = 0.97; Table 2), but litters of first parity sows had a higher incidence of diarrhea treatments (0.46 vs. 0.22) compared to multiparous sows (*P* < 0.001).

3.2 Sow performance and milk production

All sows had the same average daily feed intake (6.34 ± 0.64 kg/d; *P* = 0.36; Table 2), but as planned the daily protein intake increased from group 1 to 6 (*P* < 0.001; Table 2). Sow BW loss was minimized up till 143 g SID CP/kg where sows lost 0.58 kg/d (*P* < 0.001, Table 5, Fig. 1). Increased dietary SID CP levels increased BF loss until a breakpoint at 127 g SID CP/kg where sows lost 3.0 mm BF (*P* < 0.001, Table 5, Fig. 1). In early (day 2 - 17) and late lactation (day 17 - 26) BW loss was minimized at 121 and 132 g SID CP/kg (*P* < 0.001), respectively, and for BF loss a linear increase was seen in late lactation (*P* < 0.001, Table 5, Fig. 1). Milk fat decreased throughout lactation (*P* < 0.05; Fig. 3; Table 4) and milk protein decreased from day 3 to 10 (*P* < 0.001; Fig. 3; Table 4). Milk lactose was not affected by day of lactation (*P* = 0.15; Fig. 3; Table 4). Milk composition was only affected by dietary SID CP at day 17 of lactation (Fig. 2, Table 4 and 5). Milk fat increased linearly with increasing dietary SID CP (*P* < 0.05; Table 5) as an increase by 1 g SID CP/kg increased the fat content with 0.02 g/100 mL. Milk lactose decreased until a breakpoint at 124 g SID CP/kg (5.3 g/100 mL; *P* < 0.001; Table 5), whereas milk protein increased until a breakpoint at 136 g SID CP/kg (5.0 g/100 mL; P < 0.001; Table 5). The daily output of lactose (*P* = 0.70) and fat (*P* < 0.10) was not affected by dietary SID CP level (Table 4), but increased during lactation (*P* < 0.001). Increasing dietary SID CP increased the daily output of milk protein on day 17 until a breakpoint at 130 g SID CP/kg (663 - 670 g/d; P < 0.001; Table 4 and 5). The dietary treatment did not affect the WEI (*P* = 0.83; Table

2) and gestation length (P = 0.80; Table 2), but there was a tendency towards an increased number of total born piglets in next litter with increased dietary SID CP level (P = 0.06; Table 2).

4. Discussion

It is uncertain if the performance of the modern genotype sows is limited by the dietary intake of balanced protein (total AA) or single essential AA or a combination. Therefore the experimental diets in current study were formulated to increase in both CP and essential AA. The limitation of this design was that it was not possible to separate the effects of increasing dietary protein and Lys.

4.1 Daily Protein Requirement

Comparison with other studies using the same design to test the dietary effect of increased protein concentrations can be difficult, because these experiments rarely used sows nursing 13 - 14 piglets. The ADFI of sows at 6.3 kg/d in the current study was also higher than the ADFI in other studies ranging from 3.5 to 5.9 kg/d (King et al., 1993; Mateo et al., 2009). In addition, many other trials have used total CP concentrations varying from 195 to 240 g/kg as their highest CP group (Yang et al., 2000a; Laspiur et al., 2009; Mateo et al., 2009), which is higher than in the present study. The extent of BW changes at 13.9 to 20.6 kg in the current study was very similar to some other experiments (King et al., 1993; Yang et al., 2000b; Manjarin et al., 2012), whereas others reported BW losses below 5 kg (Jones and Stahly, 1999; Yang et al., 2000a). A similar large variation in BF loss from 0.2 to 9 mm was found by King et al. (1993) and Guan et al. (2004), with the results of the current study at 2.2 to 3.3 mm being close to the average BF loss in these trials. The discrepancies can probably be ascribed to varying production levels, large between-sow variation, different lactation length, and a variety of genetic lines used in the studies. The mentioned differences results in sows with different BW and probably also different body compositions. Additionally, it has been reported that sows with higher BW and BF at farrowing have a higher capacity for body mobilization during lactation (King et al., 1993; Dourmad et al., 1998; Quesnel et al., 2005).

In the current study the daily requirement for optimal litter ADG was estimated to 850 g SID CP/d from the average feed intake and the optimum SID CP concentration of 135 g/kg (Table 5). This was compared to simulations of the protein requirement by available requirement models (NRC, 2012; Strathe et al., 2015) for sows with similar performance as in current study. Litter growth is the best determinant of nutrient requirements of the sow during lactation (Trottier et al., 2015) and therefore the daily SID CP at the break-point for ADG of the litter was used for comparison. The 850 g SID CP/d found in current study was considerable higher than the 700 g SID CP/d estimated in the NRC model (NRC, 2012) and slightly lower than the 880 g SID CP/d (± 70 g/d) estimated in the requirement model by Strathe et al. (2015). The NRC model (NRC, 2012) seem to underestimate milk yield because the milk curve is based on weigh-suckle-weigh data (Hansen et al., 2012b), but in contrast it predicts very similar BW loss as observed in the present study. Strathe et al. (2015) might overestimate the contribution from mobilized body tissues used for milk nutrients, but in that model the requirement is given with a standard deviation so the requirement is expressed as an interval and the requirement found in the current study lies within this range. In both models, it is asserted that they are based on data that include none or only few sows nursing 13-14 piglets, and it is therefore uncertain how precise they are for prediction of requirements for hyper-prolific sows.

In addition, it is important to test new dietary protein levels on both primi- and multiparous sows because these sows are very different in size and production level, (Everts and Dekker, 1995; Yang et al., 2000a; Strathe et al., 2017) and therefore might have different daily CP requirements, which the abovementioned simulation models also suggests (NRC, 2012; Strathe et al., 2015). This is further underlined by results of the current study showing that primiparous sows reach a lower ADG of the litter at the break-point than multiparous sows indicating that they have a lower milk yield. Primiparous sows have lower litter ADG because body growth to reach mature size is prioritized in contrast to multiparous sows where milk production is prioritized above other metabolic processes (Everts and Dekker, 1995). There was no interaction between dietary treatment and parity, so primi- and multiparous sows can be fed a diet with same SID CP level, but with different feed allowances, which also was seen in the study by Yang et al. (2000a).

This leads to a discussion of how to determine the requirement, because the BW loss of the sow was minimized at a higher SID CP level than the ADG of the litter and BF loss continued to increase until a plateau at 127 g SID CP/kg. The ADG of the litter was used as determinant of the requirement, but sow BW loss and body composition should also be considered to ensure longevity and reproductive performance in the subsequent cycle. In this study it seemed reasonable to use ADG as the determinant of the protein requirement, even though BW loss and thereby muscle protein mobilization could be decreased further at higher SID CP levels. It was assumed that this level of body protein mobilization was not harmful, because only sows in the two low SID CP groups had a tendency for decreased litter size in the following reproductive cycle. Body fat mobilization increased until 3 mm back fat, and this loss seems normal compared to other studies (Mateo et al., 2009; Hansen et al., 2012a; Strathe et al., 2016) and it is not considered to affect sow reproduction negatively.

4.2 Sow and Litter performance

Milk yield in the current study was estimated using ADG of the litter, but the decrease in milk lactose at day 17 with increasing dietary SID CP concentration indicate that the higher CP intake might have increased milk yield, and thereby thinned the milk and caused the lower milk lactose concentration. This is in accordance with results by Guan et al. (2004) who found a tendency for increased milk yield and a linear decrease in lactose with increasing dietary CP concentration. Generally most experiments have shown that it is difficult to increase the protein concentration of porcine milk above 5.0 to 5.5 g/100 mL by raising dietary protein (Hurley, 2015). On the other hand the protein content can decrease to approximately 4.5 g/100 mL (Jones and Stahly, 1999; Clowes et al., 2003a; Guan et al., 2004) when dietary protein is severely restricted and sows are unable to fully compensate by muscle protein mobilization.

The CP content was slightly higher than 4.6-4.8 g/100 mL found in a previously study conducted in the same herd (Strathe et al., 2016), which indicates that increasing the dietary protein intake of the sow has increased the milk CP content. On the other hand the protein content of 5.0 g/100 mL at the breakpoint at day 17 was not unusually high but similar or lower compared to other studies with high protein intakes (Jones and Stahly, 1999; Guan et al., 2004; Laspiur et al., 2009), which indicates that sows on the low SID CP concentrations in current study were undersupplied.

The increased BF loss throughout lactation fits very well with the increased milk fat content, but it also indicated that sows fed the high SID CP diets had a higher energy deficit because of the higher demand for nutrients for milk synthesis. King et al. (1993) and Yang et al. (2000a) did also find increasing BF loss with increasing dietary protein intake concluding that the energy demand for milk production was increased. In another study by Revell et al. (1998) the BW loss and lean loss of sows fed a high protein diet was decreased drastically compared to sows fed a low protein diet. In addition, the body fat loss was also decreased indicating that the sows in this experiment (Revell et al., 1998) were not in a similar energy deficit as in the present study, where sows increased the BF loss. Sow body weight loss was minimized by increasing dietary SID CP concentration, whereas BF loss increased, which indicates that the lower BW loss was a result of decreased muscle protein mobilization. The extra dietary CP was likely used for milk protein and therefore sparing muscle protein for this purpose. In two trials with sows nursing 13 piglets it was reported that increasing dietary protein decreased BW loss (Jones and Stahly, 1999; Mejia-Guadarrama et al., 2002), whereas studies with smaller litter sizes generally stated no effect on BW loss (King et al., 1993; Yang et al., 2000a; Manjarin et al., 2012). This stresses that sows nursing large litters most likely have a higher nutrient metabolism, because of the higher milk production, than sows nursing fewer piglets and therefore probably also a higher dietary CP requirement. Low nutrient and energy intakes combined with excessive body mobilization can have a negative effect on subsequent reproduction (Zak et al., 1997; Clowes et al., 2003a), but in the current study there was only a tendency for decreased litter size in next litter in the low dietary SID CP (104.3 and 113.3 g SID CP/kg) groups. This is in accordance with other studies that only reported a negative effect of very low daily SID CP intakes on ovulation rate (428 g SID CP/d; Mejia-Guadarrama et al., 2002) or total born in next litter (500 g SID CP/d; Yang et al., 2000a).

The ADG of the litter in the present study was first affected positively by dietary CP level from mid lactation (day 10 - 17 and 17 25, Table 5), which indicated that dietary protein and mobilization of body protein first became limiting for milk production from mid lactation in sows fed a diet low in SID CP. Milk composition was unaffected by dietary treatment in mid lactation (day 10), so it could be speculated that this increase in ADG must solely be caused by a higher milk yield, whereas at late lactation the combination of a higher milk yield and increased protein and fat concentration resulted in the higher ADG, because of an increased daily nutrient intake by the piglets. The nursing piglet has a high requirement for protein and essential AA because of the high rate of protein retention in muscles and other tissues (Rehfeldt and Kuhn, 2006; Theil and Jørgensen, 2016), and therefore even small increases in the total daily output of protein in milk can be of great importance for piglet growth.

5. Conclusion

This study showed that increasing dietary SID CP to 135 g/kg feed or 850 g/d could improve litter ADG by increasing milk yield and protein output in milk. In addition BW loss and mobilization from muscle protein was minimized and reproduction in the subsequent cycle was not negatively affected by this dietary SID CP level. Future research should investigate if this effect was driven by the increase in balanced CP or lysine.

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Table 1
Dietary composition (as-fed) of diets fed from d 2 post-partum to weaning.

	Diet					
	1	2	3	4	5	6
Ingredient, g/kg						
Barley	458.9	446.3	437.2	427.0	413.0	399.2
Wheat	362.0	350.0	340.0	330.0	316.0	302.0
Soy bean meal	84.0	111.0	134.0	157.0	189.0	221.0
Wheat bran	18.0	14.4	11.4	8.4	4.2	0.0
Sugar beet pellets	20.0	20.0	20.0	20.0	20.0	20.0
Vegetable oil[1]	17.3	17.6	17.8	18.0	18.4	18.7
Monocalcium phosphate	11.5	11.3	11.1	11.0	10.7	10.5
Limestone	16.3	16.2	16.1	16.1	15.9	15.8
Salt	5.2	5.2	5.2	5.2	5.2	5.2
L-Lys (65%)	3.4	3.4	3.4	3.4	3.4	3.3
L-Thr	0.6	0.7	0.7	0.7	0.8	0.8
DL-Met	0.3	0.4	0.5	0.6	0.7	0.8
L-Val	0.0	1.0	0.1	0.1	0.2	0.2
Vitamin and mineral premix[2]	1.9	1.9	1.9	1.9	1.9	1.9
Phyzyme XP[3]	0.1	0.1	0.1	0.1	0.1	0.1
Microgrits	0.5	0.5	0.5	0.5	0.5	0.5
Composition (calculated)						
DM, g/kg	863	864	865	866	867	868
CP, g/kg	120	130	139	147	159	171
SID[4] Lys, g/kg	5.8	6.5	7.0	7.5	8.3	9.0
SID Met, g/kg	1.8	2.0	2.1	2.3	2.5	2.8
SID Met + Cys, g/kg	3.6	3.9	4.1	4.4	4.7	5.1
SID Thr, g/kg	3.6	4.0	4.3	4.6	5.1	5.5
SID Trp, g/kg	1.2	1.3	1.4	1.5	1.7	1.8
SID Leu, g/kg	6.3	7.0	7.6	8.2	9.0	9.8
SID Ile, g/kg	3.4	3.8	4.1	4.5	5.0	5.5
SID His, g/kg	2.2	2.4	2.6	2.8	3.1	3.4
SID Phe, g/kg	4.3	4.8	5.1	5.5	6.0	6.6
SID Val, g/kg	4.2	4.7	5.0	5.4	6.0	6.5
Energy, MJ NE/kg	7.9	7.9	7.9	8.0	8.0	8.0
Composition (analyzed)[5]						
CP, g/kg	128.4	138.0	146.4	154.6	166.3	177.9
Lys, g/kg	7.04	7.71	8.26	8.82	9.60	10.38
Met, g/kg	2.21	2.41	2.58	2.75	2.98	3.22
Thr, g/kg	4.80	5.26	5.64	6.02	6.55	7.08
Trp, g/kg	1.61	1.75	1.86	1.97	2.13	2.29
Leu, g/kg	8.48	9.28	9.95	10.62	11.56	12.5
Ile, g/kg	4.51	5.00	5.41	5.82	6.39	6.96
His, g/kg	2.92	3.19	3.41	3.63	3.95	4.26
Phe, g/kg	5.81	6.33	6.77	7.20	7.81	8.42
Val, g/kg	5.74	6.21	6.60	6.99	7.53	8.08
SID CP[6], g/kg	104.3	113.3	120.9	128.5	139.2	150.0
SID Lys[6], g/kg	5.85	6.46	6.98	7.52	8.25	8.98

[1] Leci E Basic, Evilec ApS, Sandbjergvej 26, 6000 Kolding, Denmark

[2] Provided per kg of the diet: 8900 IU vitamin A; 890 IU vitamin D3; 167 mg DL-alfa tocopherol, 4.24 mg vitamin K3; 2.23 mg vitamin B1; 5.57 mg vitamin B2; 3.34 mg vitamin B6; 0.02 mg vitamin B12; 16.70 mg D-pantothenic acid; 22.26 mg niacin; 1.67 mg folic acid; 89.04 mg iron ($FeSO_4$); 13.00 mg copper ($CuSO_4$); 44.52 mg manganese (MnO); 0.22 mg iodine ($Ca(IO_3)_2$); 0.37 mg selenium (Na_2SeO_3).

[3] Phyzyme XP provided 500 phytase activity (FTU) per kg of diet (Danisco Animal Nutrition – DuPont, Marlborough, United Kingdom).

[4] SID = Standardized ileal digestible (SID values used in diet formulation were from Pedersen and Boisen (2002)).

[5] Feed samples were sampled regularly during the experimental period and 12 feed samples were analyzed per dietary treatment

[6] Calculated from the analyzed composition and digestibilities used when formulating the diets.

Table 2
The effect of increased dietary standardized ileal digestible (SID) CP level on sow and piglet performance (n = 544).

	SID CP, g/kg							*P*-value		
	104.3	113.3	120.9	128.5	139.2	150.0	SE	Diet	Parity	Start[1]
Feed intake, kg/d	6.31	6.37	6.30	6.38	6.44	6.28	0.07	0.36	<0.001	-
Protein intake, g/d	811	879	922	987	1071	1116	11.0	<0.001	<0.001	-
SID protein intake, g/d	658	721	762	820	897	941	9.13	<0.001	<0.001	-
Lysine intake, g/d	43.6	48.0	51.1	55.4	61.1	64.3	0.71	<0.001	<0.001	-
SID lysine intake, g/d	37.0	41.1	44.0	48.0	53.2	56.2	0.61	<0.001	<0.001	-
Parity	2.9	2.8	2.8	2.8	2.8	2.8	0.12	0.98	-	-
Days in milk, d	24.3	24.5	24.6	24.2	24.4	24.4	0.15	<0.100	<0.01	
Litter size standardization	14.0	14.0	14.0	14.0	14.0	14.0	14.0	-	-	-
Average piglet weight standardization, kg	1.76	1.78	1.74	1.77	1.75	1.77	0.03	0.75	<0.001	-
Litter weight standardization, kg	24.7	25.0	24.3	24.8	24.6	24.8	0.37	0.75	<0.001	-
Litter size weaning	12.8	13.2	13.0	13.0	13.1	13.1	0.13	0.30	0.63	-
Litter weight weaning, kg	86.8	92.1	92.8	93.7	96.1	96.7	1.50	<0.001	<0.001	<0.001
Average piglet weight weaning, kg	6.8	7.0	7.1	7.2	7.4	7.4	0.09	<0.001	<0.001	<0.001
ADG litter (d 2-25), kg/d	2.64	2.80	2.86	2.92	3.00	3.02	0.05	<0.001	<0.001	0.13
Proportion of piglets within litters below 5 kg at weaning	0.10	0.07	0.06	0.05	0.05	0.06	-	<0.01	<0.01	-
Proportion of litters treated for diarrhea	0.20	0.19	0.17	0.19	0.17	0.21	-	0.99	<0.001	-
BW sow d -5, kg	283	283	277	282	283	280	3.07	0.51	<0.001	-
BW sow d 2, kg	252	252	251	252	254	250	2.90	0.88	<0.001	-
BW sow weaning, kg	232	234	231	236	240	237	3.04	<0.001	<0.001	<0.001
Back fat thickness sow d -5, mm	15.2	15.5	14.9	15.9	15.1	14.8	0.33	<0.100	<0.001	-
Back fat thickness sow d 2, mm	14.5	14.8	14.3	15.1	14.5	14.2	0.29	0.14	<0.001	-
Back fat thickness sow weaning, mm	12.3	12.0	11.5	12.3	11.5	10.9	0.28	<0.001	<0.001	<0.001
BW loss d2-25, kg	20.6	18.5	19.7	16.6	14.2	13.9	1.66	<0.01	0.85	<0.001
BW loss d2-25, kg/d	0.85	0.75	0.80	0.68	0.58	0.57	0.07	<0.01	0.70	<0.001
Back fat loss d2-25, mm	2.17	2.76	2.71	2.71	2.90	3.25	0.20	<0.001	0.422	<0.001
Following reproductive cycle										
Weaning-to-estrus interval, d	5.8	5.6	6.2	5.6	5.5	5.6	0.43	0.83	<0.001	-
Gestation length, d	118	117	117	117	117	117	0.16	0.81	0.68	-
Total born piglets next litter	17.7	17.8	19.0	18.9	18.8	18.8	0.41	<0.10	<0.001	-

[1] For litter weight at weaning and ADG of the litter the litter weight at standardization was used as covariate (Start) in the model. For BW and back fat thickness at weaning and BW and Back fat loss during lactation BW and back fat thickness at standardization was used as covariate (Start) in the model, respectively.

Table 3

The effect of increased dietary standardized ileal digestible (SID) CP level on sow BW and back fat (BF) and litter performance on a subsample of sows[1].

		SID CP, g/kg										
		104.3	113.3	120.9	128.5	139.2	150.0		*P*-value			
	n							SE	Diet	Parity	Time	Start[2]
Litter weight, kg									0.13	0.82	<0.001	<0.001
d 10	90	42.8	45.2	46.9	44.3	47.0	47.0	3.11				
d 17	90	61.8	66.5	69.0	66.8	70.1	70.5	3.11				
d 25	90	82.9	90.9	93.7	95.8	95.8	95.0	3.11				
ADG litter (d 2-10), kg/d	90	2.43	2.57	2.75	2.77	2.78	2.69	0.17	0.80	0.11	-	<0.001
ADG litter (d 10-17), kg/d	90	3.04	3.31	3.34	3.38	3.47	3.54	0.19	0.37	0.56	-	0.53
ADG litter (d 17-25), kg/d	90	2.66	3.07	2.90	3.19	3.02	3.02	0.20	0.38	0.88	-	<0.05
Body weight sow, kg									<0.05	<0.001	<0.001	<0.001
d 17	70	240	245	240	243	248	240	4.83				
d 25	70	231	231	232	241	241	243	4.46				
BW loss d 2-17, kg	70	15.7	12.8	8.70	8.49	12.9	5.18	3.98	0.53	0.90	-	<0.01
BW loss d17-25, kg	70	4.00	13.7	7.43	4.42	0.59	2.63	3.88	0.13	0.29	-	0.60
Back fat thickness sow, mm									<0.01	<0.01	<0.05	<0.001
d 17	70	12.5	12.2	11.8	12.4	11.3	11.7	0.43				
d 25	70	12.3	11.8	11.9	12.1	11.2	10.9	0.41				
Back fat loss d 2-17, mm	70	1.83	2.05	2.42	1.95	2.92	2.45	0.48	0.56	0.74	-	<0.01
Back fat loss d17-25, mm	70	0.11	0.29	0.07	0.19	0.11	0.58	0.35	0.54	0.77	-	0.28

[1] The interactions: Diet x Time and Diet x Parity were not significant (*P*>0.05).

[2] Litter weight, BW and back fat thickness of the sow at d 2 was used as covariate (Start) in the model, when analyzing litter weight, BW and back fat of sows, respectively.

Table 4

The effect of increased dietary standardized ileal digestible (SID) CP level on milk yield, composition and nutrient output[1].

		SID CP, g/kg							*P*-value		
	n	104.3	113.3	120.9	128.5	139.2	150.0	SE	Diet	Parity	Time
Milk yield[2] kg/d	540	10.8	11.3	11.2	11.3	11.5	11.6	0.14	<0.001	<0.001	-
Maximum milk yield[2], kg	540	12.9	13.6	13.6	13.8	14.1	14.2	0.20	<0.001	<0.001	-
Day of maximum milk yield[2], d	540	16.9	17.2	17.6	17.9	18.1	18.2	0.19	<0.001	<0.001	-
Milk composition											
DM, g/100 mL									<0.05	0.89	<0.001
d 3	37	17.9	18.9	18.5	19.2	19.0	18.4	0.62			
d 10	56	17.2	18.6	18.1	17.9	18.9	17.8	0.50			
d 17	64	16.5	17.2	17.3	17.1	17.8	17.6	0.47			
Lactose, g/100 mL									<0.10	0.46	0.15
d 3	38	5.33	5.18	5.10	5.45	4.99	5.05	0.27			
d 10	57	5.29	5.11	5.66	5.76	5.14	5.09	0.19			
d 17	59	5.58	5.47	5.22	5.47	5.44	5.15	0.19			
Fat, g/100 mL									0.14	0.92	<0.001
d 3	38	7.36	8.13	8.69	8.70	8.05	7.58	0.67			
d 10	57	6.79	7.73	7.43	7.46	7.54	7.20	0.43			
d 17	60	6.06	6.49	6.35	6.63	7.02	6.91	0.43			
Protein, g/100 mL									<0.05	0.71	<0.001
d 3	36	5.13	5.54	5.62	5.80	5.59	5.46	0.23			
d 10	56	4.66	5.02	4.99	4.97	5.08	4.95	0.16			
d 17	61	4.61	4.72	4.71	4.89	5.10	4.94	0.15			
Daily output of nutrients											
Lactose[3], g/d									0.70	0.30	<0.001
d 3	38	325	332	360	332	347	302	35.6			
d 10	57	615	621	649	621	636	591	33.5			
d 17	59	690	696	724	697	711	666	33.4			
Fat[3], g/d									<0.10	0.73	<0.001
d 3	38	431	540	572	517	581	505	50.9			
d 10	57	768	876	908	854	918	841	46.4			
d 17	60	775	883	916	861	925	848	46.3			
Protein[3], g/d									<0.01	0.19	<0.001
d 3	36	307	362	387	359	401	365	26.9			
d 10	56	520	575	600	573	615	578	25.2			
d 17	61	569	625	649	623	664	627	24.9			

[1] The interactions: Diet x Time and Diet x Parity were not significant ($P > 0.05$).[2] Estimated from Eq. by Hansen et al. (2012b).

[3] Calculated from analyzed content of nutrients and estimated milk yields at d 3, 10 and 17 of lactation.

Table 5

Dose-response data were fitted with linear line (L), linear broken-line (LB) and quadratic broken-line (QB) models. Standardized ileal digestible (SID) CP (DCP) for the individual sow was used in the models. The results are given as linear model or breakpoint (R) and confidence interval (CI) for the models.

Response	Model	AIC	-2 Log likelihood fit statistic	R[1]	CI for R	P[2]	S[3]	*P*-value
BW loss, kg/d								
	L	986	972	Y = -1.48 + 0.006 x DCP	-	-	-	<0.001
	LB	980	971	143	120; 165	-0.58	0.007	<0.001
	QB[4]	981	972	226	-468; 922	-0.38	0.00003	0.52
Back fat loss, mm								
	L	2185	2167	Y = -0.38 – 0.02 x DCP	-	-	-	<0.001
	LB	2182	2172	127	112; 142	-2.97	-0.031	<0.001
	QB[4]	2180	2170	183	-40; 406	-3.34	-0.0002	0.11
ADG litter, kg/d								
	L	757	741	Y = 1.37 + 0.008 x DCP + 5.54 x $\text{Parity}_{\text{Multi}}$	-	-	-	<0.001
	LB	748	736	135	124; 145	2.53 (primi), 3.07 (multi)	0.012	<0.001
	QB[4]	748	736	150	125; 175			<0.001
Milk CP d17, g/100 mL								
	L	48.1	40.1	Y = 3.63 + 0.01 x DCP	-	-	-	<0.001
	LB	48.4	38.4	136	115; 158	4.99	0.012	<0.001
	QB[4]	49.3	39.3	156	82; 230	5.00	0.0002	<0.001
Milk fat d17, g/100 mL								
	L	160	152	Y=4.42+0.02 x DCP	-	-	-	<0.05
	LB	-	-	Did not converge	-	-	-	-
	QB	-	-	Did not converge	-	-	-	-
Milk lactose d17, g/100 mL								
	L	46.8	38.8	Y = 6.52 – 0.01 x DCP	-	-	-	<0.01
	LB	42.7	32.7	120	110; 130	5.28	-0.026	<0.001
	QB	43.7	33.7	124	106; 142	5.30	-0.001	<0.001
Milk CP d 17, g/d								
	L	666	658	Y = 443 + 1.59 x DCP	-	-	-	<0.05
	LB	665	655	130	111; 148	670	3.09	<0.001
	QB	664	654	130	99; 162	663	0.11	<0.001

BW loss d2-17, kg								
	L	4470	4462	Y = 24.6 - 0.11 x DCP	-	-	-	<0.001
	LB	4461	4451	121	114;128	9.42	-0.31	<0.001
	QB	4464	4454	127	110; 143	9.51	-0.010	<0.001
BW loss d17-26, kg								
	L	4446	4438	Y=26.0 - 0.18 x DCP	-	-	-	<0.001
	LB	4426	4416	132	126;139	0.82	-0.29	<0.001
	QB	4441	4431	149	130; 169	0.87	-0.003	<0.001
Back fat loss d 17-26, mm								
	L	1620	1612	Y=-1.3+ 0.01 x DCP	-	-	-	<0.001
	LB	1646	1636	112	67;156	0.28	0.08	<0.001
	QB	-	-	Did not converge	-	-	-	
ADG d10-17, kg/d								
	L	154	146	Y=2.55+0.007 x DCP	-	-	-	<0.10
	LB	-	-	Did not converge	-	-	-	-
	QB	-	-	Did not converge	-	-	-	-
ADG d17-26, kg/d								
	L	150	142	Y=1.94 + 0.009 x DCP	-	-	-	<0.05
	LB	-	-	Did not converge	-	-	-	-
	QB	-	-	Did not converge	-	-	-	-

[1] R = Breakpoint for LB and QB and the regression model for L

[2] P = Intercept with y-axis

[3] S = Slope

[4] The breakpoint was outside of the upper limit of the data, and the model was regarded as inappropriate for describing data

Figure captions

Fig. 1. The effect of dietary standardized ileal digestible (SID) CP (DCP; g/kg) on sow BW loss, back fat loss and ADG of the litter was described by linear broken-line models: a) sow BW loss (kg/d) decreased until a break point at 143 g SID CP/kg at 0.6 kg/d ($P<0.001$), b) sow back fat loss (mm) increased until a breakpoint at 127 g SID CP/kg at 3.0 mm ($P<0.001$), and c) ADG of the litter (kg/d) increased until a breakpoint at 135 g SID CP/kg at 2.53 and 3.07 kg/d ($P<0.001$) for first and multiparous sows, respectively. Results are plotted as least squares means and CI.

Fig. 2. The milk composition at d 17 post-partum was affected by dietary standardized ileal digestible (SID) CP (DCP; g/kg): a) Lactose content was described by a linear broken-line model and decreased until a breakpoint at 120 g SID CP/kg and 5.3g/100 mL ($P<0.001$), b) Protein content was described by a linear broken-line model and increased until a breakpoint at 136 g SID CP/kg and 5.0 g/100 mL ($P<0.001$), and c) Fat content increased linearly: $Y = 4.4 + 0.02 \times DCP$ ($P<0.001$). Results are plotted as least squares means and CI, and the fitted linear broken-line model.

Fig. 3. The effect of stage of lactation on milk composition, which was measured on d 3, 10 and 17 post-partum: a) Lactose was unaffected by day of lactation ($P=0.15$), b) Protein was higher at d 3 compared to d 10 and 17 ($P<0.001$), and c) Fat decreased throughout lactation ($P<0.05$). $^{a\text{-}c}$superscripts indicate significant differences ($P < 0.05$) between sample times. Results are plotted as least squares means and CI, and a fitted linear broken-line or a linear model.

Figure. 1

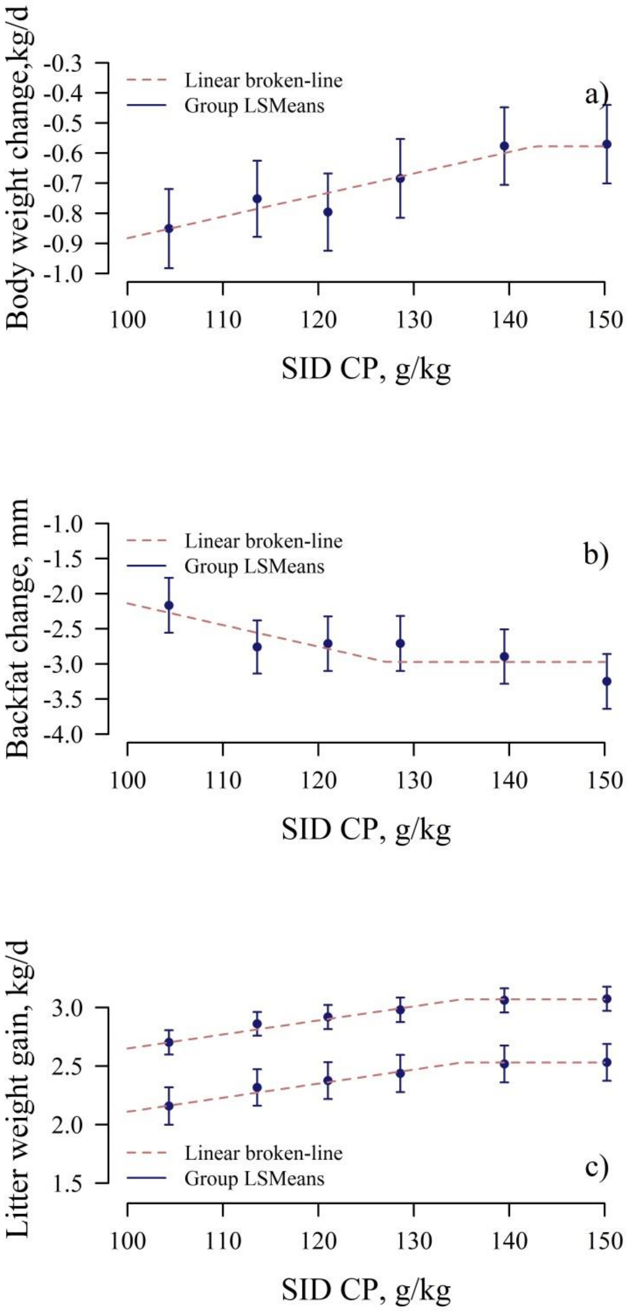

Body weight change,kg/d
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
Linear broken-line
Group LSMeans
a)
100
110
120
130
140
150
SID CP, g/kg
Backfat change, mm
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
Linear broken-line
Group LSMeans
b)
100
110
120
130
140
150
SID CP, g/kg
Litter weight gain, kg/d
3.0
2.5
2.0
1.5
Linear broken-line
Group LSMeans
c)
100
110
120
130
140
150
SID CP, g/kg

Figure 2.

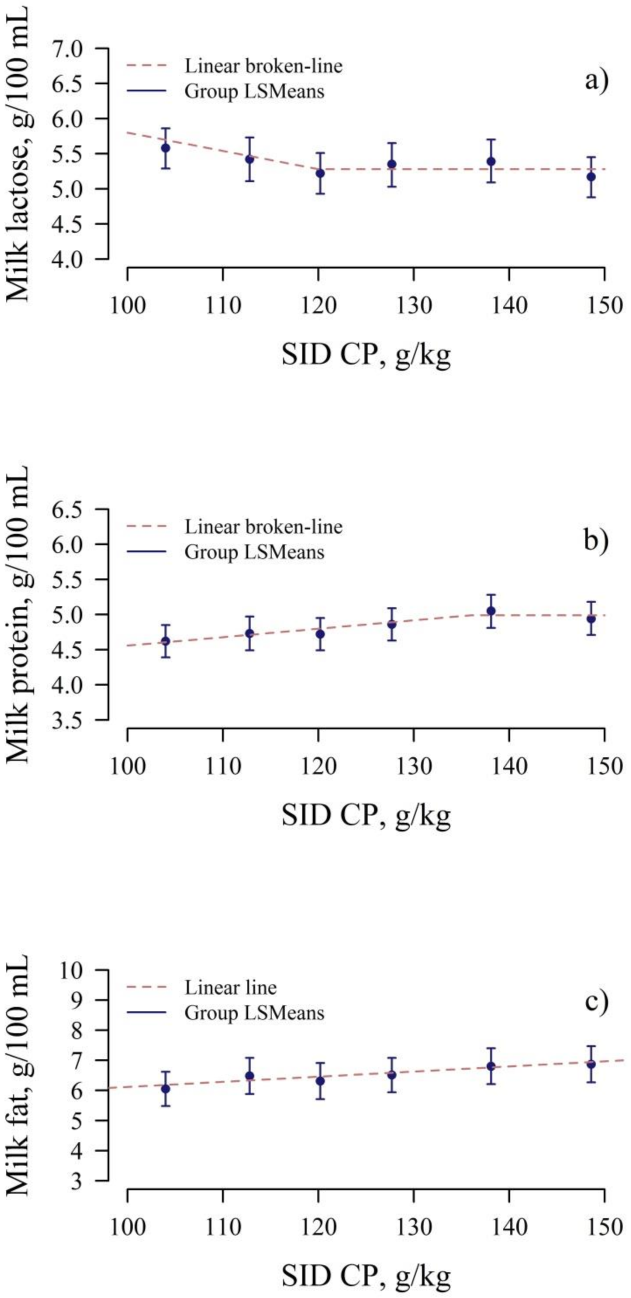
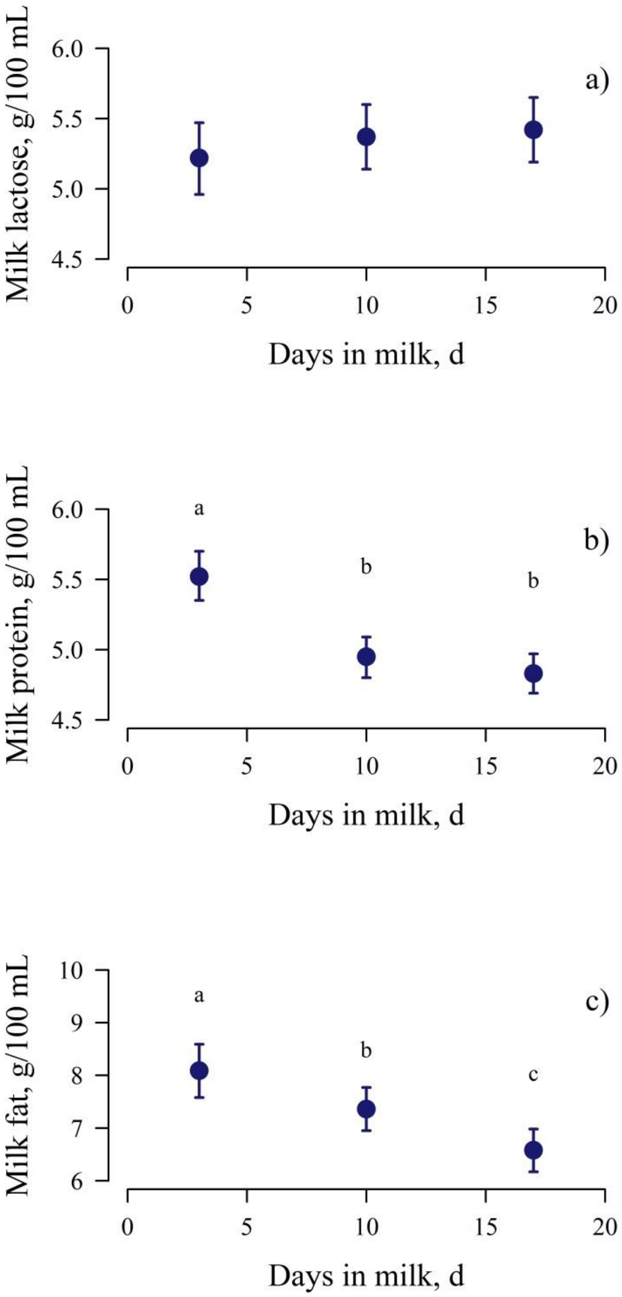

Milk lactose, g/100 mL
7.0
6.5
6.0
5.5
5.0
4.5
4.0
Linear broken-line
Group LSMeans
a)
100
110
120
130
140
150
SID CP, g/kg
Milk protein, g/100 mL
6.5
6.0
5.5
5.0
4.5
4.0
3.5
Linear broken-line
Group LSMeans
b)
100
110
120
130
140
150
SID CP, g/kg
Milk fat, g/100 mL
10
9
8
7
6
5
4
3
Linear line
Group LSMeans
c)
100
110
120
130
140
150
SID CP, g/kg

Figure 3.


a)
Milk lactose, g/100 mL
6.0
5.5
5.0
4.5
0
5
10
15
20
Days in milk, d
b)
Milk protein, g/100 mL
a
b
b
6.0
5.5
5.0
4.5
0
5
10
15
20
Days in milk, d
c)
Milk fat, g/100 mL
a
b
c
10
9
8
7
6
0
5
10
15
20
Days in milk, d

6.4 Paper IV

Increased dietary protein for lactating sows affects body composition, blood metabolites and milk production.

A.V. Strathe, T.S. Bruun, A.-H. Tauson, P.K. Theil, and C.F. Hansen

Submitted to Animal

Increased dietary protein for lactating sows affects body composition, blood metabolites and milk production

A.V. Strathe[1], T.S. Bruun[2], A.-H. Tauson[1], P.K. Theil[3], and C.F. Hansen[1]

[1]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 2, 1870 Frederiksberg, Denmark,

[2]*SEGES Pig Research Centre, Axeltorv 3, 1609 Copenhagen, Denmark.*

[3]Department of Animal Science, Aarhus University, 8830 Tjele, Denmark

Corresponding author: avstrathe@gmail.com

Short title: Metabolism of sows fed increased dietary protein

Abstract

Hyper-prolific sows give birth to more piglets putting a high demand on the nutrient supply for milk production and at the same time minimize mobilization of body protein. The effect of increased dietary protein (104, 113, 121, 129, 139 and 150 g standardized ileal digestible CP/kg) on sow metabolism was tested. From litter standardization (day 2) until weaning (day 24) sows were fed the experimental diets. Sow body composition was determined using the D_2O-dilution technique at day 3 and 24 *post-partum*. Blood and urine samples were collected weekly and milk samples were obtained at day 3, 10, and 17 of lactation. Milk composition was only affected by dietary CP at day 17. Milk fat increased linearly with increasing dietary SID CP ($P < 0.05$) and milk lactose decreased until a breakpoint at 124 g SID CP/kg (5.3 %; $P < 0.001$). The concentration of milk protein increased until a breakpoint at 136 g SID CP/kg (5.0 %; $P < 0.001$). The loss of body water from litter standardization to weaning decreased until a dietary SID CP at 130 g/kg ($P < 0.001$) and body protein loss reached a break point at 128 g SID CP/kg ($P < 0.001$). The body ash loss declined linearly with increasing dietary SID CP ($P < 0.01$), whereas the change in body fat was unaffected by dietary treatment ($P = 0.41$). In early lactation (day 3 + 10) plasma urea N (PUN) increased linearly after the breakpoint at 139 g SID CP/kg at a concentration at 3.8 mmol/L, but in late lactation (day 17 + 24) PUN increased linearly after a breakpoint at 133 g SID CP/kg ($P < 0.001$) at a concentration of 4.5 mmol/L. In conclusion, body protein loss, milk protein content, PUN, litter ADG and sow BW loss reached a breakpoint within the interval from 127 to 143 g SID CP/kg, which indicates that sows fed below this SID CP concentration , was undersupplied with dietary SID CP

Key words: Blood metabolites, Body composition, Dietary protein, Milk composition, Lactating sows.

Implications

This study showed that increased dietary protein intake of sows during lactation can prevent excessive mobilization and spare muscle protein, as dietary protein is utilized for milk production. Insufficient dietary protein intake has a negative effect on the metabolism and body condition of the lactating sow by depleting muscle and skeleton for protein and minerals. Feeding excessive dietary protein to sows increases nitrogen excretion with potential negative environmental impact. Hence, it is important that the dietary protein provision matches the actual protein and amino acid requirement of the sow.

Introduction

Modern hyper-prolific sows have become larger and leaner during the last decades and their milk yield has increased to cover the requirement of the large litters. These tremendous changes probably have changed the metabolism of the sow and increased the dietary requirement of protein and amino acids. Lactating sows often turn catabolic in order to maintain their milk production (Strathe et al., 2017a). The extent of the catabolic state might be reflected in changes in body composition, plasma concentration of metabolites and milk production (Dourmad *et al.*, 1998). These traits can be used as markers to determine the optimal dietary protein provision (Coma *et al.*, 1996, Yang *et al.*, 2000). It is important to feed the sows as close to their protein requirement as possible, because both an insufficient and excessive protein provision can be problematic. Undersupplying dietary protein can cause a high mobilization of muscle protein (King *et al.*, 1993, Mejia-Guadarrama *et al.*, 2002), since milk production is prioritized above other body functions as for instance reproduction in the following cycle. Depleted body protein pools must be restored during the following gestation, which is energetically unfavorable (Dourmad *et al.*, 1996, Bender, 2012). In addition, low protein intakes and weight loss can have a negative effect on bone health (Heaney and Layman, 2008), which can affect the longevity of the sows. Supply of protein above the requirement will cause deamination of the surplus amino acids and this will increase diet induced heat production and thereby causes an energy loss for the sow (Bender, 2012). The increased deamination also results in increased urea excretion in urine which might affect the environmental impact of pig production (Hansen *et al.*, 2014).

In another part (Strathe et al., 2017b) of the current study the dietary standardized ileal digestible (**SID**) CP concentration for maximizing litter gain was determined to be 135 g/kg. Litter gain is regarded as an important determinant of nutrient requirements of lactating sows (Trottier *et al.*, 2015), but it is also important to understand how the dietary level of CP influences different metabolic traits of the sow. Such measurements of metabolites and body composition can be used as alternative markers for the optimum SID CP, because it is not given that the metabolism of the sow is optimized at the same dietary SID CP concentration as the litter gain. This is important knowledge to gain for optimizing not only the lactational performance of the sow, but also the performance in the following cycles.

Hence, the objective of this experiment was to investigate the effect of increased dietary SID CP on body composition, changes in body composition, blood metabolites and milk production during lactation in sows. It was hypothesized that increased dietary protein would decrease body protein mobilization and improve milk production through increased yield and nutrient concentrations as well as affect plasma concentrations of metabolites related to protein metabolism.

Material and methods

The Danish Animal Experimentation Inspectorate (Authorization No. 2013-15-2934-00961) approved the completion of this trial. This experiment was carried out on a subsample of sows from a larger trial with 540 sows, and these data have been presented in Strathe et al. (2017b).

Treatments, Animals and Housing

The study was conducted in a commercial piggery using a total of 92 parity 2 and 3 sows (Danish Landrace x Danish Yorkshire, DanAvl) inseminated with Duroc semen (Hatting KS, Horsens, Denmark). The animals were studied from 4 days *pre-partum* (- 4.1±1.6) to 24 days *post-partum* (day 24.4±1.2) when the piglets were weaned. The sows were randomly allocated to 1 of 6 dietary treatments with increasing dietary levels of SID protein (Table 1) stratified for parity in a complete block design from day 2 *post-partum*. The diets were formulated to be isoenergetic on net energy basis. Sows were fed using a SpotMix feeding system (Schauer Agrotronic, Prambachkirchen, Austria) and meals for each sow were weighed and registered by the feeding system. From 4 d *pre-partum* to 2 d post-partum all sows were fed the same commercial formulated lactation diet based on wheat, soybean meal and sugar beet pulp complying with Danish recommendations (Tybirk *et al.*, 2014). From day 2 to day 10 of lactation sows were fed twice daily and from day 10 onwards sows were fed three times per day. The sows were fed 2.3 kg from day 2 *post-partum* and feed allowance was gradually increased to 8.4 kg at d 17. Sows had drinking nipples in the trough and free access to water. Feed residuals were not recorded, but the feed allowance was adjusted for the individual sows daily and the feed allowances mentioned above were used as maximum for the sows, so some sows would consume less.

Table 1 about here

Each week a batch of 6 sows was selected for the experiment, moved to the farrowing unit 4 days *pre-partum* and placed in individual farrowing crates. Twenty four hours *post-partum* (day 2) the litters of the experimental sows were standardized to 14 piglets (average piglet weight: 1.76±0.24 kg). Besides the recordings made in the experiment all animals were managed according to the general routines of the herd. Health was monitored by the stock personnel and normal practices for management, treatments and vaccinations of the herd were followed. When dead or very weak piglets were removed the date and weight of the excluded piglets were noted.

Data collection, measurements and chemical analyses

Information on collection of feed samples and analyses are given in Strathe *et al.* (2017b). On all 92 sows BW and back fat (**BF**) thickness were measured at day 2 *post-partum* and at weaning. Litter weight was recorded at litter standardization and at weaning. On two subsamples (70 and 60 sows, respectively) of the 92 sows different measurements were carried out, and some sows were included in both subsamples.

On 70 sows (10-12 per dietary treatment) blood, urine and milk samples were collected. Blood samples were collected from the jugular vein at days -4, 3, 10, 17, and 24 *post-partum* 4 hours after the morning feeding in 10 mL EDTA tubes (BD Vacutainer, Plymouth, UK). Samples were placed on ice and centrifuged for 10 min (1,560 x *g*) at room temperature and plasma was then harvested and stored at -20^{o}C in 1.5 mL microcentrifuge tubes (Kruuse, Langeskov, Denmark) until analysis. Plasma was analyzed for concentrations of plasma urea nitrogen (PUN), creatinine, glucose, lactate and non-esterified fatty acids (NEFA), triglycerides, cholesterol, total protein, albumin, bile acids, alanine amino transferase (ALT), aspartate amino transferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (Advia 1800 Chemistry System, Siemens, Denmark). Spot samples of urine were obtained on the same days as blood collection, and pH was measured immediately (FiveGo^{TM} FG2, Mettler-Toledo, Switzerland). The urine samples were stored in 1.5 mL microcentrifuge tubes (Kruuse, Langeskov, Denmark) at -20^{o}C until analysis. Urine was analyzed for creatinine (Advia 1800 Chemistry System, Siemens).

Milk samples were collected on days 3, 10 and 17 *post-partum*. Piglets were removed from the sow for at least 30 min and 2 mL oxytocin (Oxytocin "Intervet" Vet, 10IE/ml, Intervet International B. V., Boxmeer, Hollad) was injected intramuscularly to induce milk letdown. Milk was sampled from 4 to 5 teats and stored in 50 mL tubes (Sarstedt, Nümbrecht, Germany) at -20^{o}C until analysis for dry matter, lactose, fat, and crude protein (MilcoScan FT2, Foss Electric, Denmark).

On another subsample of 60 sows (10 per treatment) body water content was measured by the deuterium oxide dilution (**D_2O**) technique (Theil *et al.*, 2002) on day 3 and day 24 *post-partum*. An initial blood sample was drawn from the jugular vein into a 4 mL lithium heparinized tube (BD Vacutainer, Plymouth, UK) just before D_2O injection and then 5 hour after injection with D_2O a second sample was taken. A dose of 0.2 mL (10 % solution of D_2O, Sigma Aldrich, Brøndby, Denmark) pr. kg BW was injected *i.m.* in the neck of the sow. The exact amount of D_2O injected was determined by weighing the syringe before and after injection. The blood samples were centrifuged for 10 min (1,560 x *g*) at room temperature and plasma was then recovered and stored in 1.5 mL microcentrifuge tubes (Kruuse, Langeskov, Denmark) at -20^{o}C until analysis. The total D_2O space was determined as described by Theil *et al.* (2002).

Calculations and Statistical analysis

Calculations and statistical analyses were carried out using the statistical software R (R Core Team, Vienna, Austria) with the individual sow regarded as the experimental unit. Average daily gain of the litter was calculated from litter size and weight at standardization and at weaning, and with weights of piglets removed from the litter during the experiment added. Milk yield was estimated from ADG of the litter and litter size (data not shown) using equations by Hansen *et al.* (2012). Total nutrient output in milk was calculated from milk yield at the given day and the analyzed nutrient composition. The content of body water was estimated based on the measured concentrations of D_2O in plasma. From body water, BF and BW the body protein, fat and ash contents were computed using equations by Rozeboom *et al.* (1994).

Data were subjected to analysis of variance (ANOVA) using the nlme package in R. Feed intake, sow BW and BF, sow body composition and changes in body composition, and litter weight were analyzed using a model with diet and parity as fixed effects and block as random effect. When analyzing milk yield and composition, daily secretions, concentrations of plasma and urine metabolites and urine pH, the effect of day was also included as fixed effect and the sow as random effect. The interactions between diet and parity and diet and day were included in the model. Plasma concentrations of lactate, NEFA, bile acids, alkaline phosphatase and GGT were log transformed, because of lacking variance homogeneity. Results are given as least squares mean and **SE** (standard error). Log transformed data were back transformed and given as least squares mean and 95 % confidence interval (**CI**). Statistical significance was declared at $P < 0.05$ and tendency at $P < 0.10$.

Response variables that showed significant or tendency to effect of diet was fitted with linear broken-line and quadratic broken-line models using nonlinear mixed effects models. The random effect of block was incorporated into the plateau parameters of the models. The model output was evaluated using 3 criteria: 1) comparison of Akaike Information Criteria and -2 log likelihood fit statistics for nested models, 2) models with breakpoint outside the range of the tested SID CP concentrations was regarded inappropriate and 3) evaluation of the visual fit to data.

When the ANOVA revealed that there was no difference between sampling days, data for these days were pooled. For PUN data from day 3 and 10, and day 17 and 24, respectively, were combined. Data for plasma albumin at day 17 and 24 were united. For GGT data from day 3, 10, 17 and 24 were pooled. Urine was only collected as spot samples and there was not a full set of urine samples for all sows. Urine pH values were alike on day -4 and 3, and also days 10, 17 and 24 were similar, and therefore the data for these days were pooled to two data points per sow.

Results

Diets and sow performance

The concentration of protein was slightly higher in the six diets than planned, because wheat and barley had a higher content of crude protein than expected in the feed formulation. The average daily feed intake of the sows was similar in all groups (Table 2; $P = 0.78$), and as planned the daily intake of protein increased with increasing SID CP concentrations of the six diets ($P<0.001$). Litter size (12.8±1.2 piglets) at weaning was unaffected by dietary SID CP concentration, whereas ADG of the litters ($0.01 \times (\text{SID CP} – 135) \times (\text{SID CP} \leq 135) + 2.53_{\text{first parity}}$ or $3.07_{\text{multi parity}}$) and BF loss ($-0.03 \times (\text{SID CP} – 127) \times (\text{SID CP} \leq 127) – 2.97$) of sows increased and BW loss ($0.007 \times (\text{SID CP} – 143) \times (\text{SID CP} \leq 143) – 0.58$) decreased with increasing dietary SID CP ($P < 0.001$; data not shown; see Strathe *et al.*, 2017b).

Table 2 about here

Milk composition and nutrient output

Milk composition was only affected by dietary SID CP at day 17 of lactation. Milk fat and DM contents decreased throughout lactation ($P < 0.001$) and at d 17 Milk fat increased linearly with increasing dietary SID CP ($P < 0.05$) as an increase by 1 g SID CP/kg increased the fat content with 0.02 percentage units. Milk lactose on day 17 of lactation decreased until a breakpoint at 124 g SID CP/kg (5.3 %; $P < 0.001$), but the lactose content of the milk did not change throughout lactation ($P = 0.15$). The daily output of lactose ($P = 0.54$) and fat ($P = 0.32$) were not affected by dietary SID CP, but increased during lactation ($P < 0.001$). Milk protein decreased from day 3 to 10 of lactation ($P < 0.001$). The concentration of milk protein on d 17 increased until a breakpoint at 136 g SID CP/kg (5.0%; $P < 0.001$), whereas the daily output of milk protein on day 17 increased until a breakpoint at 130 g SID CP/kg (663-670 g/d; $P < 0.001$; Table 3).

Table 3 about here

Body composition of sows

Results of body composition measurements are given in Table 4. The loss of body water from litter standardization to weaning decreased until a dietary SID CP at 130 g/kg ($P < 0.001$) and a similar pattern was seen for the loss of protein, which reached a break point at 128 g SID CP/kg ($P < 0.001$). The body ash loss declined linearly with increasing dietary

SID CP ($P < 0.01$), whereas the change in body fat was unaffected by dietary treatment ($P = 0.41$).

Table 4 and Figure 1 about here

Plasma and urine metabolites

As expected the concentrations of metabolites in blood and urine were similar for all groups at day – 4 (Table 5; $P > 0.05$). The concentrations of PUN increased with increasing dietary SID CP intake (Figure 2; $P < 0.001$). The concentrations of PUN were similar at day 3 and 10, and at day 17 and 24, respectively and there was an interaction between diet and stage of lactation ($P < 0.05$). In early lactation (day 3 + 10) PUN increased linearly after the breakpoint at 139 g SID CP/kg at a concentration at 3.8 mmol/L, but in late lactation (day 17 + 24) PUN increased linearly after a breakpoint at 133 g SID CP/kg ($P < 0.001$, Figure 2) at a concentration of 4.5 mmol/L. Albumin concentration increased linearly in late lactation with increasing dietary SID CP (Day 17 + 24; $P < 0.05$) and GGT at all stages of lactation also increased linearly ($P < 0.05$), whereas the concentration of ALT decreased linearly at day 17 ($P < 0.01$) and 24 ($P < 0.05$) with increased dietary SID CP concentration (Figure 2).

Figure 2 about here

The urinary pH increased from early (day -4 to 3 *post-partum*) lactation to mid and late (day 10 to 24 *post-partum*) lactation ($P < 0.001$). In mid to late lactation pH showed a quadratic increase until a breakpoint at 141 g SID CP/kg, where the pH was 7.39 (Figure 3; $P < 0.001$). In early lactation there was no dietary effect (6.63 ± 0.48; $P = 0.17$).

Figure 3 about here

The dietary SID protein did not affect plasma concentrations of glucose, lactate, NEFA, triglycerides, cholesterol, creatinine, total protein, bile acids, AST, alkaline phosphatase and urinary creatinine ($P > 0.05$; data not shown), whereas all of the above mentioned metabolites were affected by day ($P < 0.001$; data not shown) except for lactate and urinary creatinine ($P > 0.05$).

Discussion

The current study investigated the impact of increasing dietary protein provision on several traits of lactating sow metabolism, which will be discussed below.

Milk production

The main purpose of the lactating sow is to produce milk with a nutrient composition and output that matches the requirements of the piglets. Dietary SID CP concentration did only affect milk composition and milk nutrient output on day 17 of lactation. The improved ADG of the litter with increasing dietary SID CP was most likely a combination of increased milk yield throughout lactation and an increased output of protein at peak lactation. Many studies have proven it difficult to increase the concentration of protein in sow milk above 5.0 to 5.5 % (Hurley, 2015), but on the other hand several studies have shown that insufficient dietary protein supply can decrease the protein content to approximately 4.5 % (Jones and Stahly, 1999, Guan *et al.*, 2004). This concurred with the results of the current study, where the milk protein content was lower in sows fed the diets with the lowest dietary SID CP concentration. In addition, the milk protein content at the breakpoint on day 17 reached only 5.0 %, which was not unusually high compared to other studies (Guan *et al.*, 2004, Laspiur *et al.*, 2009), although it was higher than shown in another recent trial with hyper-prolific sows where the milk protein content was approximately 4.5 % throughout lactation (Pedersen *et al.*, 2016). The piglet has a high rate of muscle growth and therefore a high protein requirement (Theil and Jørgensen, 2016) and the litter gain reached maximum at almost the same dietary SID CP concentration as that which resulted in maximum milk protein content.

Changes in body composition

Lactating sows often turn catabolic, and therefore milk nutrients are derived from both diet and body reserves. The decrease in BW loss in the current experiment was mainly a result of decreased muscle protein mobilization, because BF mobilization continued to increase with increased protein intake, and a similar response to increased dietary protein was reported by Dourmad *et al.* (1998). This suggests that sows fed high levels of dietary SID CP use the dietary protein as substrate for milk protein and thereby save muscle protein. Here minimum body protein loss was found at a dietary SID CP concentration close to that where milk protein was maximized. Minimizing muscle protein mobilization during lactation is important, because it costs more energy for the sow to regain protein compared to fat (Bender, 2012) during the following gestation. This is in accordance with Dourmad *et al.* (1996), who reported that sows loosing 35 kg versus 12 kg during lactation was unable to restore the body reserves, especially muscle protein, during the subsequent gestation.

Back fat loss increased with increased dietary protein, but changes in body fat estimated by D_2O dilution technique were independent of dietary protein and these results were in accordance with a study by Mejia-Guadarrama *et al.* (2002). The lacking response on body fat might be ascribed to the used equations (Rozeboom *et al.*, 1994), which were developed on data from gilts, but not developed to characterize changes in body condition during lactation. The increased BF mobilization suggests that the sow most likely was in an energy deficit and it is supported by the increased water pools in sows fed high levels of dietary SID CP. Increased energy demand will often result in increased body fat mobilization, when energy intake is not increased at the same rate as the requirement. In the current study milk fat content and BF mobilization increased with increasing dietary protein intake, and this was also seen in the experiment by King *et al.* (1993), indicating that the mobilized body fat was redirected towards the mammary glands for milk fat production.

Metabolites related to protein metabolism

In general, the concentrations of all measured blood metabolites were within the range of a normal healthy sow according to a survey on Danish sows (Thorup *et al.*, 2012).

The concentration of PUN increased both during lactation and with increasing dietary protein intake, which was also reported by Yang *et al.* (2000). The increase was expected as the higher protein intake increases the degradation of amino acids. In early lactation a higher dietary SID CP concentration was needed to increase PUN concentration than in late lactation. At the breakpoint the protein intake is at a level, where the utilization of N is most efficient and therefore has the lowest urea synthesis, whereas the increase in PUN after the breakpoint indicates that protein intake is in excess of the requirement and urea synthesis is raised (Coma *et al.*, 1995, Coma *et al.*, 1996).

In late lactation the increase in albumin concentration as response to the higher dietary SID CP concentration should more likely be seen as a decrease in sows on the low dietary protein as seen in a study on low protein intakes in pigs (Wykes *et al.*, 1996). These sows were most likely severely undersupplied with protein and therefore used albumin as a protein reserve, which shows the high priority of maintaining milk production even during periods with dietary undersupply.

The higher concentrations of GGT observed with increasing dietary SID CP intake throughout lactation reflects increased activity of this enzyme. The enzyme is involved in the uptake of amino acids by the mammary glands (Viña *et al.*, 1981b, Viña *et al.*, 1981a), which was not measured in the current study, but there was a compliance with higher concentration of GGT and a higher milk protein content due to increased dietary SID CP concentration. Increased dietary protein decreased the concentration of ALT, which might be explained by the higher muscle protein degradation in the low dietary protein groups that would also increase the activity of urea cycle enzymes (Das and Waterlow, 1974).

Bone health and urinary pH

Body ash is a measure of bone mass, and therefore the decreased mobilization of ash with increased dietary protein intake suggests that bone mobilization was reduced, so a beneficial side effect of increasing dietary protein might be improved bone health and a reduction culling due to locomotive problems (Engblom *et al.*, 2007). The change in urinary pH means that increasing dietary protein for lactating sows should include environmental considerations (Jongbloed and Lenis, 1998), and it should be ensured that this do not impact urinary tract health of the sow (Cheng *et al.*, 2015).

Conclusions

In conclusion, increasing dietary protein during lactation minimized mobilization of muscle protein and maximized milk protein content. Body protein loss, milk protein content, PUN, litter ADG and sow BW loss reached a breakpoint within the interval from 127 to 143 g SID CP/kg, which indicates that sows fed below this SID CP concentration, was undersupplied with dietary SID CP. At day 17 there was a dietary effect on milk composition, which is the time of peak milk production and therefore also the stage of lactation with the highest nutrient requirements.

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Table 1 Dietary composition (as-fed) of diets fed from d 2 postpartum to weaning.

	Diet					
	1	2	3	4	5	6
Ingredient, %						
Barley	46.0	44.8	43.8	42.8	41.4	40
Wheat	36.2	35.0	34.0	33.0	31.6	30.2
Soy bean meal	8.4	11.1	13.4	15.7	18.9	22.1
Wheat bran	1.80	1.44	1.14	0.84	0.42	0.00
Sugar beet pellets	2.00	2.00	2.00	2.00	2.00	2.00
Vegetable oil[1]	1.73	1.76	1.78	1.80	1.84	1.87
Monocalcium phosphate	1.15	1.13	1.11	1.10	1.07	1.05
Limestone	1.63	1.62	1.61	1.61	1.59	1.58
Salt	0.52	0.52	0.52	0.52	0.52	0.52
L-Lys (65%)	0.34	0.34	0.34	0.34	0.34	0.33
L-Thr	0.06	0.07	0.07	0.07	0.08	0.08
DL-Met	0.03	0.04	0.05	0.06	0.07	0.08
L-Val	0.00	0.01	0.01	0.01	0.02	0.02
Vitamin and mineral premix[2]	0.18	0.19	0.19	0.19	0.20	0.20
Phyzyme XP[3]	0.01	0.01	0.01	0.01	0.01	0.01
Microgrits	0.05	0.05	0.05	0.05	0.05	0.05
Composition (calculated)						
DM, %	86.3	86.4	86.5	86.6	86.7	86.8
CP, g/kg	120	130	139	147	159	171
SID[4] Lys, g/kg	5.8	6.5	7.0	7.5	8.3	9.0
Energy, MJ NE/kg	7.9	7.9	7.9	8.0	8.0	8.0
Composition (analyzed)[5]						
CP, g/kg	128.4	138.0	146.4	154.6	166.3	177.9
Lys, g/kg	7.04	7.71	8.26	8.82	9.60	10.38
Met, g/kg	2.21	2.41	2.58	2.75	2.98	3.22
Thr, g/kg	4.80	5.26	5.64	6.02	6.55	7.08
Trp, g/kg	1.61	1.75	1.86	1.97	2.13	2.29
Leu, g/kg	8.48	9.28	9.95	10.62	11.56	12.5
Ile, g/kg	4.51	5.00	5.41	5.82	6.39	6.96
His, g/kg	2.92	3.19	3.41	3.63	3.95	4.26
Phe, g/kg	5.81	6.33	6.77	7.20	7.81	8.42
Val, g/kg	5.74	6.21	6.60	6.99	7.53	8.08
SID CP[6], g/kg	104.3	113.3	120.9	128.5	139.2	150.0
SID Lys[6], g/kg	5.85	6.46	6.98	7.52	8.25	8.98

[1] Leci E Basic, Evilec ApS, Sandbjergvej 26, 6000 Kolding, Denmark

[2] Provided per kg of the diet: 8900 IU vitamin A; 890 IU vitamin D3; 167 mg DL-alfa-tocopherol, 4.24 mg vitamin K3; 2.23 mg vitamin B1; 5.57 mg vitamin B2; 3.34 mg vitamin B6; 0.02 mg vitamin B12; 16.70 mg D-pantothenic acid; 22.26 mg niacin; 1.67 mg folic acid; 89.04 mg iron (FeSo4); 13.00 mg copper (CuSO4); 44.52 mg manganese (MnO); 0.22 mg iodine (Ca(IO3)2); 0.37 mg selenium (Na2SeO3).

[3] Phyzyme XP provided 500 phytase activity (FTU) per kg of diet (Danisco Animal Nutrition DuPont, Marlborough, United Kingdom).

[4] SID = Standardized ileal digestible (SID values used in diet formulation were from Pedersen and Boisen (2002)).

[5] Feed samples were sampled regularly during the experimental period and 12 feed samples were analyzed per dietary treatment

[6] Calculated from the analyzed composition and digestibilities used when formulating the diets.

Table 2 The effect of increased dietary standardized ileal digestible (SID) CP on sow and piglet performance (n = 92).

	SID CP, g/kg							
	104.3	113.3	120.9	128.5	139.2	150.0		*P*-value
							SE	Diet
Parity	2.7	2.7	2.7	2.7	2.7	2.7	0.12	0.95
Feed intake, kg/d	6.31	6.24	6.52	6.62	6.42	6.34	0.22	0.78
Protein intake, g/d	808	861	951	1019	1053	1122	33.9	<0.001
SID protein intake, g/d	656	707	785	847	882	945	28.1	<0.001
Lysine intake, g/d	42.1	46.4	51.9	57.1	60.3	65.0	2.10	<0.001
SID lysine intake, g/d	35.7	39.8	44.7	49.4	52.5	56.8	1.81	<0.001
Litter weight day 2, kg	24.9	26.4	24.1	24.9	23.7	24.8	1.08	0.47
BW sow day 2, kg	239	251	252	248	253	248	6.30	0.31
Back fat sow day 2, mm	13.4	14.5	14.2	14.4	14.4	13.7	0.72	0.44
Litter weight d 25, kg	85.1	92.6	93.8	93.7	93.0	96.3	4.88	0.31
BW sow day 25, kg	221	225	227	235	237	240	6.80	0.05
Back fat sow day 25, mm	11.7	11.9	11.8	12.3	11.1	10.6	0.71	0.29

[1] The interaction Diet x Parity was not significant ($P > 0.05$).

Table 3 The effect of increased dietary standardized ileal digestible (SID) CP on milk yield, composition and nutrient output[1].

		SID protein, g/kg							*P*-value	
	n	104.3	113.3	120.9	128.5	139.2	150.0	SE	Diet	Day
Milk yield[2] kg/d	92	10.5	11.2	11.5	11.3	11.4	11.4	0.49	0.26	-
Milk composition										
DM, %									<0.05	<0.001
day 3	37	17.9	18.9	18.5	19.2	19.0	18.4	0.62		
day 10	56	17.2	18.6	18.1	17.9	18.9	17.8	0.50		
day 17	64	16.5	17.2	17.3	17.1	17.8	17.6	0.47		
Lactose, %									<0.10	0.15
day 3	38	5.33	5.18	5.10	5.45	4.99	5.05	0.27		
day 10	57	5.29	5.11	5.66	5.76	5.14	5.09	0.19		
day 17	59	5.58	5.47	5.22	5.47	5.44	5.15	0.19		
Fat, %									0.14	<0.001
day 3	38	7.36	8.13	8.69	8.70	8.05	7.58	0.67		
day 10	57	6.79	7.73	7.43	7.46	7.54	7.20	0.43		
day 17	60	6.06	6.49	6.35	6.63	7.02	6.91	0.43		
Protein, %									<0.05	<0.001
d 3	36	5.13	5.54	5.62	5.80	5.59	5.46	0.23		
d 10	56	4.66	5.02	4.99	4.97	5.08	4.95	0.16		
d 17	61	4.61	4.72	4.71	4.89	5.10	4.94	0.15		
Daily output of nutrients										
Lactose[3], g/day									0.70	<0.001
day 3	38	325	332	360	332	347	302	35.6		
day 10	57	615	621	649	621	636	591	33.5		
day 17	59	690	696	724	697	711	666	33.4		
Fat[3], g/day									<0.10	<0.001
day 3	38	431	540	572	517	581	505	50.9		
day 10	57	768	876	908	854	918	841	46.4		
day 17	60	775	883	916	861	925	848	46.3		
Protein[3], g/day									<0.01	<0.001
day 3	36	307	362	387	359	401	365	26.9		
day 10	56	520	575	600	573	615	578	25.2		
day 17	61	569	625	649	623	664	627	24.9		

[1] The interactions: Diet x Day and Diet x Parity and the effect of Parity were not significant ($P > 0.05$).

[2] Estimated from Eq. by Hansen *et al.* (2012).

[3] Calculated from analyzed content of nutrients and estimated milk yields at day 3, 10 and 17 of lactation.

Table 4 Body composition and changes in body composition in the sows measured by D_2O[1].

	SID CP, g/kg							*P*-value
	104.3	113.3	120.9	128.5	139.2	150.0	SE	Diet
Body composition day 3								
Water[2], kg	120	125	123	120	126	127	13.1	0.46
Ash, kg	7.7	8.1	8.0	7.7	8.1	8.2	0.20	0.51
Fat, kg	56.9	62.9	59.5	61.3	64.2	61.2	3.51	0.59
Protein, kg	38.9	41.0	40.0	39.4	41.3	41.3	0.97	0.50
Fat:Protein, kg/kg	1.47	1.53	1.50	1.55	1.56	1.49	0.07	0.85
Water, % of BW	52.3	49.9	51.4	50.0	50.8	50.9	1.39	0.37
Ash, % of BW	3.3	3.2	3.3	3.2	3.2	3.2	0.11	0.80
Fat, % of BW	22.1	25.1	23.1	24.2	24.1	24.0	1.23	0.40
Protein, % of BW	16.7	16.2	16.6	16.3	16.5	16.6	0.39	0.21
Body composition day 24								
Water[2], kg	116	120	123	124	126	131	3.99	0.16
Ash, kg	7.7	7.9	8.1	8.2	8.3	8.8	0.29	0.08
Fat, kg	46.5	50.6	52.2	47.7	52.8	47.9	3.70	0.55
Protein, kg	37.1	38.4	39.3	39.1	40.4	41.1	1.28	0.24
Fat:Protein, kg/kg	1.25	1.31	1.33	1.21	1.30	1.15	0.08	0.20
Water, % of BW	53.1	52.04	52.21	53.5	52.3	54.6	0.96	0.10
Ash, % of BW	3.5	3.4	3.4	3.6	3.5	3.7	0.09	0.10
Fat, % of BW	20.9	21.3	22.2	20.0	21.5	19.5	1.17	0.26
Protein, % of BW	16.8	16.5	16.7	16.8	16.7	17.1	0.19	0.13
Change in body composition, % of mass at d 3[2]								
Water loss	2.9	4.2	0.3	-3.2	0.1	-3.5	2.00	<0.01
Fat loss	19.4	20.3	16.1	23.8	20.9	22.9	4.50	0.41
Protein loss	5.8	6.3	1.8	1.0	2.9	0.7	1.92	0.06
Ash loss	0.8	2.2	-1.5	-6.8	-2.8	-7.3	2.43	<0.01

[1] The interaction Diet x Parity was not significant (*P* > 0.05). For body composition on day 3 and day 24 there was effect of Parity (*P* < 0.05), because of different BW of parity 2 and 3 sows. There was no effect of Parity on the changes in body composition (*P* > 0.05).

[2] Body water was determined by the technique described by Theil *et al.* (2002).

[3] Negative values indicate a gain.

Figure captions

Figure 1 Changes in contents of body water, protein and ash from day 3 to 24 *post partum* with increased dietary protein. The content of body water was determined using the D_2O dilution technique (Theil *et al.*, 2002). Body protein and ash were estimated from body water, BW and back fat thickness. The loss is given as the percentage of the tissue mass at day 3. a) Body water loss decreased linearly ((-0.3 x (SID CP – 130) x (SID CP ≤ 130) - 2.5) until 130 g SID CP/kg at a loss of 2.5 % ($P < 0.001$), b) body protein decreased linearly (-0.2 x (SID CP – 128) x (SID CP ≤ 128) + 1.5) until 128 g SID CP/kg at a loss of 1.5 % ($P < 0.001$) and c) body ash loss decreased linearly (Y=25.5 – 0.23 x SID CP (g/kg); $P < 0.001$). Negative values indicate a gain.

Figure 2 The effect of dietary standardized ileal digestible (SID) CP concentrations on the plasma concentrations of metabolites: a) urea N was at a stable level until a linear increase from 139 g SID CP/kg (0.09 x (SID CP – 139) x (SID CP ≥ 139) + 3.8) in early lactation (day 3 + 10) and at a stable level until a linear increase from 133 g SID CP/kg (0.09 x (SID CP – 133) x (SID CP ≥ 133) + 4.5) in late (day 17 + 24) lactation, respectively ($P < 0.001$); b) albumin increased linearly (Y=27.7 + 0.06 x SID CP(kg/d) at late (day 17 + 24) lactation ($P < 0.05$); c) gamma-glutamyl transferase (day 3 + 10 + 17 + 24) increased linearly (Y=31.2 + 0.05 x SID CP (g/kg)) with increasing dietary SID CP ($P < 0.05$); and d) alanine amino transferase increased linearly at day 17 (Y= 72.8 – 0.23 x SID CP (g/kg); $P < 0.01$) and 24 (Y= 80.1 – 0.26 x SID CP (g/kg); $P < 0.05$) of lactation, respectively.

Figure 3 The effect of dietary standardized (SID) CP concentration on pH in urine in mid and late lactation (pooled data for day 10, 17 and 24). The pH increased quadratic (0.0009 x (SID CP – 141) x (141 – SID CP) x (SID CP ≤ 141) + 7.4) until 141 g SID CP/kg ($P < 0.001$).

Figure 1.

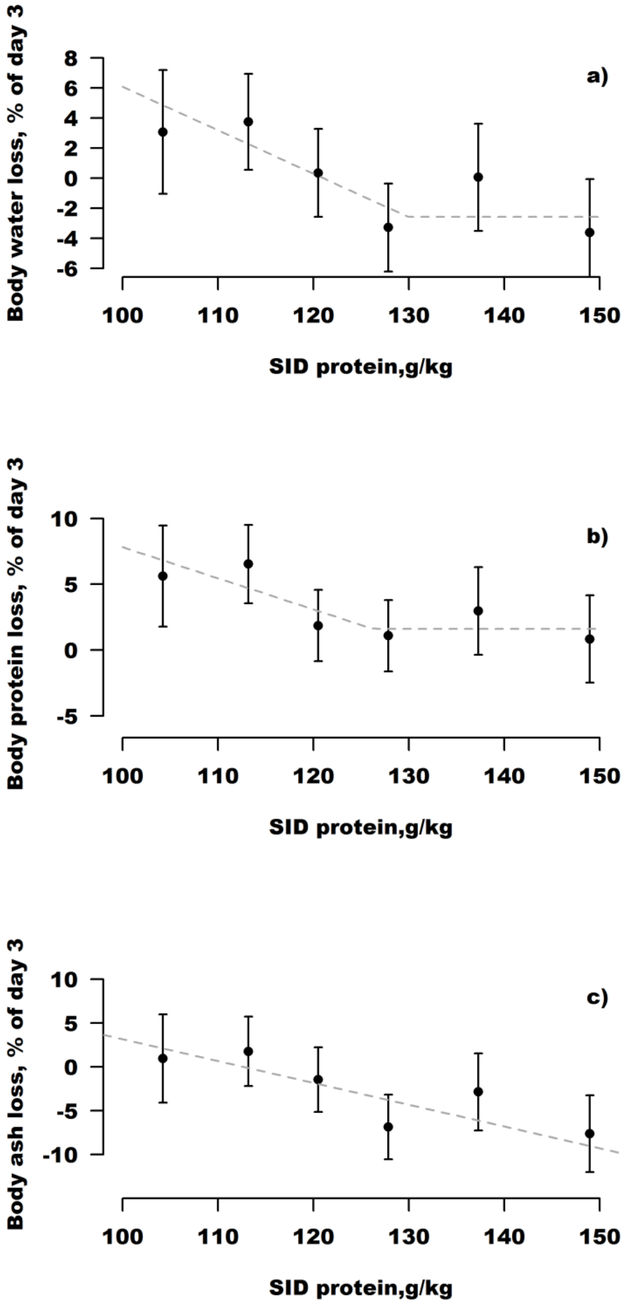

a)
Body water loss, % of day 3
8
6
4
2
0
-2
-4
-6
100
110
120
130
140
150
SID protein,g/kg
b)
Body protein loss, % of day 3
10
5
0
-5
100
110
120
130
140
150
SID protein,g/kg
c)
Body ash loss, % of day 3
10
5
0
-5
-10
100
110
120
130
140
150
SID protein,g/kg

Figure 2.

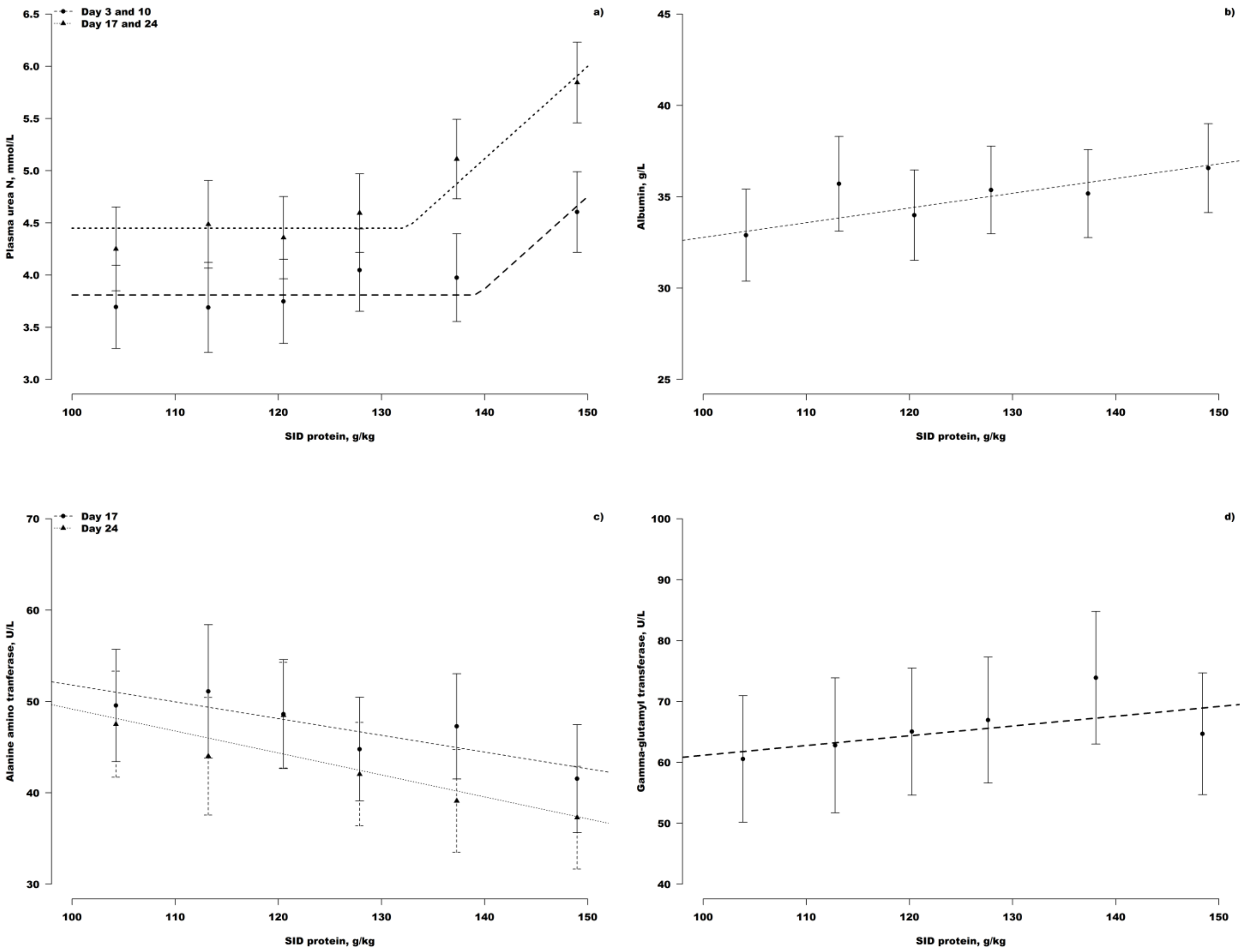
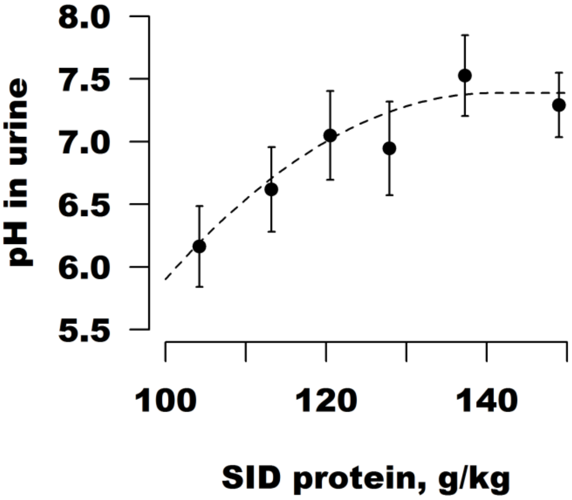

a)
Day 3 and 10
Day 17 and 24
Plasma urea N, mmol/L
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
100
110
120
130
140
150
SID protein, g/kg
b)
Albumin, g/L
45
40
35
30
25
100
110
120
130
140
150
SID protein, g/kg
c)
Day 17
Day 24
Alanine amino tranferase, U/L
70
60
50
40
30
100
110
120
130
140
150
SID protein, g/kg
d)
Gamma-glutamyl transferase, U/L
100
90
80
70
60
50
40
100
110
120
130
140
150
SID protein, g/kg

Figure 3.



7 GENERAL DISCUSSION

The overall objective of this thesis was to document the optimum Val:Lys of dietary protein (experiment 1) and the optimum concentration of dietary protein (experiment 2) for lactating sows.

7.1 Research methodology

Generally sow studies are made on few animals, because the experiments often are expensive, time-consuming and labor intensive. The experiments included in this thesis were conducted using a large number of animals, which strengthens the responses on the performance of sows and piglets. In general there is a large between-sow variation (Bergsma *et al.*, 2009, Strathe *et al.*, 2015), and therefore many replicates were required to draw conclusions from the productivity data. Power calculations were made before each experiment to ensure that enough sows were included per dietary group to detect a certain difference between groups. To my knowledge only few other recent studies on AA and CP requirements for lactating sows have included such a number of replicates, and in addition, the sows nursed and weaned 12-14 piglets. This means that the scientific results are readily usable for the Danish pig industry, because the experiments were conducted in a commercial herd reflecting the production level of hyper-prolific sows.

The experiments also, very importantly, showed that it was possible to combine large scale studies in commercial sow herds with more intensive and in-depth samplings and measurements on sow metabolism. Though there were limitations when working in a commercial herd compared to making experiments in a research facility these extra measurements were adding knowledge to the basic understanding of the metabolism of hyper-prolific lactating sows. The measurements were made on a subsample out of the total number of sows and considerable variation was observed between sows for some parameters in the experiments. In spite of that the results obtained in the herd have the same value as similar intensive measurements made in a research facility since the variation between sows would be the same.

These results, when combined with the large scale production data were supporting and helping to explain and understand the production performance results. The difficulties with performing experiments in a commercial herd were of course that not everything could be controlled or measured. In the current experiments it was for instance not possible to register feed refusals of the sows, but in general, sows were eating their ration and sows not emptying the trough had their ration reduced slightly the following days. When using single day intakes it is important to know the exact intakes of the animals, but in the current experiments only the total intake or the average daily intake for the entire lactation period were used. Therefore unregistered feed refusals for a few days would not have a great impact on the results. It was ensured that the stock person responsible for measurements of sow BW and BF thickness and litter weights was trained to get consistent measurements and additionally, the same person was responsible for carrying out these measurements during the entire experimental period. Hence the limitations of performing experimental work in a commercial herd were outweighed by the possibility of including a large number of animals in the studies.

7.1.1 Dietary treatments and dose-response trials

When conducting dose-response trials it is common to include a negative control group to make sure that it is possible to actually document a positive or negative response to the dietary treatment and also to enable identification of an optimum level either by a breakpoint or a peak, on the response curves. In both experiments it was chosen to formulate diets that were practically applicable for Danish pig producers, so they were based on the most common ingredients: barley, wheat and soy bean meal.

In experiment 1 (**Paper I**) the diet with lowest Val:Lys was formulated without the use of crystalline Val and because the diets had a slightly higher CP and Val and lower Lys content than expected the lowest total Val:Lys was 0.84 (SID of 0.80; **Paper I**), which meant that there was no negative control group. In experiment 1 no dietary effects were observed on sow and piglet performance and this can be ascribed to the relatively high Val:Lys compared to other studies that showed an effect of increasing Val:Lys when using relatively low (0.45 to 0.55) total Val:Lys (Paulicks *et al.*, 2003, Gaines *et al.*, 2006).

From a research point of view it could be interesting to define the breakpoint on the response curve, but such a low Val:Lys could only be obtained by the use of high amounts of crystalline AA, wheat as only grain source and uncommon feed ingredients, and such diets have no practical use for the pig industry in Denmark.

In experiment 2 (Paper **III** and **IV**) level of EAA necessary to fulfill the requirements determined the CP level of the dietary treatments. The CP levels could have been lowered if less wheat and soy bean meal had been used and higher amounts of crystalline AA had been included. This would result in more expensive and less practically applicable diets, but since it was intended to make diets readily applicable for the farmer this approach was not chosen.

In addition, a certain number of diet groups are important to model a meaningful dose-response curve. According to NRC (2012) at least four graded levels of the tested AA is required and there should preferably be groups both above and below the expected requirement. In experiment 1 (**Paper I**) the six applied dietary treatment groups were within a narrow range of ratios compared to other studies (Moser *et al.*, 2000, Paulicks *et al.*, 2003, Gaines *et al.*, 2006, Craig *et al.*, 2016) and it turned out that all levels were above the requirement and therefore no diet response was recorded. As discussed above it was deliberately chosen not to have a negative control in experiment 1, but because of the discrepancies in the literature regarding optimum Val:Lys it was unclear what the expected requirement should be. Hence it was anticipated that the requirement would be within the range of tested Val:Lys.

In experiment 2 it was chosen to have two dietary CP levels below the recommendation at the time of the experiment (**Paper III** and **IV**) to make sure that a response was found, one level was at the recommended CP concentration at the time of the experiment and three groups were higher than this.

In both experiment 1 and 2 it was chosen to set an upper limit for the feed allowance of the sows, which of course could have had an influence on the level of body mobilization. The applied feeding curves were however the normally applied allowances of the sows in the herd based on the experience of what most of the sows could ingest. If sows had been *ad libitum* fed there could be a risk that sows fed either a Val or CP concentration below their requirement would increase the feed intake to compensate for the undersupply. This could potentially wash out the intended differences between dietary treatments. Additionally, it has been reported that Val can stimulate the feed intake in growing pigs (Barea *et al.*, 2009, Gloaguen *et al.*, 2012).

7.1.2 Body composition measurements

Body composition can be measured either directly by slaughtering the sows or indirectly using the D_2O dilution technique (Rozeboom *et al.*, 1994) as in experiment 2 (**Paper IV**) or some type of body scan. The great advantage of the D_2O dilution technique is the possibility of repeated measurements on the same sow, because during lactation the change in body composition is more interesting than the absolute body composition after farrowing and at weaning.

This is the first time; at least in Danish pig research, that the D_2O dilution technique has been applied in a commercial sow herd. This can add knowledge on the composition of mobilized body tissue of sows used in large scale trials and thereby give a better understanding of the underlying mechanism behind the measured performance data.

The equilibration time of the sows injected with D_2O was set to 5 hours, because it has been reported that equilibrium for lactating sows was reached 5 hours after the injection. After 7 hours there would be a risk of D_2O starting to diffuse into the fat tissue, which would give wrong estimates of the D_2O space and thereby the body water pool (Theil, 2014).

The equations by Rozeboom *et al.* (1994), which were used in experiment 2 to estimate body ash, fat, protein and water content from the measured D_2O space, were developed based on data on gilts. In experiment 2 the body composition was measured on parity 2-3 sows and it is known that there can be large differences in for instance BW and body condition of first parity sows versus multiparous sows. When adding the estimated mass for ash, protein, fat and water it explained 92.8 and 93.9 % of the live BW of the sow at day 3 and 24, respectively, which is within the expected and acceptable range. The difference accounts for contents of the gastro-intestinal tract and the bladder, because equations were developed from slaughter data on empty bodies. It was hypothesized that sows fed an increased protein level would decrease the mobilization of muscle protein, and this was proven to be correct in experiment 2 (**Paper IV**), and therefore the results of body water and protein were concluded to be accurate. No changes were found in body fat as a result of the increased dietary protein intake, which was surprising, because of the clear effect on BF loss. However, the equations by Rozeboom *et al.* (1994) were based on data for sows of a fatter genotype than today, and might therefore be imprecise for predicting specifically body fat of modern lean genotype sows. The method might be imprecise in predicting the exact size of the different body pools, but in studies of lactating sows the changes in body composition from farrowing to weaning and differences between treatment groups are more informative, and therefore the method is valid when comparing such differences.

7.1.3 Milk composition

Milk composition was analyzed by infrared spectroscopy using MilcoScan (FT2, Foss Electric, Hillerød, Denmark), and in both experiments the obtained protein concentrations generally were slightly lower than reported in the literature (**Paper I** and **IV**) and it was therefore speculated that the MilcoScan might underestimate the protein content because it is developed for cow milk. Krogh (2017) compared the protein analysis from MilcoScan with the Dumas method for determination of N (CP = 6.38 x N) in sow milk and found that the milk protein content analyzed by MilcoScan was 10 % lower. This difference could also be ascribed to the fact that the Dumas method determines total N in the milk, but milk contains a non-protein N fraction including free AA, amino sugars, nucleotides and other N containing compounds (Hurley, 2015), which will be included in the N analysis by Dumas but not in the MilcoScan analysis of milk CP. Similar results were observed by Amdi *et al.* (2013) when comparing CP analysis by MilcoScan with Kjeldahl. The CP concentrations of milk found in experiment 1 and 2 were therefore most likely true. The fat and lactose content was analyzed to be 10 and 12 % higher by MilcoScan according to Krogh (2017) and with an analyzed fat content of 7% and lactose of 5.5 % in experiment 1 and 2 the overestimation will be 0.70 and 0.66 percentage units, respectively. In spite of these differences between analysis methods the values obtained by MilcoScan are valid for comparison of different treatments.

7.1.4 Metabolites as markers for protein and fat metabolism

The concentration of NEFA is often used as a measurement of body fat mobilization (Rojkittikhun *et al.*, 1993, Revell *et al.*, 1998, Valros *et al.*, 2003) and in experiment 2 an increase in BF mobilization with increasing dietary SID CP concentration was observed (**Paper IV**). A similar response for plasma concentrations of NEFA was therefore expected, but not observed. There could be several reasons for the lacking treatment effect on NEFA. First, the variation in the concentration between sows was relatively large and therefore 12 sows per group could be too small a sample to show a significant effect. Second, NEFA also increases after a meal in response to the intake and therefore concentrations are higher 2 and 4 hours after a meal compared to in a fasting sow (Mosnier *et al.*, 2010), and because NEFA can be derived from the diet it might not be the best indicator of body fatness and fat mobilization (Revell *et al.*, 1998).

The product of muscle breakdown is creatinine and plasma creatinine is therefore used as an indicator of body protein mobilization (Neil, 1996, Heo *et al.*, 2008, Yang *et al.*, 2009). In experiment 2 (Paper IV) a decrease in body protein mobilization was found with increasing SID CP intake and a similar decrease in plasma creatinine was therefore expected, but the concentration was similar for all groups. In both experiments (**Paper II** and **IV**) the concentration of creatinine declined throughout lactation and concentrations on the individual sampling days (day 3, 10, 17 and 24/26) were similar in both experiments. The differences in protein loss found in experiment 2 might be too small to actually cause a difference in plasma creatinine concentration or too few animals were included. It is also suggested that plasma creatinine most of all reflects the overall mass of body protein, because a larger muscle mass will have a higher energy requirement and therefore a higher phosphorylation of creatine to creatinine is observed in sows with higher muscle mass (Mosnier *et al.*, 2010). Therefore, the creatinine concentration may mostly reflect a difference in protein turnover related to physiological stage of lactation and not so much body mobilization in relation to feed intake (Richert *et al.*, 1996).

7.1.5 Determination of amino acid and protein requirements of sows

Prior to the experimental work included in this thesis the literature regarding the ideal dietary AA composition of lactating sows was reviewed. The conclusion was that there were only small discrepancies between the Danish recommendation and the recommendations of North America (NRC, 2012) and France (Dourmad *et al.*, 2008) and results of international studies for the EAA the exception being Val, which was somewhat lower in the Danish recommendation (**Table 3**).

It was therefore necessary to determine if the Danish recommendation for Val:Lys should be changed before it was possible to determine the optimum level of balanced dietary protein, because an unbalanced AA composition of dietary protein would result in a more inefficient utilization of the protein and increase the excretion of surplus N to the environment.

To determine the Val requirement as a ratio to Lys the Lys supply should be limiting to avoid underestimation of the optimal ratio (Boisen, 2003, Wiltafsky *et al.*, 2009). In experiment 1 it was estimated that the supplied Lys would cover 70 to 75 % of the daily requirement of the sow (Paper I). A study on weaning pigs has shown that the more limiting Lys was the higher was the determined Val:Lys (Sloth, 2010) and it can therefore be argued that the lacking response in experiment 1 was caused by a too limiting Lys level, but on the other hand the sows maintained a high production level regardless of Val and Lys levels (**Paper I**). This research approach was however developed for growing pigs, and when limiting nutrient supply of a lactating sow, the sow will use the body reserves as buffer for this undersupply. Hence, it is not possible to control a limitation of nutrient supply for a lactating sow in a similar way as for growing pigs.

Before the optimum level of balanced dietary CP was determined it could be argued that also the requirements of other EAA than Val should have been investigated on hyper-prolific lactating sows since only Lys has been extensively investigated. It was chosen not to take this approach, because of the agreement between Danish and international recommendations on these EAA (Dourmad *et al.*, 2008, NRC, 2012). However, the recommendations on other EAA are based on few and rather old studies and the sows in these studies did not have the same production level as the modern hyper-prolific sows. Therefore, future research should investigate the requirement of other EAA for lactating sows based on the optimum SID CP concentration found in experiment 2.

It is rather complicated to determine nutrient requirements of lactating sows compared to growing pigs, since not only litter growth should be considered, but also body mobilization of the sow (Trottier and Guan, 2000, Kim *et al.*, 2001a). The main purpose of the sow during lactation is to produce milk to support the nutritional requirements of the suckling piglets; therefore the main response in the experiments was chosen to be ADG of litter. However, when establishing the recommendation for dietary SID CP in experiment 2 (**Paper III** and **IV**) the responses or determined breakpoints for BW loss, BF loss, body protein loss, milk composition, PUN and reproduction results of the following cycle were also considered. This was done not only to accommodate the nutrient requirement of the litter, but also to ensure the health and longevity of the sow.

7.2 Results of the experiments

The milk production of the sow changes throughout the lactation period and accordingly the metabolism adapts to these changes, and further the litter is depending on a high milk production to optimize their ADG. It was hypothesized that improving the AA composition of the dietary protein and increasing the protein concentration of the diet would improve milk production, piglet ADG, minimize body mobilization and affect blood metabolites related to AA and protein metabolism.

7.2.1 Milk yield

Milk yield is dependent on both ADG of the litter and litter size (Hansen *et al.*, 2012b). Sows were on average nursing 13 piglets in experiment 1 and 2 and had litter gains close to 3 kg/day and it was therefore expected that the estimated milk yield would be rather high. The average milk yields were similar in both experiments within a range from 10.5 to 11.6 kg/day in the different dietary groups (**Paper I** and **III**). The day of maximum milk yield ranged from day 16.2 to 18.4 *postpartum* (**Paper II** and **III**) and a maximum yield ranging from 12.9 to 14.2 kg (**Paper II** and **III**) were also similar in experiment 1 and 2. Because of the high production level of the sows in these experiments similar milk yields were only reported in other recent Danish studies. Pedersen *et al.* (2016) and Krogh *et al.* (2016b) also found that sows nursing 12-14 piglets had the highest milk yield in week 3 of lactation and similar yields as in experiment 1 and 2 were reported.

The increasing dietary Val:Lys fed to sows in experiment 1 did not affect milk yield of the sows (**Paper I**), which was supported by several other studies testing different Val:Lys (Paulicks *et al.*, 2003, Craig *et al.*, 2016). Conversely, there was an increased milk yield with increasing dietary SID CP concentration in experiment 2 (**Paper III**), indicating that the sows redirected the extra dietary protein towards milk production. A similar response to increased dietary protein was reported by King *et al.* (1993), where milk yield increased linearly in both early and late lactation. As a result of the higher average and maximum milk yield in sows fed high dietary concentration of SID CP, days until maximum yield was obtained later in these sows than sows on the low SID CP diets. It took the sows 1-2 days extra to reach peak milk production, because milk production is increasing gradually during the first weeks of lactation (Hansen *et al.*, 2012b). This pattern was confirmed in another study where sows fed low protein (8.3 %) had the highest litter ADG at day 5-10 whereas sows fed high protein (20.7 %) had the highest litter ADG at day 15-20 of lactation (Jones and Stahly, 1999).

First parity sows produced less milk; had a lower maximum yield and milk production peaked earlier in lactation than multiparous sows (**Paper II**). Generally, the metabolism of first parity sows is slightly different from older sows, because the growth of the maternal body is prioritized and therefore fewer nutrients are available for milk production. In addition first parity sows have a lower feed intake compared to multiparous sows (Neil and Ogle, 1996, Neil *et al.*, 1996, Clowes *et al.*, 2003b).

7.2.2 Milk composition

The largest changes in milk composition are reported when transitioning from colostrum to milk (Hurley, 2015). It was therefore expected that the milk composition of the sample taken on day 3 *postpartum* in experiment 2 (**Paper III** and **IV**) would be an intermediate between colostrum and milk.

Protein

Milk protein was significantly higher on day 3 compared to day 10 and 17, which can be ascribed to the higher content of immunoglobulins in the transient milk (Theil *et al.*, 2014). The protein content on day 3 and 10 of milk samples in experiment 2 (**Paper III** and **IV**) was slightly higher than found in other studies (Huber *et al.*, 2015, Krogh *et al.*, 2016b, Pedersen *et al.*, 2016). The difference at day 3 could be ascribed to the fact that some milk samples were taken already at day 2 *postpartum* in experiment 2, as it is known that the protein content changes drastically during the first week of lactation (Csapó *et al.*, 1996).

A milk sample was obtained at day 17 in both experiment 1 and 2, and in experiment 1 no effect was observed of the increasing dietary Val:Lys on milk CP (**Paper I**). The concentrations of milk protein at day 17 in experiment 1 was similar or slightly higher than results of other recent studies (Craig *et al.*, 2016, Krogh *et al.*, 2016b, Pedersen *et al.*, 2016), where milk CP was 4.5 at day 17 in Krogh *et al.* (2016b) and Pedersen *et al.* (2016) and 4.4-4.9 at day 14 and 21 the study by Craig *et al.* (2016).

However, increasing dietary SID CP concentration in experiment 2 resulted in higher concentrations of milk protein at day 17, but not at day 3 and 10 (**Paper III** and **IV**). This increase was likely a result of the sows redirecting the extra ingested protein towards synthesis of milk protein. It is difficult to increase the milk protein content above 5 to 5.5 % (Hurley, 2015), but on the other hand the milk CP content can decrease to 4.5 % when dietary protein is severely restricted (Jones and Stahly, 1999, Clowes *et al.*, 2003a, Guan *et al.*, 2004b). The protein content of 5.0 % at the breakpoint in experiment 2 was not unusual high, but it was higher than the concentration of milk CP observed in experiment 1, where sows most likely was undersupplied with CP (**Paper I**). Extra dietary protein can increase the milk CP content to a certain level, and modern hyper-prolific sows might have a lower upper-limit for the maximum milk CP because of the higher milk yield compared to sows with a lower productivity or older genotypes.

Casein

In the review by Hurley (2015) it is stated that the casein content accounts for 50-55 % of the protein content, but in experiment 2 casein accounted for 76 to 81 % of the milk protein, which indeed is a large difference, but similar to what was observed in the study by Craig *et al.* (2016). It is uncertain why the casein content was so much higher than expected, but it might in part be explained by the analysis method, because milk samples in this thesis and in Craig *et al.* (2016) was analyzed using a MilcoScan analyzer and it might overestimate the casein content of sow milk. On the other hand Laspiur *et al.* (2009) used another analysis method, but also found rather high proportions of casein in the milk protein (66-73 %), so this might suggest that modern sows have higher casein contents in the milk than sows in the older studies cited in the review by Hurley (2015).

A higher concentration of milk casein was also obtained on day 3 in experiment 2 compared with the other sampling days, which is in accordance with the results of Csapó *et al.* (1996) where the casein concentration was higher 20 to 76 hours *postpartum* compared to the content of both colostrum and the mature milk.

The milk casein content was similar to results of Craig *et al.* (2016) and Laspiur *et al.* (2009), but higher than concentrations ranging from 3.2 % at day 3 to 2.7 % at day 20 of lactation reported by Csapó *et al.* (1996). The daily casein output was also higher than observed by Huber *et al.* (2015). The observed increase in milk casein content in experiment 2 at day 17 with increasing dietary SID CP intake indicated that a higher proportion of dietary AA was incorporated into milk casein. This indicated that the increased milk CP content was probably not caused by an increase in non-protein N in the milk, but the AA composition of the milk was not measured in experiment 2.

Amino acids

Most concentrations of AA (Met, Cys, Thr, His, Phe, Gly, Ser, Ala, Asp, Pro, Val, Leu, Ile) in the milk at day 17 in experiment 1 were within the given ranges in the review (**Table 2**) by Hurley (2015). The exception was Lys (6.9 g/100 g CP) and Arg (4.4 g/100 g CP), which was slightly lower than the expected ranges of 7-7.9 and 4.6-5.8 g/100 g CP, respectively. Dietary Lys was limited in experiment 1 (**Paper I**) and other studies with low levels of Lys have shown that the Lys content of milk can decrease. Krogh (2017) had a Lys and Arg concentration in the milk of 6.2 and 4.2 g/100 g CP, respectively, at day 17 of lactation when feeding the same dietary Lys concentration as in experiment 1. The sows in experiment 1 (**Paper I**) and the study by Krogh (2017) had similar milk yields, litter ADG, litter size and feed intake, so hyper-prolific sows might have a slightly lower Lys concentration that in older studies with lower productivity.

Glutamic acid (glutamine + glutamate) was the most abundant AA in the milk in accordance with several other studies (Wu and Knabe, 1994, Krogh, 2017), but it was much higher (36 g/100 g CP) than the expected 18.9-28.8 g/100 g CP (Hurley, 2015). Glutamic acid is taken up by the mammary gland in large quantities compared to the other AA (Nielsen *et al.*, 2002) and it has also been suggested and proven *in vitro* that glutamine is synthesized from BCAA within the mammary gland, which is supported by the relatively low contents of BCAA in the milk compared to the uptake (Wu and Knabe, 1994, Li *et al.*, 2009). There was a tendency for lower concentrations of BCAA and lower glutamic acid concentrations in the blood of the sows on day 17 in experiment 1 when higher concentrations of glutamic acid was measured in the milk indicating that these sows had a higher uptake of glutamic acid and BCAA to the mammary gland and thereby more BCAA were available for synthesis of glutamine and glutamate. In addition caseins have a high content of glutamic acid (29.5 g/ 100 CP) and therefore the high concentration in the milk could also partly be explained by the higher casein content of the milk as observed in experiment 2. However, the casein concentration of the milk was not measured in experiment 1. Interestingly, glutamine plays an important role in gut maturation of piglets (Wu *et al.*, 1996) and it is therefore beneficial for the nursing piglet if milk contains high amounts of this AA.

An increased concentration of BCAA in the milk in experiment 1 (**Paper I**) with increasing dietary Val:Lys was also observed in the study by Dunshea *et al.*, 2005 when supplementing the diet with extra BCAA. Interestingly, the concentration of all BCAA increased in the milk when only extra dietary Val was given to the sows, because interactions between the BCAA have been reported. When supplying extra dietary Leu this increases catabolism of Val and Ile (Wiltafsky *et al.*, 2010), and also decreases the uptake of the other BCAA to the mammary gland, because of the shared transport mechanisms (Jackson *et al.*, 2000, Guan *et al.*, 2002). However, extra dietary Val might not have this inhibitory effect on the uptake of the other BCAA as observed for Leu.

Urea

The increase in milk urea seen from day 3 to 17 in experiment 2 was in accordance with Huber *et al.* (2015), who found an increase in urea N from early (day 3-7) to mid lactation (day 14-18), and coincides with the increase in daily protein intake in all groups in experiment 2.

In experiment 1 the lacking correlation between milk urea and PUN could be ascribed to the fact that both PUN and milk urea was similar for all dietary groups, and in agreement with this Craig *et al.* (2016) and Roth-Maier *et al.* (2004) did not report any effect of dietary Val on urea N or urea in the milk from day 7 to 28 of lactation. In experiment 2 PUN was different between the dietary groups (**Paper IV**) and therefore sows with higher concentrations of urea in the blood would have a higher uptake to the mammary gland supported by the positive correlation between milk urea and PUN. However, there was no dietary effect on the milk urea concentration probably because of the large variation between sows for milk urea concentration in experiment 2.

Lactose and fat

Lactose is generally lower in colostrum compared to milk, which explains the numerically lower concentration observed on day 3 compared with later in lactation in experiment 2 (**Paper III**). This is in accordance with two other Danish studies showing numerically lower lactose content at day 3 *postpartum* (Krogh *et al.*, 2016b, Pedersen *et al.*, 2016). In experiment 2 (**Paper III**) the fat content of the milk decreased from day 3 to day 17 of lactation, which was in accordance with several other studies (Csapó *et al.*, 1996, Craig *et al.*, 2016, Pedersen *et al.*, 2016). The fat content of experiment 1 and 2 at day 17 ranged from 6.1 to 7.7 %, which is within the expected range of 5.5-11.5 % at this stage of lactation (Hurley, 2015) and similar to concentrations found at this day of lactation in other studies (Krogh *et al.*, 2016b, Pedersen *et al.*, 2016).

The concentration and daily output of milk fat and lactose was unaffected by dietary Val:Lys at day 17 of lactation in experiment 1 (**Paper I**). In most other studies milk fat and lactose was also unaffected by dietary Val:Lys (Moser *et al.*, 2000, Paulicks *et al.*, 2003). The milk fat content is often correlated to the degree of body fat mobilization and fat intake (Hurley, 2015), and these factors were similar for all sows in experiment 1 explaining the similar milk fat content.

In experiment 2 the milk fat and lactose content was similar in all groups at day 3 and 10, whereas at day 17 the milk lactose was higher when sows were fed low dietary SID concentration (**Paper III**), which could be ascribed to the lower milk yield making the milk slightly more concentrated which is accorded with findings by Guan *et al.* (2004b). The linear increase in milk fat at day 17 was probably more an indirect effect of the increased dietary protein as a result of a higher body fat mobilization in sows fed high dietary SID CP concentrations.

7.2.3 Litter performance

The main goal of the lactation period is to wean litters with a high weight and a high milk yield and nutrient output in milk is essential for optimizing piglet growth and survival (Theil and Jørgensen, 2016).

In both experiment 1 and 2 the highest litter ADG was observed in mid lactation (day 10-17), which coincides with the time of lactation when sows reach their maximum milk yield and the highest daily output of nutrients in milk (Hansen *et al.*, 2012b). In experiment 1 all groups had similar ADG of litters, which matches the similar milk yields and milk contents of fat, protein and lactose obtained with the six dietary Val:Lys (**Paper I**). The increased concentrations of BCAA in the milk with increasing dietary Val:Lys of the sows did not seem to affect growth of the piglets. It has been observed that Leu, but not Val and Ile, stimulates the synthesis of protein in cardiac and skeletal muscles of newborn piglets (Escobar *et al.*, 2006), but the concentrations of Leu or the range of this AA in the milk were probably too small to detect any effect on piglet growth. In addition the AA composition of milk was only measured in a limited number of sows in experiment 1. Litter ADG increased until a dietary SID CP concentration of 135 g/kg in experiment 2 (**Paper III**). This increase in ADG of the litter was likely a result of a higher milk yield and also a higher output of protein and fat in the milk at peak lactation (day 17) which is in accordance with similar results reported in other studies with increasing dietary protein (King *et al.*, 1993, Jones and Stahly, 1999).

In experiment 1 and 2 no dietary effects were observed on number of weaned piglets and sows on average weaned around 13 piglets in both experiments (**Paper I** and **III**). Accordingly, this resulted in more piglets having a BW below 5 kg in litters where sows were fed the lower SID CP concentrations in experiment 2 (**Paper III**). Sows were able to nurse the same number of piglets regardless of dietary CP intake, but the milk production was negatively affected by the low CP levels and therefore some piglets in these litters had a lower milk intake and thereby a lower weaning weight. Similarly, King *et al.* (1993) reported that sows fed increasing concentrations of dietary protein (6.3 to 23.8 %) all weaned 9 piglets, but there was a linear increase in the piglet growth rate.

Experiment 2 (**Paper III**) was not designed to test the effect of dietary CP on the prevalence of neonatal piglet diarrhea, but since feeding high protein concentrations to lactating sows often leads to concerns about neonatal diarrhea it was registered (Kongsted, 2013, Kongsted and Bækbo, 2013). Feeding high concentrations of dietary CP did not have any effects on number of diarrhea treatments of the litter. However, the higher prevalence of diarrhea treatments in first parity sows than in multiparous sows indicated that the neonatal piglet diarrhea is more related to differences in immune status between young and old sows in the herd and similar results were reported in a survey in Danish herds by Kongsted *et al.* (2014). The dietary treatments were initiated from day 2 *postpartum*, and neonatal diarrhea is mostly believed to be associated with the protein level fed up till and just after farrowing, which also could explain that no increase in diarrhea treatments were observed.

7.2.4 Body composition, body mobilization and feed intake

The highest loss of BW and BF was recorded from day 2 to 17 in both experiment 1 and 2 (**Paper II** and **III**) compared to a lower loss from day 17 to weaning. In this period the sows had the highest ADG of the litter and reached the maximum milk yield, but the maximum feed intake was not reached before after two weeks. Milk production of the sows has very high priority and therefore the sows mobilize from body reserves to maintain and support milk production with sufficient nutrients (Auldist *et al.*, 1994, Kim and Easter, 2001, Eissen *et al.*, 2003). When the sows reach the maximum feed allowance the body mobilization is lowered, because the requirement for milk production is closer to the actual intake of the sow. Krogh *et al.* (2016a) also found that hyper-prolific sows nursing 13-14 piglets had a higher BW and BF loss from day 3 to 17 (12.7 kg and 3.9 mm) compared to day 17 to 28 (9.4 kg and 1.7 mm) *postpartum*. However, in the study by Krogh *et al.* (2016a) the BF loss of sows was higher in both the first and last period of lactation compared to experiment 1 and 2. Opposite the BW loss from day 2 to 17 in experiment 1 was higher (15.9-20.4 vs. 12.9 kg) than in the study by Krogh *et al.* (2016a), which probably was caused by a lower intake in experiment 1 during week 1 and 2 of lactation (**Paper II**) and therefore sows had to mobilize more from body reserves to maintain the same litter gain as the piglets in the study by Krogh *et al.* (2016a). The regression models developed in experiment 1 (**Paper II**) described that the BW loss besides being affected by feed intake also increased with increasing BW at standardization. This indicates that sows with larger body reserves have a higher capacity for body mobilization than smaller sows. In the study by Krogh *et al.* (2016a) the BF thickness at litter standardization was higher (21 mm) compared to in experiment 1 and 2 (14.2 to 15.1 mm) and at the same time feed intake was lower during the last two weeks (6.8 to 6.9 vs. 7.3 to 7.7 kg/d [second parity sows in **Paper II**]) in the study by Krogh *et al.* (2016a). Other studies have also reported that sows with a higher BF thickness at farrowing (21.6 to 26.8 mm) have higher losses (4.3 to 9.3 mm) of BF during lactation (King *et al.*, 1993, Dourmad *et al.*, 1998, Quesnel *et al.*, 2005a), whereas studies with sows with similar BF thickness at farrowing as in experiment 1 and 2 had comparable losses (van den Brand *et al.*, 2000, Pedersen *et al.*, 2016). This illustrates the interplay between feed intake, milk production and body mobilization and the ability of hyper-prolific sows to use the body reserves as a buffer to maintain a high milk yield. It also emphasizes that strategies to minimize body mobilization should be applied during the first part of lactation, because this is where sows have the highest BW and BF losses, the lowest intakes and reach peak milk production.

In experiment 1 the increased dietary Val:Lys did not affect changes in BW and BF thickness of the sows (**Paper I**), which makes good sense since both feed intake and milk yield were similar for all sows and body reserves are generally used to compensate for a lower intake to maintain milk production. This was also observed in other studies where similar dietary Val:Lys as in experiment 1 was tested (Carter *et al.*, 2000, Paulicks *et al.*, 2003, Gaines *et al.*, 2006, Craig *et al.*, 2016).

The decreased BW loss found with increasing dietary SID CP concentration in experiment 2 was possibly mainly a result of a decrease in mobilization of body protein as confirmed by the observed changes in body protein on a subsample of the sows (**Paper IV**). Other studies have also reported that high protein diets (14.7 to 20.7 % CP) generally minimize the protein loss (Jones and Stahly, 1999, McNamara and Pettigrew, 2002). Accordingly the increasing BF mobilization indicated that fat mobilization was not minimized. Similar results were reported by Dourmad *et al.* (1998) suggesting that the extra dietary protein was used for milk synthesis. The sows given higher dietary CP concentrations in experiment 2 most likely were in an energy deficit and therefore mobilizing more from body fat reserves. This also explains the higher concentration of milk fat in these sows compared to sows in lower dietary CP concentrations. McNamara and Pettigrew (2002) confirmed that increasing dietary protein intake decreased protein mobilization and when adding more fat to the diets the fat mobilization was also reduced.

When sows turn catabolic during lactation they mobilize from both protein and fat pools of the body (Hansen *et al.*, 2014) as observed in experiment 2 (**Paper IV**), but the ratio between mobilized fat and protein differs with nutrient intake and degree of body mobilization. The body fat pool of the sows in experiment 2 at day 3 and 24 was similar to the findings by Pedersen *et al.* (2016), whereas the protein pools were slightly higher. The reason was most likely that second and third parity sows were included in the body composition measurements in experiment 2 and in the study by Pedersen *et al.* (2016) only second parity sows were used. Quantitatively the sows in experiment 1 (**Paper II**) and 2 (**Paper IV**) mobilized more fat than protein in accordance with other studies (Jones and Stahly, 1999, Quesnel *et al.*, 2005a, Pedersen *et al.*, 2016).

It is often discussed at what level of BW or BF loss the mobilization becomes harmful to the sow, however these experiments were not designed to specifically answer this question. Generally the feed or nutrient intake must be very insufficient and the mobilization high, before milk production is compromised (King *et al.*, 1993, van den Brand *et al.*, 2000). In experiment 2 (**Paper III**) it was observed that sows fed SID CP concentrations below 135 g/kg had lower litter gains in spite of increased BF mobilization, so at this dietary CP level sows were not able to compensate fully by increasing body mobilization.

7.2.5 Sow performance in the following reproductive cycle

Low feed intakes and body mobilization during lactation can have negative effects on the following reproductive cycle. The number of total born piglets in the next litter in experiment 1 was unaffected by dietary Val:Lys, which was expected because neither any differences between feed intake nor body mobilization of the sows were observed. In experiment 1 (**Paper II**) a high feed intake had a positive effect, whereas losing a high proportion of BW during lactation had a negative effect on the number of total born piglets in the next litter. Sows fed the low dietary SID CP (104 and 133 g/kg) in experiment 2 (**Paper III**) gave birth to to more than 1 pig less in the next litter compared to sows fed the higher concentrations. These sows had a higher protein loss, but lower BF loss, which indicates that the negative effect on the following reproductive cycle was mainly caused by the protein mobilization as reported in other studies (Yang *et al.*, 2000a, Mejia-Guadarrama *et al.*, 2002). However, in experiment 1 (**Paper II**) first parity sows lost the same proportion of body protein but more body fat compared to second parity sows, which resulted in a decreased litter size in the following cycle. Primiparous sows were probably more sensitive to fat mobilization than older sows, because first parity sows have less body fat at farrowing. In line with this Schenkel *et al.* (2010) observed that first parity sows dropped in number of total born piglets in the second litter when mobilizing more than 20 % of their body fat, and primiparous sows in experiment 1 lost 26 % of their body fat pool. Second parity sows did not increase litter size as parity 3 and 4 sows did in the following cycle (**Paper II**), so the higher level of fat and protein mobilization also seemed to affect second parity sows negatively. From experiment 1 and 2 it cannot be concluded specifically at what level fat and protein mobilization becomes harmful, but it is certain that both protein and fat mobilization during lactation can have negative effects on the reproductive performance in the following cycle especially in first and second parity sows. This was confirmed in the study by Quesnel *et al.* (2005b) where heavier sows were less negatively affected by a dietary protein restriction compared to lighter sows, because older and larger sows had a greater body protein pool as buffer.

The regression models developed in experiment 1 (**Paper II**) showed that feed intake and body condition during lactation had an effect on number of piglets born in the next cycle, but since the models explained only a little of the variation observed in total born piglets many other factors between mating and farrowing must also be involved.

When comparing the number of total born piglets in next litter in experiment 1 (**Paper II**) and 2 (**Paper III**) it should be noted that litter sizes in almost all sows were larger than the national average in Denmark at 17.6 total born piglets (Jessen, 2016). The only exception was primiparous sows in experiment 1 (15.2 piglets) and even the sows fed low levels of dietary protein in experiment 2 gave birth to more piglets than the average (17.7-17.8 piglets), which emphasized that this herd indeed had hyper-prolific sows.

7.2.6 Plasma metabolites

Concentrations of metabolites in the plasma are depending on nutrient intake, feeding level, stage of lactation, production level and sampling time relative to feeding. Nutrients or precursors for nutrients for milk synthesis will be transported via the blood from either the gastro intestinal tract or from body pools to the mammary gland (Bauman and Bruce Currie, 1980). In both experiment 1 (**Paper I**) and 2 (**Paper IV**) four blood samples were collected during lactation and in addition a sample was taken 4 days *prepartum* in experiment 2.

The blood glucose is narrowly regulated to maintain homeostasis and sows in both experiment 1 and 2 were fed similar levels of starch and fiber and therefore as expected no dietary effect was observed for the plasma glucose concentration. Glucose is the main substrate for milk lactose and therefore large quantities are needed, which explains that in experiment 2 (**Paper IV**) the plasma glucose concentration was higher during lactation compared to gestation as a result of the higher requirement of glucose for milk production compared to fetal growth (Rojkittikhun *et al.*, 1993, Mosnier *et al.*, 2010, Hansen *et al.*, 2012a). The increasing glucose concentration from early to late lactation in both experiments (**Paper II** and **IV**) accentuated the increasing requirement of glucose for milk lactose synthesis with increasing milk yield.

Lactate in plasma was similar at all sampling times in both experiments (**Paper II** and **IV**). Elevated levels can be observed around parturition, because of the increased production of lactic acid as a result of muscle contractions during farrowing (Mosnier *et al.*, 2010, Hansen *et al.*, 2012a), but sampling day 4 *prepartum* and 3 *postpartum* was probably not close enough to farrowing to perceive this.

In both experiment 1 (**Paper II**) and 2 (**Paper IV**) NEFA concentrations were higher at day 3 and 10 than at day 17 and 24/26 *postpartum*. The higher concentrations of NEFA in the first part of lactation fit well with the higher mobilization of BF observed in this period compared with lower BF loss in late lactation. However, the significant differences in BF and BW loss observed between dietary treatments in experiment 2 was not followed by altered NEFA concentrations with increasing dietary SID CP intake (**Paper IV**). This was in accordance with results of Rojkittikhun *et al.* (1993), whereas other studies report a connection between NEFA and BF loss (Revell *et al.*, 1998, Valros *et al.*, 2003). The differences in BF loss between the six treatment groups in experiment 2 (**Paper III**) was smaller than the difference observed from early to late lactation, therefore larger differences is probably required to detect significant changes in NEFA in relation to dietary treatments.

Non-esterified fatty acids are used as precursors for cholesterol synthesis in the liver (Rojkittikhun *et al.*, 1993), and therefore the increase in plasma cholesterol during lactation observed in experiment 2 (**Table 7**) was in line with the decrease in the concentration of NEFA.

Triglycerides are used as precursors for milk fatty acid synthesis in the mammary gland (Boyd and Kensinger, 1998), which could explain the lower concentrations observed in early lactation compared to late gestation in experiment 2 (**Table 7**) indicating an increased uptake of triglycerides by the mammary gland during lactation as suggested by Mosnier *et al.* (2010) and Neil (2000).

The decreasing plasma creatinine concentration found throughout lactation in both experiments 1 (**Paper II**) and 2 (**Table 7**) indicates a higher rate of protein turnover in the first part of lactation compared to late lactation, which is similar to results reported in other studies (Rojkittikhun *et al.*, 1993, Neil, 2000, Mosnier *et al.*, 2010). In the first part of lactation protein turnover is caused both by regression of the uterus and muscle mobilization, whereas in late lactation protein turnover is mainly a result of muscle breakdown. The plasma creatinine concentration at the individual sampling days were similar in experiment 1 (**Paper II**) and 2 (**Table 7**) even though the protein intake and protein mobilization differed both between experiments and groups within experiment 2. This indicated that plasma creatinine probably most reflects that the total protein pools of sows were similar in the experiments and therefore had the same rate of protein turnover throughout lactation. The changes in daily protein mobilization observed in experiment 2 between dietary groups (**Paper IV**) were probably too small relative to the total protein pool of the sows to cause a change in plasma creatinine concentrations. Rojkittikhun *et al.* (1993) did also report similar plasma creatinine concentrations of sows losing 10 and 33 kg during lactation, respectively, whereas Neil (2000) observed higher creatinine concentrations in sows losing 20 kg compared to zero BW loss during lactation.

The decrease in total plasma protein in late lactation in experiment 2 (**Table 7**) could be a result of the higher milk production requiring a higher uptake of AA for milk protein synthesis at this stage of lactation. Pigs severely undersupplied with dietary protein can use blood albumin as a protein reserve (Wykes *et al.*, 1996), which can explain the decrease in plasma albumin observed with decreasing dietary SID CP intake of sows in experiment 2 (**Paper IV**). Gamma-glutamyl transferase is an enzyme involved in the uptake of amino acids by the mammary gland (Viña *et al.*, 1981b, Viña *et al.*, 1981a). The increased concentrations the enzyme observed with higher concentrations of dietary SID CP agrees therefore well with the higher milk CP content detected in sows fed high dietary protein concentration (**Paper III and IV**). Reversely, the concentrations of the enzyme alanine amino transferase decreased with higher concentrations of dietary protein. This could be explained by a higher muscle protein mobilization and thereby an increased activity of urea cycle enzymes in sows fed low dietary protein concentration (Das and Waterlow, 1974).

Plasma urea N was measured in both experiment 1 (**Paper I**) and 2 (**Paper IV**) and in both experiments an increased PUN concentration was reported from early to late lactation reflecting the general increase in protein intake throughout lactation, which also was observed in several other studies (Rojkittikhun *et al.*, 1993, Neil, 2000, Yang *et al.*, 2000b, Quesnel *et al.*, 2009, Mosnier *et al.*, 2010). In experiment 1 (**Paper I**) no differences were observed in the concentration of PUN, which was similar to results of Roth-Maier *et al.* (2004) who found no response to increased Val:Lys in PUN. This indicates that the AA composition of all six diets in experiment 1 was equally good, which was confirmed by the lacking response on sow and piglet performance. On the contrary, the PUN concentrations measured in experiment 2 (**Paper IV**) showed a clear response to the increased dietary protein intake of the sows. The N-balance was optimized at the lowest PUN concentration (Coma *et al.*, 1995, Coma *et al.*, 1996b), and in late lactation (day 17 and 24) N-balance was optimized at lower dietary SID CP concentration (133 vs. 139 g SID CP/kg) than in early lactation (day 3 and 10). This difference can probably be ascribed to the lower intake in the first 2 weeks of lactation compared to the last 2 weeks, so a higher dietary SID CP concentration was required to saturate the N-balance in early lactation. It is important to mention that the requirement determined by the most optimal N-balance might not be the level of dietary protein resulting in the highest production (milk or piglet growth) of the sow (Coma *et al.*, 1996a). In experiment 1 and 2 the measured PUN concentration was a good indicator of both dietary protein quality and intake and N-balance of the sows.

Amino acids in the blood of the sows are taken up by the mammary gland and used for synthesis of milk CP and in addition the AA in the blood represents AA made available from the diet or body protein pools. In experiment 1 (**Paper I**), where whole blood concentrations of AA were measured, no effects were observed on AA concentrations when increasing the Val:Lys. The lacking response to the dietary treatments can be explained by the small variation in the daily Val intake between the six groups (41-47 g/day). Whereas, other studies with larger variation in daily Val intakes (20-64 and 47-71 g/day) have reported an effect on Val concentration of the blood (Richert et al 1996, Roth-Maier et al 2004).

7.2.7 Efficiency of utilization of dietary protein for milk

In experiment 2 (**Paper III**) it was shown that it was important that sows were fed adequate amounts of protein for optimizing milk production and at the same time prevented a high mobilization from body reserves to ensure the following reproduction. On the other hand an excessive oversupply with protein will have negative consequences for the environment through increased excretion of N and in addition it is also known that a supply with surplus protein will decrease the efficiency of utilizing dietary protein for the different life processes.

The estimated efficiency for all sows in experiment 2 (**Figure 9**) of utilizing dietary SID protein for milk protein was 0.75, but from the optimums found in the breakpoint analyzes it was assumed that sows fed the two highest SID CP concentrations (139 and 150 g SID CP/kg) were oversupplied with protein. Therefore, as expected, the efficiency increased to 0.78 when only including sows from the four lowest dietary CP groups (104 to 129 g SID CP/kg). The NRC (2012) reports an efficiency for SID CP for milk protein at 0.76 and this is close to the efficiencies found in experiment 2. Huber *et al.* (2016) also observed that the efficiency of utilizing N for milk protein decreased from 68.2 to 48.9 % when increasing dietary protein from 14.6 to 16.0 %.

Experiment 2 was not designed to determine the optimum dietary Lys concentration, but it was still interesting to investigate how efficient the utilization of dietary Lys for milk Lys was in these sows. The efficiency was 0.86 and 0.91 when including all sows (5.8 to 9.0 g SID Lys/kg) and only sows fed the four lowest SID Lys concentrations (5.8 to 7.5 g SID Lys/kg), respectively (**Figure 10**). These efficiencies are higher than 0.67 and 0.60 to 0.71 reported by NRC (2012) and Huber *et al.* (2016), respectively.

The daily SID Lys intakes used by NRC (2012) to estimate the efficiency ranged approximately from 15 to 30 g, whereas in experiment 2 the SID Lys intakes for milk Lys was 20 to 45 g which was similar to intakes in the study by Huber *et al.* (2016). NRC (2012) did not base their estimations on hyper-prolific sows as in this thesis, and despite similar Lys intakes as in experiment 2 was reported by Huber *et al.* (2016) the sows in that study had lower litter ADG, milk yields and concentration of protein in the milk.

As a result of the experimental design it was uncertain at which concentration Lys became limiting for the sows in experiment 2. However, the diet with the limiting SID CP concentration might not have the limiting Lys concentration too as reported by Huber *et al.* (2016). The study by Huber *et al.* (2016) showed that the efficiency for N and Lys utilization was highest when fed 14.6 and 15.7 % CP, respectively. Based on this the sows in experiment 2 were more efficient in utilizing dietary Lys for milk Lys than in the mentioned study (Huber *et al.*, 2016), because more Lys was excreted in the milk at similar Lys intakes. The content of Lys was not analyzed in experiment 2, so the value of 6.9 g/100 g CP obtained in experiment 1 was used. As discussed earlier the milk Lys content was lower than in other international studies on AA composition in milk, but similar to the Lys levels determined in other studies on hyper-prolific Danish sows. A higher Lys content of the milk in experiment 2 would have caused an even higher efficiency.

7.2.8 Determination of the optimum valine-to-lysine ratio and the protein requirement

There are large discrepancies in the literature regarding the optimum dietary Val:Lys for lactating sows. It was therefore important to determine the optimum Val:Lys of the diet in experiment 1 (**Paper I**) before investigating the effects of increasing the concentration of all AA in experiment 2 (**Paper III**), because an unbalanced composition of AA in dietary protein would make the utilization less efficient.

The lacking responses to increasing dietary Val:Lys in experiment 1 indicated that the optimum total Val:Lys was 0.84, or most likely even lower since there was no breakpoint within the tested range of ratios in experiment 1 (**Paper I**). If the optimum Val:Lys was actually above the tested ratios a linear increase with increasing Val:Lys in for instance litter ADG had been expected. Therefore on the basis of experiment 1 it was recommended that the dietary total Val:Lys need not to be higher than 0.84 to fulfill the requirement of the sows.

The litter ADG is the most important determinant of the dietary nutrient requirements of the sow during lactation and in experiment 2 the maximum ADG was observed at 135 g SID CP/kg (**Paper III**). It is, however, important when determining the requirement of lactating sows also to consider other parameters as body mobilization. When comparing the breakpoints for the other main response variables in **Table 8** it should be noted that the breakpoints for BF loss, body protein loss and PUN (day 17 + 24) were all below 135 g SID/kg, whereas milk CP content and PUN (day 3 + 10) were only slightly higher. The breakpoints for the main response variables were all below or close to the dietary SID CP concentration of the optimal N-balance except for BW loss, where the break-point was at a higher concentration. This indicates that the SID CP concentrations of PUN at the optimum N-balance was similar to the level where sows and piglets performance was also optimized. Although BW loss continued to decrease up till 143 g SID CP/kg no negative effects were observed on the following reproductive cycle for sows fed 135 g SID CP/kg. Since litter ADG, fat and protein mobilization, milk CP content and N-balance was optimized at a SID CP concentration of 135 g/kg it was recommended that lactating sows should be fed diets with this CP level.

8 CONCLUSIONS

Overall this thesis adds knowledge to the research gap of Val and protein requirements of hyper-prolific lactating sows nursing 13-14 piglets. Sow and piglet performance was not improved by increasing the total dietary valine-to-lysine ratio above 0.84 and sow productivity and metabolism was optimized at a dietary SID protein concentration of 135 g/kg. In addition the thesis in general contributes with new information of the metabolism of hyper-prolific sows. The main findings were:

- Increasing the dietary Val:Lys above 0.84 did not improve sow or litter performance during lactation. However, increasing Val:Lys increased the concentrations of BCAA in milk.
- Sows with the highest milk yield and litter ADG simultaneously had a high feed intake and body mobilization. Moreover, a low feed intake and high mobilization during lactation had a negative effect on the number of piglets born in the next litter.
- During the first half of lactation sows had higher body mobilization compared to late lactation. Moreover the litter reached maximum growth rate just before peak lactation.
- Plasma creatinine and NEFA concentrations were higher in early compared to late lactation indicating a higher mobilization or turnover of both protein and fat in early lactation.
- Increasing dietary SID protein concentration during lactation decreased sow BW loss and protein mobilization, but increased BF loss.
- The N-balance was saturated, expressed as the lowest PUN concentration, at a lower SID CP concentration in late lactation compared to early lactation as a result of a higher feed intake.
- Litter ADG was increased with higher dietary concentrations of SID protein as a result of improved milk yield and higher concentrations of milk protein and fat at peak lactation.
- The number of piglets born in the following reproductive cycle was negatively affected in sows fed low SID CP concentrations during lactation.

9 IMPLICATIONS AND PERSPECTIVES

9.1 Practical implications

Experiment 1 showed that there was no need to increase the total dietary Val:Lys above 0.84. The current Danish recommendation was for practical reasons kept at 0.76, because it is difficult to formulate at normal diet based on soy-bean meal, wheat and barley with Val:Lys below this level.

The results of experiment 2 clearly showed an improvement in sow and piglet performance when increasing dietary SID CP, which emphasized that the Danish recommendation was too low and it therefore resulted in new dietary recommendation in 2015 for SID CP and SID Lys (Tybirk, 2016). These recommendations are now used in practice by the feeding industry, consultants and farmers in Denmark. Increasing the dietary SID CP concentration of diets for lactating sows, based on results of this thesis, has yielded a significant economic gain for the farmers through increased weaning weights of the piglets and possibly improved longevity of the sows.

9.2 Future perspectives

The dietary recommendation of most single EAA except for Val did not differ between countries, but most of them except Lys have not been determined on hyper-prolific sows or are only the best estimate based on milk composition. Therefore, as more EAA becomes commercially available as crystalline additives future research ought to investigate the requirements of these AA.

Experiment 2 raised several new questions to be answered. First, were the improvements in sow and piglet performance caused by the increased concentration of CP or Lys or both? Recently, a major focus has been on decreasing the environmental impact of swine production by amongst others decreasing the N excretion by the pigs. Future research ought to investigate this by dose-response trials with increasing concentrations of SID CP when maintaining the concentrations of Lys and other EAA to determine if the dietary CP content can be decreased without compromising the productivity of the sows. Such trials are important to make sure that the sows are not fed excessive amounts of CP, which then is catabolized and excreted into the environment. Second, the mobilization of body protein was minimized with increasing dietary CP concentration, but at the same time the BF loss increased, which indicates that future research also needs to investigate if the sows require more energy during lactation, when protein supply is higher. Additionally, it should be examined if additional energy should be given as fat or starch. This is relevant, because it will show if sows given extra energy combined with more SID CP can further improve their milk production and litter ADG. It will also add knowledge to the gap about the interplay between energy intake, fat mobilization and milk production.

Both experiments showed that body mobilization was highest during the first half of the lactation period and at the same time litter gain and milk production peaked. Hence, new feeding strategies with for instance different protein levels, energy levels or energy sources should be explored to differentiate between early, mid and late lactation in order to optimize the overall lactational performance of the sows.

Furthermore, it was observed that primiparous sows had a lower productivity and were more sensitive to body mobilization than multiparous sows, which resulted in a lower number of piglets born in next litter. Feeding during lactation, especially of young sows, needs to focus on optimizing performance during lactation without compromising the following reproductive cycle. Therefore differentiated feeding strategies for primiparous sows compared to multiparous sows should also be explored further.

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