Cover page

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210172E022 - AP544494 - Speciale i agrobiologi

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Hand-in information

Titel: Effect of reduced dietary protein and roughage intake on metabolites in plasma, urine and milk from gestating and lactating organic sows during winter

Titel, engelsk: Effect of reduced dietary protein and roughage intake on metabolites in plasma, urine and milk from gestating and lactating organic sows during winter

Indeholder besvarelsen fortroligt materiale: No

Må besvarelsen gøres til genstand for udlån: Yes

Effect of reduced dietary protein and roughage intake on metabolites in plasma, urine and milk from gestating and lactating organic sows during winter

Master's thesis

60 ECTS, Agrobiology, Organic Agriculture and Animal Science
Submission: 7th of June 2021

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Preface and acknowledgement

This thesis was written as the final part of my master degree in Agrobiology at Aarhus University. This 60 ECTS point thesis, was carried out from September 2020 to June 2021, and includes literature review, experiment work, analysis of data, and interpretation and discussion of the analyzed data.

The practical experiment was carried out at the Organic Platform at Foulum, Aarhus University, from ultimo November 2020 to primo April 2021. During the experimental period I participated in weighing and backfat scanning of sows, sampling of blood, milk, urine and feces from sows and weighing of piglets. Moreover, I aided in the preparation of urine and feces samples for analysis and analyzed milk samples. In addition, I participated in practical work in another experiment from the current project; WI-FI. Afterwards, I organized the raw data, by preparing it for statistical analysis. At last, I performed all the statistical analysis presented in the thesis, which includes data from current experiment and data from the earlier project; EFFORT.

This thesis aims to increase the knowledge of protein metabolism of organic gestating and lactation sows, in order to adapt feeding strategies to the requirement of the organic sow. Also, light is shed upon the nutritional contribution of roughage and other potential benefits of supplying roughage to sows. This thesis is addressed to students, scientists, and advisors with interest in organic sow production and roughage, however organic feed producers and organic farmers would also benefit from current thesis.

First, I would like to show my gratitude towards my main supervisor, Peter Kappel Theil, who has put a lot of time into supervisor meetings, answering e-mails and guiding me in the right direction during the development of this thesis. Also, a special thanks to my co-supervisor Maria Eskildsen, who has overseen the experimental work and supplied me with the obtained data throughout the thesis. Moreover, thanks for participation in supervisor meetings, and reading and commenting parts of my thesis. Also, thanks to my other co-supervisor Anne Grete Kongsted, especially for helping me become a part of this project in the first place. In addition, I would like to thank Signe, Sigrid and Takele for participating in the experimental work, during early mornings and in cold weather. At last, I would like to thank my girlfriend, Celia, for proofreading and supporting me throughout the writing of this thesis.

Abstract

Oversupply of protein is an issue in organic sow production, due to disagreements between supply and requirement. This affects the metabolism and productivity of sows, and environmental impact and economy of organic sow production. Current thesis considers the effect of reducing the dietary content of protein in gestational compound feed for organic sows, specifically during winter conditions. Metabolites in plasma, urine and milk were studied to provide insight of how reducing the dietary protein concentration affects the sow's metabolism. Moreover, the nutritional significance of roughage was investigated.

Organic reared sows were fed one of two isoenergetic diets, containing 76 g or 63 g SID CP pr. kg during gestation and identical diets during lactation. Furthermore, sows were fed either clover-grass silage or barley-pea whole crop silage.

No negative effects of reducing the content of dietary protein in compound feed for gestating organic sows was found, indicated by physically measures and plasma, urine and milk metabolites. At day 60 of gestation the urinary content of urea was 23 % higher in sows fed high dietary protein. During lactation the milk yield of sows fed low protein became gradually greater as lactation proceeded. Concurrently, the feed intake and litter gain were improved by feeding low protein during gestation. Roughage proved to be a valuable nutrient contributor during gestation, and clover-grass silage contributed 8.0 MJ ME/d and 1.9 g SID lysine/d, while the contribution of barley-pea whole-crop silage was considerably lower.

In conclusion, reducing the dietary content of compound feed for organic gestating sows is recommendable. Also, the nutritional contribution of roughage should be taken into consideration when planning the feeding strategy of organic sows.

Summary

Organic sows are fed excessive dietary protein. Current thesis investigates the effect of reducing the dietary concentration of protein during gestation, on the metabolism and productivity of organic sows during winter conditions, using metabolites in plasma, urine and milk as indicators. Moreover, the nutritional contribution of roughage is studied.

Organic agriculture is shaped on a set of coherent values, namely the principle of health, ecology, fairness and care. The organic legislation is shaped by these values, which is expressed in the sow production, where sows have outdoor access, are supplied roughage, have a longer lactation period, among others. The organic production system is a contrast to the indoor conventional sow production, and consequently the requirement of the sows differs. Most noticeable organic sows have a greater requirement of energy, due to an increased need for cold thermogenesis, especially in winter conditions. The organic sow's requirement for protein is however not different from the indoor sows' requirement. Nevertheless, organic and conventional sows are fed according to the same feeding standards. Another important aspect affecting the nutrition of organic sows is the access to roughage. Besides providing several benefits in terms of welfare and health, roughage contributes to the nutrition of the sows. When fed restricted, roughage may contribute with up until half of the daily total energy intake and a substantial proportion of the amino acids required, depending on the type and quality of roughage.

In addition to physical measurements, metabolites found in plasma, urine and milk are useful indicators and provides a detailed view upon the metabolism of the sow. The five major metabolites contain information about specific parts of the metabolism, where glucose, lactate and TG are correlated to the energy metabolism, urea to the nitrogen metabolism and NEFA to body mobilization. Besides these five main metabolites, a large range of minor metabolites exists and provides even more details about the metabolism. In addition to the metabolites, milk production and chemical composition of milk are affected by the metabolism and feeding of the sows and are central in the reproductive performance of sows.

Adjusting the protein supply to sows is a matter of balance. Feeding sows too much protein, increases the amino acid oxidation and urea production, which is energy-costly and ultimately reduces the feed efficiency. Moreover, it constitutes an environmental problem. On the contrary supplying sows insufficient protein may reduce productivity and causes body mobilization to accommodate the nutritional needs for the highly prioritized reproductive traits. Thus, the dietary concentration of protein must be carefully optimized.

A total of 21 sows (Topigs Norsvin; TN70) was included in current study from day 30 of gestation until weaning at day 49 of lactation. The sows were housed under typical production conditions of outdoor

organic sows in winter conditions. During gestation sows were on one of two isoenergetic diets: An organic commercial diet containing 76 g SID CP pr. kg or a low-protein diet containing 63 g SID CP pr kg. In the lactation period all sows were fed the same organic commercial lactation diet. Besides, sows were fed either clover-grass silage or barley-pea whole-crop silage during the entire experimental period.
At day 60 and 100 of gestation blood, urine and feces were sampled and at day 5 and 20 of lactation blood, milk, urine and feces were sampled. At all sampling days, including day 30 of gestation and day 49 of lactation, sows were weighted and backfat scanned and piglets were individually weighted. Besides, plasma, urine and milk from sows fed different protein levels during lactation from a previous study, was analyzed for a range of minor metabolites.

During gestation the deposition of body reserves was not affected by dietary protein level. An interaction ($P<0.05$) showed that the concentration of urea in urine from control sows only at day 60 of gestation was 23 % higher compared to sows fed low protein diets. Moreover, the roughage intake during gestation supplied a substantial amount of nutrients, where clover-grass silage in average supplied 1.7 MJ ME/d and 0.36 g SID lysine/d, equal to 14 % ME, and 13 % and 17 % SID lysine of the total intake, for sows fed control and low dietary protein diet, respectively. The nutritional contribution of barley-pea whole-crops silage was approximately a fourth of this. During lactation the reproductive performance of the sows were very good, with an average peak milk yield of 16.2 kg/d at day 20. Consequently, the litter gain was very good, and sows weaned in average 13.3 piglets, that each weighed 19.9 kg at day 49. However, as indicated by plasma NEFA, body mobilization was high, and the sows' metabolism was under significant pressure indicated by plasma glucose. From day 5 to 20 the milk yield of sows fed low protein during gestation, became gradually higher compared to control sows ($P<0.05$). Accordingly, the litter gain was greater ($P<0.01$), thus the litter weight became gradually heavier as lactation proceeded ($P<0.001$). Despite, the higher milk production, low protein sows did not mobilize more body reserves compared to control, presumably due to a slightly higher feed intake. Of the minor metabolites, urinary urea was the only metabolite affected by dietary protein level ($P<0.05$), showing that control sows were fed excessive amounts of protein. Moreover, the metabolites provided several details of how the lactational period affected the metabolism of the sow.

In conclusion reducing the dietary content of SID CP in gestational compound feed to 63 g/kg does not affect the productivity of organic sows during winter conditions negatively. On the contrary, it seems to improve the nitrogen balance in gestation and improve the reproductive performance. Thus, there seems to be no obstacles preventing a reduction in the protein content in compound feed for gestation sows in commercial organic sow production. Moreover, clover-grass silage can contribute a substantial amount of nutrients, which should be accounted for in the feeding strategy.

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1. Introduction

The organic sector in Denmark has gone through a significant development the last decades. In 2019, 11,5 % of all agricultural land in Denmark was organically cultivated, which is more than double the percentage compared with 10 years ago (Danmarks Statistik, 2020). Looking at the organic pig production a similar positive development is seen and 1,3 % of all sows in Denmark in 2019, was to be found on organic farms (Danmarks Statistik, 2020, Landbrugsstyrelsen, 2020a). Furthermore, the market share of organic pork in Denmark was 3,2 % in 2019, which is however, relatively low compared to other organic foods (Hindborg, 2020).

Organic food production is believed by consumers to be the healthy, safe and environmentally friendly choice, in contrast to conventional farming (Harper Gemma and Makatouni, 2002). Also, looking into detail of the organic principles; the pillars of organic agriculture, sustainability is a key term used to describe organic farming (IFOAM, 2020). Organic pig production is perceived to have a higher level of animal welfare compared to conventional pig production, mainly due to differences in the production systems (Font-i-Furnols et al., 2019). Most noticeable is the outdoor access, organic feed ration not containing GMOs, roughage access, prolonged lactation, restricted medicine use and ban of pesticides, which is required by legislation (Regulation (EU), 2018). Furthermore, in Denmark organic pig producers have agreed upon stricter rules, to ensure that the organic pig production fulfills the industry's expectations (Brancheaftale, 2018).

As a result of this alternative production system, organic pigs, especially the sows, have requirements which deviate from conventional pigs. The energy requirement of organic outdoor sows is generally higher, due to markedly increased requirement for thermoregulation, potentially higher level of physical activity and prolonged lactation. It is estimated that outdoor sows require 15% to 20% higher energy intake, as compared with indoor pigs (Edwards, 2003, Close and Poornan, 1993). The outdoor conditions of organic production, do however most likely not affect protein requirement of organic sows, as compared with indoor sows.

To meet the higher energy requirement, organic sows are fed a higher quantity of compound feed. In Denmark it is common practice that organic pig producers follow conventional feed standards. Hence, organic outdoor sows are fed the same minimum ratios of protein and essential amino acids relative to energy, as the conventional indoor sows, even though the requirement of the sows most likely differ, due to the increased feed intake. The result is an oversupply of protein compared to the requirement of conventionally fed sows. In addition, since crystalline amino acids is not allowed in organic agriculture, the amino acid profile of the feed is far from being near ideal, which is in contrast to the requirement of conventional sows. An unbalanced amino acid profile can result in reduced efficiency of protein utilization, hence increased nitrogen excretion (Kim et al., 2009, van Milgen and Dourmad, 2015).

The consequence of this surplus is of environmental concern, namely in terms of nutrient leaching from the paddocks (Eriksen et al., 2002, Halberg et al., 2010), but furthermore serves as an energy expenditure for the animal, as the surplus is excreted in the urine as urea, created in an energy costly process (Pedersen et al., 2019b). In addition to the oversupply of protein through the compound feed, the pigs also consume vegetation from the pasture during the summer and various types of preserved roughages in the winter period. The nutritional contribution from this, is rarely accounted for in the diet, and might further contribute to the oversupply of dietary protein.

To accommodate the problem of protein oversupply, the ratio of protein to energy in the compound feed must be adapted to the conditions of organic sow production, namely by reducing the dietary protein content. Furthermore, the intake of roughage must be quantified and calculated within the dietary supply of nutrients for the sow.

1.1 Objectives

This master thesis is a part of the ongoing projects; *Value Added Through Efficient Organic Pig Production* (EFFORT) and *Winter Feeding of Organic Sows* (WI-FI). The purpose of the project EFFORT is to gain basic knowledge regarding the nutritional requirement of outdoor organic sows, in order to compose a feed formulation adapted to the organic conditions. As a continuation of EFFORT, the project WI-FI was initiated. WI-FI addresses, like EFFORT, the challenges regarding composing optimal feed for organic sows, with emphasis on winter feeding and the roughage supply during this period.

This master thesis considers the effect of feeding organic outdoor sows a low protein feed ration and intake of different types of roughages on the sows' metabolism, with emphasis on metabolites in blood plasma, urine and milk. The specific problem statements within this thesis is:

- How are the sow's metabolism affected by feeding low dietary protein, using metabolites in plasma, urine and milk as markers?
- How does the supplementation of roughage contribute to the nutrition of the sow?
- Is it feasible in terms of productivity to reduce the dietary content of protein in diets for gestating organic sows?

1.2 Abbreviations

AA	Amino acid
ADF	Acid detergent fiber
BF	Backfat
BWS	Barley-pea whole-crop silage
CAA	Crystalline amino acid
CP	Crude protein
CS	Clover-grass silage
DF	Dietary fiber
DM	Dry matter
EAA	Essential amino acid
FUsow	Feed unit for sow
FUgp	Feed unit for growing pig
GE	Gross energy
iNDF	Indigestible neutral detergent fiber
LCT	Lower critical temperature
LW	Liveweight
ME	Metabolizable energy
MY	Milk yield
N	Nitrogen
NDF	Neutral detergent fiber
NE	Net energy
NEFA	Non-esterified fatty acid
NFE	Nitrogen free extracts
NSP	Non-starch polysaccharide
PPE	Potential physiological energy
SID	Standardized ileal digestibility
TG	Triglyceride
UCT	Upper critical temperature
VFA	Volatile fatty acid
WSC	Water soluble carbohydrate

2. Literature review

2.1 The organic pig production system

Organic farming is not simply a specialized type of production based on a specific set of rules, allowing the farmer to marketing products as organic. It is a concept of farming founded on a set of coherent values (Vaarst et al., 2004). The international organic organization IFOAM, has defined these core values and formulated four principles of organic agriculture (IFOAM, 2020): The principle of health, ecology, fairness and care. Animal welfare, naturalness, responsibility and care for the environment and future generations are just some concepts mentioned in these principles. The organic principles should not be perceived as legislation to obey, but rather a mindset that organic farmers should possess and the framework that organic farming should operate within.

2.1.1 Organic regulation

Based on the organic core values, legislation has been formed. The legislation transforms the organic principles into a more rigid structure. These are the guidelines that organic farmers must follow to earn the right of labelling their products as organic. Depending on the geographically location the legislation might differ slightly. In EU a common set of organic regulations has been accepted, which countries belonging to the Union must follow (Commission Regulation (EC), 2008, Council Regulation (EC), 2007). January 1st 2021 a new organic regulation entered into force (Regulation (EU), 2018), serving as an update of previous rules, due to the major development the organic sector has gone through the last decades. Farmers in countries belonging to the EU must obey this regulation, however national or regional specifications might occur, but only in terms of further restricting the rules. In Denmark the national organic legislation does not differ significantly from the EU-legislation (Landbrugsstyrelsen, 2020b), however most organic pig producers have agreed upon an industry agreement, which is more strict and specific compared to the EU legislation (Brancheaftale, 2018). If organic pig producers do not follow the industry agreement, the largest slaughterhouses in Denmark; Tican, Organic Pork and Friland, do not accept their slaughter pigs.

In the following section the most important rules of organic pig production under Danish conditions are described. According to EU regulation, organic pigs should be fed an organic or in-conversion diet, that meets the requirement of the current life stage. Later it is specified that up until 5% may origin from conventional agriculture, if certain requirements are met (Regulation (EU), 2018). Danish pig farmers following the industry agreement, may however not include any conventional feedstuffs in the diet, as a 100 % organic diet is required (Brancheaftale, 2018). The feed should primarily be obtained from the farm itself or at least the same region, however only a minimum of 30 % is required. The feed must not contain any GMOs or products produced from GMOs, which includes crystalline amino acids (CAA). At last, roughage, fresh or dried fodder, or silage must by applied in the daily ration of pigs (Regulation (EU), 2018).

In the Danish regulation, this is formulated as; the animals must have permanent access to roughage (Landbrugsstyrelsen, 2020b).

Regarding housing of organic pigs, the EU regulation requires permanent outdoor access, which preferable should be pasture when conditions allow it. Open-air areas may however be partially covered by roof (Regulation (EU), 2018). The Danish regulations defines a grazing season from April 15th to November 1st, in which breeding pigs and piglets must have pasture access, if weather and the physical condition of the animal allows it (Landbrugsstyrelsen, 2020b). It is further specified by the Danish industry agreement, that farrowing may only occur on pasture in huts and the lactating sows must have access to pasture (Brancheaftale, 2018). The EU regulation adds that stocking density must not exceed 170 kg N pr. ha pr. ha, to reduce environmental pollution from animal manure (Regulation (EU), 2018). The requirement for stocking density is further restricted by Danish legislation and must not exceed 140 kg N excreted pr. ha pr. year. Farmers are however allowed to double the stocking density, when a nitrogen-demanding crop is grown the following year (Videncentret for landbrug, 1993).

At last, it is required that all piglets should be fed maternal milk in a period of minimum 40 days (Regulation (EU), 2018). This period is increased to minimum 49 days by the Danish industry agreement (Brancheaftale, 2018).

2.1.2 The production system in practice

The pig production system typically used under Danish conditions is characterized as an outdoor housing system, accordingly to Fruh et al. (2014): Lactating sows and their piglets are at pasture all year, while gestating sows are at pasture during the grazing period, according to legislation and industry agreement. However, most organic pig producers keep the gestating sows outdoors throughout the year. Hence, only one housing system is required (Kongsted and Hermansen, 2005). Outdoor sows are typically ringed under Danish conditions. The ring in the snout inhibits rooting behavior, reducing the damage to the grass sward. Keeping the grass cover intact reduces the environmental impact, as nutrient excreted from the sows, are absorbed and preserved in the sward (Eriksen et al., 2006). Weaners and fatteners are often kept in indoor stables, with access to outdoor concrete runs (Fruh et al., 2014).

Concerning feeding, organic pig production faces different challenges. First, the selection of available raw materials and feed additives is limited compared to conventional production, due to legislation. Also, since self-sufficiency is a key term in organic production, import of feedstuff from foreign countries is undesirable. The reduced range of products available makes the composing of organic pig feed difficult, especially in terms of adjusting crude protein (CP) content and amino acid (AA) composition in relation to the requirement of the animal. Suboptimal feeding reduces productivity, decreases feed efficiency and is costly for the organic pig producer, as feed is the main cost of pig production (Danielsen et al., 2000, Edwards, 2002).

Productivity-wise the organic pig production is behind conventional production. While conventional sows wean 33.0 piglets pr. year-sow, organic only wean 22.5 piglets pr. year-sow. The longer lactation period will inevitably reduce this number, due to fewer farrowings each year. Also, the piglet mortality is higher in organic pig production, where the risk of piglets being crushed by the sow is the primary explanation. This is considered a major problem in organic pig production (Rangstrup-Christensen et al., 2018). Besides weaning fewer piglets pr. year, the organic sows also consumes more feed, than the conventional sow, hence the lower feed efficiency (table 1).

Table 1, key-figures of conventional and organic sow production 2018 (Hansen, 2020, SEGES, 2019)

	Conventional	Organic
Weaned piglets pr. year-sow	33.6	22.5
Litters pr. year-sow	2.26	1.91
Piglet mortality during lactation, %	14.2	23.4
Feed usage, FUsow pr. year-sow	1500	1910

2.2 Requirement and feeding standards

2.2.1 Requirement of the gestating and lactating sow

Dealing with requirement of animals, energy is the central part and the other nutrients are often put in relation to the energy. The sow require energy for maintenance, retention and reproduction. The maintenance requirement is defined as the energy required for maintaining physical equilibrium, keeping physical activity and thermoregulation to a minimum (Theil et al., 2020). Energy-requiring processes that maintains the physical equilibrium includes essential muscular activity, active transport and synthesis of body constituents, such as hormones and enzymes (McDonald et al., 2011). These processes are highly prioritized, and the body will catabolize body depositions to continue these processes, if the maintenance energy is not met through feeding. Due to inefficiency of metabolic processes, heat is produced. The heat is utilized to keep up the body temperature, hence has value to the animal. (Theil et al., 2020). The requirement of energy for maintenance, mainly depends on the liveweight (LW) of the sow (Dourmad et al., 2008).

Following the energy requirement, is the protein requirement. Frequently this requirement is expressed as the monomers of protein; AAs, or essential amino acids (EAA) which the body cannot synthesize de novo. Lysine is most often the first-limiting AA in the diet of sows; hence this is the AA of interest. Like energy, sows require AAs for maintenance, retention and reproduction. AAs for maintenance are used to cover losses from body surfaces and the gastro-intestinal system, synthesize essential enzymes and hormones, cell renewal and the continuous process of protein turnover (Nørgaard et al., 2020).

When the maintenance requirement is met and energy are supplied above maintenance, nutrients are used for productive processes. For the sow, these changes along with age and physiological state. During gestation these processes includes fetal growth, development of uterine components, mammary growth and late in gestation; colostrum production (Feyera and Theil, 2017). In figure 2, the energy requirement of the prolific gestating sow is seen. The proportion and magnitude of the requirement differs between 1st and 4th parity, where gilts at 1st parity has a smaller, but increasing, maintenance requirement and a higher requirement for maternal gain, due to its lower bodyweight and ongoing growth (Sola-Oriol and Gasa, 2017). Maternal gain is the requirement of lowest prioritization, hence only what is feed above the maintenance and reproductive requirement are used for retention. Energy retained in body tissues during gestation is preparing the sow for the potential nutrient deficit in the upcoming lactation (Dourmad et al., 2008).

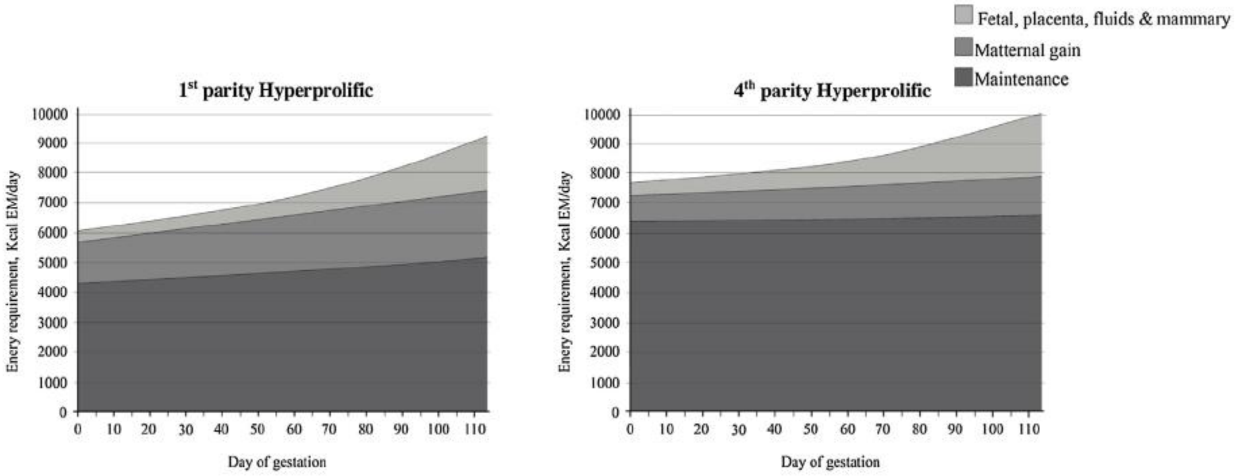

Figure 1, Energy requirement of 1st and 4th parity hyperprolific sows during gestation, expressed as Kcal ME pr. day (Sola-Oriol and Gasa, 2017)

In figure 3, the protein requirement, expressed as SID lysin, of the sow during gestation is seen. While the energy requirement for maintenance constituted a large proportion of the total energy requirement, the SID lysin requirement for maintenance, only is a small part of the entire requirement. Changes in SID lysin requirement between parity, is comparable to the changes in energy requirement; 1st parity sows have a smaller maintenance requirement and a larger requirement for protein accretion, compared to 4th parity.

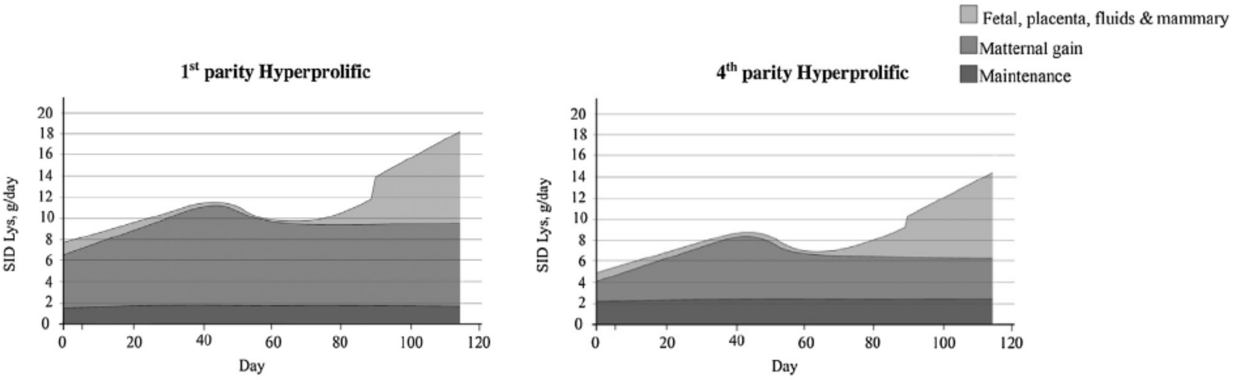

Figure 2, SID lysine requirement of 1st and 4th parity hyperprolific sows during gestation, expressed as gr. SID lysin pr. day (Sola-Oriol and Gasa, 2017)

The largest part of the fetal development and mammary growth occurs towards the end of gestation, which also is illustrated in figure 1 and 2 (Dourmad et al., 2008). Hence, the highest potential for energy and protein accretion is during the first half of gestation (Sola-Oriol and Gasa, 2017).

The transition period from gestation to lactation is an important period in the production cycle, where major changes occur in the physiological state of the sow, and the nutrient requirement of the sow changes rapidly (Theil, 2015). During the transition period the nutrient prioritization changes from fetal growth, to piglet survival at farrowing and milk production in the lactation. Milk production is extremely highly prioritized, and the sow will mobilize body reserves if the nutritional requirement is not met through the diet (Theil et al., 2012). This high priority was demonstrated by Pluske et al. (1998), that found milk production of primiparous sows did not differ when fed either ad libitum or restricted (50 % of ad libitum), due to the intense mobilization. Mobilization of body reserves is almost unavoidable when using highly productive sows, as the appetite of the sows limit the feed intake, causing the requirement not to be fulfilled (Hansen, 2012). Figure 3 shows the energy and SID lysine requirement of the sow during this period. During the last days of the gestation, the energy and SID lysine requirement increases slightly, primarily due to colostrum production. At parturition the energy requirement increases due to farrowing labor, however SID lysin requirement declines as a minimum of nutrients is retained in reproductive tissues, and colostrum and milk production are minor (Feyera and Theil, 2017). Two days after parturition, both energy and SID lysine requirement increases dramatically, as milk production truly initiates (Theil, 2015). During the first 10 days of lactation the yield increases rapidly and peak yield was estimated by Hansen et al. (2012b) to be reached in the third week of lactation. At peak lactation 95 % and 72% of SID lysine and energy are used for the milk production (Feyera and Theil, 2017).

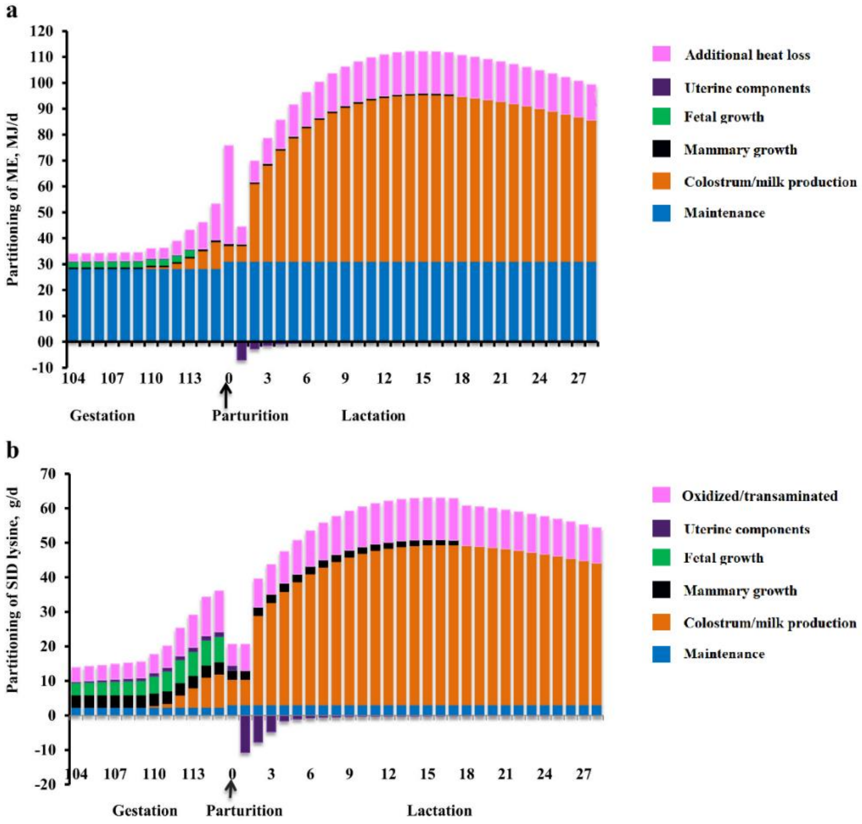

Figure 3, requirement of a) metabolizable energy and b) SID lysine (g/day) of the sow during the transition period and lactation. Edited after Feyera and Theil (2017).

2.2.2 Requirement of the organic sow

The organic pig production is in great contrast to the conventional indoor production, when looking at production conditions as earlier described. These conditions affects the sows, in terms of behavior, health, nutrition and nutritional requirement (Edwards, 2005).

Pigs are homeothermic, meaning that they keep a constant body temperature. When below a certain temperature, the heat production of the animal is increased to stabilize body temperature. This temperature is known as lower critical temperature (LCT) (McDonald et al., 2011). This limit varies depending on feeding level, body condition, housing, production stage and more (Dourmad et al., 2008). The LCT for individually housed gestating sows, were estimated by Noblet et al. (1989), to be between 20° and 23° Celsius, but decreases if supplied with straw bedding and housed in groups (Dourmad et al., 2008). During lactation the LCT decreases substantially to between 10° and 15° Celsius for individually housed sows, as feed intake increases and milk production initiates (Noblet et al., 1990, Dourmad et al., 2008). At high environmental temperatures the upper critical temperature (UCT), might be reached. Above this temperature the body temperature increases, and the animal must use energy to keep it down. Pigs ability to sweat is very limited, hence the respiratory rate is increased (Theil et al., 2020). Also, feed intake of sows have proven to be reduced, when housed at temperatures above UCT (Quiniou and Noblet, 1999). Between the LCT and UCT is the thermoneutral zone, in which no excess energy is used to maintain proper body temperature. It should also be mentioned that factors such as wind speed, humidity and various animal characteristics will affect the LCT and UCT and consequently the thermoneutral zone (Theil et al., 2020).

Under conventional production, sows use minimal energy thermoregulating, as they are indoor housed, where temperature is adjustable. However, as earlier described, organic sows are mostly housed outdoors and thus are exposed to the natural climate. Under Northern European climatic conditions, the temperature will reach values below the sows LCT during the winter season, causing energy to be spent on thermoregulation. Close and Poornan (1993) estimates that temperatures below LCT in average will cause an increase in energy requirement of 18,8 kJ ME pr. kg $LW^{0,75}$ pr. day pr. °C. This value will be lower for group housed sows and higher for individually housed thin sows (Close and Poornan, 1993). Buckner (1996) estimated the additional energy cost for thermoregulation of outdoor sows during gestation in North-East Scotland. The author found that sows with conception 15th September used between 931,7 MJ and 1049,7 MJ ME, and sows with conception 7th December used 1298,5 MJ to 1444,8 MJ ME on thermoregulation, depending on parity.

Another important property of organic sow production; roughage, this might however reduce the additional energy required during cold thermogenesis. During fermentation of fiber, heat is produced, along with VFAs and gasses. When ambient temperature is below LCT, this heat energy substitutes energy elsewise used for heat production. The contribution of heat energy from hindgut fermentation of fibers at temperatures below LCT were investigated by Noblet et al. (1985). Growing pigs were fed a low-fiber or high-fiber diet at 13° or 23° Celsius. Housed at 23° Celsius the pigs fed low-fiber had a significantly lower heat production, however at 13° Celsius the heat production of pigs fed either diet was the same, indicating that heat energy from fiber fermentation, reduces energy expenditure for heat production (Noblet et al., 1985).

Another aspect of organic production conditions, that might cause an increment in the energy requirement of sows, is the increased possibility of physical activity. The outdoor housing provides a larger area for sows to move around, but also a diverse environment, that stimulates natural behavior, such as rooting. Looking at the daily activity across different production systems, outdoor sows does however not appear to have a higher activity level compared to indoor sows (Edwards, 2003). Johnson et al. (2001) did however observe a higher activity level of lactating sows when outdoor, compared to confined indoor sows. The energy expenditure depends on the type of activity, where locomotion requires more energy compared to standing. Only few studies have investigated the daily locomotive activity of outdoor sows, where Buckner

(1996) estimates that gestating sows travels 0,1- 3.1 km/day and Eskildsen et al. (2020b) found an average travelling distance of 2.78 km/day and 2.46 km/day in mid and late gestation respectively and 0.64 km/day, 1.54 km/day and 1.68 km/day at day 5, 20 and 40 in the lactation respectively. Close and Poornan (1993) estimates that each km walked, increases the energy requirement by 7 kJ/kg LW. The excess heat produced during physical activity, will compensate for some of the additional energy required for thermoregulation during cold conditions (Close and Poornan, 1993). In contrast to this Noblet et al. (1993) investigated the energetic cost for physical activity in standing sows. The authors found that castrated sows in average stood up for 241 minutes pr. day, requiring 0.26 kJ/kg LW$^{-0.75}$/min. Thus, the energy requirement for physical activity of a sow weighing 250 kg and standing 241 minutes pr day is 3898 kJ/day. Compared to the findings of Eskildsen et al. (2020b), this is just below and above the energy requirement for physical activity of outdoor sows during gestation and lactation respectively. In conclusion the energy requirement for physical activity of outdoor sows do not substantially differ from that of the indoor sows.

The total energy requirement of outdoor sows is estimated by Close and Poornan (1993) to be 20% higher than animals kept indoors, at an average annual environmental temperature of 10° Celsius, while Edwards (2003) estimates that the increase in energy requirement corresponds to an increment of 15 % in feed requirement under Northern European conditions. An increase of 15 % of daily energy supply to organic outdoor sows was also found to be sufficient to cover the energy requirement, in a recent Danish study by Eskildsen et al. (2020b). Close and Poornan (1993) also points out, that the difference in CP or AA requirement between outdoor and indoor sows are expected to be minor, as the processes in which CP or AAs is required, is not affected by the organic production conditions.

2.2.3 Danish feeding standards

Feeding standards describe recommended dietary concentrations of energy, CP, EAAs, vitamin and minerals for different animal groups. Feeding standards are determined to ensure optimal economic productivity (Tybirk et al., 2020).

The Danish feed evaluation system is based on the energy term; potential physiological energy (PPE). This value expresses the energy available for physiological processes within the animal and is calculated based on the universal energy source, ATP. The energy value of PPE is relatively comparable to the energy term used in the Dutch and French energy evaluation system; net energy (NE) (Tybirk et al., 2006). Feed units (FU) are used to describe the energy content of feedstuff. FU for growing pigs (FUgp) contains 7.38 MJ PPE and 7.70 MJ PPE for sows (FUsow), due to their increased utilization of fermentable carbohydrates.

In previous versions of the Danish feed evaluation system other feed units has been used. Before 2002, one feed unit covered all animal groups; FUp and the energy term; net energy, was used. In 2002 the system changed, and the feed unit was divided into two; FUgp for growing pigs and lactating sows, and FUpreg for gestating sows. In 2006 the system was revised and gestating and lactating sows were collected under FUsow and growing pigs kept FUgp. Despite changes in which animal groups the feed units covers and the energy value of nutritional fractions, both FUp and FUpreg approximates to FUsow, at least for standard compound feed (Tybirk et al., 2006).

In the Danish nutrient standards CP, EAA, minerals and vitamin are set relative to the energy content, expressed as FU. CP and EAAs are specified as standardized ileal digestible (SID) and standards can be found for both gestating and lactating sows. In table 2, nutrient standards for energy density, CP concentration and the three first-limiting AA are shown. Worth noticing is the higher energy, CP and AA standards in the feed for lactating sows, which correlates to earlier section regarding nutrient requirement of sows.

Table 2, Danish nutrient standards for gestating and lactating sow (Tybirk et al., 2020)

	Gestating sow		Lactating sow	
FUsow/kg feed	0.99		1.06	
PPE/kg feed, MJ	7.4		7.9	
NE/kg feed, MJ	8.8		9.6	
SID CP, min, g/FUsow	85		118	
	g/FUsow	% of lysine	g/FUsow	% of lysine
SID Lysine	3.5	100	7.7	100
SID Methionine	1.1	48	2.4	31
SID Threonine	2.3	91	4.5	65

2.3 Roughage

Roughage is characterized by a high content of dietary fiber (DF) and many of its effects, both beneficial and detrimental, are attributable to the fibers (Bach Knudsen, 2001, Wenk, 2001). DF are primarily found in the wall of plant cells and consists of non-starch polysaccharides, which includes pectins, cellulose, hemicellulose, β-glucans and fructans, but also lignin, protein, fatty acids, waxes and more (Bach Knudsen, 2001). DF are characterized by not being affected by the enzymatic digestion in small intestine, leaving them available for bacterial fermentation in the hindgut of the animal (Jarrett and Ashworth, 2018). Beside DF, roughage also contains various amounts of digestible carbohydrates, proteins, fats, minerals, vitamins and more, that contributes to the nutrition of the animal.

Inclusion of DF in the diet of pigs reduces the digestibility of DM and energy, as they are resistant to enzymes secreted in the small intestine (Bach Knudsen, 2001). Moreover, DF encapsulates potentially available nutrients leaving them unavailable for endogenous enzymes of the pig (Lærke et al., 1997). DF also increases the rate of cell turnover in the intestinal mucosa and increases secretion of endogenous fluids, which increases the endogenous loss (Wenk, 2001).

DF are typically characterized according to their solubility. Soluble fibers have a high capacity of water binding and swells significantly when exposed to water. Soluble fibers are also highly fermentable for microorganisms in the hindgut, serving as a source of nutrition for the pig and increases microbial activity. Due to its water-binding capacity and swelling, soluble fibers increases the volume of digesta and slows the emptying of the stomach, which prolongs satiety (Wenk, 2001). In the small intestine, soluble fibers are believed to reduce absorption of nutrients, due to their effect on luminal viscosity (Bach Knudsen, 2001). Insoluble fibers, on the other hand, are not as easily fermentable as the soluble fibers (Noblet and Goff, 2001), but provides bulk to the diet, which reduces fecal transit time (Wenk, 2001, Bach Knudsen, 2001).

DF content in feedstuff are often measured using the Van Soest method, by which the fractions; neutral detergent fibers (NDF) and acid detergent fibers (ADF) are obtained. NDF includes the insoluble fibers; hemicellulose, cellulose and lignin, while ADF only includes cellulose and lignin. The soluble fibers are lost during the method and ends up in the nitrogen free extracts (NFE) fraction, together with starch and sugar (Carlson et al., 1999).

A wide variety of roughages exist, including different types of hay, silage, fresh green mass and various byproducts. In Denmark, whole-crop silage of barley and peas, silage of grass or clover-grass and fresh grass or fresh clover-grass are typically used roughages in the organic pig production (Olsen et al., 2000). Several behavioral studies investigating pig preference of roughages has been conducted (Olsen et al., 2000, Rachuonyo et al., 2005, Jensen et al., 2008). Olsen et al. (2000) investigated preference of rooting material in growing pigs. Six types of roughages were tested, and the authors found that whole crop silage of oat, vetch and lupin and chopped fodder beets were manipulated most frequently. Both roughages were characterized by high moisture content and the whole crop silage of oat, vetch and lupin was the most variable, allowing for more types of behavior (Olsen et al., 2000). In a maze-test Jensen et al. (2008) also investigated growing pigs preference of rooting material. Among other categories, three different roughages were compared, including maize silage, grass silage and sugar beets. Pigs preferred the two silages above the sugar beets, in contrast to the findings of Olsen et al. (2000). The authors point out that sugar beets instead of fodder beets were used and furthermore they were not freshly chopped (Jensen et al., 2008). It should also be kept in mind, that roughage quality may vary, depending on botanical composition, preserving technique, season, storage and more.

Roughage or DF affects animal behavior positively by allowing natural rooting behavior and providing satiety, which can reduce unwanted behavior, such as aggression towards pen-mates and stereotypies (Robert et al., 1993, Bergeron et al., 2000, Danielsen and Vestergaard, 2001, Holinger et al., 2018). Furthermore, access to roughage has shown to have a beneficial effect on animal health, where Holinger et al. (2018) found that supplementation of grass silage reduces severity of gastric ulcers in growing pigs. Also, fermentation of DF in the distal gastrointestinal tract are believed to have a beneficial effect on intestinal health, as it improves the intestinal environment in terms of reducing the pH and strengthening the mucosal barrier (Lindberg, 2014, Jha et al., 2019). At last, incorporation of dietary fiber in the diet of gestating sows, can increase the voluntary feed intake during lactation (Quesnel et al., 2008).

2.3.1 Chemical composition of roughage

Even though the nutritional contribution of roughage seldom is accounted for in the diet of the sow, the intake of roughage contributes to the nutrition of the sow. The nutritive contribution depends on the content of carbohydrates, protein and fat and the digestibility of these nutrients. These factors vary depending on the type of roughage, botanical composition, maturity and more. Roughage is primarily only used in the organic sector of pig production; hence relative few articles exist, describing the nutritional value of roughage for the sow. However, in the field of ruminant nutrition countless articles regarding roughage can be found, hence these are used in this section. It should however be kept in mind, that the composition and properties of the roughage for ruminants may vary from what is used for sows, as the digestive system of these animals differs.

Table 3, chemical composition of fresh crops typically used for roughages (NorFor, 2020)

	Italian ryegrass	White clover	Barley whole-crop	Pea whole-crop
DM, g/kg	190	130	350	320
CP, g/kg DM	210	268	102	148
Fat, g/kg DM	47	36	20	18
NDF, g/kg DM	385	262	408	315
iNDF, g/kg NDF	83	291	269	280
Starch, g/kg DM	0	20	220	100
Sugar, g/kg DM	140	46	80	120

In table 3 nutritional characteristics of the fresh crops used for preparing roughage is seen. These values apply for fresh crops, however only minor changes to the chemical composition occurs during ensiling, except for the sugar content which is heavily reduced (Søegaard et al., 2003). Important to notice is the CP content of the crops, where the highest content is found in white clover, followed by Italian ryegrass, pea whole-crop and barley whole crop. Highest NDF content is found in crops belonging to the grass family, while the legumes have a lower NDF content. Barley whole crop has the highest starch content and pea whole crop contains roughly half this amount. It should be kept in mind that these numbers merely are an indication of the nutritional composition of the crops, which varies depending on several parameters, such as maturity, fertilization, cultivar, season and more.

Table 4, chemical composition of clover-grass silages and barley-pea whole-crop silages (Boever et al., 2009, NorFor, 2020, Pursiainen and Tuori, 2006)

	Clover-grass silage		Barley-pea whole-crop silage	
	NorFor (2020)	Boever et al. (2009)	NorFor (2020)	Pursiainen and Tuori (2006)
Legume content	40 % clover	43 % clover	40% pea	74 % pea
DM, g/kg	359	481	353	255
Ash, g/kg DM	95	129	62	73
CP, g/kg DM	155	160	122	170
CF, g/kg DM	44	29	20	27
NDF, g/kg DM	419	453	380	419
Starch, g/kg DM	15	-	183	100
Sugar, g/kg DM	82	49	19	301[1]

[1] WSC (mono-, di-, oligosaccharides and fructans)

In table 4, table values of the nutritional content of clover-grass silage (CS) and barley-pea whole-crop silage (BWS) from the ruminant feed table; NorFor is seen, allowing for comparison of the two types of silages: CS is characterized by higher CP, NDF and sugar content, but lower starch content compared to BWS, at equal content of legumes, which is also confirmed by Søegaard et al. (2003). The nutritional composition of these silages also reflects the values of the fresh crops, in table 3. In table 4, roughages of similar type, used in studies is also listed. As seen the legume content might vary, causing variation in the nutritional content of the roughage. Worth noticing here is that the legume content especially affects the CP content in both types of silages and the starch content in the BWS.

Looking into detail of fiber composition of the silages Carlson et al. (1999) found a slightly higher content of DF in BWS compared to CS (table 5). The lignin content was equal and consequently BWS also had a slightly higher NSP content, yet a lower content of soluble NSP compared to CS (table 5). The CS contained 50-70 % white clover and the BWS contained about 25 % whole crop pea (Carlson et al., 1999).

Table 5, fiber composition of clover-grass silage and barley-pea whole-crop silage (Carlson et al., 1999)

	Clover-grass silage	Barley-pea whole-crop silage
DF, g/kg DM	406	428
NSP, g/kg DM	327	350
Soluble NSP, g/kg DM	37	27
Lignin, g/kg DM	79	79

Besides the overall content of CP, the AA composition is of great interest when concerned with nutrition of sows. In table 6, the content of the three first-limiting AAs for sows is shown for CS and BWS: The overall content of the three AAs is higher in CS, however this partly due to a lower CP content in BWS. Looking at the AA composition expressed as % of lysine, no general differences are seen between the two types of silage, however some overall variation is seen. Other factors such as cultivar, botanical composition and preservation technique also affect the AA composition.

Table 6, DM and CP content and AA composition of clover-grass silage and barley-pea whole-crop silage

	Clover-grass silage						Barley-pea whole-crop silage	
	Carlson et al. (1999)		Fernándéz et al. (2006)		Eskildsen et al. (2020b)		Carlson et al. (1999)	
DM, g/kg	430		331		292		320	
CP, g/kg DM	169		232		140		118	
	g/kg DM	% of lysine	g/kg DM	% of lysine	g/kg DM	% of lysine	g/kg DM	% of lysine
Lysine	6.1	100	9.0	100	6.3	100	3.7	100
Methionine	2.3	37.7	3.4	37.8	2.1	33.9	1.6	43.2
Threonine	6.4	104.9	9.7	107.8	5.8	92.9	3.9	105.4

2.3.2 Digestibility of roughage

The ability to digest DF, depends on the age and the bodyweight of the pig (Noblet and Goff, 2001). Older animals with a higher bodyweight, has a larger and more developed digestive system compared to young animals, which increases the digestibility of DF. Digestion of fiber is primarily occurring in the hindgut, however a small fraction of the soluble fibers is digested before reaching the large intestine (Jørgensen et al., 1996). Compared to the small intestine the volume of the hindgut is much larger, which decreases passage rate of digesta. This slower passage rate allows for microbial fermentation of undigested nutrients such as DF, and endogenous losses. The microbial population is affected by available substrates for fermentation, where Jensen and Jørgensen (1994) found more than five times higher microbial activity in the entire gastrointestinal tract of growing pigs fed high fiber diets, in contrast to low fiber diets. Also the age and size of the animal affects the microbial population, as sows are expected to have 6.7 times more cellulolytic bacteria in the hindgut than growing pigs, which are responsible for fiber fermentation (Varel and Yen, 1997). The products of microbial fermentation are volatile fatty acids (VFA); primarily acetate, propionate and butyrate, gasses; carbon dioxide, hydrogen and methane, urea and heat (Noblet and Goff, 2001). VFAs are rapidly absorbed across the intestinal wall in the hindgut and used for various purposes, including energy production within the pig. The VFAs, especially butyrate, also provides energy for the maintenance of the epithelium in the hindgut (Mosenthin, 1998).

The overall effect of DF in growing pigs and gestating sows on energy digestibility, were investigated by Ramonet et al. (1999). The authors found, as DF content increased, the energy digestibility in both growing pigs and gestating sows decreased linearly. The proportional decrease in growing pigs, was however greater compared to gestating sows, underlining the higher capacity of adult animals to utilize DF, compared to pigs in growth. In addition to the quantitative fiber content, digestibility of DF depends on fiber type. As earlier mentioned, the soluble fibers are much easier fermented, compared to the insoluble (Noblet and Goff, 2001). Goff et al. (2002) investigated the effect of different sources of DF on nutrient digestibility in sows. Diets contained 200 g DF/kg DM of either maize bran, wheat bran or sugar beet pulp, where wheat bran has a high content of insoluble fibers and sugar beet pulp a high content of soluble fibers (Bach Knudsen, 2001). The digestibility of nutrients in the diets reflected the fiber composition, as DM, CP and DF digestibility were higher in sows receiving the diet containing sugar beet pulp compared to wheat bran (Goff et al., 2002). Rivera Ferre et al. (2001) investigated among others, the effect of season on the herbage digestibility. The authors found considerable higher herbage OM digestibility in the spring compared to summer, as herbage quality was reduced, due to an increased content of NDF and iNDF.

In an experiment conducted by Carlson et al. (1999) growing pigs were fed CS and BWS and fecal digestibility of the nutrients in the diets, including fractions of DF, were investigated. The pigs were fed 1 kg of compound feed and roughage constituted 18-19% of the daily DM intake. A higher digestibility of DF and NSP was seen in the pigs eating CS, compared to BWS, which can be explained by the proportionally higher content of soluble NSP and lower content of cellulose in CS (Carlson et al., 1999). No overall effect on energy, DM and CP digestibility was observed, which might be due to the relatively small contribution of roughage to the total feed intake.

2.3.3 Sows intake and nutritional contribution of roughage

Quantification of roughage intake for sows, either fresh or preserved, is a relatively nonclarified scientific field. Few studies describe the intake of forage from paddocks in the summer period and even fewer describes the roughage intake during winter.

In a study by van der Peet-Schwering et al. (2010) the intake and nutritive value of different quality grass silage was investigated. Sows were fed 0.88 kg DM of compound feed and 1.43 kg DM of grass silage. The sows' energy intake from silage varied according to the quality, where sows fed the highest quality silage had a daily energy intake of 16.4 MJ ME, while the sows fed the lowest quality only had a daily energy intake of 12.5 MJ ME. Thus, the daily energy contribution of grass silage varied from 46 % to 53 %. Similarly, Bikker et al. (2014) investigated the potential nutritional contribution of grass silage for gestation organic sows. It was assumed that the silage could replace 1 kg of compound feed per day, thus the feed allowance was accordingly adjusted. The average daily energy intake from silage was 16.1 to 17.5 MJ ME, which corresponded to an energy contribution of 50 % to 60 %. The authors concluded that grass silage can at least replace 1 kg of compound fed during gestation. In agreement with these studies, Fernándéz et al. (2006) found that clover-grass silage can cover 42 % of the daily energy intake of restrictive fed sows during winter conditions. Studies investigating the intake of fresh clover-grass similarly finds that the roughage intake of gestating sows can provide up until 60 % of the required daily energy, depending on the level of compound feed supplemented (Fernándéz et al., 2006, Sehested et al., 2004).

In addition, the effect of season and compound feed supplementation on the voluntary herbage intake of clover-grass in gestating sows was investigated by Rivera Ferre et al. (2001). The experiment covered spring and summer during two periods. No overall effect on herbage intake between the groups offered 1,5 or 3,0 kg compound feed pr. day was seen, however in the spring of the second period, sows fed 1,5 kg of compound feed pr. day had a higher herbage intake than sows fed 3,0 kg compound feed. Season, however, had a significant effect on herbage intake, where the intake during summer was significantly higher than in the spring, due to poorer herbage quality. The estimated energy contribution of herbage intake in this experiment was approximately 50% of the daily requirement in all periods (Rivera Ferre et al., 2001).

In figure 4 the daily intake of fresh clover-grass relative to parturition is shown, as found by Eskildsen et al.

(2020a). Through gestation, the clover-grass intake is relatively stable and constitutes almost a tenth of the daily energy supply. In early lactation, the intake decreases significantly, probably due to limited gastric capacity and appetite. During first half of lactation clover-grass intake increases and at peak lactation, the intake reaches the highest value, however, constitutes still around a tenth of the total supply (figure 4). Late lactation the intake decreases to approximately same level as in gestation. Beside this periodic effect, an effect of dietary protein concentration was found in the study by Eskildsen et al. (2020a): Sows fed 12 % less dietary protein ate 8.4 % more clover-grass to compensate for the reduced dietary protein intake. The increased intake was however not sufficient to completely cover the reduced dietary protein intake (Eskildsen et al., 2020a). Compared to earlier described studies the relative energy contribution of roughage is rather small in the study by Eskildsen et al. (2020a), however sows were correspondingly fed a larger amount of compound feed, as is typical under practical circumstances.

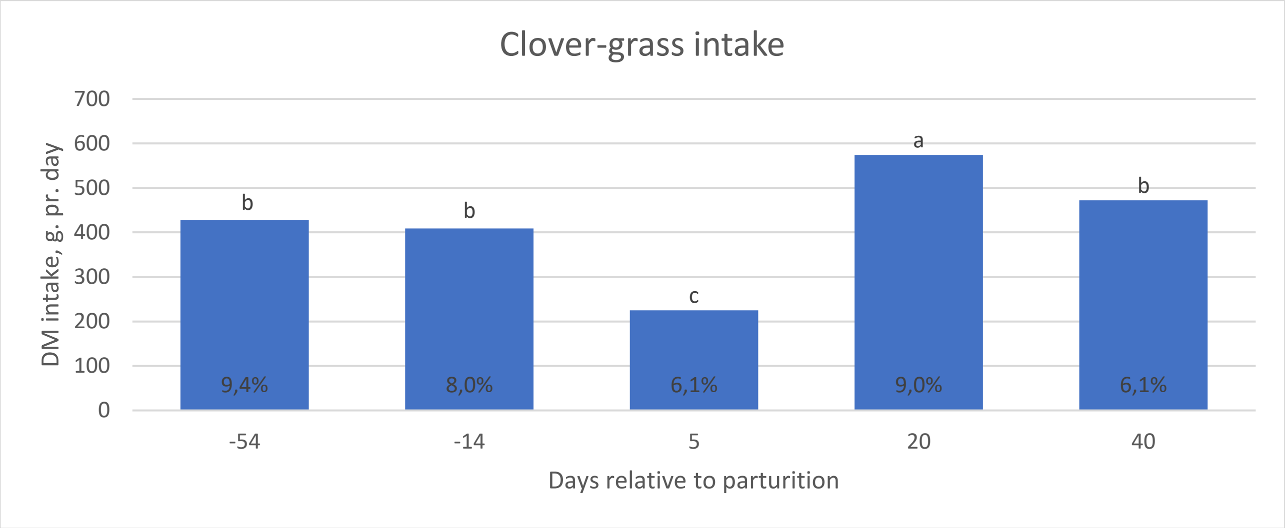

Figure 4, daily intake of clover-grass (g DM pr. day) relative to parturition. Percentages inside columns is the daily contribution of energy through roughage to the total energy intake. Different letters indicate significant different DM intake (Eskildsen et al., 2020a)

Besides supplying energy, roughage also contributes to the supply of protein and AAs to the sow. Intake of large quantities of roughage might constitute a rather large fraction of the daily requirement: In figure 5, the potential contribution of grazed clover-grass to the AA requirements of gestating sows is seen and only the requirement of lysine and methionine + cystine is not fulfilled. Such high AA contributions is however only reached at low compound feed supplementation levels and requires a high quality of roughage. In coherence with this, Fernándéz et al. (2006) found that sows fed clover-grass silage covering 42 % of the daily energy intake during winter, surely had their AA requirements fulfilled. Actually, the dietary concentration of lysine, methionine and threonine exceeded the Danish feeding standards by a relatively large margin, indicating that roughage serves as a good source of AAs.

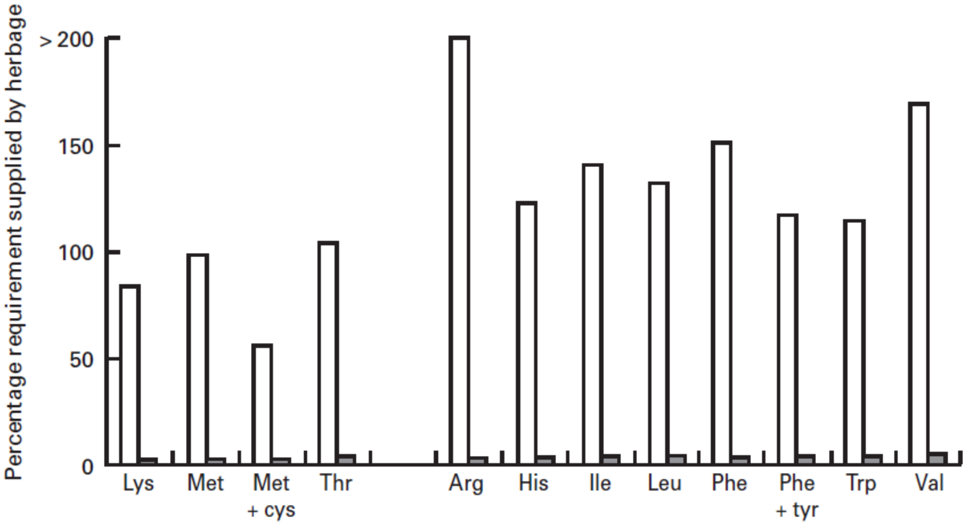

Figure 5, potential contribution of grazed clover-grass sward to the AA requirements of gestating sows (white column) and growing pigs (grey column) at an assumed ileal digestibility of 0.4, an assumed intake of 2 kg DM/day for dry sows and 0.1 kg DM/day for growing pigs offered ad libitum compound feed (Edwards, 2003)

2.4 Metabolites in plasma, urine and milk

Metabolites in plasma, urine and milk is a valuable tool for analyzing the physiological status of the sow. Countless metabolites are found within the plasma, urine and milk, where some are correlated to the nutrition and metabolic processes in the animal, others can be used to explain the health status. The focus in this thesis is the metabolites that provides the large overview of the primary metabolic processes within the sow and additional emphasis is put on the metabolites that are tightly correlated to the protein metabolism.

2.4.1 Plasma metabolites

Besides transporting oxygen and carbon dioxide between lungs and tissues, the blood vessels are used to transport metabolites such as nutrients, hormones and waste products throughout the body (Akers and Denbow, 2013). Thus, a lot of information regarding the physiological state of the animal is found in the blood, specifically the plasma. Of the countless metabolites, emphasis is put on the five major metabolites; glucose, lactate, triglycerides (TG), non-esterified fatty acids (NEFA) and urea, which each are correlated to a part of the primary metabolism of the sow.

When examining and comparing plasma metabolite concentration, sampling time is essential. Many plasma metabolites vary in concentration in relation to feeding, hence comparing samples obtained at different time after or before a meal provides invalid results (Eggum, 1970, Frayn, 1998, Serena et al., 2009). To properly compare plasma metabolites concentration between production stage, dietary treatment or other, sampling should be performed at similar time relative to feeding. Also, site of sampling must be consistent between comparisons, as the organs and tissues consume or contribute to the pool of metabolites in the plasma as it circulates. Especially the liver has an important metabolic role and concentrations of metabolites may vary significantly before and after reaching the liver (McDonald et al., 2011).

Glucose in plasma origin from various sources; digested carbohydrates, primarily starch, mobilization of glycogen depots and gluconeogenesis in the liver. Glucose has a vital metabolic role, as it supplies energy to the organs and tissues of the body. Plasma glucose is tightly regulated by the hormones insulin and glucagon produced in pancreas. Insulin promotes uptake of glucose by various tissues and storage in form a glycogen in liver and muscles. Glucagon, on the other hand, induces release of glucose from the glycogen stored in the liver and muscles. The hormones are regulated by the glucose concentration in plasma (Akers and Denbow, 2013). Thus, plasma glucose concentration is relatively stable and kept in a tight interval most of the time. Pre- and postprandial glucose concentration might exceed or fall below this interval, however is quickly regulated back to concentrations within the normal interval (Serena et al., 2009).

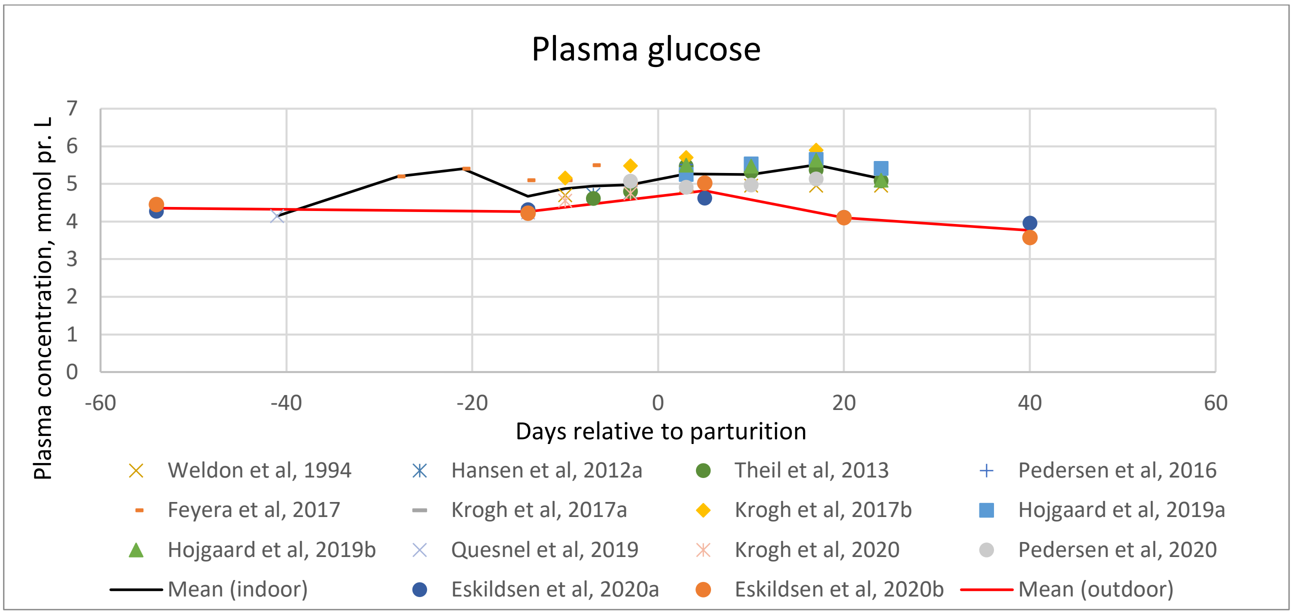

Figure 6, plasma glucose concentration of indoor and outdoor sows relative to parturition

In figure 6, plasma glucose concentrations found in previous studies are seen. The majority of plasma glucose concentrations of both gestating and lactating indoors sows, is in the interval 4.5 to 5.5 mmol pr. L. For outdoor sows more variation is seen in plasma glucose concentration and the values are generally lower than those of indoor sows. This is attributable to the sampling conditions that is associated with the outdoor experimental conditions: Blood samples is not analyzed right away, leaving time for red blood cells to consume glucose, and sampling of several outdoor sows is time consuming, stretching the sampling across a longer time interval relatively to last feeding.

Due to its precise regulation, plasma glucose concentration seldom is affected by dietary treatment and likewise no effect of dietary treatment, either DF or dietary CP, on plasma glucose was found in these studies. Thus, plasma glucose can be used as an indicator to ensure that animals thrive in terms of physiological functioning.

Lactate exists as two stereoisomers: D- and L-lactate. The lactate found in plasma is almost entirely L-lactate, as mammalian cells cannot produce D-lactate, with the exception of the methylglyoxal pathway in which extremely small amounts are produced (Ewaschuk et al., 2005). D-lactate found in mammalian plasma, thus originate from exogenous sources, such as fermented feedstuff or microbial fermentation in the gastrointestinal system. In sows the major three sources for lactate, regardless of stereochemistry, is fermentation of starch in the stomach by *Lactobacillus,* anaerobic metabolism in muscles and synthesis from propionate in the liver. As for glucose, plasma lactate concentration varies in relation to feeding: When feed is ingested and starch becomes available for fermentation in the stomach, plasma lactate concentrations increase (Serena et al., 2009). Plasma lactate concentration do however tend to be relatively stable and Serena et al. (2009) found it not to vary between dietary treatments, despite large variation in starch content. The contribution from anaerobic metabolism is highly variable and increases during physical activity (Otten and Eichinger, 1996). Also, the amount of lactate synthesized in the liver is expected to exhibit variation: Propionate is one of the major VFAs produced in the hindgut from fiber fermentation. Serena et al. (2009) found the plasma propionate concentration to be higher in fiber-rich diets compared to low-fiber diets in sows, which consequently supports a higher lactate production in the liver.

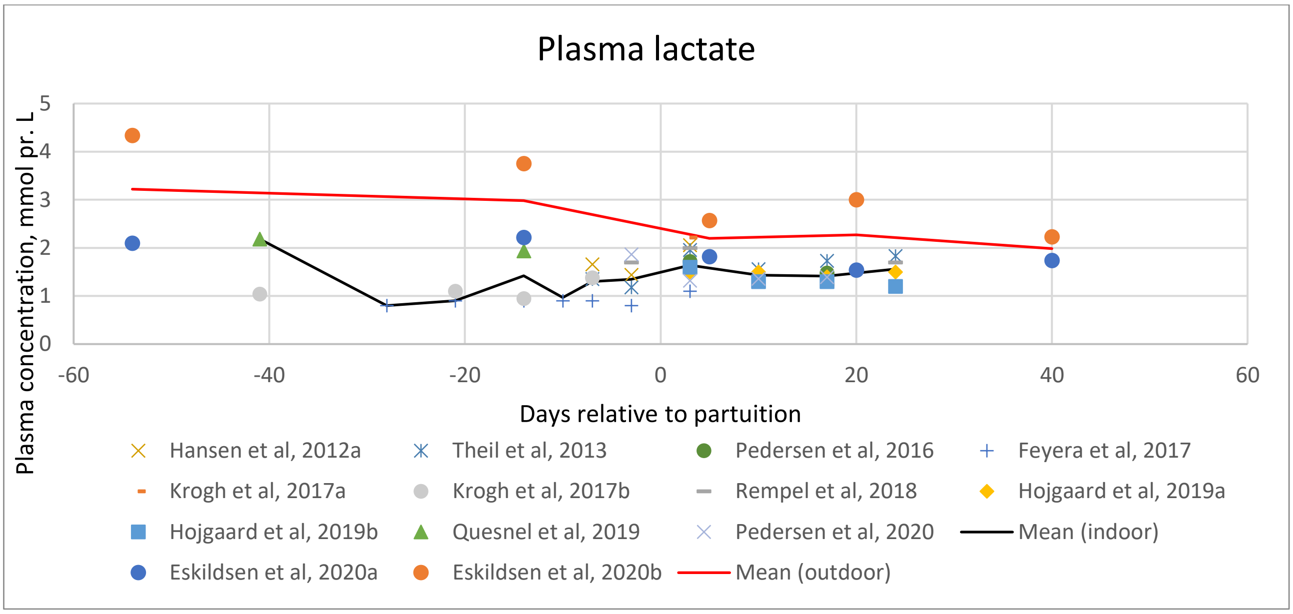

Figure 7, plasma lactate concentration of indoor and outdoor sows relative to parturition

Throughout gestation the plasma lactate is relatively steady and typically no significant intra-study variance is observed, however lactate concentration may vary between studies, depending on dietary composition and activity level (figure 7). For indoor sows, plasma lactate concentration increases prior to and during farrowing, due to nest-building behavior and uterine contractions (Hansen et al., 2012a, Feyera et al., 2018). Post-partum plasma lactate concentration also tends to be relative stable (figure 7), however Krogh et al. (2017a) found it to increase during lactation. This effect is explained by the increasing amount of feed ingested, which increases fermentation of starch in the stomach and fiber in the hindgut. In studies with outdoor organic sows, plasma lactate concentration were found to be higher than plasma concentrations of indoor sows (Eskildsen et al., 2020ab). This difference is presumable attributable to the intake of roughage by organic sows, however a higher intake of starch, diet, allowing for more starch fermentation in the stomach or the potentially higher level of physical activity of organic sows, could also be possible explanations.

Plasma TG primarily originate from feed, either compound feed or roughage, however a small amount can be synthesized by the liver. Plasma TG concentration is highly affected by the sow's productive stage, as during lactation TG in the blood is absorbed by the mammary gland and used for milk synthesis (Mosnier et al., 2010). This has been demonstrated using indoor sows by both Feyera et al. (2018) and Pedersen et al. (2020) that found considerable higher plasma TG concentrations before farrowing than after. Looking at figure 8, this difference between before and after parturition shows clearly. For outdoor sows, same legible effect of the sow's reproductive stage was not observed.

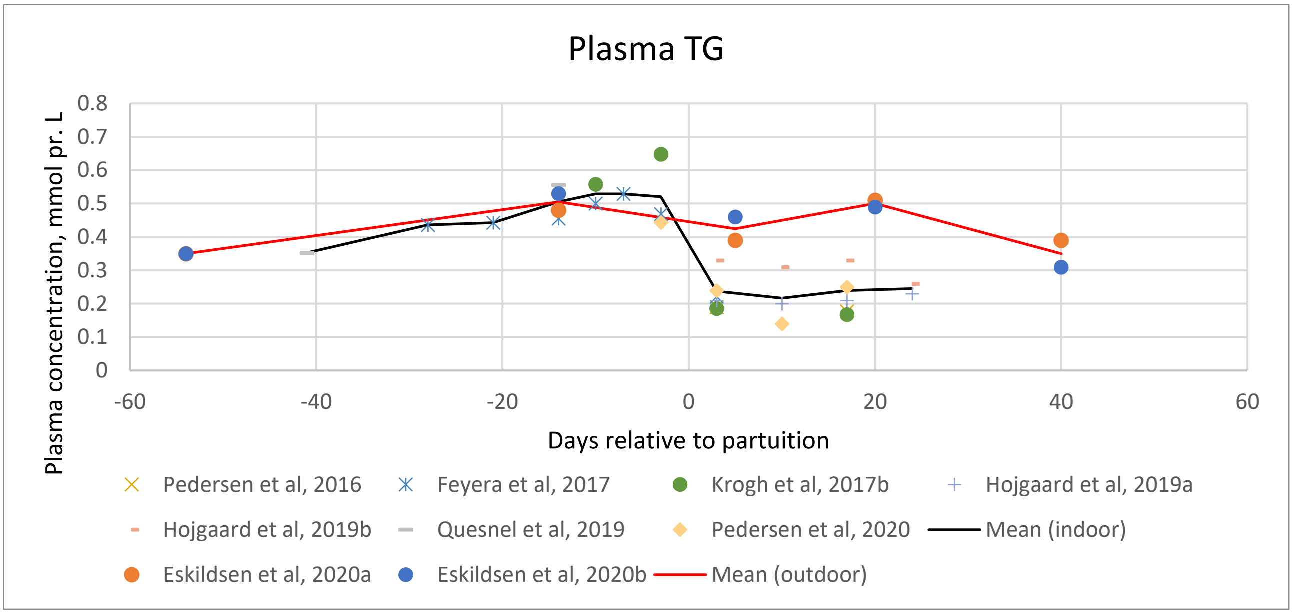

Figure 8, plasma triglyceride (TG) concentration of indoor and outdoor sows relative to parturition

Plasma NEFA originates from catabolism of body fat tissues and is an important metabolic fuel (Frayn, 1998). NEFAs is produced when adipose tissue undergoes lipolysis which is regulated accordingly to the nutritional state of the animal. When the sow experience energetic deficiency, plasma NEFA concentration increases to meet the energy requirements of the various processes within the body (Duncan et al., 2007). Variation in plasma NEFA concentration can be seen in relation to feeding, where pre-prandial plasma NEFA concentration is high and post-prandial concentrations are low (Frayn, 1998). Also, in the long term, during long-lasting energetic deficiency, plasma NEFA concentrations will be high, due to extensive fat catabolism, such as in the lactation period of the sow. Thus, concentration of plasma NEFA is often used, as an indicator for body fat mobilization.

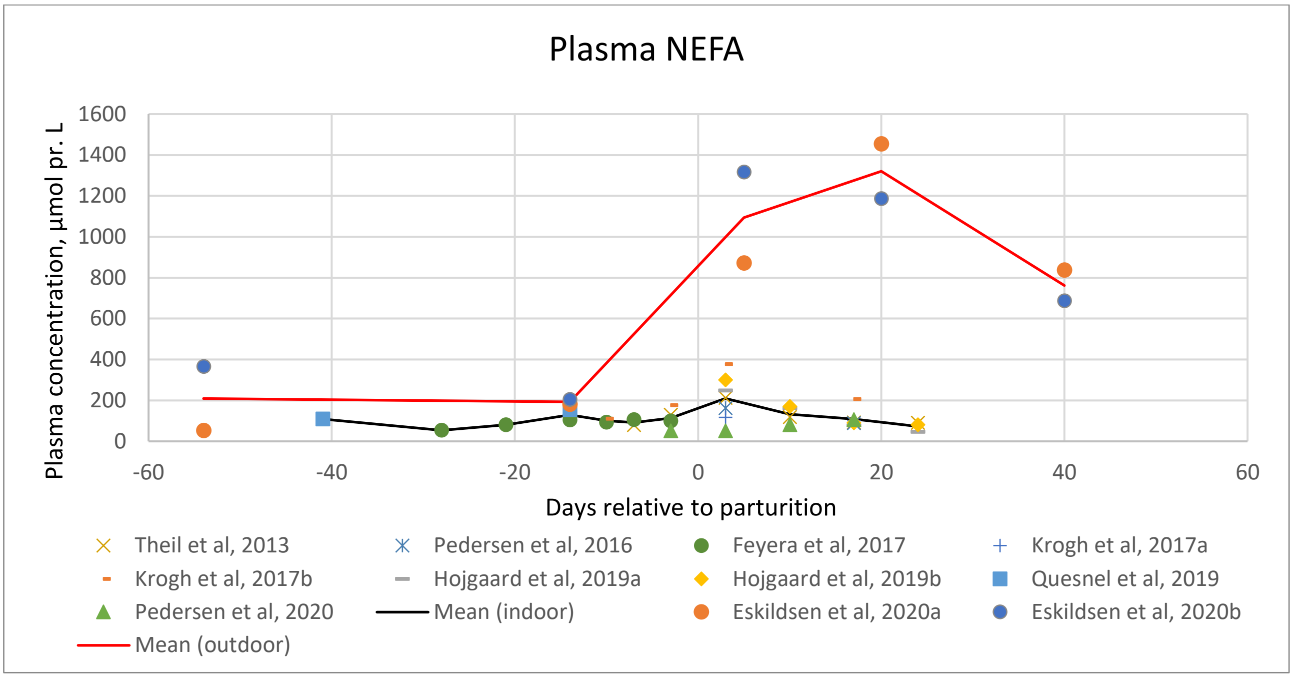

Figure 9, plasma non-esterified fatty acids (NEFA) concentration of indoor and outdoor sows relative to parturition

Plasma NEFA concentrations found in previous studies are seen in figure 9. During gestation, the plasma concentrations remains relatively stable, however during the lactational stage considerable changes occur. For both indoor and outdoor sows, the plasma NEFA concentrations increase rapidly at this stage, as the sows are nutritionally challenged. Numerically the NEFA concentration is much higher for outdoor organic sows, indicating that the dietary energy deficit and consequently body fat mobilization is higher than their conventional counterparts. As seen in figure 9, plasma NEFA concentration typically reaches maximum values in early lactation and gradually decreases as lactation progresses. This gradual decrease of plasma NEFA concentration in sows, can be explained by the energy balance of the sows. Early in lactation the nutritional deficit is very high and as lactation progresses the balance is improved and eventually becomes positive (Feyera and Theil, 2017). The findings of Strathe et al. (2017b) supports this, as the highest body fat mobilization is observed in early lactation and decreased to almost zero towards the end of lactation. Mosnier et al. (2010) and Pedersen et al. (2020) did however register increasing plasma NEFA concentrations as lactation progressed. Plasma NEFA concentration is most likely not affected by dietary treatments, such as CP and fiber (Krogh et al., 2017a, Hojgaard et al., 2019a, Eskildsen et al., 2020b, Eskildsen et al., 2020a).

Urea originates from the oxidative degradation of AAs, primarily taking place in the liver. During this process, the AAs are deaminated and the amino group are converted into ammonium ion. Ammonium ion might be used in the biosynthesis of nitrogen compounds and excess enters the energy requiring urea cycle. Finally, urea is transported in the blood to the kidneys and excreted in the urine. AA degradation thus leads to an increase of plasma urea concentration. The carbon skeleton of the oxidized AAs enters the citric acid cycle and produces energy (Tymoczko et al., 2015).

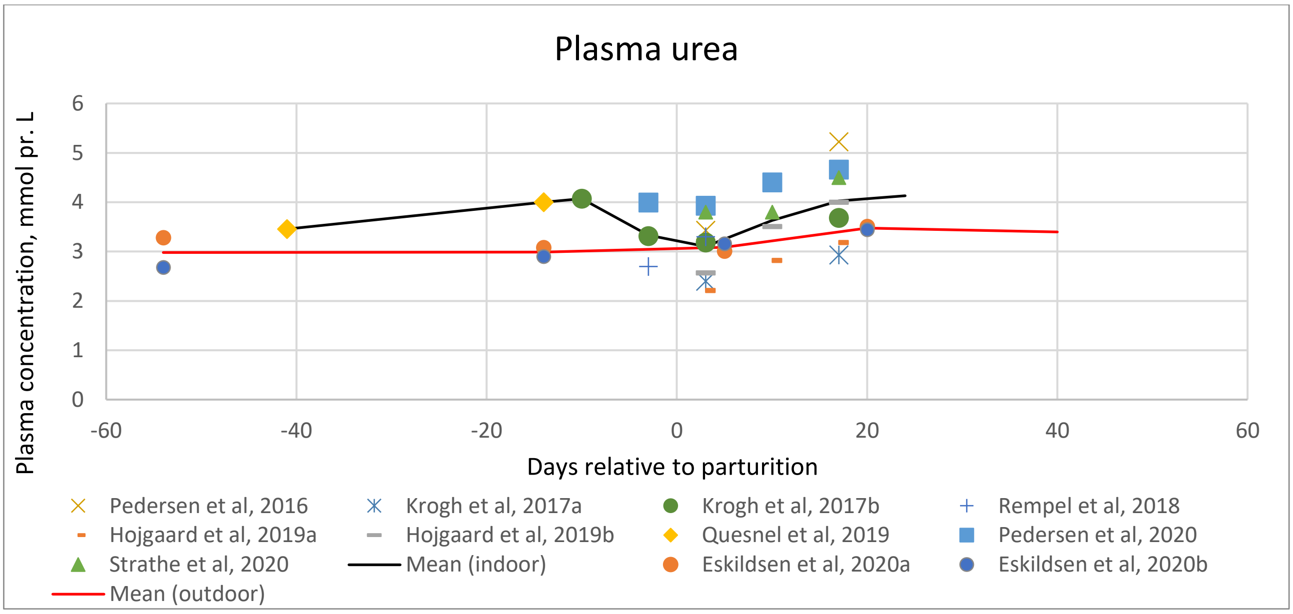

Figure 10, plasma urea concentration of indoor and outdoor sows relative to parturition

During gestation plasma urea concentration remain stable (Mosnier et al., 2010), which also can be seen in figure 10. When lactation initiates, often an increment of plasma urea concentration are seen, which tends to increase further as lactation progresses (figure 10). This gradual increment of plasma urea concentration is due to increased intake of dietary protein as feed intake increases, and mobilization of body protein due to nutritional deficit.

Also, a clear effect of dietary protein concentration on plasma urea can be found. This has been demonstrated by Hojgaard et al. (2019a); at day 3, 10 and 17 a broken-line model was used to describe the plasma urea concentration in relation to dietary protein concentration and at day 24 plasma urea concentration could be described with a linear model, increasing according to dietary protein concentration (figure 11). The higher breakpoint early in lactation is explained by a low feed intake and consequently low CP intake, allowing for higher dietary CP concentration, before a surplus is reached. Strathe et al. (2020) also investigated the effect of increasing dietary protein concentration and found a linear broken model to describe the concentration of plasma urea as a function of dietary protein well (figure 11). The increase in plasma urea concentration after the breakpoint in both studies indicates that dietary protein concentration is too high, and protein is supplied in excess of requirement. In the study by Hojgaard et al. (2019a) the breakpoints are reached at much lower dietary protein concentration compared to the study by Strathe et al. (2020), as CAAs are used to adjust the AA profile to the requirement of the sow. Thus, using CAA allows to lower the CP content and consequently reduce the plasma urea concentration. Beside the effect of protein quantity and quality, time after feeding also affects the plasma urea concentration, like many of the previous described metabolites (Eggum, 1970)

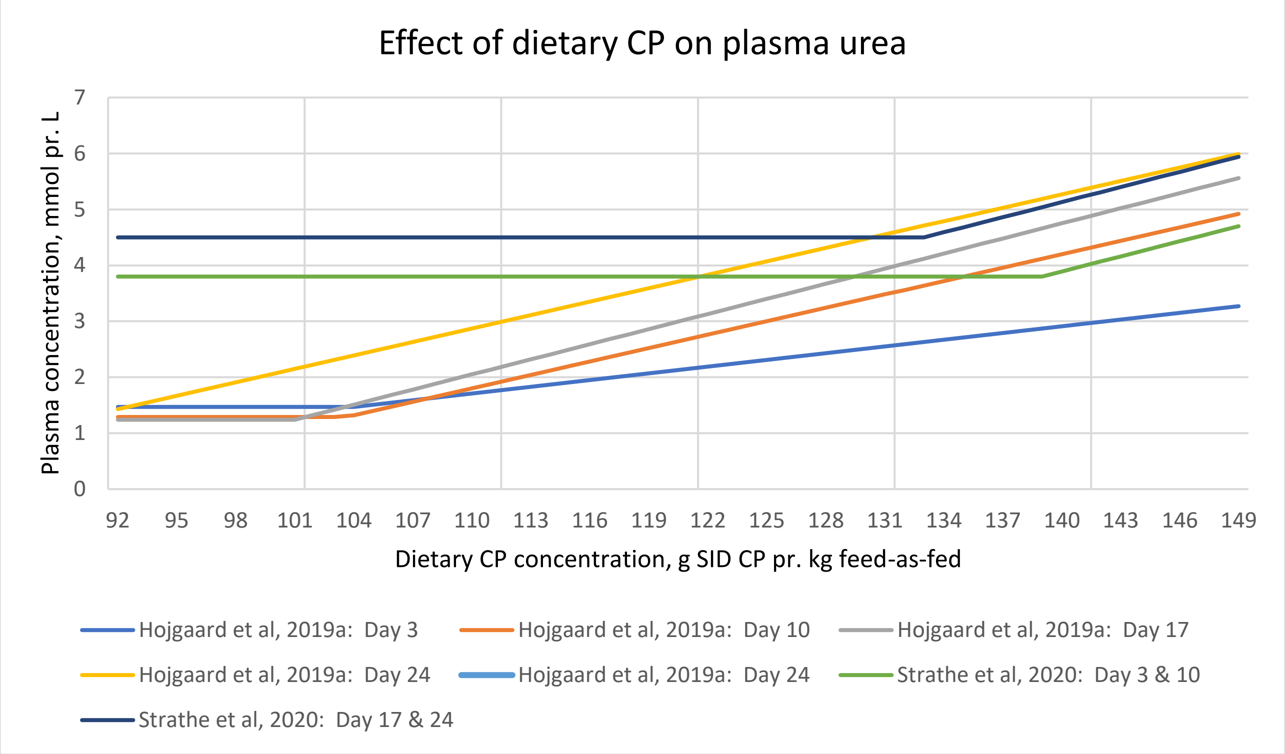

Figure 11, effect of increasing dietary protein (g SID CP pr. kg feed-as-fed) on plasma urea concentration in indoor sows

The plasma metabolites described so far, are the major metabolites describing the overall metabolism of the sow, affected by feeding, reproductive stage and more. In the following section, minor metabolites in plasma mainly associated with the N metabolism is reviewed.

Plasma AA originates from various sources, namely digestion of dietary protein, mobilization of body protein and synthesis of non-essential AAs. Different methods exist for quantifying the content of peptides and AAs in the blood or plasma. In current study free primary amino groups are measured. Each free AA have one primary amino group, located in the backbone of the AA, except for proline which have a secondary amino group. In addition, the side group of the AA may contain primary amino groups, as in case of lysine, asparagine, glutamine and arginine. Accordingly, peptides have at least one free amino group originating from the amino-terminal AA, unless this AA is proline, however the total amount depends on the AA composition.

Plasma AA increases postprandial due to the digestion of dietary protein and subsequent absorption of peptides and AAs (Krogh et al., 2017b, Hu et al., 2020, Dourmad and Matte, 2021). Also the dietary level of AA has also been proved to affect plasma AA concentration, especially the concentration of essential AAs, since these cannot be synthesizes de novo (Guan et al., 2004, Rungcharoen et al., 2011, Che et al., 2019).

Quesnel et al. (2008) found plasma AA concentration to be affected by reproductive stage: During the transition period the concentration remains steady and decreases mid and late lactation, which is explained by the high uptake of AA in the mammary gland for milk protein production (Krogh et al., 2017b)

Glutamate and glutamine are two highly abundant AAs in both feedstuff, body protein pools and milk. In plasma glutamine is the most abundant free AA (Larsen and Fernández, 2017) . Most dietary glutamate and glutamine are catabolized in the intestinal epithelium, thus the majority found within the body is of endogenous origin (Watford, 2015). Glutamate is synthesized from the citric acid cycle intermediate; α-ketoglutarate and can form glutamine by incorporation of an ammonium ion (Tymoczko et al., 2015). Conversely glutamate can be formed from glutamine when the terminal amide group is cleaved (Larsen and Fernández, 2017). Glutamate and glutamine are also synthesized from other AAs, and released during muscle proteolysis (Watford, 2015). Despite not being essential the two AAs takes part in several important processes, including the N metabolism, where they are important N donors (Watford, 2015).
The plasma concentration of glutamine can be used as an indicator of the sows energetic or protein status. During nutritional deficiencies, skeletal muscle are catabolized to accommodate the nutritional requirements. Immediately high quantities of glutamine are released, followed by a decreases of plasma concentration (Watford, 2015). Accordingly, decreasing steadily decreasing plasma concentrations of glutamine during lactation in different species of domestic animals.

Creatinine is the end-product of the creatine metabolism. Creatine and creatine phosphate is non-enzymatically converted to creatinine at an almost steady rate, depending on the body mass, and secreted in the urine (Wyss and Kaddurah-Daouk, 2000). Creatinine may also be used as an indicator for muscle degradation, as plasma and urine concentration increases during mobilization (Strathe, 2017)

2.4.2 Urine metabolites

Urea is a highly abundant molecule in urine and as described in the section about plasma urea, the metabolite is central in the nitrogen metabolism and of great concern when feeding sows. The main purpose of the urea cycle is conversion of ammonium ion into urea, which is excreted into the urine. In urine the urea is accumulated, thus higher concentration is observed compared to plasma.
During gestation urinary urea concentrations remains stable but are higher than lactational values. In lactation urinary urea concentration tend to decrease along with the progression of lactation (figure 12), indicating that the feed level and nutrient composition is better adapted the requirement of the sows at peak lactation than early lactation. Increased body protein mobilization due to nutrient deficiency, might also explain the higher N excretion in early lactation (Pedersen et al., 2019a).

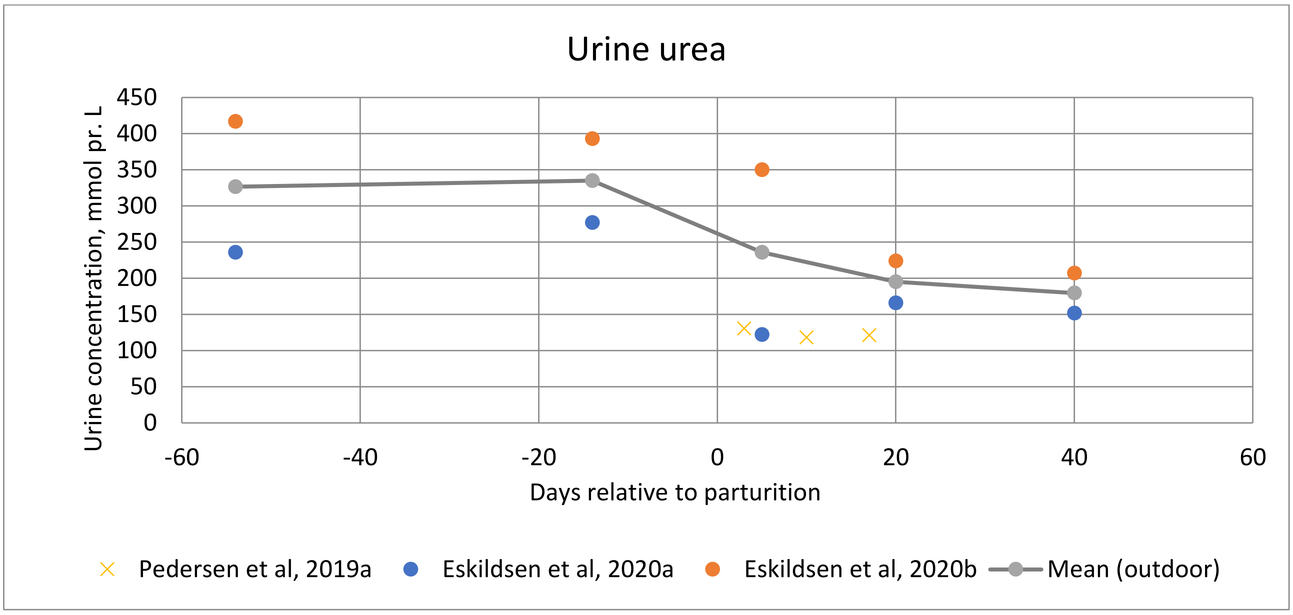

Figure 12, urinary urea concentration of indoor and outdoor sows relative to parturition

As plasma urea, urinary urea is affected by dietary CP level and AA composition of the diet. At increasing levels of dietary CP, Pedersen et al. (2019a) found urinary N excretion to increase, due to intake of CP and AAs above the requirement, leading to increased urea synthesis and excretion. Supporting this, Huber et al. (2015) found decreasing levels of dietary CP and increasing levels of CAA, the urinary N excretion to decrease, without reducing milk yield (MY) or protein content. Actually, the MY at peak lactation increased, as dietary CP content decreased, due to increased N utilization, as the dietary AA composition was better adapted the requirement of the lactating sow (Huber et al., 2015).

Urinary urea concentration is highly dependent on the general urinary concentration, affected by the sow's intake of water. To avoid the urinary concentration to affect the results regarding urinary urea concentration, the ratio between urinary urea and creatinine can be used, since creatinine is produced and excreted into urine at a rather constant rate (Eskildsen, 2020).

Besides urea and creatinine, urine contains extremely small concentrations of AAs. One important role of the kidney to avoid loss of AAs into urine and thus most AAs are resorbed. However, the filtration is not completely effective, and thus a small amount ends up in the urine. Especially when large concentrations of AAs are filtered, the filtration efficiency declines (Makrides et al., 2014). In current thesis the concentration of free primary amino groups, glutamine and glutamate in urine was investigated. Besides AAs, small peptides and proteins may contribute to the concentration of free primary amino groups, however the concentration of glutamine and glutamate presumable reflects the concentration in plasma.

2.4.3 Milk composition and yield

Mammals are characterized by producing milk, nourishing their offspring in the first period after birth. MY and milk chemical composition differs between species and breeds. During the lactation period the composition alters, however the most drastic changes occurs within the first 3 days postpartum (Theil et al., 2012). Just after farrowing colostrum is secreted. Colostrum is essential for the piglet's survival, as it contains immunoglobulins, responsible for the passive immunization of the newborn pig (Le Dividich et al., 2005). Colostrum has a very high protein content, due to the high concentration of immunoglobulins, a high fat content and a relatively low lactose content. Shortly after birth colostrum production decreases and lactation initiates, as the colostrum is diluted and gradually replaced by milk. This causes lactose content to increase, and protein and fat content to decrease. Depending on lactational stage, sows milk constitutes of 6-8 % fat, 5.1-5.8 % protein and 5.6-5.8 % lactose (Theil et al., 2012).

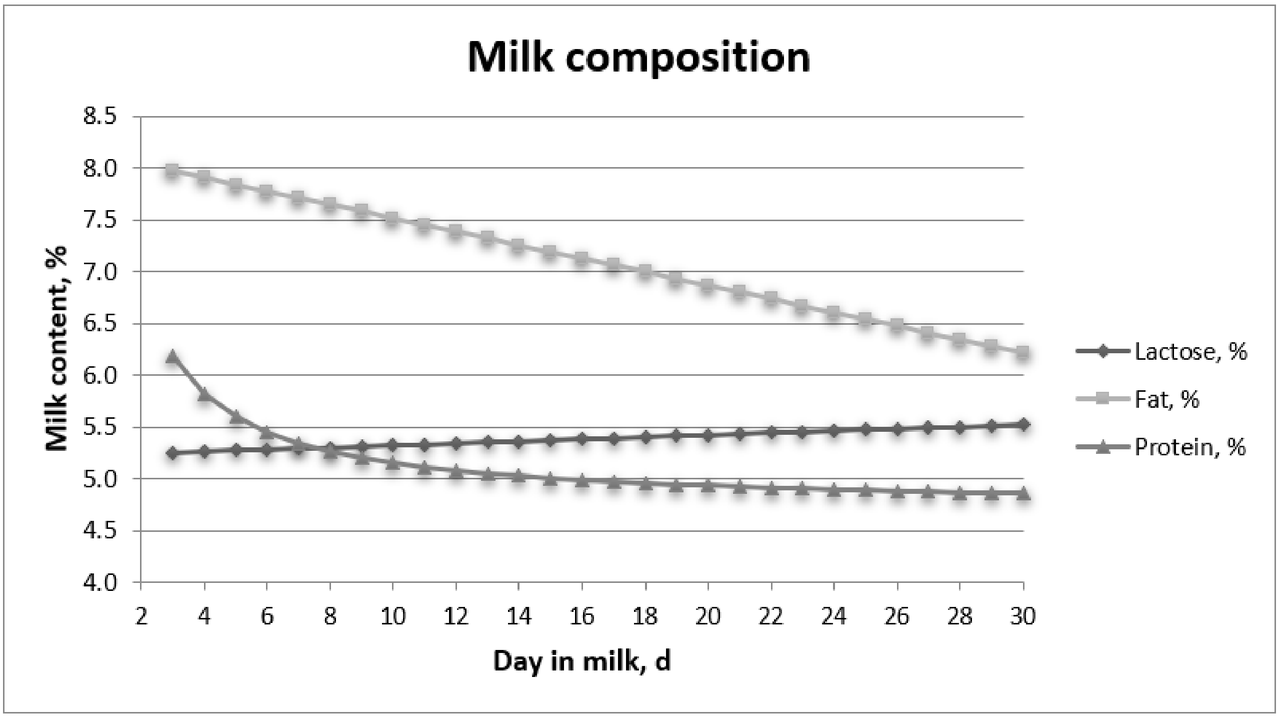

Figure 13, chemical composition of milk during the lactation period of the sow, at 16 % dietary CP (Hansen et al., 2012b)

Beside lactational stage, several other factors influence the composition of milk, such as dietary composition and energy intake. Of the three large nutritional fractions, lactose is the most constant component, due to its osmotic role of drawing water into the milk (Theil et al., 2012). This is also supported by Hansen et al. (2012b), that in the effort of making a model predicting the MY and composition, found that only stage in lactation affected milk lactose content (figure 13).

Regarding milk protein, Hansen et al. (2012b) found besides lactational stage (figure 13), dietary CP content to affect milk protein significantly, where an increasing dietary CP content increases milk protein content. This is in agreement with studies earlier described; Jones and Stahly (1999a) and Clowes et al. (2003) where sows fed diets very low in protein, had reduced milk protein content. This effect was also observed by Hojgaard et al. (2019a), who found a linear correlation between dietary CP intake, and milk protein, reaching 5.27 % protein in milk at 149 g SID pr. kg feed-as-fed. Same linear correlation was found by Guan et al. (2004), that moreover found the greatest increase in milk protein between sows fed dietary protein below their requirement and sows fed normal dietary protein levels.

The third large nutritional fraction in milk; fat, varies as lactose and protein according to lactation stage and is also affected by dietary content of fat, with exception of certain sources of fat (Lauridsen and Danielsen, 2004, Hansen et al., 2012b). In a study by Lauridsen and Danielsen (2004) different types of dietary fat, was included in diets for lactation sows. At an 8% inclusion rate, animal fat, rapeseed oil, coconut oil and palm oil significantly increased milk fat output and consequently the energy output, while fish oil and sunflower oil had no effect. Similar effect has been reported by other studies (Shurson and Irvin, 1992, Tilton et al., 1999). Other authors have contrarywise not been able to find an effect of increased dietary fat of milk fat content (Theil et al., 2004, Hansen et al., 2012b, Krogh et al., 2017a). The milk fat content might also be affected by high levels of body mobilization (Theil et al., 2012).

MY is highly dependent on lactational stage. From early lactation the MY increases until it reaches maximum, around the third week of lactation. The MY at peak lactation highly variable among sows, however yield of modern sows can reach up until 15 to 16 kg/d in productive herds (Hansen et al., 2012b, Krogh et al., 2017b, Strathe, 2017, Hojgaard et al., 2019a). Beyond day 30 of lactation, only scarce literature can be found describing the MY, as conventional piglets typically are weaning around third and fourth week of lactation (Hansen et al., 2012b). A two recent studies the MY of organic outdoor sows estimated to be 8.0, 12.2 and 9.3 kg/d for primiparous sows and 9.7, 15.7 and 11.8 kg/d for second parity sows, at day 5, 20 and 40 of lactation respectively (Eskildsen et al., 2020ab). MY varies with parity, where Beyer et al. (2007) found increasing MY at higher parities sows. A correlation between litter size and MY is also typically seen, as larger litter provides greater stimulation of total mammary tissue growth and unused gland will undergo involution (Auldist et al., 1998, Kim et al., 1999, Theil et al., 2006). Correspondingly, milk production is the major limiting factor for piglet growth. Another, but important, aspect that can affect the MY is the feeding of sows. It is however important to have in mind that sows use their body reserves as a buffer for nutrients for milk production, hence short term or slight nutritional deficiencies, will have no impact on milk production. Feeding lactating primiparous sows at either high (44 MJ NE/d) or low level (33 MJ/NE/d), van den Brand et al. (2000) found a significant effect of feeding level on MY and milk energy output. Dietary CP content was however also different in the two groups, as the sows were fed the same diet at different feeding levels. In another study, feeding lactating sows different energy levels (59.4 MJ ME/d vs 43.5 MJ ME/d), but equal dietary CP, a significant lower MY for the sows fed the low energy diet, at all stages of lactation. However, across the whole lactation no significant difference in MY was found (Noblet and Etienne, 1986). Regarding the effect of dietary CP on MY, Kusina et al. (1999) demonstrated that lactation diets very low in dietary protein (8.36 % CP and 0.33 % lysine vs 16.99 % CP and 0.88 % lysine) will reduce the MY of primiparous sows. Furthermore, the authors proved that feeding gestating sows 50% of their required CP and lysine decreased MY, due to reduced protein accretion during gestation. A similar tendency was observed in organic outdoor sows by Eskildsen et al. (2020b), where sows fed 12% less dietary protein during lactation, had reduced MY in late lactation.

2.4.4 Milk metabolites

Beside these main nutritional components in milk, many metabolites can be found and examined as well. While plasma metabolites are used to derive information about the sow's overall metabolism, affected by all the organs and tissues connected to the blood circulation, metabolites in the milk focus on the metabolism occurring at cell level in the udder. This is due to the origin of milk metabolites: As a lipid droplet is secreted into milk, the apical plasma membrane envelopes the droplet and is later pinched off. Between the plasma membrane and lipid droplet is a small space, filled out by cytosol from the secretory udder cell, containing various products and intermediates from the cell metabolism (Heid and Keenan, 2005).

Glucose is a central metabolite in the mammary gland. Approximately 70% of the arterial glucose are removed by the mammary gland and is the main precursor for lactose (Krogh et al., 2017b, Renaudeau et al., 2003). The glucose concentration in milk reflects the concentration in the mammary cell and thus is an indicator of the energy status of the mammary gland.

In the early lactation the mammary gland is exposed to oxidative stress, as body fat is mobilization to support the high energy demand of milk production. This increases the fatty acid oxidation and production of reactive oxidative species (ROS) (Turk et al., 2013). To cope with the oxidative stress, glucose is redirected towards the pentose phosphate pathway (PPP), producing the reducing agent, NADPH. Glucose-6-phosphate (Glu6P) is the first intermediate of the PPP, thus it is expected to observe high concentrations of Glu6P in early lactation, when fat is mobilized (Zachut et al., 2016). Moreover, large amounts of NADPH are used during fatty acid synthesis, which further increases the demand (Larsen, 2014)

Besides the PPP, oxidation of isocitrate, a citric acid cycle intermediate, are also believed to produce large fractions of NADPH in the bovine udder. Thus, isocitrate concentration in cows' milk are an indicator of the energy status in the udder (Larsen, 2014). Billa et al. (2020) investigated the effect of feed restriction on a range of milk metabolites in dairy cows. During feed restriction the concentration of Glu6P and isocitrate increased considerable, showing their relation to the energy status of cows.

In milk most AA as bound in protein, however a very small fraction is found as free AAs. In cows' milk free AA concentration are believed to be reflect the availability and metabolism of AA, as the concentration in milk declines during feed restriction (Billa et al., 2020). In sows, the content of free AAs are relatively low in colostrum and increases throughout lactation (Hurley, 2015).

2.5 Dietary protein level and AA composition

The nutritional composition of diets for sows should be adapted the requirements of the animal at its current state of production. Central in this context is the balance between protein and energy in the feed. At the optimal ratio of protein to energy, a minimum of AAs are oxidized and used for energy production, and rather used for productive purposes. As the content of protein increases relative to the energy above the optimal ratio, typically the feed efficiency decreases. Contrarywise, when the content of protein relative to energy is lowered below the optimum, productivity tend to decrease. In extension to this aspect is the feed intake, which ultimately decides the total protein intake. In contrast to dietary protein concentration, the feed intake does not allow for adjustment as easily, as it affects the intake of energy and remaining nutritional fractions as well. A second important aspect is the AA profile in the feed. In order to maximize productivity and utilization, the AA profile should reflect that of the designated productive purpose. Often the term ideal protein is used; a situation were all EAAs are co-limiting (van Milgen and Dourmad, 2015). Optimizing the AA profile allows a reducing of the overall protein content and reduces oxidation of AAs fed above the requirement of the animal.

CAAs are commonly used in conventional pig production, to adjust the AA profile to better fit the requirement of the animals and improve protein utilization. Several studies have found beneficial effects of including CAAs in diets for sows, as the CP content concurrently can be reduced (Huber et al., 2015, Huber et al., 2018, Hojgaard et al., 2019a, Pedersen et al., 2019b). Reducing the dietary CP content from 16,2% to 12,7 % of feed-as-fed and supplementing with CAAs for primiparous lactating sows, Huber et al. (2018) found no detrimental on milk protein yield or piglet growth rate by reducing the dietary CP content, while balancing the AA profile using CAAs. The group fed reduced dietary CP had in fact an increased utilization of N for milk production, reducing the losses of N to the environment (Huber et al., 2018). In a study by Hojgaard et al. (2019a) lactating sows were fed increasing amounts of dietary CP; 96, 110, 119, 128, 137, and 152 g SID/kg feed-as-fed, with CAA supplementation, to determine the optimal CP content for maximizing litter gain. Using a linear broken-line model, the optimal CP content was determined as 125 g SID/kg feed-as-fed, in order to maximize litter gain (figure 14). Below this dietary CP concentration litter ADG decreases (Hojgaard et al., 2019a). In addition to this, results from the study by Pedersen et al. (2019b) indicate that feed efficiency is maximized at the same dietary CP concentration and decreases as dietary CP concentration increases.

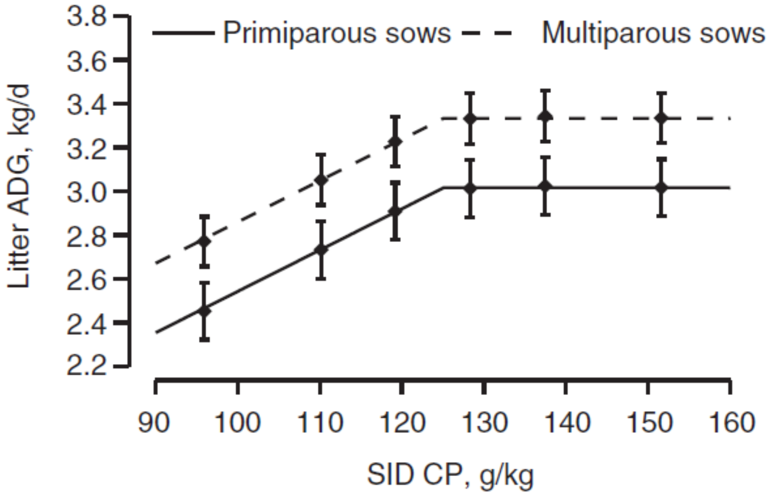

Figure 14, effect of increasing dietary CP level (g SID pr. kg) on litter average daily growth (ADG) described by linear broken model for primi- and multiparous sows (Hojgaard et al., 2019a).

In line with these studies, Strathe et al. (2020) investigated the effect of increasing dietary CP on body mobilization and milk production in lactating sows. Authors found that loss of body protein decreased with increasing dietary CP level, until reaching a plateau at 128 g SID/kg feed-as-fed. The average daily litter gain also improved, reaching maximum at 136 g SID/kg feed-as-fed. Compared to the study by Hojgaard et al. (2019a), this is 8,8 % higher dietary CP content, underlining the beneficial effect of balancing the AA profile.

Balancing the AA profile using CAAs are a very efficient tool to reduce the CP level, optimize protein utilization and consequently reduce environmentally impact. CAAs is however not allowed in organic agriculture, why organic pig producers must resort to other means. As earlier described, the requirement of organic outdoor sows, differs from that of conventional sows, as organic sows most likely have a higher requirement for energy. Fulfilling the energy requirement of organic sows, when feeding accordingly to conventional feeding standards, will indeed result in too high protein supply. Feeding a lower quantity of protein, simply by lowering the ratio of protein to energy in the feed, is one solution that organic pig producers might try. In two recent studies by Eskildsen et al. (2020ab) this was the exact approach, to attempt optimizing feeding of organic sows. In both studies a low protein diet, containing 12 % below the Danish indoor recommendations, was compared to a commercially available sow diet, during summer and winter respectively. During winter, no negative effects were observed in the gestation period and energy utilization was improved for sows fed low protein diets. In lactation however, the low dietary protein affected the sows' performance negatively, as their feed intake was low and their milk production was depressed in late lactation (Eskildsen et al., 2020b). In the summer experiment, no negative effects on productive traits were seen in both gestation and lactation, despite sows did not meet their protein and lysine requirement in the lactation period (Eskildsen et al., 2020a). A possible explanation for the different effects on lactation performance of sows during winter and summer, is the nutritional contribution from the vegetation intake form the paddocks. In the summer low protein sows consumed in average 14 % more vegetation than the commercially fed sows (Eskildsen et al., 2020a). The authors recommend that gestating organic sows are fed low protein diets, while lactating sows should be fed a higher concentration of dietary protein.

2.5.1 Oversupply of protein and AA

Oversupply of CP or AAs might be due to a high feed intake and consequently high CP intake, a not properly balanced diet in terms of CP concentration or an unbalanced AA profile. As earlier mentioned, the surplus of AAs fed above the requirement are oxidized, which increases plasma and urine urea concentration, and since the urea cycle is energetically expensive, an energy loss is associated to urea excretion (Pedersen et al., 2019b). This ultimately results in decreased feed efficiency.

Pedersen et al. (2019b) investigated the effect of increasing dietary concentration of CP on the energy utilization in lactating sows. Diets were formulated to be constant in lysine, and levels of methionine, threonine and tryptophan met or exceeded recommendations. The dietary concentration of SID CP varied from 118 to 156 g/kg DM. Results showed that when increasing the dietary CP concentration, the energy lost through urine increased (Pedersen et al., 2019b). At the second experimental week 38.7 % more energy was lost in urine in the diet with highest CP concentration, relative to the diet containing 128 g/kg DM, which is close to the optimal concentration (Hojgaard et al., 2019a). In figure 15 the impact of dietary CP concentration on feed efficiency is seen and clearly as the concentration increases, feed efficiency is reduced due to the loss through urea.

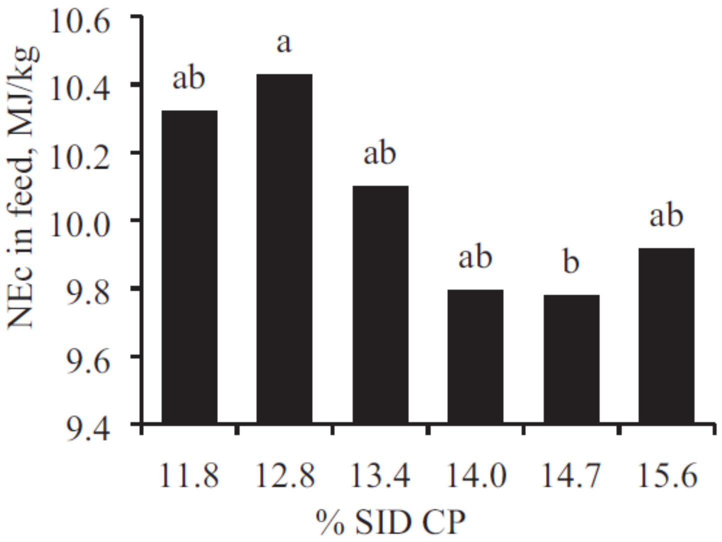

Figure 15, net energy corrected for energy mobilization in MJ/kg feed varying in dietary concentration of SID CP (Pedersen et al., 2019b)

In addition to this, Zhang et al. (2020) investigated the effect of reducing dietary CP concentration by optimizing the AA profile on energy efficiency in a recent study. Compared to the study by Pedersen et al. (2019b), the AA profile was not equal between experimental diets, as only the optimized diet contained CAAs. The optimized diet had a dietary CP concentration of 154.6 g/kg DM and the control diet 211.2 g/kg DM. In early lactation no effect of treatment was seen, however in late lactation sows fed optimized diets, had increased energy utilization efficiency due to reduced urine energy excretion (Zhang et al., 2020). Both the reduced dietary CP concentration and optimized AA profile is expected to contribute to this effect. In a study on growing pigs Le Bellego et al. (2001) observed same effect of dietary CP concentration on energy utilization, where a reduction from 189 to 123 g CP/kg DM reduced energy excreting in urine by 40%.

In addition to the energy lost through urine, additional heat is produced during oxidation of AA, increasing the energy loss associated with AA oxidation (Just, 1982). In growing pigs, heat production increased by 7%, as dietary CP content increased from 123 to 189 g/kg DM (Le Bellego et al., 2001). Zhang et al. (2020) also observed a reduced heat production associated with peak lactation, when sows were fed diets with less CP and optimized AA profile.

Another aspect that affects urea excretion, is mobilization of body reserves during nutritional deficiency, which often occurs in start lactation (Feyera and Theil, 2017). If body protein is mobilized in order to meet the energetic requirement, catabolized AAs are oxidized and urea excretion increases. Body protein may however also be mobilized if sows is in dietary deficit of CP or individual AAs, and used in highly prioritized reproductive processes, such as milk production. Under organic conditions the AA profile of the diet is seldom perfectly adjusted the requirement of the sow, due to the limited range of feedstuff and additives available. In figure 16, the EAA profile an organic compound feed for lactating sows relative to the feeding standards is illustrated. Clearly, the organic lactation diet has a skewered profile compared to the standards (figure 16). The content of lysine, methionine, tryptophan and threonine do not reach the standards, where the content of lysine is lowest and thus first limiting. Intake of roughage may supply additional lysine however if the requirement is not met through the diet, sows will mobilize body protein. In figure 18, the fate of EAA from mobilized body protein is shown; the proportion of AAs marked by red boxes are oxidized and excreted in urine as urea, while black boxes indicate the number of AAs utilized, primarily for milk production in lactation. It is assumed that the sows eat approximately 7 FUsow pr. day at peak lactation, while the recommended minimum daily intake is 8 FUsow (Eskildsen et al., 2020ab, SEGES, 2020). As seen in figure 17 all mobilized lysine is used for productive purposes, as it is the first limiting AA, and most of leucine, isoleucine, valine, histidine and phenylalanine are oxidized, as these are in surplus in the organic lactation diet according to figure 16. Concerning figure 16 and 17, it should be mentioned that only limited knowledge exists regarding the requirement of the EAAs, except lysine for which the requirement is experimental documented (Hojgaard et al., 2019b). Thus, the amount utilized and oxidized is merely an estimate based on the Danish feeding standards.

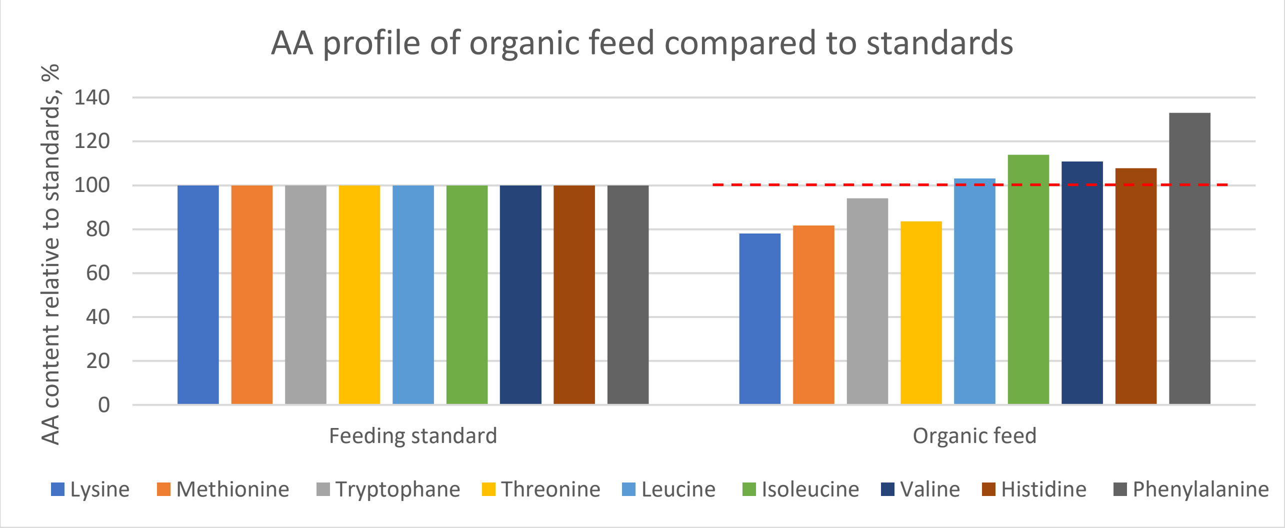

Figure 16, profile of EAA of organic compound feed for lactating sows ("green sow") relative to AA profile of feeding standards. The red line is the optimal AA composition of feed. Bars above and below indicate surplus and deficit of AA respectively (Tybirk et al., 2020)

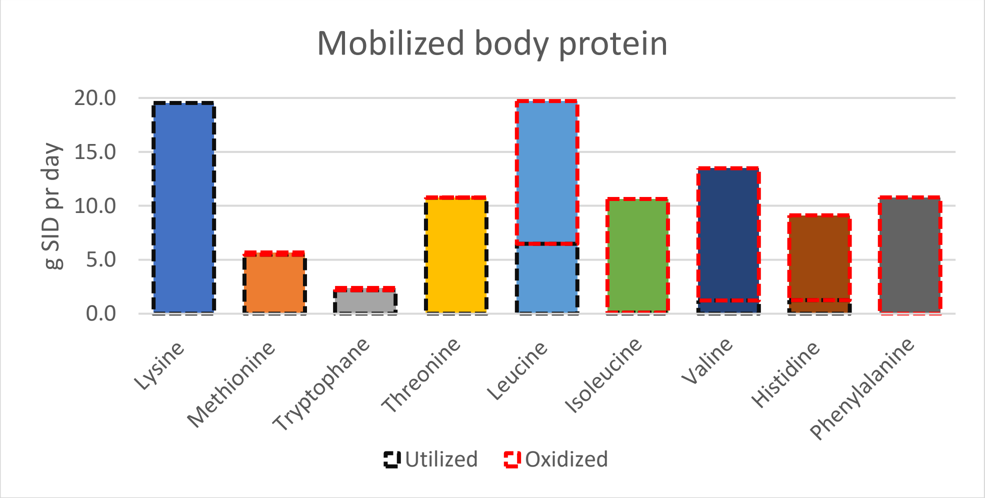

Figure 17, daily mobilized essential amino (g SID pr. day) at peak lactation when feeding "green sow" organic lactation compound feed (7 FUsow pr. day) relative to feeding standards and recommended minimum feed intake (8 FUsow pr. sow). Black and red boxes indicates the estimated proportion of the AA utilized and oxidized respectively (van der Peet-Schwering and Bikker, 2019, SEGES, 2020, Eskildsen et al., 2020b)

A consequence of oversupply of protein is the potential environmental impact. As nitrogen excretion increases, so does the environmental risk, especially when combined with outdoor housing. Hence, organic sow production is often connected to a high environmental burden (Halberg et al., 2010). Excess amounts of N in the soil, might lead to the formation of gasses such as nitrous oxide; a highly potent greenhouse gas contributing to the global warming, or ammonia, causing eutrophication in natural environments. Nitrate leaching may also occur, potentially polluting human drinking water or damaging aquatic environments (Tuomisto et al., 2012). An experiment by Eriksen et al. (2002),the authors intended to describe the fate of nitrogen in outdoor pig production. Nitrogen balance in an organic production system with lactating sows and their piglets was investigated and ammonia emission, denitrification and nitrate leaching were estimated. For six months of grazing the N surplus was estimated to 490 kg N pr. ha, however soil N had only increased by 179 kg N pr. ha. Surplus N was either retained in organic form or lost to the environment and during the following winter additional 150 kg N pr. ha was lost from the soil (Eriksen et al., 2002). Furthermore, mapping of N distribution in the soil, revealed inhomogeneity deposition of excreta, creating hotspots of N around the feeding sites, enhancing the risk of losing the N. Is was estimated that 114, 69 and 141-308 kg N pr. ha, had been lost through ammonia evaporation, denitrification and nitrate leaching respectively (Eriksen et al., 2002).

2.5.2 Deficiency of protein and AA

A negative effect is also observed, if the sow contrariwise is fed below its requirement of either CP or EAA. While oversupply decreases feed efficiency, a deficiency reduces the productivity of the animals.

The sow has a certain priority of nutrients, which changes during the productive cycle. As earlier mentioned, the highest prioritized process during gestation is fetal growth, which changes in the lactation to milk production (Theil et al., 2012). When the requirement for CP or EAA is not met, the sow will mobilize its body protein to provide adequate nutrients to support milk production (Clowes et al., 2003). Hence, the loss of body protein reserves is high in sows fed diets low in CP or lysine (Dourmad et al., 1998). If the deficiency of dietary protein becomes too high, mobilization of body reserves cannot supply sufficient nutrient to keep the milk production at the same level as sows receiving required levels of protein. This was demonstrated by Jones and Stahly (1999a) where primiparous sows were fed isoenergetic diet either high or low in protein, containing 100% and 30% of lysine requirement respectively. During the lactation period the liveweight of all sows declined, however the sows receiving low protein diet had a significant higher loss. In addition, sows on low protein diet lost body protein, while high protein sows gained. Milk composition were also affected significantly by diet, and sows on low protein had lower DM, energy and CP content, which consequently reduced litter growth (Jones and Stahly, 1999a). Sows receiving the low protein diet, furthermore, had extended weaning-to-estrus interval (Jones and Stahly, 1999b). These results are supported by Clowes et al. (2003) that found primiparous sows fed low protein diets during lactation, with a higher body protein loss (17.3 % vs 10.6 %) had reduced litter growth rate towards the end of lactation, compared to sows fed protein levels closer to the requirement. The authors also demonstrated that higher body mass at gestation, will delay the onset of negative effects of to low protein diets in the lactation (Clowes et al., 2003). This negative effect on litter performance is however unlikely to occur if sows are fed close to their requirement. Clowes et al. (2003) suggests that a mobilization of body protein below 9-12 % will not affect litter growth negatively. Protein reserves lost during lactation should be gained during following gestation but since it is a energetically costly process, the lactational loss should be minimized through proper feeding regimen (Strathe et al., 2020).

3. Manuscript to research article

In the following section the manuscript to a research article are presented. The manuscript is based upon the experimental part of current thesis.

Effect of reduced dietary protein in gestation and roughage supplementation on plasma, urine and milk metabolites in organic sows during winter conditions

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3.1 Abstract

Oversupply of protein is an issue in the Danish organic sow production. Currently, organic sow feed is composed in accordance to feeding standards of conventional sows, which do not take the higher feed allowance and nutritional contribution from roughage of organic sows into consideration. The objective in current study was to investigate the effect of reducing the dietary content of protein in gestational compound feed for organic sows during winter conditions, using metabolites in plasma, urine and milk as indicators. Moreover, the nutritional contribution of roughage was studied, thus this can be taken in account when adapting the feeding strategy to the requirement of organic sows.

In total 21 sows (Topigs Norsvin; TN70) was included in the experiment, lasting from day 30 of gestation until weaning at day 49 of lactation under outdoor organic conditions during winter. During gestation, sows were fed one of two isoenergetic diets, containing 76 g SID CP pr. kg feed (control) and 63 g SID CP pr. kg feed (low protein). In lactation all sows were fed the same diet. Moreover, sows were supplied either clover-grass silage or barley-pea whole-crop silage.

Plasma and urine samples were collected at day 60 and 100 of gestation, and plasma, urine and milk were collected at day 5 and 20 of lactation. On all collection days, including day 30 of gestation and 49 of lactation, sows were weighted, and backfat scanned. In lactation piglets were weighted individually.

Sow liveweight and backfat thickness was not affected by dietary protein level. An interaction indicated that sows fed control diets had 23 % higher urinary urea concentration at day 60 of gestation. Peak milk yield was in average 16.3 kg at day 20 and sows weaned in average 13.3 piglets, each weighing 19.9 kg at day 49. During lactation the milk yield of sows fed low protein in gestation, became gradually greater compared to sows fed control diet. Concurrently, the litter gain of these sows was better, and their litters was heavier at day 49 compared to control sows. Roughage intake was highest during gestation, where sows ate more clover-grass silage than barley-pea whole-crop silage. Clover-grass silage contributed in average 1.7 MJ ME/d and 0.36 g SID lysine/d, equal to 14 % ME, and 13 % and 17 % SID lysine of the total dietary intake during gestation, for sows fed control and low dietary protein diet, respectively.

In conclusion, organic outdoor sows benefitted from being fed reduced dietary protein during gestation in winter conditions, indicated by urinary urea concentration, milk yield and litter gain. Roughage, especially clover-grass silage proved to contribute with a considerable amount of nutrients during gestation, which is important to take into consideration when adjusting the feeding strategy to the organic sow production.

3.2 Introduction

The production conditions of organic sows are considerable different from that of indoor conventional sows and consequently, the requirement of the organic and conventional sow also differ. Most noticeable, organic sows have a higher energy requirement, mainly due to a higher demand for thermoregulation, especially during winter conditions (Close and Poornan, 1993, Buckner, 1996). Under Northern European conditions the energy requirement of outdoor sows is estimated to be 15-20 % higher than indoor sows and this requires a higher feed allowance. The protein requirement is however seemingly not affected by production conditions (Close and Poornan, 1993, Edwards, 2003).

Currently, organic sow feed is composed in accordance to the indoor feeding standards, without taking the higher feed allowance into consideration. Consequently, the organic sows are oversupplied with protein. This is unfavorable, as it increases the energy output in urine through urea, which decreases feed efficiency (Pedersen et al., 2019b). On the contrary, supplying inadequate amounts of protein is equally as undesirable, as this will negatively affect the productivity of the sows (Hojgaard et al., 2019a).

Organic sows are required by EU-legislation to have permanent access to roughage (Regulation (EU), 2018). This serves to improve animal welfare, as it allows for manipulation and increases satiety which reduces unwanted behavior (Bergeron et al., 2000, Danielsen and Vestergaard, 2001), and enhances gastro-intestinal health of the sows (Lindberg, 2014, Jha et al., 2019). The consumption of roughage also has a nutritional value to the sow and can contribute with a considerable amount of nutrients (Fernándéz et al., 2006). Not taking this nutrient input into consideration could increase the protein oversupply.

Not only is oversupplying the sow with protein physiologically unfavorable, it also increases the environmental impact of organic pig production (Eriksen et al., 2002). Thus, it is highly relevant to adjust and optimize the dietary protein concentration in the compound feed to the conditions of organic production and requirement of the organic sow, to avoid the undesirable consequences of feeding excess dietary protein. In addition, it is important to consider the nutritional contribution from roughage and benefit from this contribution.

The aim of this study is to investigate how reducing the concentration of dietary protein in organic compound feed for gestating sows, affects the metabolism and productivity of the sows during their production cycle in winter conditions, using metabolites in plasma, urine and milk as indicators. Besides, the study will consider the nutritional contribution of clover-grass silage and barley-pea whole-crop silage.

Key words: dietary protein, metabolites, organic sows, roughage

3.3 Materials and methods

The animal experiment procedures were performed in accordance to the Danish Ministry of Justice, Law no. 474 of 15/02/2014 regarding animal experiments issued by the Danish Ministry of Environment and Food. Moreover, the animal experiments comply with the ARRIVE guidelines and follows Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Rearing, housing and sampling has been in coherence with Danish laws for care and use of animal in research purposes (Animal Experimental permit No. 2017-15-0201-01484).

3.3.1 Animals and housing

Twenty-one sows (Topigs Norsvin; TN70) of mixed parity (2nd to 5th) were inseminated with commercial DanBred Duroc production semen. The sows were randomly assigned to one of two iso-energetic gestation diets; a standard organic feeding regime (Control sows, n=10) or low dietary protein feeding regime (LP sows, n=11), and fed one of two roughages; clover-grass silage (CS, n=11) or barley-pea whole-crop silage (BWS, n=10). However, during lactation the sows were fed the same commercial standard organic lactation diet. Sows originate from a Danish commercial organic herd with a high productivity. Insemination was performed between October 22nd and 27th and the sows arrived at the experimental location November 24th after a positive gestation scan. Here the sows were reared outdoors under organic conditions in the winter 2020-21 at the Organic Platform at Aarhus University, Denmark. During gestation sows were housed in two groups depending on the type of roughage assigned. The gestation paddocks measured 4000 m^2 ($40 \times 10\ \mathrm{m}$) and two 12 m^2 isolated gestation huts were located in each paddock. Ten days prior to expected farrowing sows were moved to individual paddocks measuring 480 m^2 ($30 \times 16\ \mathrm{m}$). Two types of farrowing huts were used: Three four-compartment communal huts, where each individual section measured $2.4 \times 2.5\ \mathrm{m}$ and four two-compartment communal huts, where each induvial section measured $2.0 \times 2.0\ \mathrm{m}$. A heated piglet creep area was associated to each individual section. All huts were supplied with chopped straw as bedding; approximately 12,5 kg/m^2.

Gestation and lactation paddocks were sown with a commercial clover-grass mix (ForageMax 22A, DLF Trifolium, Roskilde, Denmark), consisting of 15% Trifolium Repens (white clover) and 85 % Lolium Perenne (perennial ryegrass). Sows were carrying nose-ring to reduce rooting and keep the sward intact.

The health conditions were monitored daily, and animals were treated if necessary, in compliance with normal procedures. According to Danish law, animal health was monitored by the herd veterinarian at monthly visits.

During the entire experimental period the animals had ad libitum access to drinking water and possibility for wallowing, when temperatures were above 15 °C.

3.3.2 Diets, roughage and feeding

A commercially available organic compound feed, based on cereals, rapeseed cake and peas, was used as control diet during the gestation period (table 7). The control diets were formulated to supply the recommended amount of standardized ileal digestible (SID) amino acids, except lysine, for gestating sows, according to Danish feeding standards (Tybirk et al., 2020). A customized compound feed, based entirely on cereals, was used as low-protein diet during gestation. The protein content of the low-protein diet had been reduced to a degree that was assessed would not affect sows feed intake. The control and low-protein diets were formulated to be isoenergetic and contained 12.4 MJ ME pr. kg feed. The control and low protein gestation diets contained 76 and 63 g SID CP pr. kg feed, respectively (table 8). A commercial available lactation compound feed, based on cereals, peas and soy beans, was fed to all lactating sows (table 7), as a previous study has proven that productivity is reduced, when outdoor sows are feed low-protein diets during lactation (Eskildsen et al., 2020b). The lactation diet was formulated to supply the recommended amount of standardized ileal digestible (SID) amino acids, except lysine, for lactating sows, according to Danish feeding standards (Tybirk et al., 2020). The lactation diet contained 13.2 MJ ME and 108 g SID CP pr. kg feed (table 8). All diets were formulated to fulfill the sow's requirements of vitamins and minerals at the given production stage. Roughage was supplied according to the appetite of the sows, to ensure maximal intake and to reduce residues.

The compound feeds were manufactured at a commercial feed company (Vestjyllands Andel, Vildbjerg, Denmark) and delivered two times throughout the study; at the arrival of the sows and before farrowing.

Table 7, ingredients of experimental diets

Ingredients, g/kg	Gestation		Lactation
	Control	Low protein	
Barley	334	722	330
Rye	200	100	100
Oat	150	150	102
Wheat	100		250
Wheat bran	100		
Rapeseed cake	42.6		25.2
Peas	34.4		60
Starfish meal	10		20
Potato protein	3.8		20
Calcium carbonate	11.3	13.1	30
Mono calcium phosphate	6.7	8.6	20
Sodium chloride	5.1	4.8	20
Vitamin and mineral mix[1]	1.6	1.1	5,9
ME, MJ/kg[2]	12.1	12.3	13.1
FUsow/kg	0.98	1.00	1.05
SID CP, g/kg[3]	83.0	62.3	118.7
Lysine, g/kg	4.89	3.35	7.50
SID lysine, g/kg	3.74	2.36	6.18

[1] Pr. kg: 8000 IU vitamin A, 800 IU vitamin D3, 58.99 mg E-vitamin, 2.00 mg vitamin B1, 5.00 mg vitamin B2, 3.00 mg vitamin B6, 0.02 vitamin B12, 2.00 mg vitamin K3, 15.00 mg D-pantothenic acid, 20.00 niacin, 0.4 mg Biotin, 1.5 mg folic acid, 80.00 mg iron ($FeSO_4$), 2.00 mg iodine, ($Ca(IO_3)_2$), 15.00 mg cupper ($CuSO_4$), 40.00 mg manganese (MnO), 100.00 mg Zink (ZnO), 0.30 mg selenium (Na_2SeO_3).

[2] The content of metabolizable energy (ME) was calculated from the feed units (FUsow) in accordance to Theil et al. (2020)

[3] Standardized ileal digestible

Through gestation and during lactation sows were fed according to recommendations for indoor sows, specifically the feeding curve for lean sows and the feeding curve for highly productive sows respectively (Sørensen, 2019, Bruun et al., 2017).

Gestating sows were fed compound feed and silage twice per day at 9.00 AM and 14.00 AM. A feeding system was used, allowing for individual feeding of the compound feed. 45 mins after feeding leftovers were collected and weighed. Silage was supplied in open throughs and leftovers were collected and weighed at the following feeding session. During lactation, sows were fed compound feed and silage once per day at 9.00 AM. The compound feed was provided in covered feeders, reducing the feed loss to rodents and birds. Silage was supplied in open throughs. Once per week feed and silage residues were collected and weighed at individual level.

One sample of each experimental diet was taken during the study. Each feed sample was split into two subsamples and analyzed for CP and amino acid composition (Eurofins Steins Laboratory A/S, Vejen, Denmark). Five representative samples of each silage were taken throughout the experimental period, pooled and stored at -20° C until analysis. The chemical composition of the diets and roughages are presented in table 8. The roughage samples displayed no systematic variation through the experimental period, thus the values presented in table 8, is a simple mean of the five samples. Also, as no standardized ileal digestibility was available for CP and amino acids in the roughages, it was assumed that the digestibility of these fractions was identical to the enzymatically digestibility of organic matter at ileum (EDOMi) (Tybirk et al., 2006).

Table 8, chemical composition of experimental diets and roughages

	Gestation		Lactation	Clover-grass silage	Barley-pea whole-crop silage
	Control	Low protein			
DM, g/kg	865	866	864	394	226
FUsow/kg DM[1]	1.17	1.17	1.23	0.65	0.63
ME, MJ/kg DM[2]	14.33	14.30	15.30	9.83/10.06[4]	9.67/9.88[4]
Chemical composition, g/kg DM					
SID CP[3]	118	98	153	132	125
Fat	45	37	50	37	30
Ash	54	48	58	69	68
Amino acids, g SID/kg DM[3]					
Lysine	5.23	3.93	7.48	5.13	4.39
Methionine	2.21	1.56	2.70	1.61	1.56
Cystine	2.65	2.42	3.04	1.00	1.06
Threonine	4.23	3.26	5.83	5.25	4.70
Tryptophan	1.49	1.24			
Isoleucine	4.00	3.42	6.08	5.37	4.37
Leucine	7.72	6.65	10.89	9.28	7.47
Histidine	2.76	2.11	3.38	2.10	2.03
Phenylalanine	5.03	4.42	6.68	5.90	4.52
Tyrosine	3.29	3.05		3.15	1.32
Valine	5.51	4.86	6.98	7.10	6.15

[1] Danish feed units for sows (Tybirk et al., 2006)

[2] The content of metabolizable energy (ME) was calculated from the feed units (FUsow) in accordance to Theil et al. (2020)

[3] Standardized ileal digestible.

[4] For gestating and lactating sows respectively

3.3.3 Recording and sampling

Samplings were performed at day 60 and 100 of gestation, day 5 and 20 of the lactation period and at weaning at day 49. Sampling and measuring began at sunrise. Sows were caught in the huts and transported to a wagon, where the experimental procedures were performed. First the liveweight of the sows was measured on a walk-in scale and simultaneously the backfat thickness were measured. Backfat was measured using the digital ultrasound scanner LEAN MEATER (Baltic Korn A/S, Naestved, Denmark) in the point P2; approximately 70 mm from either side of the spine at the last rib. Next, with exception of the weaning sampling, sows were fixated using a snare restraint around the snout and blood was collected from the jugular vein. If this was not possible, a blood sample from the ear vein was collected. The blood samples were immediately centrifuged, and plasma was harvested and frozen at -20 °C and -85 °C until analysis. At lactational sampling days sows were injected 0.3 ml oxytocin into the ear vein to induce milk letdown and manually milked from random teats, while standing fixated. The milk samples were filtrated through gaze and stored at -20 °C until analysis. At last sows were intramuscularly enriched with deuterium in the neck. The following day a spontaneous spot urine sample and fresh feces were collected at sunrise. Urine samples was collected in the middle of the excretion, if possible, in a 200 ml collection pot.

At day 1 of lactation live piglets were ear tagged. No litter equalization was performed. At day 1, 5, 20 of lactation and at weaning the piglets were individually weighed. Male piglets were castrated at day 5. Dead piglets were collected and registered on a daily basis. Piglets had access to a commercial weaning diet inside the covered creep area from day 10 and feed intake was registered at litter level on a daily basis from day 12.

3.3.4 Chemical analysis

Samples of compound feed, roughage, plasma and urine were analyzed in duplicate, except for the amino acid analysis, for which a single sample was used. Milk samples were analyzed in triplicates.

DM content of compound feed and roughage samples were determined by oven drying at 103° C until reaching a constant weight and ash content were determined by oven drying at 525° C for six hours.

Crude protein content was calculated by multiplying the nitrogen content of the sample by 6.25. Nitrogen content was determined by the international Dumas method.

The amino acid content in compound feed and roughage samples were analyzed according to Commision Regulation (EC) (2009): Samples were hydrolyzed for 23 hours at 110° C, with (Cys and Met) or without (Arg, His, Ile, Leu, Phe, Tyr and Val) prior oxidation using performic acid. Following, AA were separated using ion exchange chromatography and quantified by reacting with ninhydrin and using photometric detection.

Plasma glucose, lactate, triglycerides and urea and urine urea and creatinine concentrations were analyzed according to standard procedures (Siemens Diagnostics Clinical Methods for ADVIA 1650) using an auto analyzer (ADVIA 1650 Chemistry System, Siemens Medical Solutions, Terrytown, NY). Plasma NEFA concentration was determined using the Wako, NEFA C ACS-ACOD assay method (Wako Chemicals GmbH, Neuss, Germany).

The chemical composition of milk; DM content, fat, lactose, protein and casein, was determined by infrared spectroscopy using a Milkoscan 4000 instrument (Foss Milkoscan, Hillerød, Denmark).

3.3.5 Calculations and statistical analysis

Milk yield (MY) from day 1 to day 20 was predicted according to the model of Hansen et al. (2012b), developed to quantify MY of conventional sows, using litter gain and litter size as predictors. MY after day 20 was purposely not estimated, due to piglet's intake of compound feed. Milk energy content was calculated based on the gross energy (GE) values of the constituents of milk (38.1 MJ/kg fat, 24.5 MJ/kg protein and 16.5 MJ/kg lactose (McDonald et al., 2011). Daily output was calculated as the energy content of milk multiplied by the daily estimated MY.

Sows body protein pool in kg (BP) were estimated accordingly the prediction equations of Rozeboom et al. (1994), using sows liveweight in kg (LW) and backfat thickness in mm (BF):

$$BP = \ 2.5 + 0.146 * LW - 0.110 * BF$$

Plasma and urine metabolites were analyzed using the following statistical model:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + (\alpha\gamma)_{ik} + A_m + \varepsilon_{ijklm}$$

Where Y_{ijkl} is the observed trait, μ is the overall mean of observations, α_i is the effect of dietary protein (i = control or low protein), β_j is the effect of roughage supplementation (j= clover grass silage or barley-pea whole-crop silage), γ_k is the effect of day in gestation or lactation (k= 60, 100, 5 or 20), δ_l is the effect of parity of sows (l= 2 or >2), $(\alpha\gamma)_{ik}$ is the interaction between dietary protein and day in gestation or lactation, $A_m$is the random effect of sow (m= 1, 2, … , 20) and ε_{ijklm} is the residual random components. MY and milk chemical composition was analyzed using the same model, however only with day 5 and 20 of lactation. Piglet weight and litter weight and growth were also analyzed using some model, however with day 0, 5, 20 and 49 of lactation. Compound feed intake, ME intake, SID CP intake, SID lysine intake, liveweight gain, backfat gain, body protein gain and litter gain were analyzed using the same model, except day were replaced by five periods; day 30-60, 60-100, 100-5, 5-20 and 20-49. Finally, sow liveweight, backfat and estimated body protein was analyzed using same model as described above, however with day 30, 60 and 100 of gestation and day 5, 20 and 49 of lactation.

Plasma NEFA concentration did not fulfill the assumptions of the model and where transformed using the natural logarithm. Variables that have been transformed to fulfill the assumptions for the model, has been transformed back.

Statistical analyses were performed using the software “R”. Effects were considered significant if P-values were below 0.05 and tendencies were accepted at P-values below 0.10.

3.4 Results

The experimental period lasted from ultimo November 2020 to primo April 2021. The temperature was 1.9 °C, wind speed was 4.0 m/s and it rained or snowed 1.58 mm/d, on average (DMI, 2021).

The analyzed SID crude protein content of the low protein gestation diet was 18.1 % lower than the control gestation diet. This is in accordance with the experimental design and a prerequisite for the conduction of the study.

One sow and her piglets were removed shortly after farrowing, due to severe metritis.

3.4.1 Sow characteristics and intake of nutrients

Descriptive statistics for sow characteristics during the experimental period are summed up in table 9 and 10. On average sows gained 62.7 kg LW and 4.42 mm BF during gestation and lost 54.6 kg LW and 4.86 mm BF during lactation. Dietary protein level and roughage supplementation did not affect the LW or BF gain, however sows fed low dietary protein weighted in average significantly less, throughout the experimental period ($P<0.05$). Consequently, the estimated body protein pool was significantly higher for control sows throughout the experimental period ($P<0.05$). Second parity sows gained significant more liveweight during the entire experimental period, compared to older sows ($P<0.05$). On average sows had 17.2 liveborn piglets, each weighing 1.67 kg in average and weaned 13.3 piglets, each weighing 19.9 kg in average at weaning, summing up to a litter weight of 256 kg on day 49. Litters from LP sows gained significantly more weight during lactation ($P<0.01$), thus gradually became heavier than litters from control sows as lactation proceeded ($P<0.001$). In addition, the piglets from LP sows had a significantly higher intake of solid feed ($P<0.05$), however the intake until day 20 of lactation was negligible.

The sow's intake of compound feed was in accordance to the feeding curve throughout the experiment. No feed residues were registered during gestation. An interaction between dietary protein and reproductive stage was found to affect the sow's daily intake of SID CP ($P<0.01$) and SID lysine ($P<0.001$) from compound feed, showing that LP sows had a lower intake during gestation, but higher during lactation (figure 18). In compliance, the average daily intake of compound feed and ME from compound feed tended to be higher in LP sows, compared to control sows ($P=0.10$ and $P=0.08$).

The intake of roughage during gestation was lower than expected and during lactation the intake was even lower. During gestation the intake of clover-grass silage and barley-pea whole-crop silage was on average 817 g DM/d; equal to 8.0 MJ ME/d and 1.9 g SID lysine/d, and 179 g DM/d; 1.7 MJ ME/d and 0.36 g SID lysine/d, respectively. This changed in lactation, where sows fed clover-grass silage in average ate 99 g DM/d; equal to 1.0 MJ ME/d and 0.23 g SID lysine/d, while sows fed barley-pea whole-crop silage ate 161 g DM/d; equal to 1.6 MJ ME/d and 0.32 g SID lysine/d ($P<0.001$). Consequently, the nutritional contribution of barley-pea whole-crop silage was significantly higher, compared to clover-grass silage in lactation. During gestation clover-grass silage contributed in average with 14.4 % and 14.6 % of the total intake of ME and SID lysine respectively, while barley-pea whole-crop only contributed with 3.5 % and 3.1 %, respectively. From day 100 of gestation until day 5 of lactation, the intake of roughage was almost absent. In lactation the contribution was much smaller: 1.1 % and 0.6 % for clover-grass silage and 1.6% and 0.8 % for barley-pea whole-crop silage of total ME and SID lysine intake, respectively.

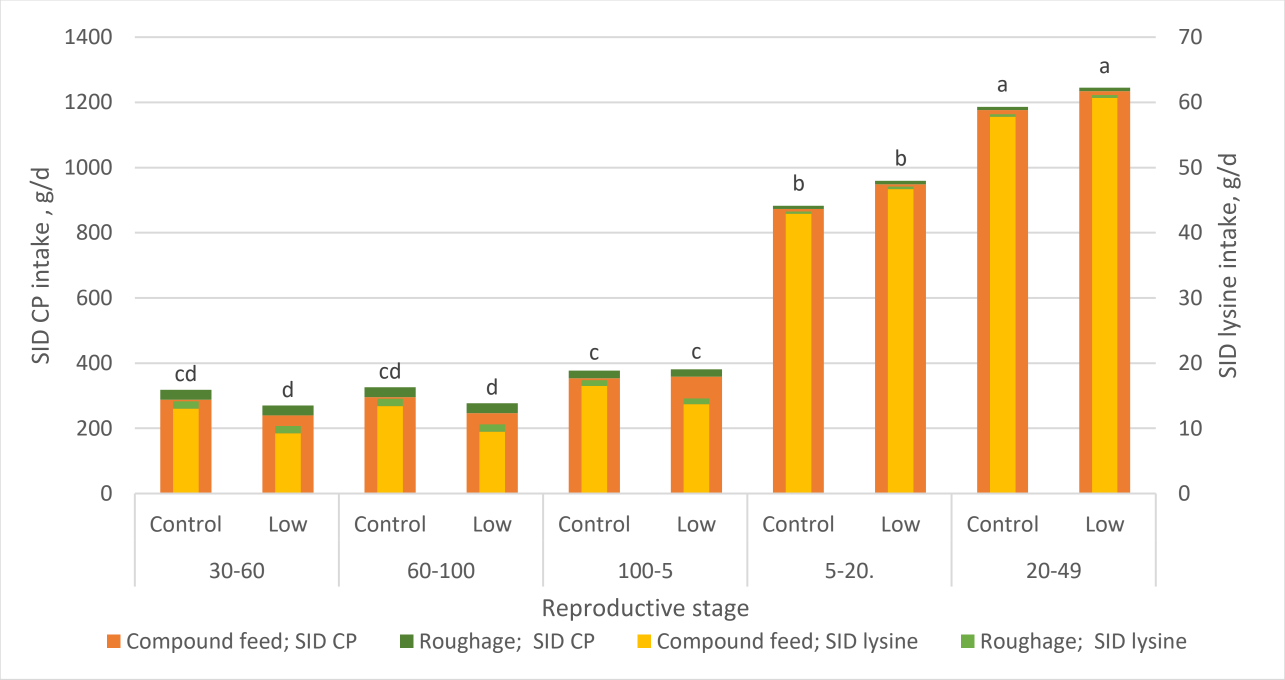

Figure 18, effect of the interaction between dietary protein level and reproductive stage on sow's daily intake of SID CP and SID lysine from compound feed and roughage

Table 9, nutritional intake of compound feed, changes in body composition and litter gain of sows fed diets varying in dietary protein level and two types of silage: Clover-grass silage (CS) and barley-pea whole-crop silage (BWS)

	Protein level			Roughage			Parity		
	Control	Low	SEM	CS	BWS	SEM	2	Older	SEM
Compound feed intake, kg/d	6.12	6.41	0.12	6.18	6.35	0.11	6.26	6.27	0.13
ME intake, MJ/d	79.4	83	1.34	80.3	82	1.24	81.7	80.7	1.45
SID CP intake, g/d	597	606	10.9	594	609	10.1	606	597	11.8
SID lysine intake, g/d	28.7	27.9	0.54	28	28.7	0.5	28.5	28.1	0.58
Liveweight gain, kg	0.89	1.55	1.58	1.38	1.05	1.48	3.79^{a}	-1.36^{b}	1.73
Backfat gain, mm	-0.18	0.01	0.32	-0.14	-0.04	0.30	0.15	-0.33	0.36
Body protein gain, kg	0.15	0.23	0.022	0.22	0.16	0.2	0.54	-0.16	0.24
Litter gain, kg/d	3.57^{b}	4.22^{a}	0.15	3.77	4.02	0.14	3.95	3.84	0.16

Table 9 (continued), nutritional intake, changes in body composition and litter gain of sows fed diets varying in dietary protein level and two types of silage: Clover-grass silage (CS) and barley-pea whole-crop silage (BWS)

	Reproductive stage[1]						P-value				
	30-60	60-100	100-5	5-20	20-49	SEM	Protein	Roughage	Parity	Stage	Protein x stage
Compound feed intake, kg/d	3.78^{c}	3.9^{c}	4.13^{c}	8.4^{b}	11.12^{a}	0.15	0.10	0.30	0.92	<0.001	0.12
ME intake, MJ/d	46.9^{c}	48.3^{c}	52.3^{c}	111.2^{b}	147.1^{a}	1.45	0.08	0.32	0.61	<0.001	0.14
SID CP intake, g/d	264^{d}	272^{d}	356^{c}	911.0^{b}	1205^{a}	13.9	0.59	0.32	0.61	<0.001	<0.01
SID lysine intake, g/d	11.1^{d}	11.4^{d}	15.1^{c}	44.80^{b}	59.2^{a}	0.68	0.32	0.31	0.61	<0.001	<0.001
Liveweight gain, kg	27.2^{a}	33.5^{a}	-27.2^{c}	-15.1^{b}	-12.3^{b}	2.24	0.77	0.88	<0.05	<0.001	0.14
Backfat gain, mm	2.22^{b}	1.32^{b}	0.88^{b}	-2.14^{a}	-2.72^{a}	0.48	0.68	0.82	0.31	<0.001	0.91
Body protein gain, kg	3.76^{a}	4.76^{a}	-4.05^{c}	-1.97^{b}	-1.53^{b}	0.32	0.8	0.85	<0.05	<0.001	0.16
Litter gain, kg/d				4.16^{b}	5.71^{a}	0.16	<0.01	0.23	0.59	<0.001	0.29

$^{a-d}$Within a row, values without common subscriptions differ (P<0.05)

[1] 30-60 covers day 30 to 60 in gestation, 60-100 covers day 60 to 100 in gestation, 100-5 covers day 100 in gestation to day 5 in lactation, 5-20 covers day 5 to 20 in lactation and 20-49 covers day 20 to 49 in lactation

Table 10, body composition and reproductive performance of sows fed diets varying in dietary protein level and two types of silage: Clover-grass silage (CS) and barley-pea whole-crop silage (BWS)

	Protein level			Roughage			Parity		
	Control	Low	SEM	CS	BWS	SEM	2	Older	SEM
Liveweight, kg	269^{a}	248^{b}	6.06	257	259	5.23	229^{a}	287^{b}	6.65
Backfat, mm	14.3	12.7	0.86	12.9	14.1	0.76	12.1^{a}	15.0^{b}	0.95
Body protein, kg	40.2^{a}	37.3^{b}	0.81	38.6	38.9	0.71	34.6^{b}	42.8^{a}	0.89
Piglet weight, kg	7.46	8.02	0.55	8.11	7.37	0.52	7.97	7.51	0.59
Litter weight, kg	93.3^{b}	111^{a}	4.34	101	104	4.08	103	101	4.66
Piglets pr. sow	14.6	14.7	1.23	14.5	14.9	1.16	14.3	15.1	1.32

Table 10 (continued), body composition and reproductive performance of sows fed diets varying in dietary protein level and two types of silage: Clover-grass silage (CS) and barley-pea whole-crop silage (BWS)

	Reproductive stage[1]							P-value				
	30	60	100	5	20	49	SEM	Protein	Roughage	Parity	Stage	Protein x stage
Liveweight, kg	235^{d}	262^{b}	294^{a}	267^{b}	252^{c}	239^{d}	4.59	<0.05	0.70	<0.001	<0.001	0.24
Backfat, mm	11.9^{a}	13.7^{b}	14.9^{bc}	15.8^{c}	13.6^{b}	11.2^{a}	0.67	0.21	0.22	<0.05	<0.001	0.97
Body protein, kg	35.6^{d}	39.2^{b}	43.9^{a}	39.8^{b}	37.7^{c}	36.1^{d}	29.8	<0.05	0.75	<0.001	<0.001	0.21
Piglet weight, kg[2]				2.32^{c}	7.11^{b}	19.9^{a}	0.49	0.49	0.33	0.57	<0.001	0.9
Piglets pr. sow[3]				14.7^{a}	13.6^{ab}	13.3^{b}	0.86	0.95	0.81	0.68	<0.001	0.22
Litter weight, kg[4]				32.7^{c}	92.8^{b}	256^{a}	3.82	<0.01	0.62	0.73	<0.001	<0.001

[a-d] Within a row, values without common subscriptions differ (P<0.05)

[1] Day 30, 60 and 100 of gestation and 5, 20 and 49 of lactation

[2] Birth weight of piglets was in average 1.67 kg

[3] Liveborn piglets was in average 17.2

[4] Litter weight at birth was 25.3 kg for sows fed control feed and 29.4 kg for sows fed low dietary protein (P=1.00)

3.4.2 Metabolites in plasma and urine

Sow plasma and urine metabolite concentrations are seen in table 11. No effect of dietary protein and roughage supplementation was found on plasma glucose, lactate, triglycerides and NEFA. Plasma urea concentration was significantly higher and plasma creatinine tended to be higher in sows fed barley-pea whole-crop silage compared to sows fed clover-grass silage ($P<0.05$). An interaction between dietary protein and reproductive stage, tended to affect plasma urea concentration ($P=0.08$), showing that LP sows have a slightly lower plasma urea concentration at day 60 of gestation, but slightly higher concentration during lactation. Reproductive stage affected all plasma metabolites.

An interaction between dietary protein and reproductive stage was found to affect the ratio of urinary urea to creatinine ($P<0.05$), indicating that control sows had a 23 % higher urinary urea concentration at day 60 of gestation compared to LP sows (figure 19).

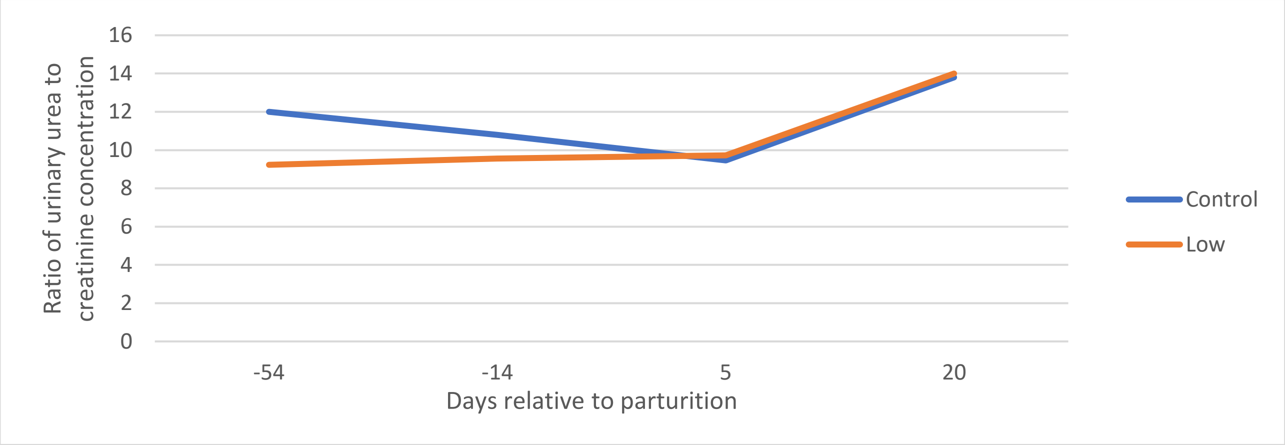

Figure 19, effect of the interaction between dietary protein level and reproductive stage on the ratio of urinary concentration of urea to creatinine

3.4.3 Milk yield and composition

MY, milk energy output and milk chemical composition are presented in table 12. An interaction between dietary protein and days in lactation was found on MY ($P<0.05$), displaying that MY from LP sows became gradually greater than MY from control sows as lactation progressed. A significant effect of roughage was found on milk DM and fat concentration, revealing that milk from sows fed clover-grass silage had a higher DM and fat concentration compared to milk from sows fed barley-pea whole-crop silage ($P<0.05$). Through the lactation, MY and energy output increased significantly, reaching 16.7 kg/d and 87.3 MJ GE/d at day 20. Milk protein concentration tended to decrease along lactation ($P=0.07$).

Table 11, plasma and urine metabolites from sows fed diets varying in dietary protein level and two types of silage: Clover-grass silage (CS) and barley-pea whole-crop silage (BWS)

	Protein level			Roughage			Parity		
	Control	Low	SEM	CS	BWS	SEM	2	Older	SEM
Plasma									
Glucose, mM	4.37	4.29	0.17	4.29	4.37	0.16	4.36	4.30	0.18
Lactate, mM	3.20	3.00	0.31	3.25	2.94	0.29	3.56	2.63	0.34
TG, mM [2]	0.489	0.524	0.032	0.484	0.528	0.031	0.559^{a}	0.454^{b}	0.035
NEFA, mM [2]	311 (253-383)	314 (256-386)		324 (269-390)	299 (248-360)		330 (264-413)	296 (236-370)	
Urea, mM	2.97	3.01	0.08	2.88	3.1	0.07	3.05	2.94	0.09
Creatinine, µM	134	143	5.52	132	145		137	140	6.05
Urine									
Urea: Creatinine[3]	11.5	10.6	0.48	10.6	11.6	0.45	11.7	10.5	0.53

Table 11 (continued), plasma and urine metabolites from sows fed diets varying in dietary protein level and two types of silage: Clover-grass silage (CS) and barley-pea whole-crop silage (BWS)

	Reproductive stage[1]					P-value				
	60	100	5	20	SEM	Protein	Roughage	Stage	Parity	Protein x Stage
Plasma										
Glucose, mM	4.75^{a}	4.60^{a}	4.28^{ab}	3.69^{b}	0.18	0.73	0.69	<0.001	0.79	0.48
Lactate, mM	3.98^{a}	2.58^{b}	2.75^{b}	3.08^{ab}	0.31	0.66	0.46	<0.005	0.05	0.51
TG, mM [2]	0.451^{b}	0.576^{a}	0.407^{b}	0.591^{a}	0.036	0.45	0.31	<0.001	<0.05	0.41
NEFA, mM [2]	79.0^{c} (61.7-101)	91.8^{c} (71.7-118)	863^{b} (674-1105)	1510^{a} (1180-1933)		0.98	0.57	<0.001	0.50	0.10
Urea, mM	2.55^{c}	2.59^{c}	3.12^{b}	3.71^{a}	0.10	0.7	<0.05	<0.001	0.36	0.08
Creatinine, µM	140^{ab}	136^{ab}	148^{a}	130^{b}	4.62	0.28	0.08	<0.01	0.64	0.19
Urine										
Urea: Creatinine[3]	10.6	10.2	9.59	13.9	0.51	0.21	0.14	<0.001	0.10	<0.05

[a-c] Within a row, values without common subscriptions differ (P<0.05)

[1] Day 30, 60 and 100 of gestation and 5, 20 and 49 of lactation

[2] TG: Triglycerides, NEFA: Non-esterified fatty acids

[3] Ratio between concentration of urinary urea and creatinine

Table 12, milk yield, energy output and composition of sows fed diets varying in dietary protein level and two types of silage: *Clover-grass silage (CS) and barley-pea whole-crop silage (BWS)*

	Protein level			Roughage			Parity		
	Control	Low	SEM	CS	BWS	SEM	2	Older	SEM
Milk yield, kg/d	12.4	13.8	0.80	12.7	13.5	0.76	13.3	12.9	0.86
Milk output, MJ GE/d	62.9	71.8	5.7	67.8	67	5.4	71	63.7	6.2
DM, %	18.3	18.5	0.39	18.9^{a}	17.8^{b}	0.38	18.9	17.8	0.42
Protein, %	5.28	5.22	0.16	5.4	5.1	0.15	5.33	5.18	0.15
Casein, %	4.24	4.18	0.15	4.31	4.11	0.14	4.27	4.16	0.16
Fat, %	7.33	7.78	0.36	8.08^{a}	7.03^{b}	0.35	8.13^{a}	6.98^{b}	0.39
Lactose, %	5.03	4.93	0.07	4.90	5.07	0.06	4.9	5.07	0.07

Table 12 (continued), milk yield, energy output and composition of sows fed diets varying in dietary protein level and two types of silage: Clover-grass silage (CS) and barley-pea whole-crop silage (BWS)

	DIM			P-value				
	5	20	SEM	Protein	Roughage	Parity	DIM	Protein x DIM
Milk yield, kg/d	9.96^{b}	16.2^{a}	0.56	0.25	0.42	0.78	<0.001	<0.05
Milk output, MJ GE/d	49.9^{b}	84.9^{a}	4.3	0.29	0.91	0.39	<0.001	0.48
DM, %	18.4	18.4	0.37	0.69	<0.05	0.05	0.96	0.28
Protein, %	5.45	5.06	0.15	0.80	0.16	0.52	0.07	0.87
Casein, %	4.32	4.10	0.13	0.76	0.32	0.63	0.2	0.98
Fat, %	7.43	7.68	0.34	0.37	<0.05	<0.05	0.58	0.22
Lactose, %	4.99	4.98	0.06	0.29	0.07	0.08	0.98	0.36

$^{a-b}$Within a row, values without common subscriptions differ ($P<0.05$)

3.5 Discussion

3.5.1 Effect of dietary protein level during gestation

The sows gained liveweight (LW), backfat (BF) and body protein (BP) from day 30 to 100 of gestation and continued to gain BF until day 5 of lactation. No difference in nutrient deposition between sows fed control and low protein diet was observed, when reducing the concentration of SID CP 18 %; from 76 to 63 g/kg and SID lysine 30 %; from 3.5 to 2.4 g/kg in compound feed. This indicates that current reduction in dietary SID CP and lysine content, does not affect the gestating organic sow's ability to deposit nutrients, when fed according to the feeding curve of lean indoor sows (Sørensen, 2019). This also indicates that LP sows were not supplied protein below their requirement during gestation, since this would have affected the sows ability to deposit protein negatively (Kusina et al., 1999, Rehfeldt et al., 2011). In agreement with these observations, the daily intake of SID lysine of LP sows complies well with the requirement throughout most of gestation (Samuel et al., 2012). On the contrary, this implies that control sows are supplied protein above their requirement. Plasma and urinary urea are useful indicators for amino acid (AA) oxidation, followed by production of urea; processes which are increased if dietary protein is supplied above the requirement of the sow (Pedersen et al., 2019a, Hojgaard et al., 2019a). Feeding the sow surplus protein is undesirable, as urea synthesis is an energy-costly process, thus reduces the feed efficiency and applies unnecessary metabolic stress to the sow (Pedersen et al., 2019b). In current study an interaction between dietary protein and reproductive stage, tended to affect plasma urea concentration: At day 60 of gestation LP sows had lower plasma urea concentration and during lactation they had higher plasma concentrations, compared to control sows. This agrees with the literature, where similar effects have previously been reported for both gestating and lactating sows fed reduced dietary CP (Jang et al., 2014, Hojgaard et al., 2019a). The differences between the groups were merely numerically, however the effect is yet interesting to consider, as it supports the assumption, that control sows are supplied excessive dietary protein. A similar pattern was observed in the urinary urea concentration. Due to the high correlation between urinary hydration and urinary urea concentration, the ratio of urinary urea to creatinine was investigated in current study. This reduces the impact of urinary hydration on urea concentration, since creatinine is excreted into urine at a constant rate (Wyss and Kaddurah-Daouk, 2000). At day 60 of gestation control sows tended to have a 23 % higher ratio of urea to creatinine compared to LP sows, while during the remaining experimental period no differences were found. Again, this indicates that control sows are supplied protein above their requirement during gestation. Towards the end of gestation, the sow's requirement of protein increases considerably due to growth of fetus, placenta and mammary gland and colostrum production, whereas the energy requirement only increases slightly (Feyera and Theil, 2017, Sola-Oriol and Gasa, 2017). Consequently, the feed supply is increased to meet the sow's increasing nutritional demand, however the dietary composition does not change. Thus, late gestation the sows may be challenged by a low CP intake relative to their requirement and this could explain, why we find no effect of dietary protein content on plasma and urinary urea at day 100 of gestation. Furthermore, this explains why sows in current study kept gaining BF from day 100 of gestation to day 5 of lactation, but lost LW and BP.

An unbalanced dietary AA composition relative to the requirement of the sow, will increase the AA oxidation and thus increase urea production (Huber et al., 2015, Hojgaard et al., 2019b). In current study, lysine was the first limiting AA in all diets, thus the ratio of the other essential AAs relative to lysine, was higher compared to the feeding standards (Tybirk et al., 2020). This is not unusual in organic sows' diets, due to the limited range of feedstuff and feed additives available, particularly crystalline AA (Regulation (EU), 2018). While the SID CP content was reduced 18 %, the SID lysine content was reduced 30 % in the low protein diet and consequently, the AA imbalance was most pronounced in this diet. This prevents from fully benefitting from the reduced CP content, as the AAs in surplus relative to lysine are increased. Also, the relative low SID lysine content in the low protein diet, may prove to be an obstacle, preventing further reduction of dietary CP.

3.5.2 Mobilization during lactation

Mobilization of body reserves is typically seen in lactating sows, due to insufficient voluntary feed intake to cover the nutritional demands, combined with a high prioritization of nutrients into milk production (Hansen, 2012, Theil et al., 2012). Regardless of production type, studies finds that sows mobilizes body reserves during lactation (Maes et al., 2004, Lavery et al., 2019, Weissensteiner et al., 2018, Eskildsen et al., 2020ab). In current study sows lost in average 27.4 kg LW, 3.50 kg BP and 4.86 mm BF from day 5 of lactation until weaning at day 49. This is in agreement with the findings of Weissensteiner et al. (2018), who also studied organic sows. Feed intake and MY are the two main factors affecting body mobilization. Increasing feed intake decreases mobilization and increasing MY increases mobilization (Strathe et al., 2017a). This explains why we find no differences in body mobilization between dietary protein level, as besides having a higher MY, the sows fed low protein during gestation also tends to eat more compound feed. Supplying high yielding sows with insufficient dietary nutrients to cover their requirement, are undesirable and will inflict severe metabolic deprivation (Eskildsen et al., 2020a). Losses of body reserves during lactation are often lower in conventional sows, compared to current study: Lavery et al. (2019) found an average liveweight loss of 32.9 kg and backfat loss of 2.7 mm and Strathe et al. (2017b) found that sows mobilized 22.1 kg of liveweight and 2.9 mm backfat in average. Organic sows are required by legislation to have at least 40 days of lactation, which in Denmark is extended to 49 days, according to industry agreements (Regulation (EU), 2018, Brancheaftale, 2018). In current study sows continued to lose body reserves from day 20 to 49, indicating that the longer lactation period is demanding on the sow, despite that the metabolic burden presumably declines towards the end of lactation, as the nursing frequency and milk production decreases and the piglets intake of solid feed increases (Kongsted and Hermansen, 2009).

Plasma NEFA concentration is an indicator of body fat mobilization, as NEFA is released when adipose tissue undergoes lipolysis (Duncan et al., 2007). In current study, plasma NEFA concentration remained low in gestation and increased drastically during lactation, correlating well to the observed backfat loss. At peak lactation plasma NEFA concentration reached 1510 mM in average. This is in agreement with the findings of Eskildsen et al. (2020a) in high productive organic sows, who registered an average plasma NEFA concentration of 1454 mM at day 20 of lactation. However, compared to the observed plasma concentrations in organic sows by Weissensteiner et al. (2018) and in conventional indoor sows, considerable higher values was found in present study (Theil et al., 2004, Pedersen et al., 2016, Krogh et al., 2017a, Hojgaard et al., 2019b, Hojgaard et al., 2019a, Pedersen et al., 2020). Plasma NEFA concentration varies relative to feeding, where pre-prandial plasma NEFA concentration is high and post-prandial concentrations are low (Frayn, 1998). Thus, the timing of blood sampling explains part of the differences observed, since blood samples in current study was taken on fasting sows. Nevertheless, the contrast between production type, indicate that organic sows indeed are metabolically challenged during lactation, which is aggravated by the very high milk production in current study.

Excessive mobilization should be avoided, as it affects the sows welfare and reproductive efficiency negatively, and rebuilding of body reserves following gestation is expensive for the sow (Maes et al., 2004, Strathe et al., 2017b). Dourmad et al. (1996) found that a liveweight loss of 12 to 35 kg and BF loss of 2.5 to 4.0 mm during lactation, can be regained in the succeeding gestation if sows are fed a high energy level. This indicates that the sows in current study approaches the limit of reasonable body mobilization.

3.5.3 Milk production and reproductive performance

MY at day 5 and 20 was estimated according to Hansen et al. (2012b), using litter size and gain as inputs. The model has an upper limit of litter size and gain of 14 piglets and 4.2 kg/d, respectively. The performance of several sows in current study surpassed these limitations, however MY was still estimated using the same equation, as it was assessed that the estimated MY of these very high performing sows was reasonable. According to Hansen et al. (2012b), milk production peaks around third week of lactation: At day 20 in current study, average MY was estimated to 16.2 kg/d. This is a very high peak MY, however not unreasonable taking the high litter size and litter gain into considaration (Auldist et al., 1998). Also, a minimal intake of compound feed was observed in piglets until day 20 (unpublished data), supported by the findings of Middelkoop et al. (2019), indicating that piglets intake of solid feed did not result in

overestimation of MY. In line with current study, Eskildsen et al. (2020a) estimated the MY of second parity organic sows to reach 15.7 kg/d in average at day 20 of lactation. Compared to high performing indoor herds, the sows in current study performed very well, as Krogh et al. (2017b), Strathe (2017) and Hojgaard et al. (2019a) found MY of 15.3, 14.1 and 15.1 kg/d respectively, at peak lactation.
Milk production is the major factor affecting piglet growth (Auldist et al., 1998, Hansen et al., 2012b). Correspondingly, the litter growth in present study was very impressive. Litters gained in average 4.16 kg/d from day 5 to 20 and 5.71 kg/d from day 20-49 and in average the sows weaned 13.3 piglets, each weighing 19.9 kg. The very high litter gain from day 20-49 is partly explained by the piglets' intake of solid feed, which increased rapidly towards the end of lactation (unpublished data). In comparison, the sows in the study by Eskildsen et al. (2020a) had an average litter gain of 3.45 kg/d from day 5 to 20 and 3.28 kg/d from day 20 to 40, despite having almost similar MY. Also compared to highly productive indoor sow herds, the sows in current study performs extremely well, as Hojgaard et al. (2019a) found a maximal litter growth of 3.33 kg/d for multiparous sows.

Milk production is highly prioritized in lactating sows and almost all dietary nutrients are used by the mammary gland, afflicting considerable pressure on the metabolism of the sow (Strathe et al., 2017a, Feyera and Theil, 2017). Plasma glucose concentration is an indicator of the sows energy metabolism. Due to its tight regulation, plasma glucose concentration normally remains steady within tight interval, despite reproductive stage and dietary treatments (Pedersen et al., 2016, Krogh et al., 2017a, Hojgaard et al., 2019a, Pedersen et al., 2020). During lactation, the plasma concentration of glucose decreased significantly, reaching 3.69 mmol pr. L at day 20, due to the removal of glucose into the mammary gland (Farmer et al., 2015). This underlines the immense stress that the high MY applies to the sow's general metabolism.

In current study an interaction between dietary protein and DIM was found to affect milk production: As lactation proceeded the MY of LP sows became increasingly higher compared to control sows. Correspondingly, the gain of litters from LP sows were 15,4 % higher compared to control sows, however a higher intake of compound feed of piglets from LP sows co-explains this effect. A high feed intake is a prerequisite for a high milk production (Strathe et al., 2017b) and correspondingly, a tendency of higher daily compound feed and energy intake by LP sows was found. Furthermore, it was found that LP sows, had a lower daily intake of SID CP and lysine during gestation, but higher during lactation, compared to control sows. These effects are supported by the interaction affecting plasma urea concentration, showing that plasma urea concentration are lower during gestation, but higher during lactation for LP sows, compared to sows fed control diet. As mentioned earlier, control sows are seemingly supplied CP above their requirement during gestation. Oversupply of dietary CP content are known to reduce feed efficiency, as the urea synthesis is energy-costly (Pedersen et al., 2019b), which could have affected the mammary gland development during gestation. Also, despite only being a numerical difference, litters from LP sows was heavier than control sows' litters. Larger litters and greater piglet weight provides greater stimuli to the mammary gland, improving the development of the gland during lactation (Auldist et al., 1998, King, 2000). These factors could explain, why LP sows have a better milk production, compared to control sows.

3.5.4 Nutritional contribution of roughage

During gestation the intake of roughage was considerable higher compared to lactation, were it was almost neglectable. The decline in roughage intake, can be explained by the very high intake of compound feed during lactation, presumable close to the maximal physical capacity of the sow (Strathe et al., 2017b). Gestating sows fed clover-grass ate by far most roughage and it contributed with 14 % of the total daily ME intake, and 13 % and 17 % of the total SID lysine intake for control and LP sows respectively. Meanwhile, the nutritional contribution of barley-pea whole-crop silage was only a fourth of this contribution. In comparison Eskildsen et al. (2020a) found that the ME contribution of fresh clover-grass was only half the amount compared to clover-grass silage in current study, however the SID lysine contribution was equal, as the fresh clover-grass had a considerable higher CP content (Eskildsen et al., 2020a). A considerable higher energy contribution of grass silage to organic gestating sows was found by Bikker et al. (2014), however the sows were also fed more restrictive compared to current study.

In previous studies legumes have been preferred above grasses, however in current study both silages contained legume and grass species (Sehested et al., 2004, Rachuonyo et al., 2005). Rachuonyo et al. (2005) proposed this preference could be due to the fibrous character of grass, which the findings of Aubé et al. (2019) supports. Indeed, the barley-pea whole-crop silage had a rougher structure and a larger content of stems (personal observation), which could explain the larger intake of clover-grass silage during gestation. Interestingly, during lactation it changed and sows fed barley-pea whole-crop silage had the largest intake. What exactly caused this effect, was not clarified in current study.

Plasma lactate is among others, an indicator of fiber fermentation in the hindgut of the sow and thus an indicator of roughage intake. Despite, a considerable higher intake of clover-grass during gestation, only a numeric difference in the plasma lactate concentration was found between the roughages. However, on average plasma lactate concentration during the experimental period was in accordance to the findings of Eskildsen et al. (2020a) and considerable higher compared to indoor sows, due to the intake of roughage (Krogh et al., 2017b, Feyera et al., 2018, Hojgaard et al., 2019a, Pedersen et al., 2020).

Despite the higher nutritional contribution, we found no evidence that the body deposition of sows fed clover-grass silage was improved during gestation, compared to barley-pea whole-crop silage. Thus, it could be speculated that the sows fed barley-pea whole-crop silage had a higher intake of the clover-grass pasture in the paddocks. In support of this speculation, a higher plasma concentration of urea was found in sows fed barley-pea whole-crop silage, despite not having a higher intake of compound, compared to sows fed clover-grass silage. Eskildsen et al. (2020a) found that fresh clover-grass harvested in September had a CP content of 190 g/kg DM, which is considerable higher compared to the content in the experimental silages. Thus, it seems likely, that the high plasma concentration of urea in the sows fed barley-pea whole-crop silage was due to a higher intake of fresh clover-grass. In support of this assumption, Aubé et al. (2019) found that sows fed fresh forages consumed double the amount of DM, compared to sows fed dry forages. Also, this would explain why we found no difference in plasma lactate concentration.

An interesting side-effect of roughage supplementation was that, milk DM and fat content were found to be higher in milk from sows fed clover-grass silage compared to barley-pea whole-crop silage. No difference in feed intake, liveweight, backfat, MY or metabolites related to the metabolism was observed between the groups, indicating that the effect possibly is attributable to the chemical composition of the roughage, e.g. fatty acid composition (Hurley, 2015). This was however not studied in sufficient detail in current study, to conclude what exactly caused this effect.

3.6 Conclusion

In conclusion, reducing the dietary SID CP concentration of gestational compound feed with 18 %, does not affect the sow’s ability to deposit body reserves during gestation, indicating that sows are not supplied dietary protein below their requirement. Furthermore, an interaction showed that control sows have 23 % higher urinary urea concentration at day 60 of gestation, but not the remaining period, implying an improved nitrogen balance of LP sows in mid-gestation. During gestation the intake of roughage proved to contribute with a substantial nutritional value to the sow. Clover-grass silage supplied 14 % ME, and 13 % and 17 % SID lysine of the total dietary intake during gestation, for sows fed control and low dietary protein diet, respectively. Moreover, results indicate that a reduction in dietary protein during gestation, has a carry-over effect to lactation, increasing the milk production, feed intake and consequently the litter gain, without further increasing the body mobilization. In addition to the physical measurements and observations, metabolites in plasma and urine proved to be valuable indicators regarding the metabolic state of the sow.

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4. Additional results

In addition to the results obtained in current study, selected results from the project; EFFORT was analyzed. Specifically, a range of minor metabolites in plasma, urine and milk samples was analyzed, correlated to the energy and nitrogen metabolism of the lactating sow. Plasma metabolites included free primary amino groups (free NH_2), glutamine, glutamate and D-lactate. From urine free NH_2, glutamine, glutamate, creatinine and urea were analyzed. And finally, from milk; glucose, glucose-6-phosphate (Glu6P), isocitrate and free NH_2. The experimental procedures are described in Eskildsen et al. (2020a). In the following section, the statistical analysis and main results of the analysis are described.

Plasma, urine and milk metabolites from the EFFORT project was analyzed using the following model:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\gamma)_{ik} + A_l + B_m + \varepsilon_{ijklm}$$

Where Y_{ijkl} is the observed trait, μ is the overall mean of observations, α_i is the effect of dietary protein (i = control or low protein), β_j is the effect of day in lactation (j= 5, 20 or 40), γ_k is the effect of breed (k= DanBred or Topigs Norsvin), $(\alpha\gamma)_{ik}$ is the interaction between dietary protein and day in lactation, A_l is the random effect of sow (l= 1, 2, … , 47), B_m is the random effect of periodic block (m= spring or summer), and ε_{ijklm} is the residual random components. Plasma D-lactate, urine free NH_2 and glutamate, and milk glucose and glu6P concentrations did not fulfill the assumptions of the model and where transformed using the natural logarithm. Variables that have been transformed to fulfill the assumptions for the model, has been transformed back.

Sow plasma, urine and milk metabolite concentration are presented in table 13. The effect of breed was not discussed in current study. No overall effect of dietary protein was observed on plasma metabolites. Days in lactation was found to affect plasma free NH_2 and D-lactate concentration.
An interaction between dietary protein level and days in lactation was found on urinary free NH_2, thus the concentration of free NH_2 from sows fed control diet was significantly higher at day 5, than at day 20 and 40, while no significant differences at different days was found in urine from sows fed low protein feed.
No overall effect of dietary protein was observed on urinary metabolites, however an effect on the ratio of urinary urea to creatinine was observed. In average sows fed the control feed had a ratio 18.1, while sows fed the low protein diet had a ratio of 15.1 ($P<0.05$).
Days in lactation was also found to affect concentration of urinary free NH_2 and ratio of urea to creatinine.
No overall effect of dietary protein was found on milk metabolites; however, a tendency was observed on the concentration of isocitrate and free NH_2. Both the concentration of isocitrate and free NH_2 tended to be higher in milk from sows fed control diets; 0.100 mM and 24.8 mM respectively, compared to low protein diets; 0.088 mM and 23.1 mM ($P=0.06$ and $P=0.05$).

Table 13, plasma, urine and milk metabolites from sows fed diets varying in dietary protein level

	Protein level			Breed		
	Control	Low	SEM	DanBred	Norsvin	SEM
Plasma						
Free NH_2, µM[1]	2423	2432	118	2423	2431	117
Glutamate, µM	257	249	20.2	256	249	20
Glutamine, µM	432	448	23.7	420	460	23.1
D-lactate, µM	32.8 (14.1-76.2)	35.5 (15.3-82.5)		33.1 (14.3-76.9)	35.5 (15.3-82.5)	
Urine						
Free NH_2, µM[1]	713 (607-838)	685 (584-805)		713 (613-830)	757 (651-881)	
Glutamate, µM	40.0 (31.9-42.8)	34.8 (30.1-40.3)		34.5 (30.0-39.6)	37.3 (32.5-42.9)	
Glutamine, µM	20	17.3	2.09	17	20.3	1.99
Urea: Creatinine[2]	18.1^a	15.1^b	0.83	17.9^a	15.3^b	0.79
Milk						
Glu6P, mM	0.023 (0.020-0.026)	0.023 (0.020-0.026)		0.022 (0.020-0.025)	0.023 (0.020-0.026)	
Glucose, mM	0.11 (0.10-0.13)	0.13 (0.11-0.16)		0.11^b (0.09-0.14)	0.14^a (0.12-0.16)	
Isocitrate, mM	0.100	0.089	0.005	0.097	0.091	0.005
Free NH2, µM[1]	24.8	23.1	1.03	23.4	24.5	1.01

$^{a\text{-}b}$Within a row, values without common subscriptions differ ($P<0.05$)

[1] Glu6P: Glucose-6-Phosphate, Free NH_2: free primary amino groups

[2] Ratio between concentration of urinary urea and creatinine

Table 13 (continued), plasma, urine and milk metabolites from sows fed diets varying in dietary protein level

	Days in lactation				P-value			
	5	20	40	SEM	Protein	Day	Breed	Protein x Day
Plasma								
Free NH$_2$, µM[1]	2568^{a}	2297^{b}	2417^{ab}	120	0.91	<0.01	0.91	0.29
Glutamate, µM	253	250	255	19.8	0.5	0.88	0.59	0.28
Glutamine, µM	441	431	448	23.2	0.47	0.65	0.07	0.93
D-lactate, µM	27.7 (11.9-64.3)	38.5 (16.6-89.4)	37.3 (16.1-86.7)		0.55	<0.05	0.63	0.56
Urine								
Free NH$_2$, µM[1]	854^{a} (736-991)	556^{b} (479-645)	713^{a} (614-828)		0.75	<0.001	0.15	<0.01
Glutamate, µM	38.5 (33-4-44.3)	35.2 (30.5-40.5)	34.1 (29.6 -39.3)		0.55	0.37	0.45	0.14
Glutamine, µM	22.7	16.8	16.5	2.21	0.33	0.06	0.25	0.06
Urea: Creatinine[2]	15.0^{b}	15.1^{b}	19.7^{a}	0.85	<0.05	<0.001	<0.05	0.94
Milk								
Glu6P, mM[1]	0.027^{a} (0.024-0.031)	0.019^{c} (0.017-0.022)	0.023^{b} (0.020-0.026)		0.88	<0.001	0.38	0.33
Glucose, mM	0.15^{a} (0.13-0.18)	0.12^{b} (0.10-0.14)	0.11^{b} (0.09-0.12)		0.12	<0.005	<0.05	0.29
Isocitrate, mM	0.098	0.093	0.092	0.005	0.08	0.63	0.31	0.35
Free NH2, µM[1]	26.5^{a}	21.9^{b}	23.4^{b}	1.09	0.05	<0.001	0.22	0.16

$^{a-b}$Within a row, values without common subscriptions differ (P<0.05)

[1] Glu6P: Glucose-6-Phosphate, Free NH$_2$: free primary amino groups

[2] Ratio between concentration of urinary urea and creatinine

5. Discussion

5.1 Energy and protein balance during gestation

The gestation diets were isoenergetic and contained 1.01 FUsow pr. kg, which is in agreement with the Danish feeding standards (Tybirk et al., 2020). The control and low protein diet contained 76 and 62 g SID CP pr. FUsow, respectively, thus the content of SID CP was reduced by 18 %. This content is considerably lower than the recommended concentration, according to Danish feeding standards, of at least 85 g SID CP pr. FUsow (Tybirk et al., 2020). The SID lysine in the control gestation diet content was 3.42 g/FUsow, which is in accordance with the recommendation of 3.5 g/FUsow in the Danish feeding standard, while the low protein diet only contained 2.37 g SID/FUsow, which is equivalent to a reduction of 30 %. Thus, the content of lysine was reduced relatively more than the CP content, which is disadvantageous, as lysine already was the first limiting AA in the control diet. Consequently, the content of the other AAs is higher relative to lysine, increasing the proportion of AAs being oxidized, compared to the control diet (Hojgaard et al., 2019b) (figure 20).

Ideally, the roughages should have a high content of lysine to counter the low content in the diets. This is not the case, as lysine the second or third limiting AA in the roughages. Furthermore, the AA profiles of the two roughages are very unbalanced, compared to the recommendations (figure 20).

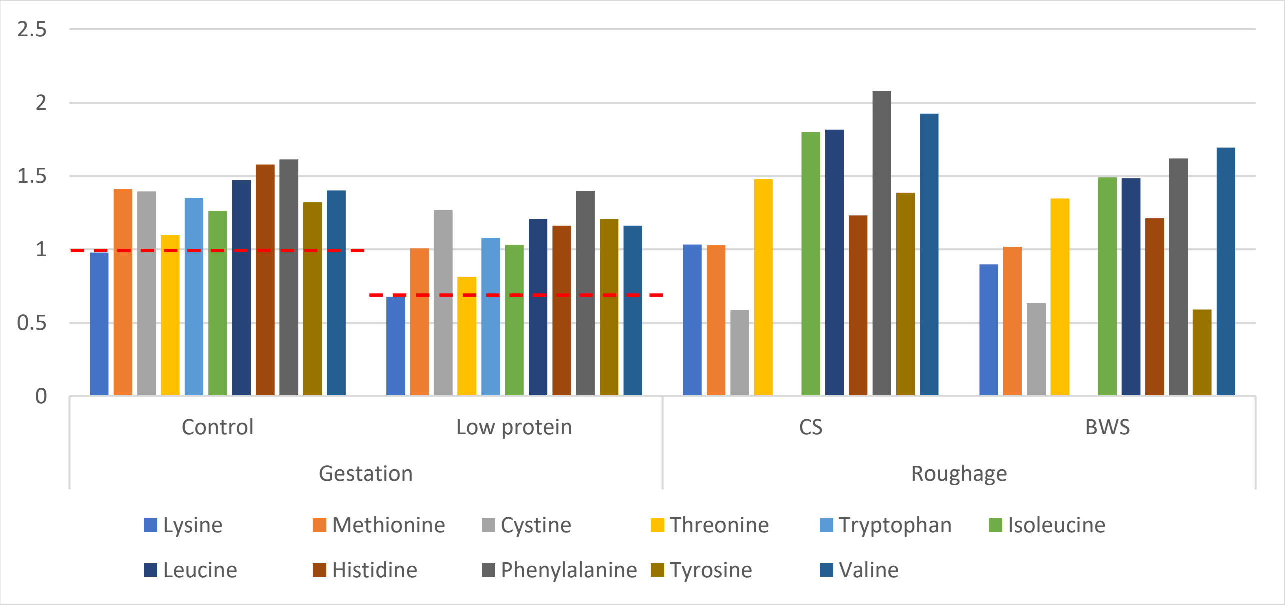

Figure 20, amino acid profile of experimental diets, clover-grass silage (CS) and barley-pea whole-crop silage (BWS), expressed relative to the feeding standard recommendations (Tybirk et al., 2020). The red lines indicate the amount of AA supplied in excess relative to the lysine content

Fulfilling the recommendations of nutrient concentration does not mean that the sows fed the diet will perform good. The feed supply and intake are equally important, to ensure the sows daily requirement are met, since these are quantitively expressed. Danish feeding standards are targeted indoor conventional sows, which requirement differs from the outdoor organic sows. As earlier stated, the energy requirement of organic sows are estimated to be 15 to 20 % higher, than indoors sows (Close and Poornan, 1993, Edwards, 2003). Thus, in order to increase the energy supply, gestating sows in current study was according to the feeding curve of lean indoor sows. The energy requirement of the organic outdoor sow during gestation mainly comprises of maintenance, maternal gain, reproductive gain (reproductive organs and fetus), physical activity and thermoregulation. The maintenance requirement was calculated according to Dourmad et al. (2008) using LW as input. Maternal and reproductive gain was calculated according to NRC (2012) from the average daily LW gain from day 30 to 100 of gestation. The requirement of thermoregulation and physical activity was estimated to correspond to the findings of Buckner (1996) and Eskildsen et al. (2020b) respectively, also studying outdoor gestating sows during winter conditions. In figure 21 the intake and requirement of ME during gestation of the outdoor organic sows from current study, are presented.

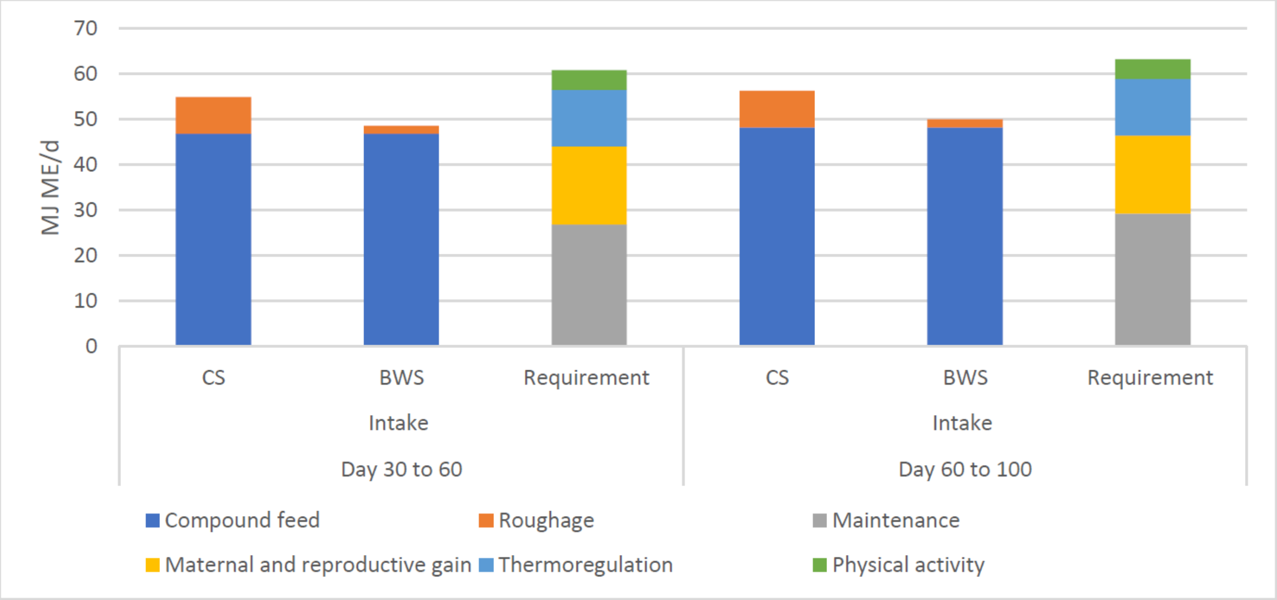

Figure 21, ME intake and requirement of gestating organic sows fed clover-grass silage (CS) or barley-pea whole-crop silage (BWS) during winter conditions (Buckner, 1996, Dourmad et al., 2008, NRC, 2012, Eskildsen et al., 2020b)

As clearly seen, there is a disagreement between the intake and requirement. As discussed earlier, especially the sows fed barley-pea whole-crop silage, may have had an additional intake of fresh clover-grass from the paddocks, which could account for the difference between intake and requirement. Also, as described earlier the heat produced during physical activity and during fermentation of roughage, presumable reduces the requirement for cold thermogenesis

While the energy requirement is higher for organic sows, the requirement for protein is not assumed to be different from the indoor sow's (Close and Poornan, 1993). In figure 22, the intake and requirement of SID lysine during gestation for the control and LP sows from current study, fed either clover-grass silage or barley-pea whole-crop silage is presented. Compared to the SID lysine requirement for indoor sows (Samuel et al., 2012), sows fed control compound feed were oversupplied with lysine from day 30 to 60 and this surplus is only increased when taking the roughage intake into consideration. This supports our earlier assumption; that control sows are fed to much protein during early and mid-gestation. In the same period LP sows fed clover-grass silage are fed slightly above their requirement and LP sows fed barley-pea whole-crop silage merely fulfills the SID lysine requirement. Towards the end of gestation, the requirement of SID lysine increases relatively, compared to the energy requirement (Sola-Oriol and Gasa, 2017). This is clearly seen from figure 22, as only the control sows fed clover-grass have their requirement fulfilled. The deficit is most pronounced in the LP sows fed barley-pea whole-crop silage, which underlines the importance of a high intake of roughage, preferable of good quality. As for the ME balance, it should be kept in mind, that the intake of nutrients from roughage described here, only accounts for the intake of supplied silage. Also, the ileal digestibility of CP and AAs in the roughages was approximated to be equal to the ileal digestibility of organic matter, which could have led to an underestimation of the SID lysine contribution.

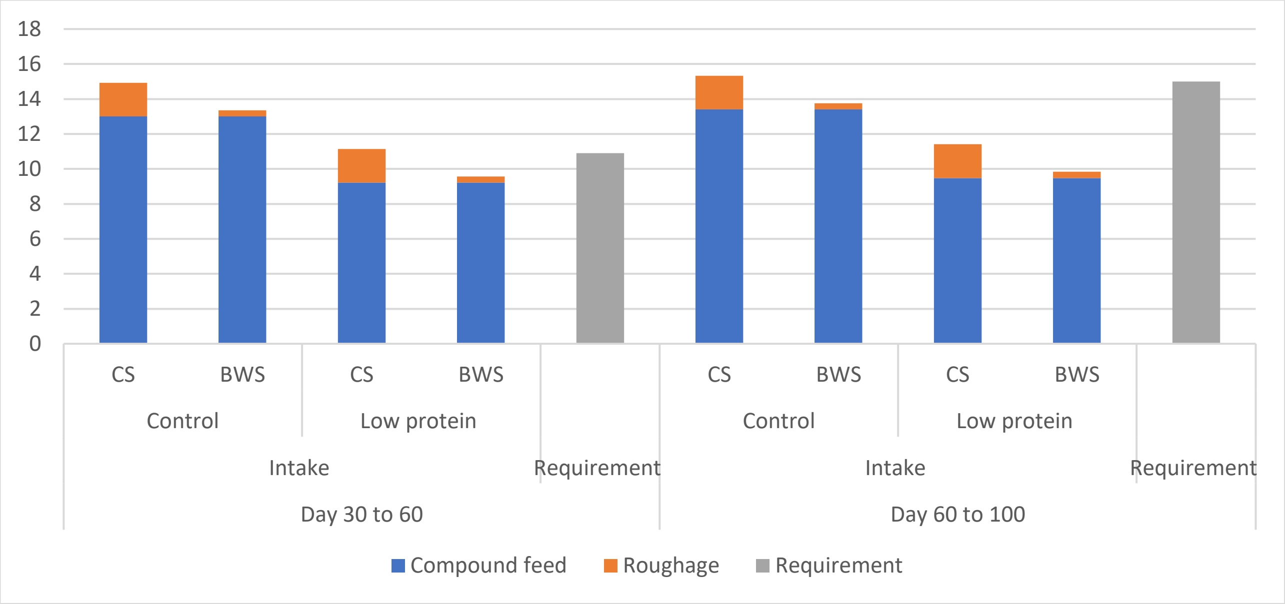

Figure 22, SID lysine intake and requirement of gestating organic sows fed control or low protein diets, and clover-grass silage (CS) or barley-pea whole-crop silage (BWS) during winter conditions (Samuel et al., 2012)

According to figure 22, sows fed low dietary protein in current study, was fed minimum 20 % below their SID lysine requirement from day 60 to 100 of gestation. If the dietary protein level was to be reduced even further in future studies, it should be considered to introduce feeding techniques that allows alteration of the ratio of energy and protein, for instance two-component feeding (Feyera et al., 2020).

In conclusion, the ME and SID lysine balances indicates that by reducing the dietary protein content to 62 g SID CP and 2.37 g SID lysine pr. FUsow, we approach the limit of what is possible without compromising the sows intake of SID lysine relative to the requirement. Furthermore, the balances indicates that roughage supplementation, especially clover-grass silage, contributes with valuable nutrients needed to fulfill the requirement of the sow, when fed low dietary protein. It should however be kept in mind, that the nutrient balances estimated in current study, were based on table values of the requirement, which can lead to misestimation. A proper evaluation of the sow's requirement must be conducted to fully understand the nutritional balances.

5.2 Minor metabolites in plasma, urine and milk

Current project, WI-FI, was created as a continuation of the project EFFORT, with emphasis on roughage supplementation during winter conditions. Consequently, some changes in the experimental design was made. A few things should be kept in mind when comparing the studies, such as impact of the natural environment, as sows were studied during both summer (Eskildsen et al., 2020a) and winter conditions (Eskildsen et al., 2020b). Also, the effect of sow parity must be considered, as sows in EFFORT were younger compared to current study, affecting both the requirement and productivity (Sola-Oriol and Gasa, 2017, Lavery et al., 2019). At last, sows in EFFORT were fed normal or low dietary protein level during both gestation and lactation. It was however, experienced that lactating sows fed low protein during winter conditions were severely challenged, and consequently only gestating sows were fed low dietary protein in current study (Eskildsen et al., 2020b).
Besides the major metabolites examined in current study, a range of minor metabolites, associated with the energy and nitrogen metabolism, was investigated in plasma, urine, and milk of lactating sows from the summer study of EFFORT (Eskildsen et al., 2020a). Certain results are important to have in mind, when studying these metabolites and comparing to current study: The daily intake of compound feed was only two thirds in early and peak lactation, presumable due to their low parity, leaving the sows in negative energy and protein balance (Lavery et al., 2019). Despite the low intake of dietary nutrients, the milk production and litter gain were high, due to immense mobilization of body pools (Eskildsen et al., 2020a).

Only few of the minor metabolites in plasma, urine, and milk, was affected by the level of dietary protein during lactation. Some effects have presumable been concealed by the inadequate dietary intake and high degree of body mobilization. Nevertheless, the urea concentration in urine, expressed by the ratio of urea to creatinine was higher in sows fed control diets, due to the higher CP content in the diet (Pedersen et al., 2019a). Also, concentration of free primary amino groups in milk, which are believed to be an indicator of AA availability in dairy cows, tended to be higher in sows fed control diet. This supports the finding in gestating sows in current study. Besides, the concentration of free primary amino groups in urine, was found to be higher in sows fed control diet in early lactation, but not differ later in lactation. It seems likely, that the difference in early lactation is attributable to the higher dietary CP content, however later in lactation the effect diminished, due to the severe nutrient deficiency. The urinary concentration of glutamine tended to be affected by the same interaction, however glutamine is also the most abundant AA in plasma (Larsen and Fernández, 2017).

During lactation the feed intake gradually increased to provide nutrients for the increasing milk production (Eskildsen et al., 2020a). Similarly, the concentration of D-lactate in plasma increased from early lactation to mid where it stabilized. D-lactate origins from exogenous sources, primarily from starch fermentation in the stomach by *Lactobacillus*, thus can be used as an indicator of feed intake (Serena et al., 2009). This correlates well to the plasma urea concentration, that also increases during lactation due to the increasing feed and protein intake (Eskildsen et al., 2020a, Hojgaard et al., 2019a).

Several of the minor metabolites in plasma and urea can be used to explain the progression in milk production and body mobilization during lactation. As earlier mentioned, milk production is a highly prioritized process and removes large amounts of nutrients from the blood (Krogh et al., 2017b). Plasma and urine concentration of free primary amino groups, an indicator of AA concentration, was found to be inversely proportional to MY, which can be explained by the intensive removal of nutrients from the blood into the mammary gland. An effect we also observed on plasma glucose in current study. A similar effect was observed in urine concentration of glutamine, however the concentration remained low from day 20 to 40. This could be attributable to the negative SID lysine balance at day 5 and 20, triggering mobilization of body protein, which is known to cause a drop in plasma glutamine concentration (Watford, 2015, Eskildsen et al., 2020a). Besides, large amounts of body fat was mobilized during lactation, which clearly affected plasma NEFA concentration (Eskildsen et al., 2020a). Fat mobilization is associated with oxidative stress in the mammary gland (Zachut et al., 2016), indicated by high levels of Glucose-6-Phosphate in early lactation, which emphasizes the negative energy balance of the sows.

5.3 Challenges of outdoor experiments

Performing experiments in outdoor conditions are associated with certain challenges, primarily due to a less controllable and unpredictable environment.

Sows intake of roughage was estimated by weighing back the residues and subtracting this from the supplied amount. The roughage was supplied in open troughs and consequently the roughage could be spread across a large area, due to the rooting and manipulating behavior of sows. This complicated the collection of residues and presumable led to underestimating the amount of residue. Furthermore, the roughage spread around the trough, would be spoiled in earth and mud, increasing the weight. Similarly, on rainy or snowy days the residues would be soaked, increasing the weight substantially. Consequently, on certain days the weight of the residues exceeded the amount of roughage supplied and the roughage intake these days could not be estimated.

Taking blood samples from outdoor sows is also associated with certain challenges. Loose outdoor sows are very difficult to catch, thus the sows were confined in the feeding system during gestation and huts during lactation before sunrise on the sampling days. Typically, blood samples are taken four hours postprandial, as several metabolites are affected by feeding (Eggum, 1970, Frayn, 1998, Serena et al., 2009). In current study, sows were fed at afternoon the day before, however, might have consumed roughage or leftovers of compound feed in the lactation during the evening and night. Nevertheless, the sows were assumed to be fasting when blood samples were taken. Thus, this difference must be kept in mind when comparing concentration of blood metabolites to other studies.

Conducting outdoor experiments with sows requires a lot of manual work and space, thus are expensive. Financial resources are not unlimited, so unfortunately the number of experimental units often is relatively small in outdoor sow studies. Correspondingly, 21 experimental units were used in current study and this was even reduced to 20 in the lactation, due to disease. In comparison the studies of Eskildsen et al. (2020a) and Weissensteiner et al. (2018) included 40 and 36 organic outdoor sows, respectively. The statistical power of an experiment is determined by three main factors: The effect size, amount of random variation and number if replicates or experimental units (Ruxton and Colegrave, 2011). The effect size is the magnitude of the effect that we are measuring; the larger the magnitude, the easier it is to detect. Random variation is the variation between individuals, that cannot be accounted for by the factors investigated and is caused by natural differences between individuals. Less random variation makes differences between treatments, easier to detect. Increasing the amount of replicates, reduces the noise from the random variation, allowing to easier determine what is an effect of the experimental treatment. Also, as it improves the power of the statistical analysis, making it easier to detect effects of low magnitude. Thus, the low numbers of replicates in current study, reduces the statistical power and makes it more difficult to detect effects of low magnitude. Consequently, tendencies or even numerically differences have been considered and discussed in current study, with precaution, especially when corresponding well to other effects observed.

6. Conclusion

Reducing the dietary SID CP concentration 18 % in compound feed for organic gestating sows, from 76 g SID CP/kg to 63 SID CP/kg, did not affect the sow's metabolism and productivity negatively, under current feed allowance. This indicates that we have not gone below the lower limit for dietary protein concentration in compound feed for organic gestating sows, leaving room for future studies to reduce the concentration even further. During gestation, the N metabolism of LP sows displayed improvement, using plasma and urine urea as indicators. Most important, an interaction indicated that control sows had 23 % higher urinary urea concentration at day 60 of gestation compared to LP sows. Thus, the high intake of protein could have reduced the feed efficiency of control sows. The SID lysine balance indicates that towards the end of gestation, especially the LP sows could be challenged by a low SID lysine intake relative to their requirement. However, in current study, this did not negatively affect the gestating sows' ability to deposit nutrients. During gestation sows ate considerably more roughage compared to the lactation period, and the intake of clover-grass silage was noticeable higher than barley-pea whole-crop silage. Clover-grass silage contributed with 14 % ME, and 13 % and 17 % SID lysine of the total dietary intake during gestation, for sows fed control and low dietary protein diet, respectively. This shows that roughage has an important nutritional value, especially to gestating sows. This should be taken advantage of, especially when reducing the dietary content of protein. On the contrary the nutrient contribution from roughage also increased the oversupply of dietary protein seen in control sow, especially in early and mid-gestation. This underlines the importance of including roughage in the feed planning of organic sows. In future studies it would be interesting to consider optimizing the quality of roughage, to fully exploit the nutritional advantages of roughage when feeding low protein diets.

The reproductive performance of sows in current study was very good, with an average peak MY of 16.2 kg/d and an average litter size of 13.3 at weaning at day 49, where each piglet weighed 19.9 kg. Consequently, lactating sows experienced considerable body mobilization, indicated by high plasma NEFA concentrations. As lactation proceeded the MY of LP sows became gradually greater, compared to control sows. Concurrently, an interaction showed that the intake of compound feed was equally greater in LP sows, compared to control sows, thus the LP sows avoided further mobilization of body reserves. As a result of the higher MY, litters from LP sows gained more weight during lactation resulting in a considerable higher litter weight at weaning. Besides the major metabolites in plasma, urine and milk, the minor metabolites provide a more detailed view upon the metabolism of the lactating sow from EFFORT. It should be considered to analyze plasma, urine and milk from current project for these minor metabolites, as this would contribute with valuable knowledge of the sow's metabolism.

In conclusion, it should be considered to reduce the SID CP concentration to 63 g/kg in organic compound feed for gestating sows feed during winter conditions in the commercial organic sow production.

7. Perspectives

In recent years, the attention on environmental protection and global warming has gradually intensified. It is estimated that the livestock sector produces 18 % of the total greenhouse gasses emitted ((FAO), 2006). Thus, a large responsibility lies upon the livestock production to increase the sustainability of current systems. The organic pig production is no exception. Due to the outdoor access and uncontrollable excretion of nitrogen, organic sow production is often associated with a large environmentally burden, in terms of producing the greenhouse gas; nitrous oxide and causing eutrophication of natural environments (Eriksen et al., 2002, Halberg et al., 2010). In current study it was found that a reduction of 18 % in the content of SID CP in gestation compound feed, tended to reduce the urinary content by 23 % of urea at day 60 of gestation. Despite only being a tendency, this is a considerable reduction in the urea excretion. Not only is it expected to increase the feed efficiency by reducing the sow's energetic cost for producing urea, it also reduces the environmental impact of the gestating organic sow substantially. In addition it might also help the organic sow production meet the consumer expectation; that organic agriculture is environmentally friendly (Harper Gemma and Makatouni, 2002).

The low protein diet used in current study was entirely based on cereals. Consequently, the crops for the diet can be produced on the farm itself or nearby farms under Northern European conditions. This eliminates the need to import feedstuff from other parts of the world, in great coherence with the organic mindset. Also, it reduces the feed cost, which constitutes a very large proportion of the total cost in organic pig production (Edwards, 2002). Composing a diet entirely on cereals does however come with certain challenges. As earlier discussed, this diet had a particular low content of lysine. If the protein content were to be reduced further, it would be advantageous to make same adjustment in the ingredient composition of the diet, to increase the lysine content. For instance, leguminous crops, such as fava bean *(Vicious Fava)* or field pea *(Pisum sativum)* have a higher content of lysine relative to the CP content compared to cereals (McDonald et al., 2011). Also, it would still be possible to be self-sufficient, as these crops also can be grown under Northern European conditions.

In current study clover-grass silage proved to be the preferred roughage of gestating sows and contributed with a substantial quantity of nutrients during gestation. Clover-grass is already a highly utilized crop in organic pig production and has an important role in building of soil fertility, thus easy to incorporate in the organic crop rotation. In the future, focus should be laid upon developing clover-grass silage of very high quality, in order to maximize the sows' intake and nutritional value of roughage. In current study, the nutritional benefits of supplying outdoor organic sows with roughage was considered; however, this does not mean it cannot be applied elsewhere. Compared to compound feed, roughage is a cheap way of supplying nutrients to sows and furthermore it provides several benefits, both in terms of welfare, health and even productivity. Thus, it should be considered if roughage also could be applied in the conventional sow production.

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