NUTRITION OF ORGANIC SOWS:

IMPACT OF ENERGY AND PROTEIN SUPPLY

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In loving memory of Sigurd Eskildsen

† May 6th 2020

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“Done is better th~~e~~an perfect”

PREFACE

This thesis is the result of three years PhD study within the project "*EFFORT: Value added through resource efficient organic pig production*". The report is to meet the criteria dictated for the PhD program in Animal Science under the Graduate School of Science and Technology (GSST) at Aarhus University. The project was partly funded by Innovationfund Denmark (2/3) as part of the EFFORT project and partly by GSST (1/3). In accordance with GSST rules, parts of this thesis were also used in the progress report for the qualifying examination in January 2018.

The thesis aims to clarify nutritional questions and challenges related to protein- and energy requirements for pregnant and lactating organic sows in Denmark. The PhD study began the 1st of August 2016 and due to ten months of maternity leave, timely completion is May 2020.

The PhD period was based on two individual experiments with the following purposes:

Experiment 1: To develop a method for quantifying protein intake and protein intake from grass-clover in sows. The experiment was performed in the intensive care unit at AU Foulum in September and October 2016.
Experiment 2: To quantify energy and protein requirements for physical activity, thermoregulation, lactation and maintenance in outdoor pregnant and lactating sows under influence of season. The data collection took place from September 2016 to September 2017 in the fields of the Organic Platform at AU Foulum.

This thesis will present:

- An introduction to nutrition of organic sows
- An account of methods, results and conclusions
- Three submitted papers
- An overall discussion, conclusion and perspectives on feeding organic sows

The target audience is professionals working with organic pig production; pig producers, the feed industry, pig consultants and the examination committee.

Foulum May 2020

Maria Eskildsen

ACKNOWLEDGEMENT

This PhD study has been a great journey, with a steep learning curve and only very few serious obstacles on the way and I wish this opportunity for everyone, who is interested in immersing themselves into a single subject. For this experience to be so positive, many good people have contributed:

First and foremost, I thank my main supervisor Dr. Peter Kappel Theil. I knew from the very first phone call, that I would enjoy working with You. It has been “freedom with responsibility” and You have trusted me with the overall responsibility for planning and conducting the experiments, which I am very grateful for. Our supervisor meetings have always been fruitful and in a nice atmosphere. Your immense knowledge, motivation and patience has given me the spirit to excel within animal science.

Apart from my main supervisor, I wish to express my gratitude to my co-supervisors Dr. Jan Værum Nørgaard and Dr. Anne Grete Kongsted. You and my other co-authors Dr. Martin Tang Sørensen and Dr. Mette Skou Hedemann have played an important role in polishing my research writing skills.

I am also pleased to thank the technical staff in the intensive care unit and not least the technical staff at the Organic Platform; Henrik Tauber Sørensen, Kurt Preben Jensen, Uffe Schmidt, Birgit Storm Hansen and Mikkel Jaquet. Thank You for Your willingness to solve any of my practical challenges at all times and for forgetting about working hours and getting up in the middle of the night to catch pigs in the mud through a whole year ☺

I also wish to acknowledge my talented and hardworking colleague, Post Doc Uffe Pinholt Krogh, with whom I shared many hours collecting body fluids in all kinds of weather. Surely, a great research career is waiting for You.

Doing animal experiments is time- and labor consuming and performing them on free range animals makes the workload even bigger. However, I have been lucky to persuade friends, family, neighbors and colleagues to help me, which I am very grateful for. Also several AP degree-, bachelor- and master students have joined the project in shorter or longer periods. In this respect, I am indebted to Natasha Brandt, Stine Lindgren, Jesper Vodder, Sigrid Jost Wisbech Skovmose, Prince Chisoro, Johanne Dalsgaard and Daniela Vega Sampedro for Your

contributions - big or small. I would also like to express my appreciation to Dr. Sara-Lina Aagaard Schild with whom, I had a very constructive collaboration about sharing the sows at the organic platform.

I will always remember my fellow students Camilla Højgaard, Takele Feyera, Liang Hu, Pan Zhou, Sigrid Jost Wisbech Skovmose and Signe Emilie Nielsen from the Theil Lab for the good time we spent together. A special thanks to the warmhearted Trine Friis Pedersen for always making sure, that everyone is ok. Though, I have had no less than five different offices at AU Foulum within the last four years, it has always been a pleasure to have my cup of afternoon tea with Tina Skau Nielsen, Lene Stødkilde, Anne Krogh Ingerslev, Saman Lashkari, Søren Krogh Jensen, Janne Funch Adamsen, Anette Møjbæk Pedersen, Elsebeth Lyng Pedersen, Winnie Østergaard Thomsen, Stina Handberg, Lisbeth Märcher, Cecilie Vangsøe, Knud Erik Bach Knudsen, Geonil Lee, Yetong Xu and all other members of the previous Molecular Reproduction and Nutrition Group at the Department of Animal Science.

During the last six months of the PhD period, two major events changed my private life; My father became terminally ill and, less dramatic; I became a dog owner. In both cases, Dr. Helle Lærke has been a great source of comfort and good advice. Thank You.

Above all, I am very grateful for my husband Kristian, who despite a substantial pay cut and a lot of interference with the family planner, encouraged me to follow my dreams and start as a PhD student at “an old age”.

On the very same day, I started writing this thesis, the Corona virus changed every agenda in Denmark and the rest of the world, reminding us all, why science is so important. Suddenly I was stuck at home writing with four kids around all day and a working husband. At that time, I did not believe, that punctual assignment would be feasible at all, but with the love and support from Kristian, my mother Tove Poulsen and her husband Jon Poulsen, a couple of creative residencies at Ørslevkloster Arbejdsrefugium and not least, patient well-behaved children, it became possible to obtain timely completion after all. Villiam, Ellen, Peder and Agnes Eskildsen; The four of You will always be my greatest achievement.

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SUMMARY

Organic sows are exposed to a wide range of changes in the environmental conditions (photoperiod, thermoregulation, locomotive activity, and grass/roughage intake), and their lactation period is twice as long compared to indoor sows. Nutrient recommendations for organic sows are, however, based on knowledge obtained for indoor sows.

Two experiments were included in this Ph.D project to determine the energy- and protein intake from grazing and to quantify the energy needed for maintenance, maternal retention, mobilisation, milk production, thermoregulation, and locomotive activity in organic sows.

The first experiment included sixteen sows fitted with urinary catheterized and kept in metabolic cages to study the effect of increasing intake of fresh grass-clover on plasma - and urine metabolites, estimate the grass-clover digestibility in sows, and to identify possible biomarkers for grass-clover intake in organic sows.

The second experiment included forty-seven free-range gestating and lactating sows, who were randomly assigned into either a control group with “normal” protein concentration in the compound feed or a low protein group, where the protein content was reduced by 12% in both gestation and lactation diets. Two parities were studied, which were confounded with winter and summer seasons, and therefore published in separate papers. The two dietary strategies were adjusted to be iso-energetic and were of 100% organic origin. To meet the extra demand for thermoregulation and physical activity, the energy allowance from both treatments was increased by adding 15% to the recommended energy level for indoor sows in winter and + 10% in summer.

Fresh grass-clover intake of sows was highly correlated with plasma pipecolic acid and plasma bisnorbiotin concentrations. Apparent total tract digestibility of dry matter, organic matter, nitrogen, and energy of grass-clover was 64%-72% using the regression method, and the excretion of nitrogen in urine was highly correlated with grass-clover intake.

The main challenge during lactation was a limited feed intake capacity. The actual feed intake was only 2/3 of the fed amount in the first three weeks of lactation, and sows mobilised more than 1 kg of body fat per day in the period from day 5 to day 20 in lactation in both seasons. Body fat loss was particularly critical during winter as sows need fat for insulation.

and their energy intake was not sufficient for gilts under Danish winter conditions, even though it was a mild winter.

In pregnancy, sows walked 1.9-2.5 kilometers per day and spent 3.9-4.8 MJ ME on physical activity. The covered distance in early lactation was 704 meters/d, at peak lactation 1482 m/d, and in late lactation 1644 m/d. Energy consumption for thermoregulation during winter was much higher than for physical activity, and the energy requirement for locomotive activity in organic sows was relatively low compared to that of indoor animals.

For pregnant sows, the intake of grass-clover dry matter was 409 g and 428 g per sow on d60 and d100 as determined by the profile of plasma pipecolic acid. The SID lysine contribution from grass-clover was 3 g/d corresponding to 23% and 17% of the daily SID lysine requirement on d60 and d100 of gestation.

In lactation, sows had a daily energy expenditure of 1.4-3.0 MJ ME for locomotive activity, and the mean intake of grass-clover dry matter varied between 225 g/d on day 5 and 574 g/d at peak lactation. The SID lysine intake from grass-clover increased from 1.7g/d to 4.5 g/d with progress of lactation. The total energy requirement at peak lactation was 130 MJ ME/d, and the average energy requirement for thermoregulation was 13.5 MJ ME/d in a mild Danish winter and 5.2 MJ ME/d in a typical summer, which corresponds to 1.0 and 0.4 kg extra feed, respectively.

Counterbalancing the contribution of energy and protein from fresh grass-clover enables a deduction of approximately 12% of crude protein in gestation compound feed during summer, without impairing sow productivity, provided that sows have access to a rich grass-clover sward. The crude protein content in lactation compound feed should not be reduced to more than 148 g/kg, unless daily feed intake is above 10 kg/d in lactation, as the intake capacity of lactating sows limited the intake of energy and SID lysine in lactation and in turn milk yield and piglet daily gain.

In conclusion, it was possible to reduce the dietary protein concentration to pregnant organic sows when the animals had ad libitum access to fresh clover grass in summer and grass silage in winter. Still, dietary protein should not be decreased during lactation.

SAMMENDRAG (DANISH SUMMARY)

I forhold til konventionel sohold, afviger forholdene for økologiske søer på flere afgørende punkter; dagslængde, temperaturudsving, plads til fysisk aktivitet, indtag af græs/grovfoder samt en diegivningsperiode, der er minimum syv uger mod tre til fire uger i indendørs svineproduktion.

Fodernormerne til økologiske søer er imidlertid baseret på anbefalingerne til indendørs svin, selvom øko-søer på daglig basis sandsynligvis har brug for mere energi men ikke mere protein end konventionelle søer. Normerne for protein er angivet i forhold til energi og når Øko-søer i gennemsnit fodres med 30% mere foder med den samme protein koncentration, er der risiko for protein overforsyning, særligt når søerne samtidig indtager protein fra grovfoder i form af ensilage om vinteren og kløvergræs om sommeren. Der er derfor behov for at justere forholdet mellem protein og energi i kraftfoderet til økologiske søer, så det bedre matcher det øgede energibehov uden at overforsyne med protein og dermed belaste miljøet med overskydende kvælstof.

Dette PhD projekt er baseret på to forsøg, som havde til formål at bestemme energi- og proteinindtaget fra afgræsning om sommeren samt kvantificere energibehovet til vedligehold, tilvækst, mælkeproduktion, termoregulering og fysisk aktivitet hos udendørs søer.

I det første forsøg indgik seksten søer i balance bure. Det havde til formål at undersøge effekten af stigende tildeling af frisk kløvergræs på plasma- og urin metabolitter, estimere fordøjeligheden af kløvergræs, samt identificere mulige biomarkører for økosøers daglige indtag af kløvergræs.

Det andet forsøg omfattede 47 drægtige og diegivende søer på friland. Søerne blev inddelt i to grupper og fodret efter to protein strategier; enten kontrol, som blev fodret efter de nuværende anbefalinger for protein indhold og en lav protein gruppe, hvor protein indholdet i drægtigheds- og diegivingsfoder var reduceret med12%. Foderet indeholdt samme mængde energi i de to grupper og var 100% økologisk. For at imødekomme det ekstra behov for energi til termoregulering og fysisk aktivitet, blev energiindholdet i alle blandinger øget med 15% i forhold til normerne for indendørs søer om vinteren og +10% om sommeren. Søerne fik to kuld grise; det første i vinterperioden og det andet i sommerperioden.

Resultater

Indholdet af pipecolsyre og bis-norbiotin i plasma var stærkt korreleret med indtaget af kløvergræs. Totalfordøjeligheden af tørstof, organisk stof, kvælstof og energi i kløvergræs blev estimeret til 64%-72% ved hjælp af regressionsmetoden. Udskillelsen af kvælstof i urin steg med stigende indtag af frisk kløvergræs.

Søer på lavt protein åd 14% mere frisk græs end kontrol gruppen om sommeren. Generelt var foderindtaget kun 2/3 af den udfodrede mængde de første tre uger af diegivningsperioden og søerne mobiliserede mere end 1 kilo kropsfedt per dag i perioden fra dag 5 til dag 20 efter faring – både sommer og vinter. Det samlede daglige energibehov på top-laktation var 12.2 FEso for 1. lægssøer om vinteren og 2. lægs søer om sommeren og det gennemsnitlige daglige behov for energi til termoregulering var 1.05 FEso om vinteren og 0.40 FEso på en normal dansk sommerdag. Vinteren 2016/2017 var mild, også efter danske forhold.

Drægtige søer gik 1,9-2,5 kilometer om dagen og havde et energiforbrug på cirka 0.35 FEso per dag til fysisk aktivitet. For drægtige søer var det gennemsnitlige daglige indtag af kløvergræs mellem 409 g og 428 g tørstof per so, bestemt ved hjælp af koncentrationen af pipecolsyre i plasma. Det daglige bidrag af SID lysin fra kløvergræs var cirka 3 g, hvilket svarer til 23% af normen i tidlig drægtighed og 17% i sen drægtighed. I diegivningsperioden gik søerne i gennemsnit 0,7-1,7 kilometer om dagen og brugte således 0,1-0,2 FEso om dagen på fysisk aktivitet. Hos diegivende søer varierede indtagelsen af tørstof fra kløvergræs mellem 225 g på dag 5 og 574 g på top laktation og bidraget af SID-lysin fra kløvergræs varierede mellem 1,7-4,5 g/d. Der blev ikke observeret forskelle i koncentrationen af næringsstoffer i plasma, urin, mælk eller for soens produktivitet (levende fødte og mælkeydelse) mellem de to protein niveauer.

Disse resultater bidrager til en bedre forståelse af økologiske søers energi- og proteinbehov og bekræfter, at drægtige, og til dels diegivende søer, er i stand til at udnytte nogle af næringsstofferne fra marken, såfremt der opretholdes rigelig græsdække. Yderligere forskning er nødvendig for at bestemme energi- og protein bidraget fra grovfoder om vinteren.

MY OWN CONTRIBUTION TO THE RESEARCH

The overall aim of the EFFORT project was determined before I was employed in August 2016 and the research idea must be attributed to Dr. John E. Hermansen and Dr. Anne Grete Kongsted from department of Agrobiology and Dr. Peter K. Theil from Department of Animal Science, Aarhus University Foulum

Planning experiments

The experimental plan for experiment 1 was written by me under the guidance of Dr. Jan Værum Nørgaard in September 2016. The experimental plan for experiment 2 was compiled by me in the autumn of 2016, with inputs from Uffe Krogh and Peter K. Theil.

I shared the animals in Experiment 2 with a behavioral study performed by Dr. Lene Juul Pedersen. Therefore, I had a close cooperation with Ph.D student Sara-Lina Schild to ensure that my handling of the animals did not interfere with their study. Every Monday from October 2016 to September 2017, I had a meeting with the technical staff at the Organic Platform to plan the sample collection of that particular week.

Data collection

The experimental work included the collection and handling of samples and data which involved:

Experiment 1

Thirty-two blood samples, thirty-two urine samples, and thirty-two fecal samples were collected by me. Compound feed and clover grass samples + residues were compiled by the technical staff in the intensive care unit.

Experiment 2

For the sows, it amounted to 435 individual weighings and BF scannings. I also collected 435 blood-, milk- and urine samples and performed 435 D2O enrichments on sows in cooperation with post-doc Uffe Krogh with the help from technical staff and other colleagues. In total, we conducted 6,530 individual piglet weighings, and 144 blood samples were drawn on piglets by me in cooperation with the technical staff at the organic platform, Uffe Krogh and Sara-Lina Schild.

Compound feed samples were collected by technical assistant Kurt Preben Jensen, and grass-clover samples were collected by the technical team at the Organic Platform. Silage samples were collected by me. Data on exact birth time of the piglets was provided by Sara-Lina Schild. Data form the GPS trackers and the weather stations were collected by me just as macro-chemical analysis of the composition of milk was performed by me with help from EM-

SANF student Daniela Vega Sampedro. Laboratory technicians performed all other laboratory analyses.

Sub-sample preparation

All preparation and agreements concerning laboratory work on samples were done by me.

Laboratory work

- Laboratory assistant Hanne Berg has analyzed energy content and chemical composition of grass-clover, urine and feces in Experiment 1
- Laboratory assistant Casper Poulsen did the LCMS analyses of plasma
- Dr. Mette Skou Hedemann did the interpretation of the LCMS data in both experiments. Mette Hedemann also wrote the LCMS section + prepared Figure 1 and 2 in Manuscript I.
- Laboratory assistant Lisbeth Märcher and laboratory assistant Stina Handberg has done energy and carbohydrate analyses on compound feed, urine, and grass-clover.
- Chemical composition of grass-clover, silage, and compound feed were determined at Eurofins Lab., DK-6630 Vejen.
- Laboratory assistant Carsten Berthelsen performed analyses of the chemical composition of plasma, urine, and micronutrients in milk from Experiment 2.
- Laboratory assistant Anne Krustrup performed deuterium analyses on plasma, and urine in Experiment 2.

Data handling

Student worker Johanne Dalsgaard typed individual winter feed consumption data from Experiment 2. All other typing and statistical analysis of data was performed by me under the guidance of Dr. Peter Kappel Theil.

Authorship

My main contributions to this thesis included the planning and execution of the experiments, sub-sample preparation, data analysis, drafting the three scientific manuscripts, and revising them based on inputs from my co-authors. Apart from the LCMS part and Figure 1 and 2 in Manuscript I, I declare that I have written the present Ph.D thesis, and that all work included is my own. The assistance I have received during this Ph.D project has been duly acknowledged, and the work presented has not been submitted for any other degree or professional qualification.

I had no non-university supervisors or co-authors and see no conflicts of interest within this PhD project.

LIST OF INCLUDED MANUSCRIPTS

The thesis includes three scientific manuscripts which will be referred to by the following Roman numerals:

Manuscript I

Eskildsen, M., M.S. Hedemann, P.K. Theil, J.V. Nørgaard. Impact of increasing fresh clover grass intake on nitrogen metabolism and plasma metabolites of sows. Submitted to Livestock Science in November 2019. Revised in September 2020.

Manuscript II

Eskildsen, M., U. Krogh, M. T. Sørensen, A.G. Kongsted, P.K. Theil. Effect of reduced dietary protein level on energy metabolism, sow body composition and metabolites in plasma, milk and urine from gestating and lactating organic sows during temperate winter conditions. Submitted to Livestock Science in February, 2020. Published in Livestock Science in May 2020.

Manuscript III

Eskildsen, M., U. Krogh, J. V. Nørgaard, M. S. Hedemann, M. T. Sørensen, A. G. Kongsted, P. K. Theil. Grass-clover intake and energy requirements for physical activity, thermoregulation, lactation and maintenance in second parity organic sows fed two levels of dietary protein during the summer period. Published in Livestock Science in August 2020.

LIST OF OTHER SCIENTIFIC CONTRIBUTIONS

Abstract/posters:

Eskildsen, M., JV Nørgaard, MS Hedemann, PK Theil. Grass intake of sows quantified by plasma metabolites.14th International Symposium on Digestive Physiology of Pigs. Brisbane, Australia, 2018.

Eskildsen, M., DV Sampredro, U Krogh, T Larsen, PK Theil. Effect of dietary protein level on milk yield, milk composition and blood metabolites in organic sows on pasture summer and winter. EAAP Scientific Series, 2285-2298. 6th EAAP International Symposium on Energy and Protein Metabolism and Nutrition. Belo Horizonte, Brazil 2019.

Krogh, U., M Eskildsen, MT Sørensen, H Jørgensen, PK Theil. Heart rate as predictor of heat production at different reproductive stages in second parity free-ranging sows. Abstract from 14th international symposium in Digestive Physiology in Pigs. Brisbane, Australia 2018.

Krogh, U., M Eskildsen, MT Sørensen, H Jørgensen, PK Theil. Heat production in free-range sows estimated by heart rate recordings. 14th International Symposium on Digestive Physiology of Pigs. Brisbane, Australia 2018

Eskildsen, M., U Krogh, AG Kongsted, PK Theil. Fresh grass-clover intake and energy metabolism in organic sows fed a control or a low protein compound feed in winter and summer. Accepted for the Pre-Conference on Animal Husbandry linked to the 20th Organic World Congress in Rennes Organized by IFOAM Animal Husbandry Alliance France on 8-10 September 2021.

Magazine reports:

Hvor meget næring kan søer hente på marken? Svinefokus, Effektivt Landbrug 6:7. September 2016

Foulum-forsker skal reducere foderforbruget hos økosvin. Magasinet Svin. 14:15. October 2016

Mad og motion skal kortlægges for øko-søer. Viborg Stifts Folkeblad. October 2016.

Økologiske svin skal fodres anderledes. Økologi og Erhverv. 12:13. October 2018.

70 pct. fordøjelighed af frisk kløvergræs hos søer. Ny forskning anbefaler nye fodersammensætninger til økologiske søer og gylte. Fagmediet Økologisk 33-34 January 2020.

LIST OF ABBREVIATIONS AND CLARIFYING TERMS

Amino acid(s)	AA
Atomic fraction	AF
Back fat	BF
Body weight	BW
Crude protein	CP
Deuterium oxide	D_2O
Digestible energy	DE
Feed unit(s) for sow	FUsow
Kilo gram	kg
Kilo joule	kJ
Metabolisable energy	ME
Standardized ileal digestible	SID
Non-esterified fatty acids	NEFA
Glucose-6-phosphateuric	Glu-6P
β-hydroxybutyrate	BHBA
N-acetyl-beta-d-glucosaminidase	NAGase
Lactate dehydogenase	LDH

Definition of reproductive stages relative to sample days in Experiment 2

Mid Gestation	Day 60 in gestation
Late gestation	Day 100 in gestation
Early lactation	Day 5 after parturition
Peak lactation	Day 20 after parturition
Late lactation	Day 40 after parturition

Compound feed: Mix of ingredients (energy and protein sources, vitamin and minerals)

Sow productivity: Liveborn, piglet gain, weaned piglets, milk yield weaning to estrus interv

INTRODUCTION

With “Økologisk handlings-plan 2020” from the Ministry of Environment and Food, it became a Danish national plan to double the area grown organically from 2007 to 2020, which was achieved and at present, 10.5 % of the agricultural land in Denmark is grown after organic principles. The Danish organic exports set a record ten years in a row in 2019, and Denmark retains its world-leading position, as the nation, where organic farming has the largest market share, followed by Sweden and Switzerland (FIBL & IFOAM, 2020). The number of organic pigs has also been rapidly increasing the last decade, and although the market share of organic pork is rather low (3.2 % in 2019), the market for organic pork is also expanding quite rapidly in Denmark, Figure 1. The turnover reached a value of approximately 180 million Danish kroners in 2018 (Hindborg, 2020).

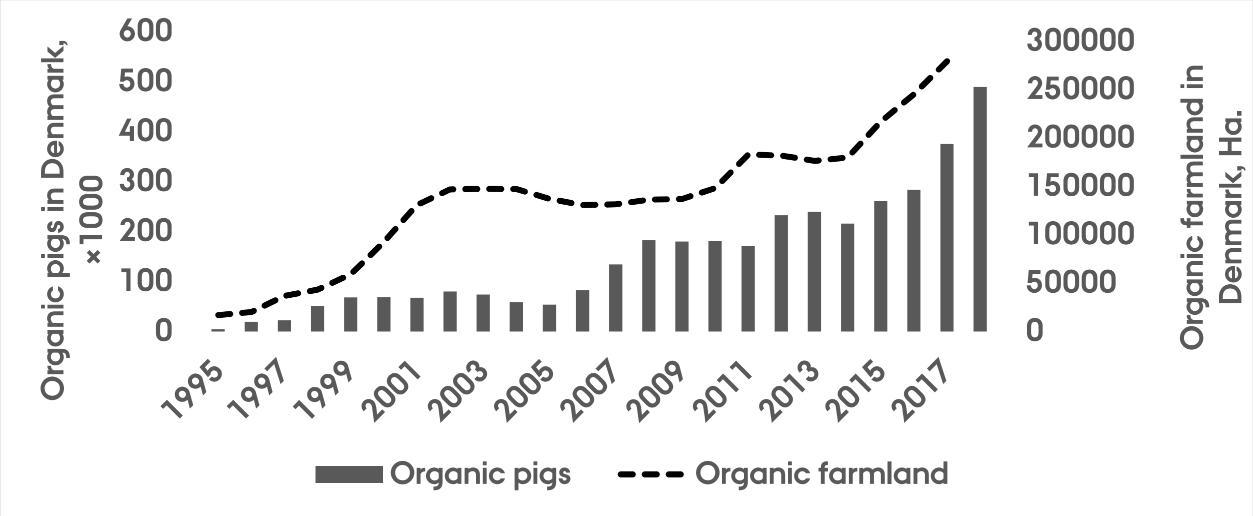


Figure 1. The number of organic pigs and the total area grown organically in Denmark for the last 25 years (Danmarks Statistik, 2020).

These figures indicate an increased consumer demand and interest of the retailers to approach into food production that takes sustainable, environmental, and animal welfare considerations into account. However, increasing the organic pork production is not without challenges. In terms of nutrition, the organic farmers are currently facing a legal requirement of 100% organic feed and increased public focus on nitrogen and phosphorous leaching to the environment.

In order to achieve maximum efficiency and profitability, the establishment of accurate formulation and rationing of the feed is critical. Based on an intensive and continuous acquisition of knowledge, feeding strategies for indoor pigs have become extremely sophisticated. Much of the experience gained indoor has been transferred into an organic context - but leaving aside some essential considerations (Blair, 2005).

Feed for use in organic pig production can contain ingredients from two categories only:

- Agricultural products that have been produced and handled organically and locally – preferably on-farm or in the same region (EU can be considered a region).
- Non-synthetic substances, such as vitamins, minerals, enzymes, probiotics, and others considered to be natural ingredients that have been approved for use in organic pig production. All feed materials must be non-GMO and listed in Annex II of Council Regulation No. 1804/1999.

As relevant data on feeding organic pigs have to be extrapolated from conventional indoor pig production practices, the limitations in the choice of feed leads to at least three significant challenges in feeding organic sows:

1) 100% locally produced protein and the environment

Supplemental crystalline amino acids are prohibited, and import of protein (mainly soybean meal) from South America and Asia is also not an option as from January 2019. Hence, European organic pig farmers must rely on on-farm production of protein (e.g. faba-beans, rape-seed, hemp, and line-seed). Many of these feeds have a non-optimal amino acid profile, probably followed by an inefficient protein utilization. Because of this, organic pig farmers are facing numerous challenges concerning feed efficiency and nitrogen-leaching to the environment.

2) Unknown intake of protein and energy from pasture

Unlike conventional indoor sows, Danish organic sows must have access to grazing areas for a minimum of 150 days in the summer period. For now, the daily intake of grass is unknown; therefore, it is very complicated to set off the contribution of nutrients from pasture against the total daily requirement of energy and protein. This results in unbalanced diets.

3) Increased energy requirement

Organic sows live under varying climate conditions, weaning age is more than twice as long, and the sows have an opportunity for increased locomotory activity compared to conventional indoor sows. These factors presumably lead to an increased demand for energy, but at present, the additional energy required for thermoregulation, physical activity, and the prolonged lactation period is an unknown quantity.

Some of the challenges to nutritionists within the research area of organic pig production is to help rectify these three constraints in a way compatible with the sustainable ideas of organic farming.

Data on feeding organic pigs are minimal (Blair, 2018; Close and Poornan, 1993), and Jakobsen and Hermansen (2001) conclude that research is highly needed to establish the requirements and supply of energy and essential amino acids under organic farming conditions.

This thesis aims to clarify nutritional questions and challenges related to protein- and energy requirements for pregnant and lactating organic sows in Denmark. It contains a State-of-the-art section unfolding relevant literature on this topic together with three submitted scientific manuscripts based on two animal experiments - performed to estimate grass-clover intake, and the energy and protein requirements of organic sows during winter and summer.

There is a short introduction to the aims and hypotheses, a brief description of the applied methods, and concise summaries of the three manuscripts. In the manuscripts, summer and winter have been separated, but in the overall results section in this thesis, data are summarized to create an overview of the overall effect of season (which for practical reasons was confounded with parity), protein level and reproductive stage on the protein- and energy requirements of organic sows.

The current thesis focuses on energy and protein supply to organic sows under Danish weather conditions. Vitamin- and mineral requirements will not be discussed, just as topics concerning behavior, rearing, meat quality and health – or nutritional issues concerning weaners, growers and finishers, will be left out. Data on breed (DanBred vs. TN70 from Topigs Norsvin) are presented in the overall results section, due to considerable interest from the agricultural industry. However, they will not be discussed any further as there was no breed/protein level interactions – and genetic differences, how interesting they may be, are not a theme for this dissertation.

STATE-OF-THE-ART

One major challenge within organic sow nutrition is that the animals are fed using recommendations for indoor pigs, even though especially the energy requirement differ substantially between these production systems. And this has, in turn, consequences for the optimal concentration of dietary protein, because the recommendation is expressed relative to dietary energy and because organic produced sows have a higher annual feed consumption than indoor sows. This paragraph seeks to unfold the three introduced challenges within the field of energy and protein supply of outdoor organic sows.

100% LOCALLY PRODUCED PROTEIN AND THE ENVIRONMENT

Because of a lack of organic protein sources, the transition to 100% organic feed ingredients for organic livestock has been postponed repeatedly in the European Union since 2015. Until December 31st 2020, it is still lawfully allowed to use 5% non-organic protein feeds for organic pigs in Denmark {Ministry of Environment and Food of Denmark, 1584/2018). However, in practice, 100% of the feed has been organic since 2018 according to a voluntary industry agreement that must be complied with, if organic farmers wish to have their animals slaughtered in Denmark at the slaughterhouses Friland A/S, Tican or Organic Pork. These three companies together slaughter about 99% of the certified organic pigs in Denmark.

In Denmark, the climate does not allow self-sufficiency in the protein needs of an increasing organic pig production, and organic soy has been imported to fill the protein gap. This import conflicts with the organic approach, where the feed supply should be predominantly farm-owned production, and the nutrient cycle within a farm system should be closed.

When organic farmers are prevented from using imported soy protein, deficiencies of limiting amino acids are likely to occur due to difficulties in the supply of protein-rich ingredients in sufficient quantities/qualities and the prohibition on crystalline amino acids. As a consequence, organic sows are fed excessive amounts of protein and other nutrients to comply with the nutritional requirements of the animals (Hermansen et al., 2015; Jakobsen et al., 2015).

Since crystalline amino acids may not be added to organic feeds, the concentration of crude protein must be increased to comply with the requirements of the first limiting amino acids. This, together with the higher feed consumption, results in a significantly higher nitrogen content in organic manure compared to that of conventional indoor pigs (Tybirk, 2018).

This oversupply, feed waste, and the excretory behavior of pigs may create nitrogen hotspots in the paddocks and increase the environmental impact from organic pig production. Nitrogen is present in urine in the form of urea and as different nitrogenous compounds in feces. In organic fattening pigs with access to outdoor runs, a substantial proportion of nutrients (43-95%) was found to be concentrated in an area of arable land representing 4-24% of the total pen area (Salomon et al., 2007). Eriksen et al., (2001) investigated the distribution of nutrients in lactating organic sow paddocks and observed a correlation between soil inorganic nitrogen and the distance to feeding sites after the paddocks had been used for six months. Under Danish conditions, (Larsen et al., 2000) have shown a surplus of 330-650 kg nitrogen per hectare of land used for organic grazing sows. Eriksen et al. (2001) found that the nitrogen input from organic lactation compound feed could be accounted for in piglets (44%), as ammonia evaporation (13%), as denitrification (8%), or as nitrate leaching (16 - 35%).

Sommer et al., (2001) found that ammonia evaporation was highest near the feeding area and the huts, where the sows tended to urinate. Ammonia evaporation was related to the amount of feed given to the sows, and the annual ammonia loss was 4.8 kg NH_3–N from each organic sow.

At present, Danish organic pig producers are exempted from restrictions on ammonia emission due to difficulties in complying with this regulation. This is, however, a potential future prospect, and it would be of high value to the organic pig industry if improved feeding strategies could reduce the environmental impact of organic pigs and also help to solve the challenges with 100% organic feed.

To a great extent, the environmental load of organic pig production is related to the amount of nutrients in the compound feed (Hermansen et al., 2005), and the nitrogen content of the compound feed is the most potent factor in reducing the nitrogen excretion from pigs (Everts et al., 2010). If the contribution of energy and protein from direct foraging was counterbalanced and the protein content in the compound feed thereby reduced by 10% on average, it would be possible to reduce the climate footprint from organic pig production by 4% points relative to a baseline situation. This scenario would cause a 9% point lower eutrophication and a10 % point less acidification of the environment.

Danish organic pig farmers should preferably identify alternative protein sources in adequate amounts, which are not only in line with the organic certification rules, produced regionally or at least within Europe; the composition of amino acids and the content of crude protein must comply with the nutritional requirements of the animals without challenging the environment more than necessary.

Homegrown forages and direct foraging in the paddocks is suggested as a reliable way to improve nutrient efficiency at farm level, as the need for protein input into the system via supplemental compound feed is reduced (Kelly, 2007 ; Smith, 2014; Jakobsen, 2015). Among others, the nutritional contribution from grazing depends on voluntary feed intake, the nutritional value, and the digestibility of grass-clover, which until now is unknown.

UNKNOWN INTAKE OF PROTEIN AND ENERGY FROM PASTURE

Rearing systems for organic pigs in the European Union are to be based on maximum use of grasslands according to the availability of pastures in the different periods of the year (Regulation, EC No 1804/1999). According to the Danish organic regulations, sows must have access to grazing in the period from April 15th to November 1st. If the weather conditions and the animals physical condition allow it, they should preferably be on pasture in the entire period (195 days). However, most of the organic producers have chosen to keep the sows on pasture throughout the year (Kongsted and Hermansen, 2005). The palatability, intake, and nutrient content of pasture vary with season, climate, and plant content (Rivera Ferre et al., 2000), but information about grass-clover intake and the feeding value of grazing is difficult to obtain. This, probably because reliable measurements of grass intake by pigs on pasture are challenging to achieve (Blair, 2018).

The recovery of n-alkanes of herbage and a known dose of artificial alkanes in feces is known as the n-alkane method. This method enables unbiased estimates of grass intake in animals receiving supplementary feed (Mayes et al., 1986). N-alkanes are components of the plant cuticular wax and the n-alkane method was developed to find markers for the estimation of grass intake and digestibility in grazing ruminants, but the method is also validated for estimation of grass intake of sows on pasture (Gannon, 1996; Sehested et al., 1999a; Rivera Ferre et al., 2001, Kanga et al., 2012)

With the n-alkane technique, it is possible to estimate the grass-clover intake of sows without compromising the behavior or welfare of the animals. However, it may be difficult to rely on the method in outdoor pig production, since feed waste is considerable (Lauritsen, 1998; Nissen, 2019) and a complete marker intake is essential to avoid overestimation of the clover grass intake. Another challenge could be fermentation. Little is known of the fate of n-alkanes in the hindgut of the sow, because some bacterial species and yeast have been shown to utilize n-alkanes (Dostalek et al., 1968; Yamada and Yogo, 1970).

It might be possible to estimate voluntary grass intake by bite-size and bite rate, short term changes in live weight, or total feces collection with a chromium marker in the basal diet, if the clover grass digestibility is known. (Sehested et al., 2004) found a daily grass intake of 14-16 MJ ME in a well-established field and 18-19 MJ ME in a recently established field, i.e., with highly digestible grass. Others have found a voluntary grass-clover intake of 22 MJ ME/d on the basis of the growth of restrictively fed pregnant sows (Fernandez et al. 2006), or 5.8 kg and 7.3 kg of fresh grass per day in spring and summer, respectively (Rivera Ferre et al., 2001). Observing measurements of weight gain and ingested bites taken by continuously monitored Iberian pigs, a grass intake of 0.38± DM/d and 0.49 ± 0.04 was found in two different herds (Rodríguez-Estévez, 2009; Rodríguez-Estévez, 2010).

Pasture consumption of grass-clover was estimated by cutting pasture samples pre- and post grazing and weekly weighings of growing wild boars (initial weight 14.4 kg) with ad libitum access to compound feed for one hour and access to different amounts of grass-clover DM available per day (0 g, 400 g, 600g and 1200 g). The consumption of supplemental compound feed tended to be less in animals with greater herbage allowance (P=0.16) and animals with access to pasture had higher weight gain than those without access to pasture. The daily consumption of DM from grazing was 0 g, 107 g, 139 g, and 229 g in the four dietary groups, respectively (Rivero et al., 2013a). In another experiment with fattening wild boars (18.3 kg ± 0.45 kg), the average pasture consumption of DM from grass-clover was 242±18 g/d (Rivero et al., 2013b).

The nutritional contribution from pasture also depends on the available vegetation. In a video surveillance study of gilts, the animals preferred grazing white clover and alfalfa, and rooting and eating white clover compared with buffalo grass or tall fescue (Rachuonyo et al., 2005).

Edwards (2003) conclude that grazed herbage can contribute 50% of the maintenance energy requirement and a high proportion of the amino acid requirements of dry sows. Using calculations based on live weight gain, Fernandez et al., 2006 found that pregnant sows on pasture could cover up to 61% of their nutritional need by grazing in the summer period. Using sward structure, herbage quality, and live weight changes, Danielsen et al.

(2001) reported that pregnant sows on pasture could cover a significant part of their nutritional need by grazing. Still, the same did not apply for lactating sows. As a rule of thumb, the energy contribution from grass-clover in summer has been set to one Scandinavian feed unit per day for pregnant sows, which is approximately 12.2 MJ ME/d (Danielsen et al., 2001; Serup, 2008)

The nutritional contribution made by grazing will depend on many factors as pasture availability, nutrient composition, intake and the grass quality, i.e., the digestibility of nutrients and energy. The different techniques mentioned are either expensive, time-consuming or difficult to apply on outdoor sows. Thus, research is needed to estimate both grass intake, and the nutritional contribution of protein and energy from grass-clover in organic pig production.

INCREASED ENERGY REQUIREMENT

The environment of organic sows comprises several variable factors such as temperature, wind speed, radiation, humidity, rainfall, snow, the absence/presence of bedding, shelter, shade, and the opportunity to wallow. All these factors affect the daily energy requirement, and Close and Poornan (1993) conclude, that the total energy requirements of organic sows are some 20% higher than corresponding animals kept indoor. Edwards (2003) suggests, that the total energy requirement of outdoor pigs is increased by approximately 15% under Northern European conditions and Close (1989) estimated the additional energy required to be 17% higher than those for indoor animals.

During pregnancy, maintenance represents 75 to 85% of the total energy requirements, but this is affected greatly by environmental temperature and locomotive activity (Noblet et al., 1990).

Thermoregulation

The biological optimum temperature constitutes an ambient temperature, at which the sow is exposed to minimum thermal stress, so that, on average, the highest overall performance can be expected. As a rule of thumb, the thermoneutral zone of sows is often said to be around 18 °C – 25 °C depending on many factors as wind speed, humidity, etc. (SEGES, 2011).

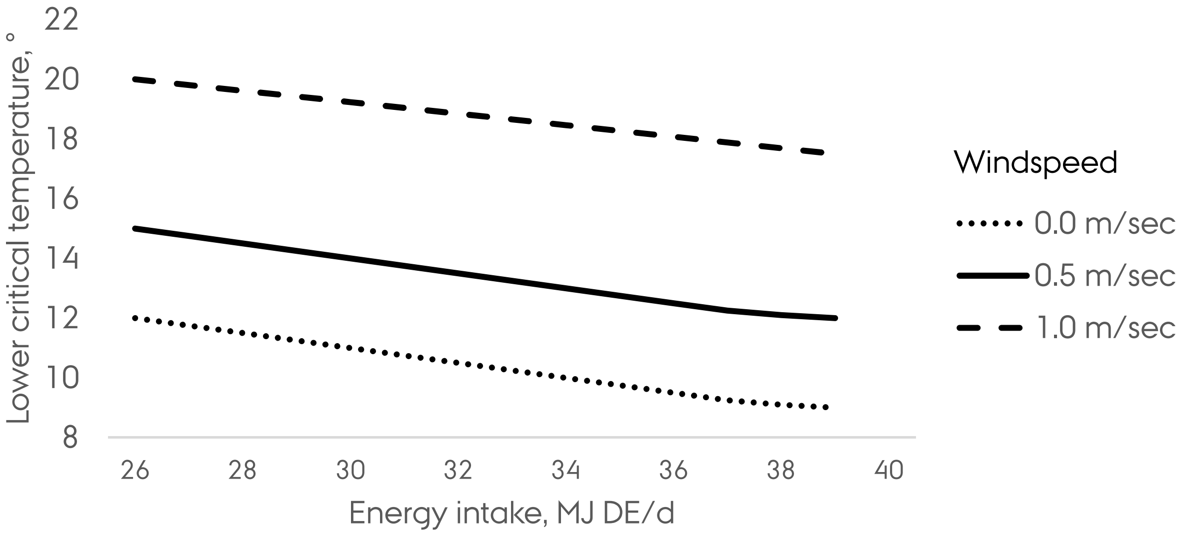
In outdoor pig production, ambient temperature, air movement, and downpour is nearly impossible to control. Under Danish conditions, the average minimum temperature in the coldest month (February) was -1.2 °C and the average maximum temperature in the warmest month (July) was 21.8 °C in the period from 2006-2015 (Meteorological Institute, 2020).

The average normal rectal temperature of pigs is 39.2 °C with a range from 38.7-39.8 °C (Cunningham, 1997). Body temperature in healthy animals is maintained within relatively narrow limits, despite significant variations in ambient conditions. Given the wide range of temperatures and other meteorological variation that outdoor pigs experience throughout the year, there are practical implications for managing the energy requirement in terms of thermoregulation.

Cold conditions can be expected to increase the need for energy in organic pigs (Blair. 2005), Under Danish weather conditions, energy required for thermoregulation is highest during winter, as we have few days with average temperatures above 25 °C, which is the upper limit before sows experience heat stress.

In pregnant and individually housed sows, the lower critical temperature is approximately 20° C, and the daily heat production is increased by approximately 15 kJ/kg BW.75 for each degree below the lower critical temperature .(Noblet et al., 1997). In group-housed sows the lower critical temperature is 14°C (Geuyen et al., 2010). Plastic curtains are placed in the entrance of most organic huts and plenty straw supplied during winter in order to decrease the lower critical temperature. The energy lost for thermoregulation also depends on energy intake and wind speed. As feed intake increases, the critical temperature decreases, whereas in a draughty environment, the lower critical temperature increases, Figure 2 (Close, 1989).

Figure 2. The effect of energy intake and wind speed on the lower critical temperature of a 160 kg group-housed sow in good condition and with straw bedding. {Modified after unpublished data in Close, 1989)



The additional energy requirement for thermoregulation also varies with body weight, so for heavy sows, the critical temperature is lower than for light sows (Verhagen et al., 1986). The additional heat production of a 200 kg sow is approximately 3.8 MJ ME/d or 0.7 MJ ME/d for each 1°C below 15°C to maintain a constant body temperature, Table 1 This represents a substantial amount of feed under Danish outdoor conditions where the average temperature through 30 years is 8.3 °C (Meteorological Institute, 2020).

Table 1. Additional energy required for pregnant sows fed restrictively, assuming 1600 degree days below a base temperature of 15 °C. {Close, 1989)

Bodyweight, kg	Additional energy requirement		
	MJ ME/d/1 °C	MJ ME/year	MJ ME/d
120	0.5	784	2.6
160	0.6	976	3.3
200	0.7	1149	3.8

Locomotive activity

Organic sows are housed outdoor with more space, and therefore, physical activity might have an impact on their energy requirement. There are typically two daily peaks of activity; one in the morning and another in the afternoon or evening with a resting period around midday (Marchant-Forde, 2009).

Buckner et al., (1998) estimated the time spent outside the hut by outdoor sows, Figure 3

Figure 3. Estimated proportion of the day spent outside by outdoor sows. The range is presented in brackets (Bucker et al., 1998)

Season	Stage of reproductive cycle			
	Pregnant	Pre-farrowing	Post-farrowing	Lactating
Autumn	**22.9%** (10.5%-33.8%)	**24.4%** (12.5%-37.1%)	**7.7%** (7.0%-8.4%)	**18.3%** (8.1%-35.3%)
Winter	**15.0%** (10.2%-34.9%)	**14.8%** (9.6%-41.1%)	**4.8%** (3.3%-7.6%)	**13.8%** (6.9%-31.7%)
Spring	**28.4%** (15.3%-38.7%)	**29.2%** (19.4%-36.2%)	**9.3%** (7.1%-19.0%)	**28.8%** (12.5%-45.9%)
Summer	**28.2%** (20.9%-51.0%)	**30.2%** (22.4%-42.4%)	**11.1%** (7.7%-17.7%)	**25.9%** (8.4%-50.9%)

Activity level and traveled distances depend on the distribution of feed in the paddocks and environmental factors as well as the nutritional status and age of the pigs (Edwards 2003). In an observational study on wild boars, the animals spent 42.4% of their daytime "grazing", being more active in the first three hours of daylight, and 45.4% of their time lying down - especially from 11.30 AM to 2.30 PM (Rivero et al., 2013a).

In terms of traveled distances, (Kurz, 1972) used radio telemetry of wild boars and reported mean ranges of 2.5 km/d with 2.9 km/d as the longest. In a two year study of feral hogs, (Barret, 1978) estimated home ranges of 694 boars and 731 sows in Californias Dye Creek to be 50 km^2 and 10 km^2, respectively. Males moved up to 11 airline kilometers in a day or two. Sows with piglets younger than three weeks rarely moved more than 0.5 kilometers from their nest.

Buckner (1996) showed pedometer values indicating a range of 0.1-3.1 km/d for pregnant sows, and (Rodríguez-Estévez et al., 2010) found Iberian pigs walking 3.9 ± 0.18 km within a total of 6.25 hours of activity/d. This was at the cost of 6.3 ± 0.15 MJ ME/d. The energy cost for walking constituted 8.0% and standing 16.4% of the daily energy expenditure of ingested energy.

Close and Poorman (1993) calculated, that the additional expenditure of energy by growing pigs for walking was 7 kJ of ME/kg of body weight for each kilometer. They state, that an outdoor 240 kg sow walking 1 km/day would dissipate an additional 2,1 MJ ME/day for locomotive activity, use 5,2 MJ ME for thermoregulation (15°C) and hence require an additional 7,3 MJ ME/day compared to an indoor sow of the same size.

To summarize, the travel distance of outdoor domesticated pigs seems to be 0.5 to 3.9 km/d depending on age, sex, and reproductive stage. The energy expenditure ranges from 1.6 to 2.1 MJ ME/km.

Prolonged lactation period

Another major difference in the energy requirements in organic and conventional pig production systems is the length of the lactation period. By law, lactation lasts three to four weeks for indoor sows and at least 40 days for organic sows {Regulation, (EC) No

1804/1999), but according to the Danish amendment, organic piglets must have the opportunity to suckle until they are seven weeks. Interestingly, neither milk yield nor milk composition has previously been measured in organic sows.

Milk energy output has been estimated to be comparable in 1st parity sows (43.2 MJ/d) and in multiparous sows (42.2 MJ/d) {Pedersen et al., 2019). In indoor sows, {Krogh et al., 2017) find an average milk yield of 7.7 kg/d on d3 and 15.3 kg/d on d17 of lactation in 2nd and 3rd parity sows. Also in indoor sows, milk yield has been estimated to 8.66 -10.7 kg/d in 1st parity and 8.61-11.4/d in multiparous sows (Pedersen et al., 2019; Strathe et al., 2017), where these values represent the mean yield for a 4-week lactation period. At SID CP levels of 128.5 g/kg and 150 g/kg, {Strathe et al., 2017) find average daily milk yields of 11.3 kg/d and 11.6 kg/d in high producing multiparous indoor sows. Whereas (Hojgaard et al., 2019a) find 12.8 kg/d and 12.9 kg/d at SID crude protein levels of 135 and 149 g/d respectively.

Optimal energy supply during lactation is important because milk production is associated with a massive drainage of nutrients each day (Theil et al., 2004). The ME requirement of indoor sows increases from approximately 36 MJ ME/d in late gestation to 77 MJ ME/d and 112 MJ ME/d in early lactation (Krogh, 2017). In late gestation, the energy requirement for maintenance constitutes 83% of the total daily energy requirement. On d5 and d20 in lactation, these numbers are as low as 34% and 28%, illustrating the rapid increase in energy demand for milk production as lactation progresses (Feyera and Theil, 2017; Figure 4).

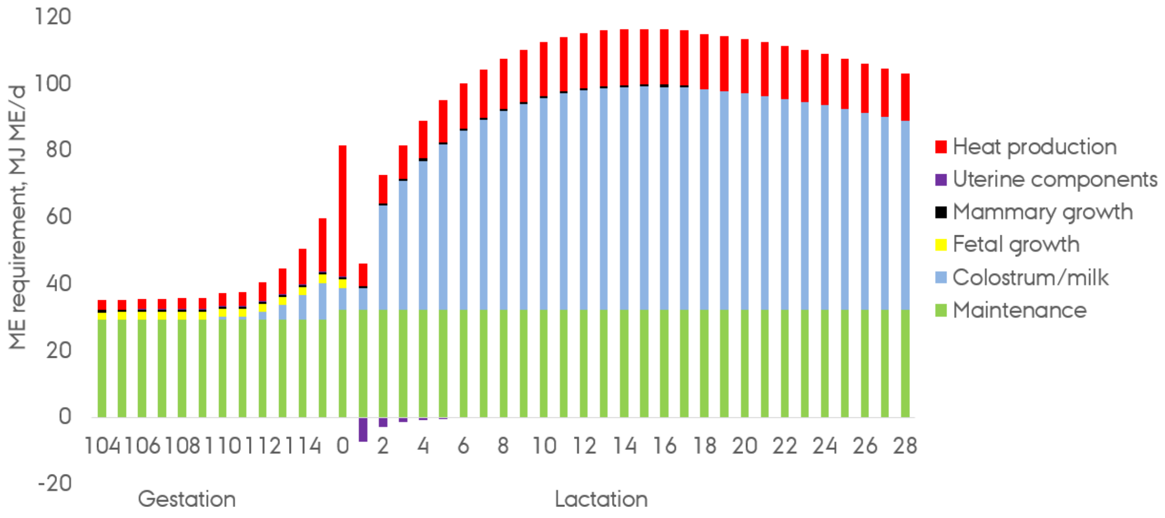


Figure 4. Estimated daily energy requirement of a 250 kg multiparous indoor sow throughout transition and 28 days of lactation. Adapted from Feyera and Theil (2017).

The dietary energy intake is insufficient to cover the rapid increase in energy demand for milk production. Consequently, indoor sows are often mobilising in early and peak lactation. Mobilisation for milk production is energetically unfavorable as the efficiency of utilising body fat and body protein for milk production is 88% (Noblet, 1987), but these body reserves need to be restored in the subsequent pregnancy from gestation compound feed. Restoring body reserves occur at an efficiency of 80% when fat is retained and 60% when protein is retained in gestation (Noblet, 1990). If body protein and body fat are retained in equal amounts, the actual efficiency of using body depots for milk production is therefore closer to 62% (88%* (80%+60%)/2). Instead, if feed intake was sufficient to cover the daily energy demand for milk production, the direct conversion of metabolisable energy from feed to milk occurs at an efficiency of 78% (Theil, 2004).

Therefore, to optimize feed efficiency, it is essential that energy intake as close as possible meet the energy requirement. In the model reported by Feyera and Theil (2017), the energy requirement for milk production at peak lactation was found to be 66.7 MJ ME/d in a 250 kg sow. This is in line with Close and Poornan (1993), who states that a 240 kg outdoor sow with 12 piglets has a calculated energy requirement of 69,6 MJ ME/day only for milk production with a daily piglet weight gain of 200 g.

Protein requirement

During early and at peak lactation, as much as 700–800 g of protein is secreted in milk daily by high yielding sows. Sows are not normally able to consume sufficient dietary protein to account for this protein output, and therefore, they mobilise body protein to support their milk production (Theil and Hurley, 2016).

The nitrogen requirement of lactating sows can be expressed as total N, as digestible N or as digestible amino acids. Lysine has been found often to be the first limiting amino acid, and therefore, the requirement of lactating sows is often expressed in terms of SID lysine (Everts and Dekker, 2010). The supply of SID lysine is required for maintenance, development of uterine tissues, fetal growth, mammary growth, colostrum- and milk production.

In gestation, the SID lysine requirement is equivalent to 35 mg/kg$^{0.75}$ × metabolic live weight, which increases to 46 mg/ kg$^{0.75}$ × metabolic live weight right after parturition (NRC, 2012). Thus, the maintenance requirement for lysine amounts to only 2-3 g SID lysine daily for adult sows. Using a factorial approach on indoor lactating sows Feyera (2017) estimates an overall requirement at peak lactation of 60.9 g SID lysine/d (46.3 g to be secreted in milk, 11.5 g to be lost and 3.1 g for maintenance), Figure 5.

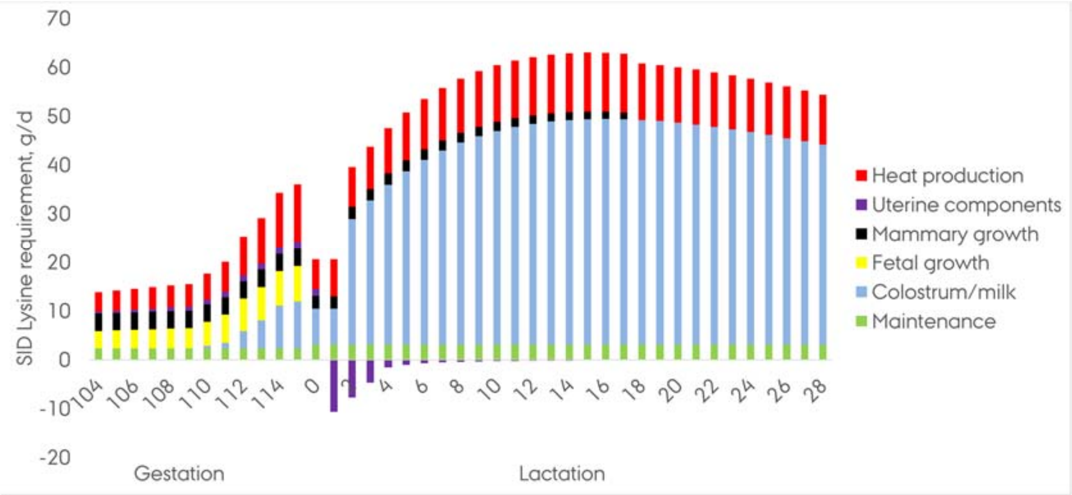


Figure 5. Estimated daily SID lysine requirement of a 250 kg multiparous indoor sow throughout transition and 28 days of lactation. Adapted from Feyera and Theil (2017).

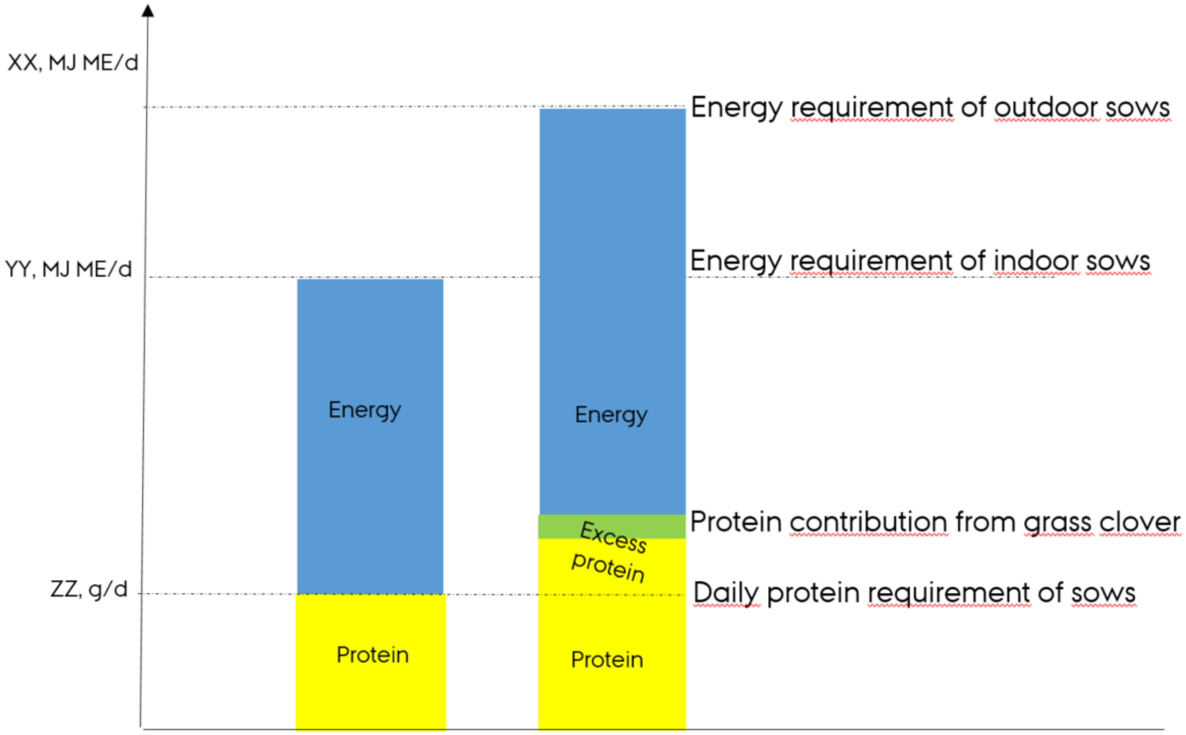
Others find that high yielding indoor sows have a lysine requirement of 68 to 70 g SID lysine/d at peak lactation (Gourley et al., 2017; Hojgaard et al., 2019b).

The total energy and protein requirements of outdoor sows, can be estimated as the sum of the needs of animals kept indoor plus the requirements for locomotive activity and to compensate for the outdoor environment an increased feed wastage and a prolonged lactation period.

Increased thermoregulation, physical activity, and prolonged lactation influence the energy demand, and feed consumption in organic pig production is higher, whereas feed efficiency is considerably lower than in conventional indoor pig production. A comparison in thirteen Danish organic herds (4806 sows in total) showed an average feed consumption of 25 700 MJ ME per sow per year (SEGES Økologi, 2016). In comparison, the national average in conventional pig production (416.481 sows) was 18 900 MJ ME per sow per year. Indeed, organic produced sows ingest about 1/3 more compound feed than indoor sows, even though they also consume grass and roughage.

When the need for energy is higher, while the daily protein requirement most likely is comparable with that of indoor sows, there will be an oversupply of protein if the protein-to-energy ratio in the compound feed is the same as recommended for indoor production. In addition, sows concomitantly ingest (unknown amounts of) protein from grass or silage. If the protein contribution from grass-clover was known, and the protein-to-energy ratio reduced, it should be possible to optimize feed efficiency and reduce the risk of nitrogen leaching from organic sows, Figure 6.

a) With comparable protein to energy ratio in compound feed



b) With reduced protein to energy ratio in compound feed

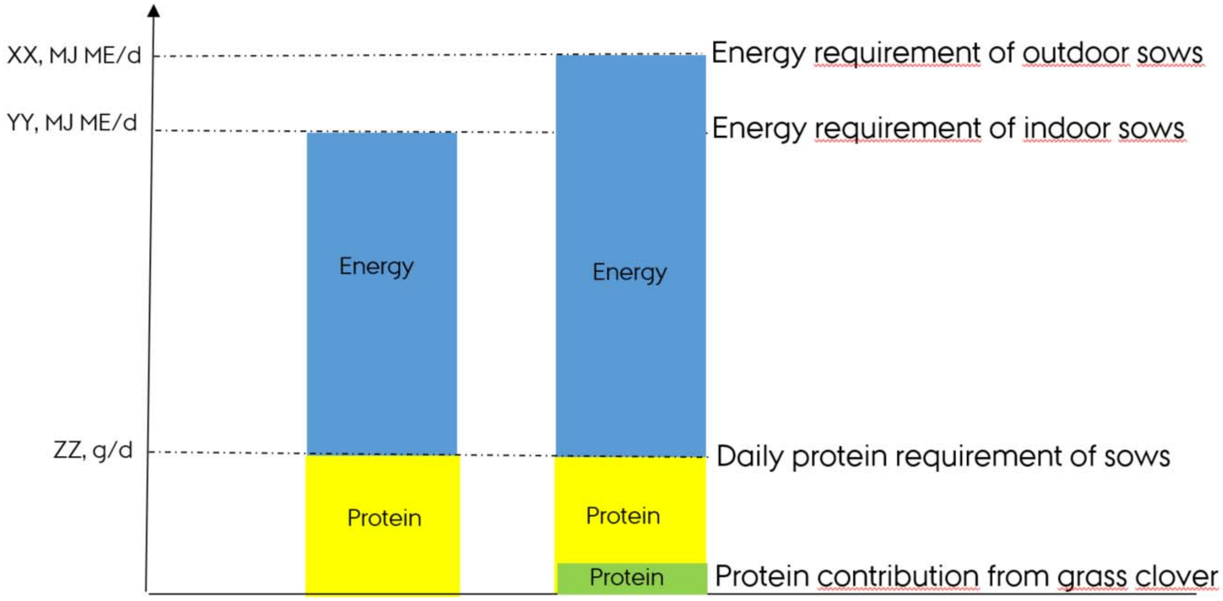


Figure 6. When organic sows are fed the protein-to-energy ratio recommended for indoor sows, there is a risk that sows are fed excessive amounts of protein from the extra kilos of compound feed and from the intake of grass-clover (panel a). An optimized situation, where the protein concentration in compound feed is reduced, and the protein contribution from grass-clover counterbalanced to 100% match the energy and protein requirements and reduce the amount of excess protein (panel b)

AIMS AND HYPOTHESES

Energy requirements of outdoor pigs are generally higher because of increased climatic energy demand, better possibilities for locomotory activity, and prolonged lactation, while daily protein requirements are relatively unaffected (Close & Poornan, 1993; Jakobsen & Danielsen 2006). All crude protein and amino acid recommendations in Denmark are expressed relative to dietary energy concentration (Tybirk, 2017). However, the protein-to-energy ratio formulated for conventional pigs is not optimal for organic production, as organic sows, on average, need more energy per day. It seems that organic sows are able to cover part of their nutritional needs through grazing (summer) and roughage consumption (winter), which exacerbates the challenge in supplying compound feed to match their total energy and protein requirements.

The theoretical energy requirement of organic sows has been calculated in several publications (Close & Poornan, 1993; Buckner, 1996; Jacobsen & Danielsen, 2006; Fernandez et al., 2006, Kongsted et al., 2015), but it has not been empirically quantified. Therefore, it is unknown exactly how physically active organic sows are or how much energy they spend on thermoregulation and the prolonged lactation period in different seasons. Information on grass-clover intake and digestibility is also scarce, and in practice, the nutrient supply of organic sows is probably imbalanced, which may negatively affect feed efficiency and the environmental load of organic pig production.

The overall aim of this Ph.D project was to establish basic knowledge on energy- and protein requirements of organic outdoor sows to increase the resource efficiency of organic pig production in Denmark.

Main research questions:

- Is it possible to develop a method to quantify grass-clover intake in organic sows based on biomarkers in blood or urine?
- What is the digestibility of grass-clover in organic sows?
- What are the energy and protein contributions from grass-clover in summer?
- Compared to indoor sows, what are the additional energy requirements for:
    - Thermoregulation?
    - Physical activity?
    - Prolonged lactation?
- Is it possible to reduce the content of protein in the supplied compound feed without compromising the productivity of outdoor gestating and lactating sows under the influence of season?

These questions were answered by the completion of two individual animal experiments. Apart from myself, another Ph.D student and one Post-Doc was involved within the EFFORT project.

The other Ph.D student was engaged in other work packages of the project to test more optimal dietary strategies under commercial conditions in 2019/2020.

In 2021 the Post-Doc will work on a factorial approach on the sow data and predict milk yield by use of a mathematical model developed to quantify milk yield of conventional sows using a deuterium dilution technique on selected piglets from Experiment 2.

HYPOTHESIS - EXPERIMENT 1

"*It is possible to identify metabolites in blood or urine that are linked to the intake of grass-clover, and use the biomarker(s) to predict the grass-clover intake in organic sows* kept on pasture.*"*

Experiment 1 also aimed to determine the digestibility of grass-clover in sows. The biomarker should be selected and used to predict total intake of energy and dietary protein from grass-clover in pregnant and lactating sows in Experiment 2.

HYPOTHESIS - EXPERIMENT 2

"*It is possible to reduce the protein concentration in organic compound feed for sows by counterbalancing energy and protein intake from grazing in summer without compromising the nutritional requirements or impair sow productivity"*

Experiment 2 also aimed to quantify the following parameters in pregnant and lactating sows in winter and summer:

- Fresh grass-clover intake of sows fed two different protein levels. Based on the biomarker selected in Experiment 1
- Energy used for thermoregulation in winter and summer
- Energy used for physical activity in winter and summer
- Energy and protein requirement for milk production
- Chemical composition of milk
- Total sow heat production
- Retention/mobilisation of body fat and body protein
- Overall energy balance of organic sows with access to pasture in summer and winter

BRIEF PRESENTATION AND JUSTIFICATION

Before addressing the overall objective and test the hypotheses, this chapter gives an introduction to the two experiments and a short description of central methods used for the determination of biomarkers, locomotive activity, body composition, milk production, milk composition, heat production, and selected physiological measures of sow metabolism.

Experiment 1 was carried out in the indoor experimental herd at AU Foulum. The sixteen sows came from different herds, were of different parities, and were not housed under organic conditions, as it would be impossible to conduct total urine and feces collection outdoor.

In an organic context, Experiment 2 was a large scale experiment. It was initially thought to be conducted in a commercial organic herd. Still, due to the invasive character of the samplings with frequent collection of blood, it was decided to buy in a relatively large herd of twenty-two conventional gilts and rear them under organic conditions at Aarhus University, Foulum. By chance, another organic behavioral study with twenty-five sows was carried out at the Organic Platform the same year. As the two studies did not interfere, it was decided to combine them, which doubled the number of animals in both studies – in total, forty-seven sows were studied, and eighty-eight reproductive cycles were monitored.

The compound feed used in Experiment 1 was a commercial organic gestation concentrate, also used for pregnant sows in Experiment 2. The compound feeds were of 100% organic origin and based on the four common Danish cereals wheat, barley, rye, and oats and without any soybean meal. The protein-restricted mixture that was used for “protein dilution” in Experiment 2 contained barley and oats primarily. The fresh grass-clover used in Experiment 1 was cut on the gestation fields used in Experiment 2.

The data collection took place in two seasons; winter (November 2016-March 2017) and summer (April-September 2017) to vary clover grass intake and the need for thermoregulation.

The fact, that data collection was run in an experimental environment, provided the opportunity to plan working days to be very efficient as it became possible to conduct the sampling without interfering with the day-to-day operations in a commercial herd.

The experimental work was based on live weight measurements, back fat scannings, feces-, blood-, urine and milk samples, GPS data, and data from a local weather station.

A variation in ± 1 day was accepted, except for weaning, which was performed on d47 ± 3d.

Materials and methods used in the experiments are presented in the included manuscripts (I-III). Manuscript I includes data from Experiment 1. Manuscript II and Manuscript III include data from Experiment 2.

Manuscript I: Experiment 1

Manuscript II: Experiment 2 - Winter, 1st parity

Manuscript III: Experiment 2 - Summer, 2nd parity

EXPERIMENT I

Sixteen sows housed in an experimental environment were divided into four dietary groups and supplied increasing levels of freshly cut grass-clover; from 0 to 6 kg per sow per day. Besides the grass-clover, they were offered a commercial organic gestation compound feed, and the daily energy supply was aimed to be 25.7 MJ ME/d in all four dietary groups, Figure 7.

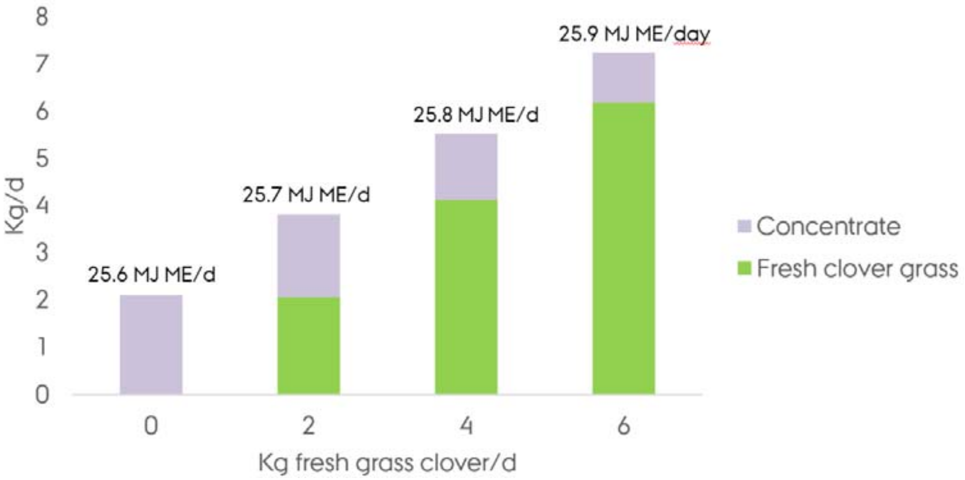


Figure 7. Grass-clover and compound feed supply in the four dietary groups. Sows were fed twice a day. Diets were supposed to be iso-energetic. Still, the total energy supply per day differed a little, because the grass contained slightly more energy than expected.

Sows were placed in metabolic cages with total urine and feces collection for two times five days, with a five-day break in between for the sake of animal welfare, Figure 8.

Figure 8. Multiparous sows in metabolic cages fed increasing amounts of freshly cut grass-clover.

Blood sampling was performed on the last day of each collection period, Table 2.

Table 2. Experimental setup of Experiment 1 with six-teen sows in metabolic cages for a total of ten collection days.

	D1	**D2**	**D3**	**D 4**	**D5**	**Break**	**D1**	**D2**	**D3**	**D4**	**D5**
Bodyweight	x				X		X				x
Total urine collection	x	x	x	x	X		X	x	x	x	X
Blood sample					X						X
Fresh urine sample					X						x
Total feces collection	x	x	x	x	X		X	x	x	x	X
Compound feed- and grass intake	x	x	x	x	X		X	x	x	x	x

Blood and urine samples were analyzed by LCMS to identify and quantify metabolites, that relate to the grass-clover intake and apparent total tract digestibility of fresh grass-clover in sows was quantified.

Urine was analysed for urea and creatinine content. Plasma was analysed for the content of glucose, NEFA, triglycerides, lactate, and creatinine.

EXPERIMENT 2

Forty-seven gilts were bought at twenty-two weeks of age in august 2016. Twenty-two were Danavl crossbred LY-gilts from the Danish SPF herd "Kristiansminde" and the remaining twenty-five were Topigs Norsvin L x Large White (TN70) from Norway.

All gilts were placed in quarantine for six weeks, and subsequently SPF blood sampled and tested for MRSA. During quarantine, the animals were inseminated twice in their second estrus with semen from three known Danbred Duroc boars. The gilts were randomly assigned into two dietary treatments - either a standard (**Control**) or minus 12% (**Low protein**) protein strategy, Table 3.

Table 3. Distribution of animals on dietary treatments and breeds in winter and summer

Season	No of sows	Parity	Control	Low protein	DanBred	Topigs norsvin	Farrowing
Winter	47	1	24	23	22	25	Nov. 2016 - Jan. 2017
Summer	41	2	23	18	20	21	May 2017- Aug. 2017

The low protein gestation compound feed contained minus 11%, and the low protein lactation compound feed contained minus 12% protein compared to the control group, Table 4.

Table 4. Energy and protein content in the control and the low protein dietary strategy. The gestation control diet was the same as used in Experiment 1.

	Gestation		Lactation	
	Control	Low protein	Control	Low protein
Energy, MJ ME/kg	12.1	12.3	12.2	12.2
FU_{sow}/kg	1.00	1.01	1.00	0.99
Crude protein, g/FU_{sow}	128	113	148	131
SID Crude protein, g/FU_{sow}	98	80	118	103
Lysine, g/FU_{sow}	6.3	4.6	6.8	6.1
SID Lysine, g/FU_{sow}	4.9	3.5	5.6	4.7
Recommendation indoor sows , SEGES				
SID Crude Protein, g/FU_{sow}	90		118	
SID Lysine, g/MJ ME	4.0		7.7	

The supplied compound feeds were iso-energetic in the two dietary strategies. To meet the extra demand for thermoregulation and locomotory activity, both treatment groups were supplied with 15% more ME than recommended in the feeding curves for indoor sows from the Danish Pig Research Centre in winter and 10% more ME during summer.

Throughout the two parities, recordings and samples were collected for sows and piglets as outlined in Table 5.

Table 5. Overview of the protocol of Experiment 2

	Gestation		**Lactation**			
88 reproductive cycles	**Day 60**	**Day 100**	**Day 5**	**Day 20**	**Day 40**	**Weaning**
Sows						
Weight	X	X	X	X	X	X
Backfat scan	X	X	X	X	X	X
Urine collection	X	X	X	X	X	
Deuterium	X	X	X	X	X	
Blood sampling	X	X	X	X	X	
Activity tracker	X	X	X	X	X	
Milk sampling			X	X	X	
Functional tits			X	X	X	X
Individual feed intake	Registered daily		Registered once a week			
Piglets						
Individual weight		Birth X	X	X	X	X
Blood samples for milk intake			3/litter	3/litter	3/litter	

Pregnant sows were group housed and fed in individual feeding stalls to determine individual feed intake, Figure 9a and 9b. Lactating sows were individually housed and fed individually in troughs with a lid to prevent feed waste, Figure 10a and 10b.

Figure 9a. Gestation huts with maximum twelve pregnant sows per hut

Figure 9b Individual feeding stalls for pregnant sows on pasture

Figure 10a Communal hut with room for four individually housed lactating sows

Figure 10b “So-stub” feeding trough for individually fed lactating sows

METHODOLOGY

METHOD USED FOR IDENTIFICATION OF BIOMARKER FOR GRASS-CLOVER INTAKE

A biomarker is a measurable indicator of a biological state or condition. Biomarkers are used in many scientific fields, where they are identified based on insight from primarily animal physiology and biochemistry. In animal science, biomarkers are often used to examine normal or pathogenic biological processes in the body. An example of a biomarker could be the discovery of creatinine as a marker of kidney function.

There are many new molecular biologic techniques that enable rapid identification of relevant biomarkers; genomics, glycomics, proteomics, secretomics, and lipidomics are some technologies used in this context. Metabolomics is one of the most recent "omics" and is the science of studying a considerable number of metabolites in a biological sample – "the metabolome," in one run. The idea originates from the concept, that minor modifications in fluxes through pathways may induce significant changes in the concentration of metabolites in the body due to up-concentration in e.g., plasma or urine (Soumeh, 2015). Hence, metabolomics makes it possible to discover all changes in the metabolome caused by relatively small changes in feed, health status or other environmental or management factors. In Experiment 1, metabolomics was used to identify a possible biomarker for grass-clover intake in either plasma or urine from dry sows receiving increasing amounts of grass-clover. The aim was to discover a metabolite that increases linearly with increasing grass-clover intake, Figure 11.

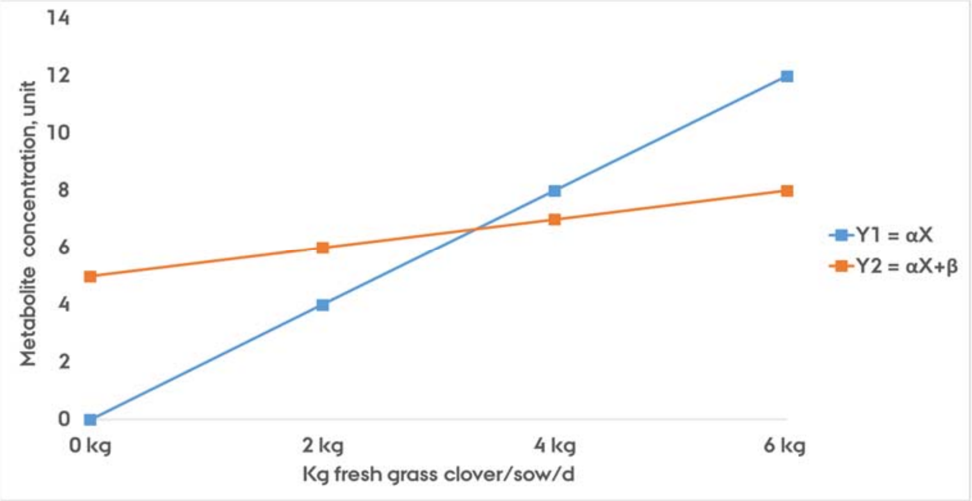


Figure 11: Schematic illustration of the concentration of two potential biomarkers for predicting the daily grass-clover intake in sows.

Of the two illustrated prediction equations, the Y1 equation is preferable, as there is no intercept and therefore no contribution of the molecule, if sows do not consume grass-clover. It is also desirable to have the molecule in certain amounts in the plasma/urine and that it is possible to detect the identified molecule directly in the original grass-clover distributed to the animals.

The practical performance of the method is described in details in Manuscript I, but shortly the principle is as follows :

Preparation; Sample of grass-clover, plasma and urine has been stored at –80°C and are defrosted and deproteinized

Separation; Analytes are separated by ultra-high pressure liquid chromatography

Ionization; The eluent is imparted with a charge and transferred to gas by electrospray ionization and analyzed in positive and negative ionization modes

Detection; Mass spectra are calibrated to identify metabolites. Loading plots are used to identify metabolites discriminating sows eating 0 kg grass-clover from sows eating 6 kg grass-clover. Plasma pipecolic acid and plasma bisnorbiotin were identified using accurate masses and fragmentation patterns based on searches in online databases

Validation; The identification of plasma pipecolic acid was confirmed with a standard. However, bisnorbiotin is apparently a costly compound (that is > 30.000 EUR for 20 mg) due to the complex and lengthy process of manufacturing the product. This was, unfortunately not compatible with the allocated resources within the project. Hence, only plasma pipecolic acid was validated as a biomarker for grass-clover intake.

Prediction equations were studied with and without intercepts (depending on whether this was significantly different from zero) to express the linear correlation between consumption of grass-clover and the level of pipecolic acid in plasma.

In Experiment 2, plasma samples was analysed using the metabolomics approach and plasma pipecolic acid concentration was used to estimate the daily intake of fresh grass-clover during summer based on the prediction equation for plasma pipecolic acid found in Experiment 1.

METHOD USED FOR ESTIMATING LOCOMOTIVE ACTIVITY

For indoor sows, total energy requirements are generally considered to be the sum of requirements for maintenance, retention, growth of conceptus, and milk production. In an outdoor system, the sows are able to walk around more freely, and some estimate has to be made of the energy expended due to locomotive activity under these circumstances. In the literature, estimates on locomotive activity of pigs range from one to ten kilometers per day, and has been done with radio telemetry and pedometers. Nowadays, many different positioning systems for animals are available on the market, some with RFID tags for 'line of sight' for satellites and for this project, pet GPS trackers have also been considered.

After some research on battery time, waterproofness and weather-resistance characteristics, the Polar Team Pro GPS-based athlete tracking system was purchased. The equipment is developed for human football players and combines high precision GPS-derived movement data, inertial sensor metrics, and integrated heart rate monitoring into a mobile tracking system. The system provides heart rate, heart rate zones in percent of max heart rate, the traveled distance and travel speed with one-second data recording but has not previously been used in pigs. The practical performance of obtaining locomotive activity data is described below:

- Sensors were fully charged and prepared with sow no (player no) keyed in on beforehand. Tape was wrapped around the sensor and the belt to prevent mud from floating in and other pigs from eating the device.
- Belt was wrapped around the belly of the sow. A belt for an XL man fits most sows
- Gel for gestation scan was placed underneath the two measuring points to ensure the electrical recording of the heart rate through skin contact
- All data was either broadcasted live via Bluetooth LE technology to an iPad with a 200-meter range or stored on the sensor with a battery lifetime of up to 12 hours, Figure 12.
- After twelve hours, the belt was removed, and data were downloaded from the individual sensors to an IPad, from where data could be directly transferred to Excel/SAS

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3	28 %	01:50:55	3	6 %	0.01
2	48 %	03:14:51	2	4 %	0.01
1	1 %	00:03:45	1	1 %	0.00

Figure 12. Illustration of method for estimation of sow locomotory activity with the Polar Team Pro equipment. The activity gauges were wrapped around the sows belly, and gel for ultrasound gestation scan was used to create permanent contact with the device. The system is initially developed for football players and provides heart rate, an activity map, and covered distance in different predefined velocity zones.

The data reveal a noticeable difference in heart rate, when sows are physical active and when they are resting, Figure 13

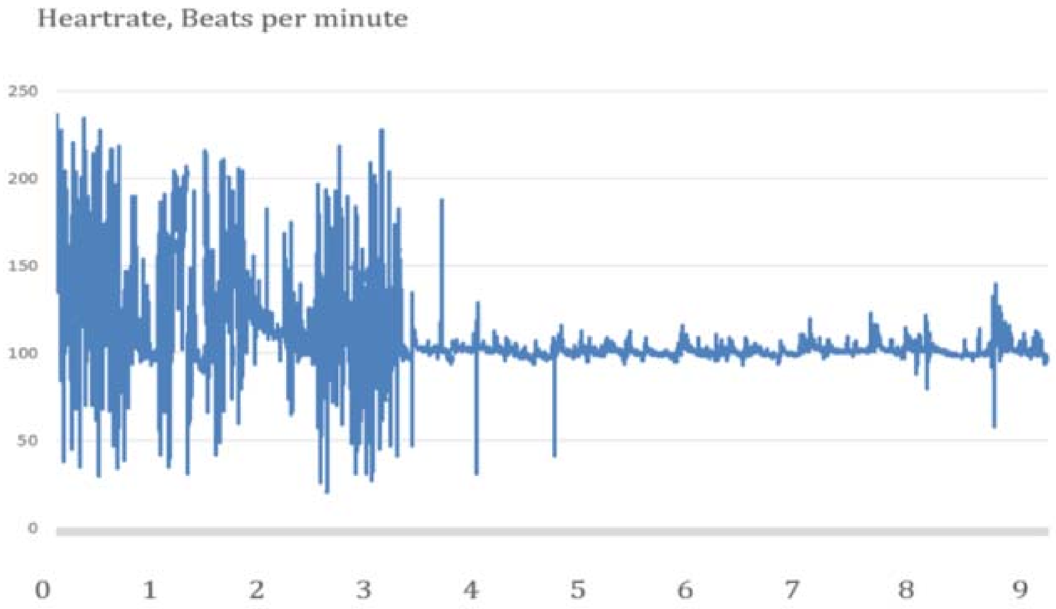
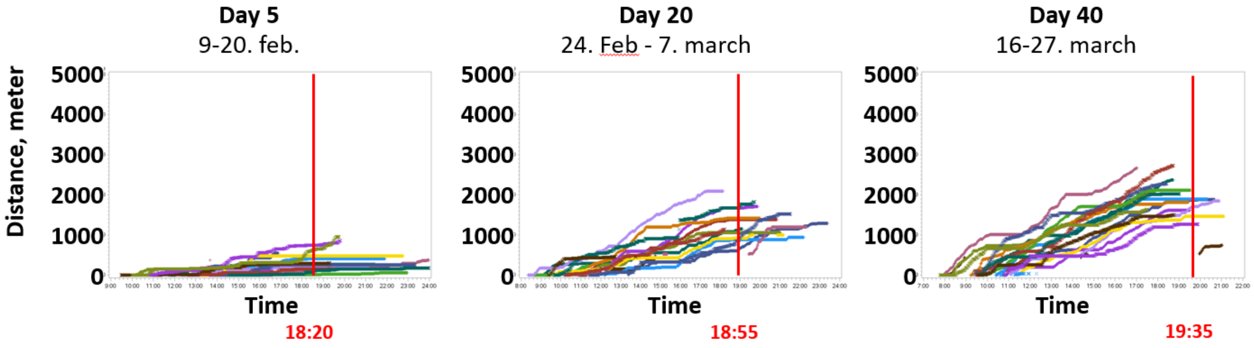


Figure 13. Heart rate of a random sow measured with the Polar Team Pro equipment

The system provides large amounts of data, that can be used to investigate not only energy expenditure but also circadian rhythm and other behavioral patterns of sows on pasture, Figure 14.

During Experiment 2, there was a loss of two sensors.

Figure 14. Traveled distance and heart rate of twelve random lactating sows in winter. Every line represents one sow.



	8 Hours	8 Hours	8 Hours
Distance, m:	**280**	**1151**	**1683**
Pulse, Beats/min.:	**96**	**122**	**118**

METHOD USED FOR DETERMINATION OF MOBILISATION/RETENTION

The obvious method for determining retention/mobilisation are recordings of changes in live weight and back fat depth over time. If the energy intake is lower than the energy expenditure, mobilisation of mainly body fat but also body protein will occur (Noblet and Etienne, 1987b) – typically in lactation, where the daily ME requirements are particularly high and generally are not being met by voluntary feed intake (Noblet et al.,1990).

In practice, mobilisation/retention is often estimated via live weight or back fat scanning with an ultrasound- or doppler scanner. Also, alterations in body composition is a common method to determine mobilisation/retention. Under more experimental conditions, sow body composition may be determined directly by dissection or by use of a body composition monitor (bio-electrical impedance, dual-energy X-ray, dexter scanners etc.). Another option is to make use of the deuterium dilution technique, which was done in Experiment 2 to investigate mobilisation of fat and protein to evaluate the impact of decreased dietary protein intake. The deuterium dilution technique estimates the deuterium dilution space, and based on this, live weight and back fat it is possible to estimate the body pools of water, fat, protein, and ash in individual sows. With the deuterium dilution method, it is possible to obtain repeated measurements of the same animal, and it is not necessary to euthanize the sows as in the comparative slaughter technique.

The sows are enriched with a known dose and concentration of D_2O after collection of a pre-dose sample that is used to correct for the natural abundance of ^2H in body fluids. When equilibrium of deuterium within the body water pool is achieved, the extent of the dilution can be determined with a post-dose sample of body water (urine, plasma, saliva). The amount of ^2H above the pre-dose level is the excess enrichment of ^2H in body water.

In Experiment 2, the practical performance of the method is described below:

- A 10% solution of D_20 was prepared for every sow based on individual live weight at the previous weighing (15-30d prior to the sampling day). The individual syringe was weighed. The enrichment was 0.2 mL of the infusate per kg bodyweight. A sample of the infusate was stored to determine the exact concentration of infused D_2O
- A pre-dose blood sample was drawn from the jugular vein to determine the background level of D_2O in the blood
- The infusate was injected intramuscularly in the neck of the sow.
- The syringe was weighed (on the same scale) after the injection to determine the exact weight of the injected infusate
- A spontaneous post-dose urine sample was collected the next morning at sunrise
- Infusate, plasma, and urine were analyzed for the fraction of hydrogen found as D_2O. The results are given as atomic fractions (AF)

From the atomic fractions of D_2O in infusate, plasma and urine, the D_2O space of the sows were calculated as in Theil et al., 2002:

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

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

The body pools of water, fat, ash, and protein were estimated using prediction equations from Rozeboom et al. (1994a), Table 6. The equations were developed for Yorkshire × Landrace gilts using live weight, back fat thickness, and D_2O space. The pools represent empty body pools including all organs, which generally accounts for 90-95% of the body weight, because the empty body weight does not include gut and bladder fill as the live body weight does.

Table 6. Prediction equations for sow body composition in Yorkshire x Landrace gilts (Rozeboom et al., 1994).

Body protein, kg	=	$1.3 + 0.103 \times \text{BW (kg)} + 0.092 \times D_2O \text{ space (kg)} - 0.108 \times \text{BF (mm)}$
Body fat, kg	=	$-7.7 + 0.649 \times \text{BW (kg)} - 0.610 \times D_2O \text{ space (kg)} + 0.299 \times \text{BF (mm)}$
Body water, kg	=	$5.4 + 0.08 \times \text{BW (kg)} + 0.613 \times D_2O \text{ space (kg)} - 0.11 \times \text{BF (mm)}$
Body ash, kg	=	$0.04 - 0.001 \times \text{BW (kg)} + 0.054 \times D_2O \text{ space (kg)} - 0.034 \times \text{BF (mm)}$

Retention or mobilisation of fat and protein may then be calculated as;

$$\frac{\text{Final pool} - \text{initial pool}}{\text{Number of days}}$$

METHOD USED TO ESTIMATE MILK YIELD

Apart from the nutritional state of the sow, milk yield is linearly related to litter size (Auldist et al., 1998). Litter size and litter gain are usually positively related (Hansen et al., 2012), but increased litter size may reduce the average growth rate of individual piglets. Environmental factors such as ambient temperature and photoperiod may also affect milk yield in well-fed sows. However, the influence from the physical environment on milk yield will often be through interactions with the suckling behavior and the ability of the piglets to extract more milk from the mammary gland (King, 2000).

The mammary gland of sows does not contain cisterns for milk storage (Cunningham, 1997). Hence, milk yield of sows is difficult to measure, as it is not possible to regularly milk the sow in the same way that can be done with goats or dairy cows. However, there are several methods used to estimate milk yield in sows. This paragraph describes methods used to estimate milk yield and measure milk composition.

The first method is called "Weigh – suckle – weigh". This method is performed by weighing the piglets before and after suckling bouts. The weight difference should then correspond to the milk intake of the litter. This method is known to underestimate the milk yield by approximately 13% (Theil et al., 2002) due to substantial disturbance of the sow – piglet interactions.

Another method is the deuterium dilution technique. This method aims to measure the rate of total water turnover in the piglets. It must be ensured that the piglets do not eat or drink any water apart from that originating from sow milk, which is very difficult under organic conditions as piglets exploit the free-range conditions from about day fourteen, where they start to eat sows compound feed, grass-clover, silage, etc. Another aspect is that metabolically produced water must be considered, as this also leads to dilution of the deuterium and thereby an incorrect increased milk uptake.

A third method is based on the weight gain of piglets. The piglets are weighed at birth and again at another age, and a feed conversion ratio is then assumed and used to convert the weight gain into milk intake, and intake of all littermates can be summed to estimate the sow milk yield.

The piglets in Experiment 2 were weighed at birth, d5, d20, d40, and at weaning on d49. The milk yield estimated by the method of Theil et al. (2002) surely would have been more accurate, with many more weighing of all individual piglets. The "Weigh-suckle-weigh" method is suitable to estimate milk yield, because the sow nurse the litter in regular intervals – at least in the period from d5-d20. Still, under outdoor conditions, this method would cause severe stress in both animals and humans, as all piglets must be separated from the sow twice within a short time period several times daily.

To get a more accurate quantification of the milk intake of selected piglets and derive a milk conversion factor for outdoor piglets, the deuterium dilution technique was used on fourty-eight individual piglets (twenty-four winter and twenty-four summer) from sixteen different litters in Experiment 2. These data will be analyzed in 2021.

Different equations have been developed to predict milk yield – either from piglet gain or litter gain and litter size (Noblet and Etienne, 1989; Theil et al., 2002; Hansen et al., 2012). A fourth method is also based on piglet weight gain. Hansen et al. (2012) developed a lactation curve prediction model based on results from eighteen scientific studies published after 1980, where piglets were not supplied with additional nutrients from feed or milk replacer.

The model estimates milk yield at different stages in lactation from d3-d30 based on litter gain and litter size inputs. In manuscript II and III, this model was extrapolated to include prediction of milk yield until d40 of lactation. The estimates of the natural logarithm to the daily milk yield was calculated as:

$$\ln(\text{Milk yield d5}) = 1.93 + 0.07 \times (\text{litter size d5} - 9.5) + 0.04 \times (\text{litter gain d5, kg/d} - 2.05)$$

$$\ln(\text{Milk yield d20}) = 2.23 + 0.05 \times (\text{litter size 20} - 9.5) + 0.23 \times (\text{litter gain d20, kg/d} - 2.05)$$

$$\ln(\text{Milk yield d40}) = 2.15 + 0.02 \times (\text{litter size d40} - 9.5) + 0.31 \times (\text{litter gain d40, kg/d} - 2.05)$$

The lactation curves illustrate a rapidly increasing milk yield the first week of lactation, with a peak around d20, Figure 15.

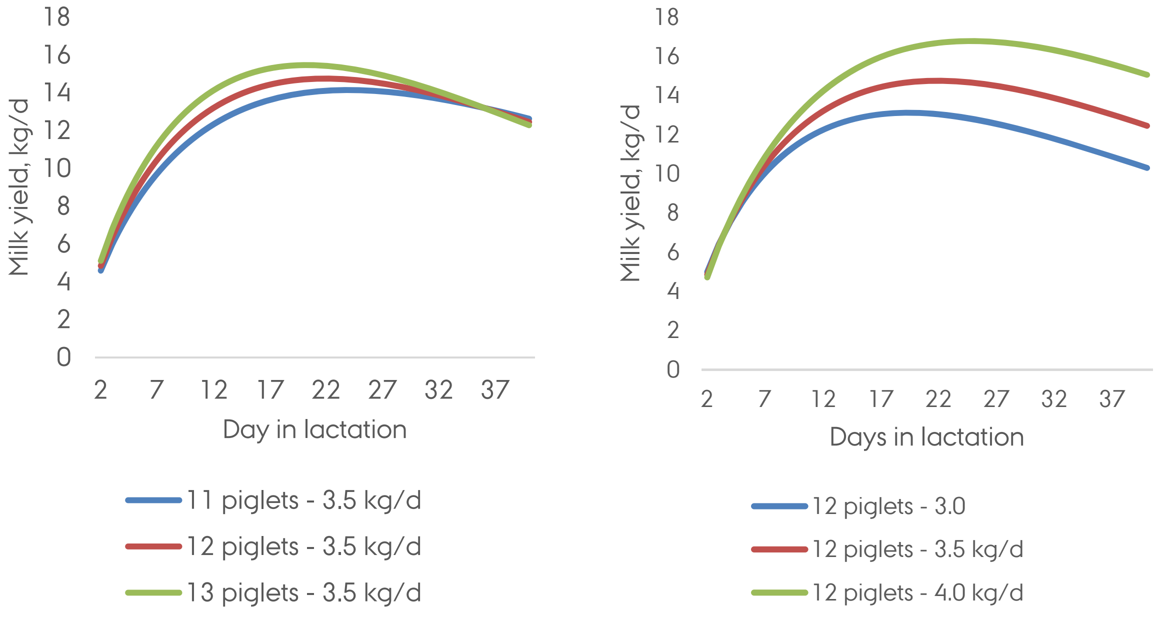


Figure 15. Prediction of organic sow lactation curves illustrating the effects of a) litter size and b) litter gain. Based on the model developed by Hansen et al., (2012)

Milk samples from Experiment 2 were obtained from the sows and analyzed for nutrient composition, to determine the effect of reduced protein on both quantitative and qualitative aspects of lactation. The practical performance of obtaining milk samples is described below:

- Sows were caught before sunrise in the huts
- Piglets were removed from the sow for approximately thirty minutes and individually weighed
- The sow was restrained with a wire snare
- The sow was given a 0.3 ml injection of oxytocin intravenously in the ear to induce milk letdown
- Milk was collected from a standing position from six to eight random teats to get at least 50 ml., Figure 16.
- Milk yield was estimated by the prediction model reported by Hansen et al. (2012)

Figure 16. Milk sampling of outdoor sows. The chemical composition of milk for DM content, protein, casein, lactose, and fat was analyzed in triplicate through infrared spectroscopy using a Milkoscan 4000 instrument.

METHOD USED TO ESTIMATE HEAT PRODUCTION

In lactating sows, heat production has been found to account for 43-48% of total gross energy intake (Theil et al., 2004). Heat production is often estimated as loss of heat by direct- or indirect calorimetry. While direct calorimetry is achieved through direct measurement of total body heat produced, via a thermally sealed chamber, indirect calorimetry measures inspired and expired gas flows, i.e., volumes and concentrations of O_2 and CO_2. Both methods are non-invasive and accurate and used to determine energy requirements and response to nutrition over time. It would have been interesting to have had the dry sows from Experiment 1 in a calorimeter to study the effect of increasing grass-clover intake on heat production. However, these techniques are laborious and quite costly, and therefore, unfortunately, it was not possible within the budget of this project.

Indirect calorimetry is not an applicable approach to estimate heat production in free-ranging sows. However, oxygen consumption is a main determinant of heat production and linearly related to heart rate, unless animals perform maximal work. Krogh et al., (2018) recorded heart rate and heat production in eight second parity indoor sows during gestation (day 30, 60, 80, and 104) and during lactation (day 10, 17 and 24) using indirect calorimetry. These data were used to investigate the relationship between heart rate and heat production as a method to estimate the heat production of outdoor sows using heart rate monitors. Heart rate during inactivity was obtained as an average of heart rate when distance = 0 meters/minute.

The 24-hour heart rate was then estimated as a weighted average of the daily heart rate from sunrise to sunset and average heart rate during the night (sunset to sunrise represented by heart rate during inactivity).

In gestation, total heat production (MJ/d) could be predicted as

$$HP_{Gestation}\text{, MJ/d } (R^2=0.62) = 0.323\ (\pm 0.025;\ P<0.001) \times \text{heartrate, bpm} - 2.4\ (\pm 2.3;\ P=0.33)$$

In lactation, total heat production could be predicted as

$HP_{Lactation}$, MJ/d ($R^2=0.25$) = 0.118 (± 0.034; $P=0.003$) × heartrate, bpm + 26.7 (± 3.4; $P<0.001$)

More variation in heat production was explained by the heart rate in gestation than in lactation (greater slope, higher R^2), and also that a large intercept (which explains no variation) was fitted in the model for lactating sows. This shows that most of the variation in sow heat production cannot be reliably predicted from heart rate for lactating sows.

Heat production can, however, also be estimated otherwise. The total heat production in manuscript III was calculated using a factorial approach as follows:

$HE_{factorial}$, MJ/d = HE for maintenance + HE associated with milk production +HE for locomotory activity + HE for thermoregulation + HE for protein retention + HE for fat retention.

Contributions from mobilisation and milk production in gestation is zero, while in lactation, thermoregulation, physical activity, and fat and protein retention were considered included in the maintenance requirement in summer (Manuscript III). Still, HE for locomotive activity, thermoregulation and retention in summer is calculated and shown in the overall results section.

HE for maintenance was estimated as 0.459 $MJ/kg^{0.75}$ × metabolic live weight for pregnant sows (Theil et al., 2004) and 0.482 $MJ/kg^{0.75}$× metabolic live weight for lactating sows (Theil et al., 2002). Heat produced due to protein and fat retention was calculated by assuming that they occur with efficiencies of 60% and 80%, respectively, mobilisation occurs with 88% efficiency, and milk production occurs with 78% efficiency. The HE calculated due to efficiencies below 100% was done with the following general equation using milk production as an example:

HE milk, MJ/d = Milk energy output MJ/d /0.78 – Milk energy output, MJ/d.

The HE associated with milk production was estimated from the estimated energy output in MJ/d. Milk energy concentration can be analysed in wet or freeze-dried milk by combustion in a bomb calorimeter (Noblet and Etienne, 1986; Dourmad et al., 1998; Theil et al., 2004). In Experiment 2, the energy concentration was calculated from the analysed concentrations of fat, protein, and lactose. The energy values were obtained from (Weast, 1984), where the values were 23.86, 39.76, and 16.51 kJ/g for protein, fat and lactose, respectively, and the milk energy output was calculated as milk yield x milk energy concentration. The comparison of analysing milk gross energy in a calorimeter versus calculating it from nutrient energy values is reported by Pedersen (2019) on the basis of six studies. These studies reveal differences of -4% to +7% when comparing measured with calculated energy.

METHOD USED TO EVALUATE PHYSIOLOGICAL MEASURES OF SOW METABOLISM

Apart from the calculated energy balance and changes in live weight, back fat and body pools, changes in different urine, milk, and plasma metabolites can be used as indicators of the sows physiological status. This section will describe selected urine, plasma and milk metabolites and their potential as physiological biomarkers.

URINE

Total urine collection can be done in metabolism cages, respiration chambers, or in calorimeters, where separation of urine and feces is possible. Collection of urine without catheter will be biased due to different amount of urine in the bladder at the beginning and at the end of the experiment. Thus, prolonging the collection period improves precision and accuracy. Still, the period in which the animals may be housed under these conditions is restricted by Danish law to five consecutive days due to ethical concerns.

Urine production can also be measured by infusing an external flow marker as para-amino hippuric acid (Krogh et al., 2016; Feyera et al., 2018). After intravenous infusion, the kidneys will clear the marker from the blood and excrete it into the urine at a given rate. This rate provides a reasonably precise measure of urine production. The urine production of sows measured with total collection, catheter or by infusion of a marker is 6.24 -9.58 kg/d, or 260-399 g/hour (Pedersen, 2019).

In experiment 1, total urine was collected for two times five days. Inserting urine catheters has the advantage that feces and urine can be kept 100% separate. A significant disadvantage is the risk of infection, when the catheter slides in and out, when the sow changes posture. Of the sixteen sows in Experiment 1, two sows got severe pelvic inflammatory disease and had to be excluded from the experiment due to fever and massive feed refusals.

In Experiment 2, it was not possible to insert catheters in the bladder of outdoor sows, and urine production could therefore not be quantified. Instead, a 100 ml urine sample was collected from the first spontaneous urination at sunset on d60 and d100 in gestation and d5, d20, and d40 of lactation. Hence, it was only possible to obtain an analysis of the concentration of urinary metabolites at a given time – a concentration that was highly dependent on an unknown water intake, i.e., the hydration status of the sow.

The metabolism of protein and amino acids results in the production of nitrogenous end products, and the primary nitrogenous constituent of porcine amino acid degradation is urea {Reece, 1997). The synthesis of urea depends of the daily protein intake and the protein metabolism (MacDonald, 2002; Verlander, 2007), and in a well-balanced diet (i.e., using crystalline amino acids and high level of knowledge on the animal amino acid requirement) the urea synthesis is expected to be lower than in un-balanced diets.

UREA IN URINE

Urea is formed by the liver from ammonia produced during amino acid metabolism, and the body expends considerable energy in producing urea, so that the toxicity of ammonia can be avoided (Reece, 1997). Hence, the energy loss in urine and probably also as heat, increase if the protein-to-energy ratio in sow compound feed is unbalanced (Pedersen, et al., 2019). In addition, excess urea excreted in urine is a challenge from an environmental point of view.

In older studies, the output of nitrogen in urine accounts for 41% to 54% of the nitrogen intake on d1-21 in lactation (Noblet and Etienne, 1987a; Dourmad et al.,1998). However, the amino acid profile of compound feed has improved over the years, and Pedersen et al. (2019) find, that 17-25% of the nitrogen intake is excreted in the urine of sows. In the first week of lactation, {Pedersen et al., 2019) found a daily urine production of 6.88 kg/d and 8.98 kg/d with a urinary urea concentration of 198 mM and 117 mM in first and multiparity sows, respectively, (P<0.001). This indicated that older sows had a better utilization of protein than 1st parity sows.

To quantify the urinary output of urea, the urine production must be quantified along with the concentration of urea in urine, which was done in Experiment 1 but not in Experiment 2.

CREATININE IN URINE

In healthy animals with normal renal function, creatinine in urine is a waste product from muscle activity and protein digestion. The kidneys remove creatinine from the blood, and creatinine is excreted via urine. Creatinine can be used as an indicator for muscle catabolism; hence in periods with protein mobilisation an increase in the daily excretion of creatinine in urine would be expected. In Experiment 1 it was possible to calculate the daily excretion of creatinine, but not in Experiment 2, as water intake and urine volume were unknown.

On d 4 in lactation, Pedersen et al. (2019) find a urinary creatinine concentration of 11.8 mM and 9.26 mM in first and multiparity sows, respectively (P=0.19). On d18, urinary creatinine concentration was 6.36 and 6.33 mM (P=0.98).

BLOOD

Securing a representative sample is the key to obtain reliable results for compositional analysis. In Experiment 1, blood samples were drawn four hours after the morning feeding to represent sows in a fed state and to ensure resonably stable levels of all metabolites. In Experiment 2, this procedure was more than challenged, as sows and piglets could only be rounded up in the huts before sunset i. e. sows were in a fasting state and they had probably also not been drinking for at least 8 hours, as GPS data collected during selected nights revealed, that they seldomly left the huts during the nights. All urine, plasma and milk analysis from Experiment 2 must therefore be interpreted with that in mind.

PLASMA GLUCOSE

Plasma glucose increase rapidly after feeding a then decrease from 45 minutes until 2.5-3 hours after feeding and then stabilizes, but typically plasma glucose is within 4.5 to 6.5 mM in sows. Insulin lowers blood glucose after feeding, while glucagon keeps the blood glucose constant in the fasting state by stimulating glucose release from glycogen depots in the liver. Hence, plasma glucose will only drop when the liver glycogen depots are depleted, as in sows exposed to prolonged fasting (Serena et al., 2009).

The plasma glucose concentration is seldomly affected by dietary treatment. Still, when feeding a diet high in soluble fibers, a decreased and delayed glucose response in the portal vein was observed by Serena et al. (2009) in indoor sows. The concentration of glucose in the portal vein throughout the day was also more stable when feeding a diet with high soluble fibers compared with low fiber or high insoluble fiber content.

Average plasma glucose concentration in indoor pregnant sows vary from 5.0-5.5 mM (Feyera et al., 2018) and in lactating sows the average is 5.60 mM ranging from 4.52-6.67 mM (Petersen et al., 2016; Krogh et al., 2017; Strathe et al., 2017; Hojgaard, 2019). Serena et al. (2009) find an average portal glucose concentration of 3,96mM in sows fed high soluble fibers 0-10 hours post-feeding.

Glucose in plasma was measured in Experiment 1 and 2 to determine a possible stabilizing effect of grass-clover intake on blood glucose levels and as an indicator of whether the nutritional status of individual sows is fine or not. Indeed, this measure may reveal if sows have not been eaten well for some days prior to the blood sample.

PLASMA UREA

The concentration of nitrogen in plasma increases with increasing protein intake, and urea excretion is decreased with a more efficient nitrogen utilization. Hence, the level of urea in plasma reflects the protein intake and the biological value of the amino acid composition, but it is also affected by the time after feeding (Eggum, 1970). Sows fed the compound feed with the best amino acid composition, will have the lowest content of urea in plasma (Brown and Cline, 1974).

Apart from the feed, urea can also originate due to protein catabolism from body tissues. If the protein balance becomes negative, or if the energy balance becomes negative, body pools of protein are broken down to supply either AA or energy to the sow. In case of the latter, increasing the amount of energy supplied by digestion in relation to the requirement will therefore decrease protein catabolism and result in lower concentrations of plasma urea. Excess concentrations of urea in the blood are believed to have detrimental effects on milk production, reproductive efficiency, embryo survivability and immune function in cows (Phillips, 2020). In sows, it was recently shown that excess dietary protein decreases the feed efficiency of lactating sows (Pedersen et al., 2016

Under indoor conditions, plasma urea concentration was found to be 2.7 ± 0.09 mM in 1st and 2nd parity sows. Day one after farrowing, levels were 3.3±0.09 mM and at weaning 4.4±0.09 mM (Rempel, 2018). Typical values of plasma urea in multiparous indoor Danish sows are 2.5-6 mM with a mean of 4 mM. (Thorup et al., 2012). In elderly organic sows (average parity = 4.6) with more than ten piglets, Weissensteiner et al. (2018) reported an effect of dietary crude protein level (179 g/kg vs. 151 g/kg) on plasma urea concentrations as high protein sows had 5.7 mM, and low protein sows had 4.3 mM (P=0.003).

The concentration of urea in plasma changes within 24 hours after changes in dietary protein or amino acids, and the most suitable time for blood sampling is 4-5 hours after feeding (Eggum, 1970). This was applied in Experiment 1 but not in Experiment 2 due to practical reasons.

Plasma urea concentration may be used as an indicator of the N-balance, as sows had an increased protein intake with increasing level of fresh grass-clover in Experiment 1, and two different levels of dietary protein in compound feed in Experiment 2.

Plasma lactate

Plasma lactate has to origins; approximately 5% is produced from the decomposition of starch in the stomach by *Lactobacilli* (Serena et al., 2009), whereas the greater part derive from anaerobic metabolism in the muscles. An increase in lactate production is typically caused by reduced tissue oxygenation, as seen with locomotive activity above a certain oxidative threshold. Plasma lactate concentration not only result from the production but also from its clearance, which occurs predominantly in the liver and kidney, whereas during hyperlactatemia, muscles also metabolize lactate (Reece, 1997; Cunningham 1997). Plasma lactate concentrations increase after farrowing due to nesting activity and increased uterine contractions prior to and during farrowing (Hansen et al., 2012 ; Mosnier et al., 2010).

Under indoor conditions, plasma lactate concentration was found to be 1.7 ± 0.08mM in 1st and 2nd parity sows. Day one after farrowing levels were 2.0±0.08 mM and at weaning 1.7±0.09 mM (Rempel et al.,2018). The average plasma lactate concentration in conventional Danish lactating sows is 1.50 mM (0.36mM-2.64 mM) (Pedersen et al., 2016; Krogh et al., 2017; Strathe et al., 2016; Hojgaard, 2019).

PLASMA TRIGLYCERIDES

Plasma triglycerides originate primarily from the feed and grass, although the liver may synthesize some triglyceride as well. The concentration of plasma triglycerides is typically much lower during lactation (0.18 to 0.22 mM) than in late gestation (0.42 to 0.49 mM; Pedersen et al., 2020) because milk is rich in fat and the high secretion of milk fat causes a drainage of plasma triglycerides from the mammary gland. This is supported by Feyera et al. (2018) who found arterial plasma triglycerides in a concentration of 0.44-0.53 mM on d 88 to d 113 of gestation, 0.28 mM at farrowing and 0.22 mM post-farrowing.

PLASMA CREATININE

Creatinine is an end product formed in the muscles due to the continuous build-up and breakdown of protein (creatine phosphate may be split into creatinine and phosphate to build ATP). When body pools of protein are mobilized, an increase in plasma or urine creatinine can be expected (Reece, 1997).

In late gestation, plasma creatinine concentration of indoor sows was found to be 338.9±5.25 μM in 1st and 2nd parity sows. Day one after farrowing, plasma levels were 337.6±5.26 μM and at weaning 303.3±5.27 μM (Rempel et al., 2018). Plasma creatinine is not affected by time relative to feeding in fasting lactating sows, as reported by (Mosnier et al., 2010).

First parity sows have a mean value of 167 μM and multiparous sows a creatinine concentration in plasma of 202 μM with a variation within the range 120-260 μM the difference could be due to a larger protein pool in older sows. (Thorup et al., 2012). Strathe et al. (2016) find higher plasma creatinine concentration in early compared to late lactation, indicating a higher protein turnover in early lactation.

Urine- and plasma creatinine is measured in both experiments as an indicator of changes in body protein mobilisation.

PLASMA NON-ESTERIFIED FATTY ACIDS (NEFA)

Plasma NEFA originates mainly from body fat depots when feed intake is inadequate or for instance after overnight fasting. Thus, plasma NEFA is a good indicator of short term changes in feed supply as it decreases rapidly after a meal is consumed due to insulin secretion (Theil et al., 2011). Sampling time relative to feeding is therefore essential to standardize, and in many recent sow studies, the plasma samples have been taken four hours (variation three to five hours) after feeding, but this was not possible in Experiment 2 due to practical considerations. Plasma NEFA level is in general regarded a useful biomarker for sow fat mobilisation (Valros et al., 2003; Mosnier et al., 2010). Strathe et al (2016) found higher plasma NEFA concentrations in early compared to late lactation, indicating a higher mobilisation of fat in early lactation.

Plasma NEFA concentration in lactating sows four hours after feeding is 210 μM (49 μM - 422 μM) (Pedersen et al., 2016; Strathe et al., 2016; Krogh et al., 2017; Hojgaard, 2019).

MILK

Little is known about milk composition in organic sows or from lactation after day thirty. In indoor sows, the composition of porcine milk varies with stage of lactation and nutritional status of the sow (Hurley and Theil, 2011; Krogh et al., 2017; Hojgaard, 2019), but type of production and season might also have an effect. First parity sows have a lower milk yield than older sows (King, 2000; Beyer et al., 2007), but young and older sows have broadly similar milk composition profiles of fat, protein, lactose, and net energy concentrations throughout the period from d0 to d21 of lactation (Craig et al., 2019).

In Experiment 2, milk subsamples were analyzed for dry matter, protein, fat, lactose, and casein using infrared spectroscopy on a Milcoscan FT2 developed for bovine milk. Other milk subsamples were analyzed for glucose-6-phosphate, glucose, uric acid, β-hydroxybutyrate, isocitrate, N-acetyl-beta-d-glucosaminidase, and lactate dehydrogenase by fluorometric assays (Larsen, 2005a; Larsen, 2005b; Larsen and Moyes, 2010; Larsen et al., 2010; Larsen and Aulrich; Larsen, 2014; Larsen, 2015).

In general, milk composition in conventional sows with a four week lactation period is 17-20% DM, 6-8% fat, 5-6% protein, 4-5% lactose and 0.6-1.0% ash (Hurley, 1997). In an older study of normal milk for an average lactation period of eight to twelve weeks, DM content was 17.98%, fat content was 6.77%, and protein and ash content were 6.22% and 0.97% respectively (Hughes and Hart,1935). The transition from colostrum to milk occurs within the first five days of lactation (Gallagher et al., 1997). Hence, only selected components in normal milk will be described here, because colostrum and transient milk was not collected in Experiment 2.

GLUCOSE IN MILK

Milk yield depends on the production of lactose. Lactose is synthesized from glucose and galactose in the mammary gland, and lactose regulates milk secretion because of high osmolarity (Reece, 1997). Glucose may not be the sole carbon source for lactose synthesis, but it is estimated that 80-85% of lactose carbon is of glucose origin in cows (Larsen, 2015). Krogh et al. (2017) has shown, that 36% of the glucose taken up by the mammary gland was used for lactose synthesis in sows.

In Experiment 2, milk glucose was measured as an indicator of the energy status of the sow.

URIC ACID IN MILK

Uric acid is an intermediate in the composition of purines. Uric acid in cow milk is believed to reflect the microbial nitrogen flow to the duodenum, which may be used as a potential indicator of rumen microbial protein yield in high-yielding dairy cows (Larsen, 2010).

Postpartum dysgalactia syndrome (PDS) is defined as insufficient milk yield in sows 72 hours after farrowing. Kaiser et al. (2020) found higher concentrations of uric acid in sows with no PDS after farrowing, suggesting that healthy sows have a higher level of microbial activity of beneficial gut bacteria.

Milk uric acid concentration was measured in Experiment 2 for evaluation of the gut microflora and the sows energy status, as high levels indicate adequate energy supply

Β-HYDROXYBUTYRATE IN MILK

β-Hydroxybutyrate (BHBA) is synthesized by ketogenesis of liver cells in the conversion of long-chain fatty acids from body fat mobilisation and absorbed butyrate in the rumen epithelium of ruminants (Cunningham, 1997). Determination of β-hydroxybutyrate in plasma and milk are important tools in the diagnosis of ketosis in dairy cattle (Larsen, 2005b). Ketosis may develop, whenever there is a change from carbohydrate metabolism to fat metabolism, which typically occurs in lactation.

Primary ketosis as a metabolic disease is generally not recognized in pig production (Alsop et al., 1994; Bruun et al., 2011). Still, secondary ketosis is said to occur “not uncommonly” two to three weeks after farrowing (Penny, 1970). Even when there are no visible clinical signs, ketosis can negatively affect milk production, reproduction and immunity in cows (Enjalbert et al., 2001). The symptoms of secondary ketosis in sows include reduced appetite, weight loss, hypoglycemia and general weakness (Penny, 1970)

In Experiment 2, BHBA was measured in milk as an indicator of body fat mobilisation.

ISOCITRATE IN MILK

Isocitrate is an intermediary metabolite in the citric acid cycle. Oxidation of isocitrate is believed to deliver large fractions of energy used for fatty acid and cholesterol synthesis in the udder of cows. Isocitrate in milk is correlated to milk protein and milk fat and Larsen (2014) showed, that citrate and isocitrate were directly proportional to day in lactation, but inversely proportional to milk yield of cows.

In Experiment 2, Isocitrate was measured to reflect the energy status in the mammary gland.

UREA IN MILK

Urea diffuses from the blood into the milk and shortly after milking, the concentration of urea in milk found in the mammary gland closely parallels the concentration of urea found in the blood of cows {Phillips, 2020).

Urea in milk was measured in Experiment 2 as an indicator of the nitrogen balance, and increasing levels in milk may reflect increasing nitrogen available for milk protein synthesis. Still, high level of urea in milk may also indicate an imbalanced amino acid profile of the feed. In a dose-response trial, Hojgaard et al., (2019) found that milk urea decreased until the sows reached an optimum protein supply, thereafter milk urea increased with increasing protein consumption.

MANUSCRIPTS

The scientific results from Experiment 1 are presented in Manuscript I. The results from the first parity (winter trial) are presented and discussed in Manuscript II, and the results from the 2nd parity (summer trial) are outlined in Manuscript III.

Only very few studies have been conducted on the nutritional requirements of organic sows. Thus, to generate further knowledge and to be able to discuss findings in paper 2 and paper 3, the combined results of summer and winter were further subjected to statistical analyses and presented in Tables 7, 8 and 9 to show the main effects of reproductive stage, season, protein level and breed throughout the year. The overall results section also presents correlations between grass-clover intake in summer and selected parameters from both Experiment 2 in Tables 10 and 11.

These overall results will also constitute the background to the subsequent general discussion.

MANUSCRIPT I

Impact of increasing fresh grass-clover intake on nitrogen metabolism and plasma metabolites of sows

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Abstract

The purpose of this study was to identify biomarkers for fresh grass-clover intake and determine the digestibility and energy value of fresh grass to be able to estimate voluntary fresh grass-clover intake in organic sows on pasture. A total of sixteen multiparous dry sows (Danish Landrace x Danish Yorkshire) were housed in metabolism cages for two periods of five days. To study dietary effects, sows were fed one of four daily mixed rations where increasing proportions of a basal commercial organic sow compound feed was replaced with 0, 2, 4, or 6 kg fresh grass-clover collected three weeks after the previous cut. Sows were fed similar amounts of ME. Total collection of urine and feces was performed on a daily basis for five days and blood samples were collected from the jugular vein on the last day of feeding. Plasma metabolites were analysed using a non-targeted liquid chromatography-mass spectrometry (LC-MS) approach. Analysed plasma metabolites (area under the curve in arbitrary units from the LCMS), were screened for correlation with grass intake. Data on nutrient digestibility and plasma metabolites were analysed using a MIXED procedure while accounting for repeated measurements. Apparent total tract digestibilities of DM, OM, nitrogen, and energy of fresh grass-clover were 72%, 64%, 71%, and 68%, respectively, using the regression method. Nitrogen intake increased linearly with increasing fresh grass-clover intake ($P<0.001$). Nitrogen deposition and nitrogen utilisation were not affected by grass intake within the range investigated in this experiment ($P>0.05$). There was a linear increase in plasma urea content (2.64 to 4.39 mM) when grass intake increased from 0 to 6 kg per day ($P=0.02$). Plasma glucose, lactate, creatinine, and NEFA and triglycerides were not affected by increased grass-clover intake. The daily ME contribution from fresh grass-clover (MJ/d) was found to be highly positively correlated with plasma pipecolic acid ($Y = 0.0289 X$; $r^2=0.91$; $P<0.001$) and a metabolite tentatively identified as plasma bisnorbiotin ($Y = 0.482 X$; $r^2=0.92$; $P<0.001$). In conclusion, fresh grass-clover intake of sows was highly correlated with plasma pipecolic acid and plasma bisnorbiotin concentration and apparent total tract digestibility of DM, OM, nitrogen, and energy estimated for 100% fresh grass-clover intake in dry sows was 64%-72% using the regression method.

Keywords: Biomarkers, Digestibility, Energy, Organic, Urine

Introduction

According to the European organic regulations, organic sows must have access to grazing for at least some part of the year (Council regulation No. 1804/1999). The nutritional contribution from pasture will depend on the availability, nutrient composition, intake, and digestibility of herbage, roots, worms etc., and the nutritive value of foraging is thus difficult to estimate (Edwards, 2003; Blair, 2018). In Denmark, it is common practice to nose ring outdoor sows to prevent them from rooting and thereby damaging the grass sward (Eriksen et al., 2006), hence, grass species are probably the largest contributor to the daily feed intake from pasture in Danish organic sows in the grazing period from May to October. Good quality grass-clover has the potential to supply a substantial proportion of the energy, protein, and nutrients needed by organic sows from May to October (Edwards, 2003). This exacerbates the challenge in supplying a gestation- and lactation diet to match their total daily requirements for nutrients and reduce the environmental load in terms of nitrogen leaching from dietary excess. Therefore, it is of relevance to find credible methods to assess the daily intake of fresh grass-clover from pasture in outdoor sows. N-alkanes are components of the plant cuticular wax, and they have been used as markers for the estimation of grass intake and digestibility in grazing ruminants – an approach known as the n-alkane method (Mayes et al., 1986). The n-alkane method was developed for ruminants, but is also validated for estimation of grass intake of sows (Gannon, 1996; Rivera Ferre et al., 2001; Sehested, 1999b; Kanga et al., 2012). With the n-alkane technique, it is possible to estimate the grass intake of sows without compromising the behavior or the welfare of the animals. However, it may be difficult to apply the method in outdoor pig production, since a quantification of total marker intake is essential to avoid overestimation of the grass intake. Another challenge could be fermentation. Little is known of the fate of n-alkanes in the hindgut of the sow, but some bacterial species and yeast have been shown to metabolise n-alkanes (Dostalek et al., 1968; Yamada and Yogo, 1970). It might be possible to estimate voluntary grass intake by bite-size and bite rate, short term changes in live weight, or, if the grass-clover digestibility is known, by total feces collection with a chromium marker in the compound feed. However, these techniques are both expensive, time-consuming and difficult to apply on outdoor sows.

The aim of the present study was to develop a simple and fast method to assess the voluntary daily intake of grass-clover on pasture. We hypothesised that it is possible to identify metabolites in blood, which are linked to the intake of fresh grass-clover, and that these biomarkers can be used to predict grass-clover intake in organic sows kept on pasture. This, to allow prediction of total intake of energy and dietary crude protein (CP) from fresh grass-clover in organic sows. Such knowledge may in the future enable optimisation of the composition of organic gestation- and lactation compound feed and ultimately reduce the feed costs and nitrogen leaching from organic pig production.

Materials and methods

The animal experimental procedures were carried out in accordance with the Danish Ministry of Justice, Law no. 253/08.03.203 concerning animal experiments and care and license issued by the Danish Animal Experimental Inspectorate, Ministry of Food, Agriculture and Fisheries, the Danish Veterinary and Food Administration. The animal experiment complies with the ARRIVA guidelines and was performed in accordance with the legislation for the protection of animals used for scientific purposes **(**EU Directive 2010/63/EU for animal experiments).

Animals

A total of sixteen 2nd - 6th parity dry sows (Danish Landrace x Danish Yorkshire) with a mean live weight of 258 (range 191-320) kg were randomly selected from the experimental herd at Aarhus University, Foulum. The sows were divided into four dietary groups (0, 2, 4, and 6 kg fresh grass-clover/d) with four animals in each group. Every sow was studied during two balance periods of five days. During a preparation period of three weeks, animals were stratified for willingness to consume freshly cut grass-clover, to ensure that feed allowances were within the appetite capacity of the individual animal. Some sows were consuming no or very small amounts of fresh grass-clover in the preparation period, and these sows were allocated to group 1, who were supplied with only compound feed. Other sows were very eager to consume the grass-clover in the preparation period. These sows were put in group 4 and were supplied with 6 kg grass-clover/d. The remaining sows were distributed by chance in group 2 and 3 and received 2 kg/d or 4 kg/d of grass-clover, respectively. There was no correlation between sow body weight or parity and the willingness to consume large amounts of fresh grass-clover in the preparation period. Individual sows remained on the same dietary treatment in the two subsequent experimental periods.

Experimental diet

A basal organic gestation compound feed based on barley, rye, oat, and rapeseed cake was formulated according to Danish recommendations to ensure recommended supply of nutrients and net energy for gestating sows (Tybirk, 2016) and purchased from a commercial supplier (Vestjyllands Andel, Ringkøbing, Denmark; Table 1). The four groups were offered 0, 2, 4 or 6 kg freshly cut grass-clover, while the amount of compound feed was reduced concomitantly to aim at iso-energetic ME supply to all groups on a daily basis. Prior to the experiment, the energy concentration of the grass-clover was calculated using tabulated values (Møller et al., 2000). The grass-clover was a commercial mix (ForageMax55, DLF Trifolium, Roskilde, Denmark) of 10% Trifolium Repens (white clover, Rivendel), 50% Lolium Perenne (perennial ryegrass, Humbi 1), 15% Lolium Perenne (perennial ryegrass, Masai) and 25% Festuca Rubra (red fesque, Gandolin). The feeding trial started October, 3rd, 2016 during a period with mild autumn climate, when the grass was three weeks post-cut. The fresh grass was harvested every morning between 0500 and 0600 h. The experimental rations were offered in two equally sized portions with eight-hour intervals during the two balance periods with total feces and urine collection. The morning ration was offered at 0800 h. Grass-clover for the afternoon feeding was stored at 5 °C until supplied at 1600 h.

Housing and husbandry

At each meal and for each sow, fresh grass-clover and compound feed were weighed and distributed manually. The daily supply of freshly cut grass-clover was offered on the solid floor with side partitions to reduce lateral loss in the balance cage, and the compound feed was fed in a trough. Leftovers were collected and weighed just before the next feeding to determine intake of feed and fresh grass-clover, which along with analyses of the composition of feed and grass-clover, allowed estimation of the daily nutrient intake. The sows had ad libitum access to fresh water from a nipple drinker attached to a water meter.

Each sow was individually housed in a metabolic cage (2.4 m × 0.8 m) made of stainless steel, with no edible bedding materials. The metabolic cages allowed separate total collection of feces and urine, as bladder catheters were inserted at the beginning of each experimental period. Between the two collection periods, the sows remained on the experimental dietary treatments. Stil, they were group-housed for five days in a traditional free access feeding stall, where they were locked inside the feeding stall for two hours from the feeding time.

Room temperature was kept at 20 °C, and the light was turned on from 0700 to 1830 h. Besides the experimental recordings, the sows were managed according to the general routines in the experimental herd. Health was monitored on a daily basis by the stock personnel and weekly by the herd veterinarian.

Measurements

Sows were weighed on a walk-in scale before and after the experiment. Individual water intake was registered every day. Feed intake corrected for refusals, was recorded on a daily basis for both fresh grass-clover and compound feed. Subsamples of the compound feed were taken daily and pooled for analysis. Samples of fresh grass-clover were taken twice daily and stored at -20°C. The grass-clover was freeze-dried before analysis. Prior to the beginning of the 5-day balance period, a balloon urinary catheter was inserted through the urethra into the bladder. The daily amount of urine was collected in sealed plastic jars containing 50 ml 30% H2SO4, weighed, and 10% was subsampled over the five days for N analysis. Sulphuric acid was added to the container to prevent evaporative loss of ammonia. Total feces was collected and pooled on a weekly basis. Prior to analysis, feces was thawed, homogenised, subsampled, and freeze-dried. Blood was collected from vena jugularis four hours after the morning feeding on day five of each balance period. Samples were drawn in heparin vacuum tubes, placed on ice, and plasma was harvested shortly after by centrifugation at 1558 × g for 12 min at 4°C. Subsamples of plasma and fresh urine were stored at −20°C until analysis. Subsamples of plasma and fresh urine sample was stored at -80°C for metabolomics analysis.

Analytical methods

Apart from amino acids, all chemical analyses of compound feed, fresh grass-clover, feces, urine, and plasma were performed in duplicate. The DM content of all feed and feces samples was determined by oven drying at 103 °C. Ash was determined by oven drying at 525 ºC for 6 hours. The CP content was calculated as nitrogen × 6.25 as reported by Eggum (1991). The nitrogen content of grass, compound feed, feces and urine was determined by the modified Kjeldahl method (Method 984.13; AOAC Int, 2000) using a KjeltecTM 2400 (Foss, Hillrød, Denmark). Amino acids (AA) were analysed in product samples hydrolysed for 23 hours at 110 °C with (Cys and Met) or without (Arg, His, Ile, Leu, Lys, Phe, Tyr, Thr, Trp, Val) performic acid oxidation, and AA were separated by ion-exchange chromatography and quantified by photometric detection after ninhydrin reaction. Compound feed, grass-clover, and fecal gross energy (GE) was determined with a bomb calorimeter (Parr 6300 Instrument Company, Moline, Illinois, USA). Starch and non-starch polysaccharides (NSP) were analysed as described by Knudsen (1997). Plasma samples were analysed for glucose, lactate, creatinine, non-esterified fatty acids (NEFA), triglycerides, and urea. Glucose, lactate, triglycerides, and urea were analysed according to standard procedures (Siemens Diagnostics Clinical Methods for ADVIA 1650) on an auto analyser (ADVIA 1650 Chemistry System, Siemens Medical Solution, Tarrytown, NY). Plasma content of NEFA was determined using the Wako, NEFA C ACS-ACOD assay method (Wako Chemicals GmbH, Neuss, Germany). Urea and creatinine concentrations of urine were determined according to standard procedures (Siemens Diagnostics Clinical Methods for ADVIA 1650) using an auto analyser (ADVIA 1650 Chemistry System, Siemens Medical Solution, Tarrytown, NY). For metabolomics, plasma samples were deproteinised and prepared for analysis as described by (Soumeh et al., 2016). Freeze dried samples (50 mg) of the compound feed and the fresh grass-clover was mixed with 400 µl acetonitrile (10 %, v/v) containing p-chlorophenylalanine and glycocholic acid (Glycine-1 13C) (0.01 mg/ml) as internal standards. The suspension was vortexed for 15 min at 4°C and centrifuged (20800 × g, 10 min, 4°C). The supernatants were transferred to vials. Chromatographic separation of the samples was performed using a Dionex UltiMate 3000 (Dionex, Sunnyvale, CA, USA) ultra-high pressure liquid chromatography system (UHPLC) equipped with an HSS T3 C18 UHPLC column, 1.8 µm, 100 × 2.1 mm (Waters Corporation, Milford, MA) equipped with a VanGuard Pre-column, 100Å, 1.8 µm, 2.1 mm × 5 mm (Waters Corporation, Milford, MA). The column was maintained at 30°C and the samples were placed in an autosampler kept at 10°C during the entire run. The mobile phases were 0.1% formic acid in Milli-Q water (A) and 0.1% formic acid in acetonitrile (B). The flow rate was 0.4 mL/min. The gradient program was as follows: 0-12 min, linear gradient from 5 to 90% B; 12-12.3 min, 90% B, and return to initial conditions in 0.2 min. Corresponding changes in A were made. The column was re-equilibrated at 5% B for 2 min at the beginning of each run. The eluent was introduced into an Ultra-High Resolution Qq-Time-Of-Flight mass spectrometer (Impact HD, Bruker Daltonics GmbH, Bremen, Germany) by electrospray ionisation and analysed in positive and negative ionisation modes using the instrumental parameters described by Hedemann (2017). To evaluate the analytical performance of the system blank samples (5% acetonitrile) and quality controls (QC, a pooled plasma sample) were analysed after each five or 10 samples, respectively, to check for potential cross-contamination from samples and system reproducibility during the run.

Calculations

The 24 h losses of energy via feces and urine were quantified using a total collection. Undigested DM was calculated from average daily feed intake (ADFI) and DM apparent total tract digestibility (ATTD), and the fecal GE output was then quantified by multiplying undigested DM with the analysed GE concentration in feces. The ATTD was calculated using both the regression and difference methods. In the identification of metabolites from fresh grass-clover, mass spectra were calibrated and converted to mzXML-spectra and preprocessed using an R-based XCMS package (Smith et al., 2006). The exported data tables were filtered to eliminate features present in blanks, retention times were truncated to contain only portions with chromatographic peaks, and masses higher than 800 m/z were discarded. The data tables were imported to LatentiX 2.10 (Latent5 Aps) and were Pareto-scaled, which reduces the importance of high intensity peaks but retains the variability in the data structure partially intact (van den Berg et al., 2006) then Principal Component Analysis (PCA) was performed. Loadings plots were used to identify metabolites discriminating sows eating 0 kg grass from sows eating 6 kg grass-clover, and plots were made to check for linear correlation between intake of grass and the level of the metabolite in plasma. The relevant metabolites were identified using accurate masses and fragmentation patterns based on searches in online databases: the METLIN (http://metlin.scripps.edu/), Human Metabolome Database (http://www.hmdb.ca/), and LIPID MAPS (http://www.lipidmaps.org/). The identification of the annotated compounds was confirmed with standards, when available, on the same analytical system under the same conditions (validation based on retention time and mass spectra).

Statistical analysis
The statistical analysis was performed using the MIXED procedure of SAS (Ver. 9.4; SAS Institute, 2012) with a statistical model including dietary treatment as fixed effect. Plasma NEFA was subjected to a logarithmic transformation to stabilise the residual variance. All variables were considered significant when $P < 0.05$ and tendencies were accepted at $P \leq 0.10$. Mean values are represented as least squares means ± standard error of mean (SEM) or back-transformed mean with lower and upper confidence intervals. In addition, linear and quadratic effects of grass intake were tested within the model.

Results

Two sows (one from group 2 and one from group 3) were excluded from all analyses, as they developed fever and did not consume their daily rations of grass-clover 100%. Apart from these two sows, all sows in generally consumed their full rations of grass-clover. In group 2, the actual grass intake was 1.905 kg/d (SEM =0.03), in group 3 the grass intake was 3.997 kg/d (SEM = 0.04) and in group 4 it was 6.009 kg/d (SEM = 0.04).

Chemical composition of fresh grass-clover
Increasing the allocation of grass-clover from 0 to 6 kg/d increased the CP content of the total ration from 129 to 209 g/kg DM (Table 2). The lysine concentration in the total mixed ration increased from 6.3 g/kg DM in the 0 kg grass group to 11.1 g/kg DM in the group receiving 6 kg grass/d. Methionine increased from 2.0 to 3.3 g/kg DM, and threonine from 4.7 to 8.7 g/kg DM in the 0 kg and 6 kg total rations, respectively. Cysteine was fairly constant across treatments, whereas the remaining AA in the total rations also increased gradually from 0 to 6 kg daily grass intake. The AA accounted for 92% and 85% of the dietary CP in the 0 kg and 6 kg total rations, respectively.

Voluntary grass intake
Three sows lost weight during the experiment (average -2.4 kg). The remaining eleven sows had a weight gain (average +4.4 kg; Table 3). The individual weight changes were not affected by grass-clover consumption. The fresh grass-clover contained 16% DM, which was 3%-units less than expected (19%) in the dietary formulation. Unintendedly, the intake of ME therefore decreased linearly from 25.7 MJ/d to 22.1 MJ/d ($P<0.02$), with increasing allocation of fresh grass-clover. There was no effect of grass-clover inclusion on water intake or feces output.

Digestibility of fresh grass-clover for sows
The ATTD of energy in the total ration declined linearly from 80.4% to 73.6% with increasing grass-clover inclusion ($P<0.05$; Table 4). The ATTD OM was reduced from 79.8% to 72.6% ($P<0.05$). There was no effect of increasing grass inclusion on ATTD N in this study. The ATTD DM tended to decrease with increasing grass-clover intake ($P=0.09$).

Using the regression method, the ATTD of DM, OM, nitrogen and energy of 100% grass-clover were 70.0 ± 3.4%, 67.0 ± 3.5%, 74.7 ± 2.7% and 68.7 ± 3.4%, respectively. Using the difference method, DM digestibility of 100% fresh grass-clover ranged between 68.6%-72.2%, digestibility of OM of grass ranged between 58.9%-62.9%, digestibility of nitrogen of grass was between 70.4%-79.9%, and digestibility of grass-clover ranged between 67.4%-69.5%.

Biomarkers for grass-clover intake

The PCA-scores plot of plasma in positive mode showed a clear separation between sows fed no grass-clover and sows fed 6 kg grass-clover per day, and especially principal component 2 was high when sows did not consume any grass and decreased with increasing grass-clover intake (Figure 1). Inspecting the loadings plot and correlating the area of the peaks to the intake of grass-clover showed that three metabolites important for the discrimination between the groups correlated to intake of grass-clover. The metabolites were identified as pipecolic acid (m/z 130.0864), and a fragment of pipecolic acid (m/z 84.0808) and m/z 217.0686 was tentatively identified as bisnorbiotin. Inspecting the chromatograms of grass-clover and the compound feed showed that fresh grass-clover contained a clear peak of pipecolic acid, whereas the compound feed only showed a minor peak (Figure 2). Searching the chromatograms for m/z 217.0686 showed a minor peak in the compound feed, whereas no peak was found in grass-clover. The daily ME (MJ/d) contribution from fresh grass-clover was found to be highly positively correlated with plasma pipecolic acid ($Y=0.0289 X$ ($SE = 0.0011$); $R^2 = 0.91$; $P<0.001$) and plasma bisnorbiotin ($Y=0.482 X$ ($SE = 0.019$); $R^2 = 0.92$; $P<0.001$; Figure 3). The daily intake of digestible OM from grass-clover in g/d may be predicted as $Y = 0.871 X$ ($SE = 0.033$); $R^2 = 0.91$, where X is plasma pipecolic acid or $Y = 14.5 X$ ($SE = 0.7$); $R^2 = 0.89$, where X is the metabolite tentatively identified as plasma bisnorbiotin.

Plasma metabolites

Plasma concentrations of urea ($P=0.04$), pipecolic acid and bisnorbiotin ($P<0.001$) increased linearly when grass intake increased from 0 kg/d to 6 kg/d. Plasma creatinine decreased with increasing grass intake in a curvilinear manner. Moreover, a tendency for a quadratic effect was observed on NEFA with the highest concentrations observed in sows fed 0 or 6 kg/d and lowest in sows fed 2 kg/d of grass. There was no effect of fresh clover grass intake on plasma lactate, glucose, or triglycerides.

Nitrogen balance

Nitrogen intake increased linearly with increasing fresh grass-clover intake ($P<0.001$; Table 5). Nitrogen excretion in urine and feces in g/d increased with increasing grass-clover intake ($P<0.003$). Nitrogen retention was not affected by grass intake within the range investigated in this experiment, neither expressed in g/d nor in % of intake.

Discussion

Voluntary grass-clover intake

There was a large between-animal variation in voluntary grass-clover intake during the three weeks of training period. The animals were housed indoor and had never experienced fresh grass before. A few sows in the adaptation period would not ingest any grass-clover at all and were therefore assigned to the 0 kg grass group. This grass rejection by some sows was also reported in another experiment (Rivera Ferre, 1999). Edwards (2002) suggested that the daily herbage intake of sows range from 2 to 10 kg/d with an average of 6-7 kg/d. Gannon (1996) reports individual variations in herbage intake in the range between 1.1 and 10.5 kg/d of fresh herbage in the spring, and 4.3 to 11.8 kg/d in the summer. Therefore, the contribution of grass-clover to the daily nutrient intake, and the degree to which grass-clover can substitute a compound feed, might also vary among individuals within a herd. We saw no relationship between sow weight and tendency to voluntarily consume 6 kg of fresh grass-clover.

The contribution of energy and CP from the compound feed might affect the grass-clover intake of the sows. Still, in this experiment, there were no grass-clover residuals, suggesting the daily upper limit might be higher than 6 kg fresh grass-clover per day. Using live weight measurements (Fernandez, 2006) also found no effect of allocated concentrate on the intake of grass-clover in 15 pregnant sows on pasture in May-June and August-September. Gaseous energy losses, i.e. methane emission, was not considered when calculating dietary ME in the present study, and may marginally affect an overestimation of the reported ME values.

Biomarkers for voluntary grass intake

Plasma piperidine-2-carboxylic acid, more commonly known as pipecolic acid, increased linearly with increasing DM from fresh grass-clover. L-pipecolic acid is identified as a constituent of legumes (Broquist, 1991) and (Morrison, 1953) isolated pipecolate (500 mg) from 500 g of cut leaves of white clover. Hence it is likely that the increase in plasma pipecolic acid shown in this experiment, derives from the 10% white clover in the grass mix. However, we also saw a very small amount of pipecolic acid in the 0 kg grass-clover group. Pipecolic acid is an AA first identified in plasma of cows and goats, and Onodera and Kandatsu (1969) showed that pipecolic acid was produced from L-lysine by rumen ciliate protozoa but not by rumen bacteria. Chavatte et al. (2016) demonstrated that protozoa are common in the gastrointestinal tract of pigs. Therefore, lysine intake from the compound feed can explain the intercept in plasma pipecolic acid, and it seems likely that the small amount of pipecolic acid in the 0 kg grass group is produced by protozoa in the hindgut of the sows. The metabolite tentatively identified as plasma bisnorbiotin, increased linearly like pipecolic acid with increasing grass-clover intake. Bisnorbiotin is a major biotin metabolite and has been detected in pig plasma after intravenous administration of physiologic amounts of biotin (Wang et al., 2001). Biotin is synthesised from cellulose by microbial fermentation in the hindgut. Since the daily intake of cellulose was increased by 75% when increasing grass supply from 0 to 6 kg grass-clover per day, this likely explains the highly positive correlation between plasma bisnorbiotin and fresh grass-clover intake. There is one argument saying that plasma bisnorbiotin might be a more applicable marker for grass-clover intake in organic sows than pipecolic acid since there was no intercept (i.e., no bisnorbiotin at 0 kg grass-clover). On the other hand, there was a very high content of pipecolic acid in the grass-clover but not in the compound feed, and the metabolite tentatively identified as bisnorbiotin is found in a much lower concentration in plasma than pipecolic acid, which makes pipecolic acid a reliable marker for fresh grass-clover intake. It is possible that the relative plasma concentrations of pipecolic acid and bisnorbiotin may vary with sex, age, breed, and reproductive state of the pigs due to varying conditions and the microbial composition in the digestive tract. Different grass varieties differ in the relative proportion of CP and carbohydrates, and it also seems likely that changes in lysine and cellulose content during the growing season of grass-clover may affect plasma concentrations of the studied metabolites in pigs. The validity of grass intake estimates by using plasma bisnorbiotin or pipecolic acid as a marker might also be affected by the composition of the supplementary diet, and ingredients such as grass meal containing clover must be avoided when formulating the supplementary diet. Further investigations are needed to evaluate how robust these metabolites are, when predicting grass-clover intake.

Digestibility of fresh grass-clover for sows

The ATTD of energy in the total ration decreased from 80.4% to 73.6%, when fresh grass-clover intake increased from 0 kg to 6 kg/d. This is also seen in other studies, as dietary fiber is associated with impaired nutrient utilisation and reduced net energy values (Lindberg, 2014). The level of dietary fiber can linearly reduce the ATTD of DM, OM, energy, CP, and non-fibrous carbohydrates in sows (Noblet and Le Goff, 2001; Oelke et al., 2018). The ATTD of OM in the total ration was also affected by the level of grass inclusion ($P<0.05$). The ATTD of grass-clover diet was estimated using both the regression and the difference method. Energy digestibilities around 68% indicate, that the grass was reasonably digestible, which corresponds well with (Carlson et al., 1999), who found total GE digestibilities of a ration with compound feed and freshly cut grass-clover (18% of DM) to be 82% in 30-kilo gilts. The fresh grass-clover constituted 53% of DM in the 6 kg group in our study. The digestibility of nitrogen in the total ration appeared not to be affected by the level of fresh grass-clover intake within the studied range.

Plasma components

All plasma glucose concentrations were within the normal range of pigs, but there was a tendency to decreased plasma glucose concentrations with increasing grass-clover intake ($P=0.11$). This is in accordance with (de Leeuw et al., 2004) who found, that fermentable dietary fiber stabilised anteprandial blood glucose levels in pregnant sows, and Serena et al. (2009) who finds lower net absorption of glucose two hours after feeding in multicatherised sows fed high fiber diets as compared with a low-fiber diet. The dietary effect on NEFA was probably also related to the shift in energy being net absorbed from the small intestine (mainly as glucose) to hindgut fermentation (mainly uptake of short chain fatty acids) when comparing sows without any grass intake with the sows receiving substantial amounts of grass. It should be emphasized that the blood samples in this study were taken approximately four hours after feeding and thus represent a fed state although most of the glucose from digested starch is already net absorbed at this time (Serena et al., 2009). Most likely, this pattern would not be consistent throughout the day because fluctuations in net energy absorption is much more pronounced when fiber and roughage intake is low.

Nitrogen balance

As the CP content in the diet increased with increasing allocation of fresh grass-clover, there was an increase in nitrogen intake from 43.6 g/d to 65.2 g/d. Grass-clover substitution caused an oversupply of all essential AA compared to the nutrient recommendations (Tybirk, 2016). Urinary N losses were 27.3 g/d in the 0 kg grass group, and this increased to 48.4 g/d in the 6 kg grass group. As this cannot be explained by a less optimal AA profile in fresh grass-clover (5.5 g lysine/16 g N) as compared with the corn-based compound feed (4.9 g lysine/16 g N), it is most likely due to the greater N intake in the 6 kg/d group. Plasma urea increased linearly with increasing grass intake, indicating that sows were supplied with excess CP when grass replaced the compound feed. A negative consequence of CP oversupply is that metabolism and excretion of excessive CP reduces the energy utilisation by up to 6% (Pedersen et al., 2019). Hence, sows will require more feed to reach the same productivity level, if the contribution of CP from grass is not balanced relative to the intake of net energy in the total ration for sows on pasture. Increasing fresh grass-clover inclusion in the diet doubled the faecal nitrogen output. Increased faecal nitrogen output was also reported by Lindberg et al. (1995) and Vestergaard (1996), who increased forage meal inclusion in a barley-based diet for finishing pigs. The increased grass-clover intake did neither affect nitrogen deposition in g/d nor when expressed in percent of intake in our study. Oversupply with CP and AA is seen to result in an increased nitrogen content in the slurry and consequently increased environmental pollution due to nitrogen emission and leaching (Nørgaard et al., 2014).

Conclusion

Increasing grass-clover intake linearly increased the daily CP intake but decreased the apparent total digestibility of dry matter, organic matter, N, and energy. The daily ME contribution from fresh grass-clover was found to be highly positively correlated with plasma pipecolic acid and plasma bisnorbiotin. The present data confirmed that fresh grass-clover is a good source of both CP and energy in organic sow production. To avoid CP oversupply and nitrogen leaching, it is important to counterbalance the nitrogen contribution from fresh grass-clover when formulating compound feed for grazing sows.

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Table 1

Dietary ingredients of the four experimental rations containing a basal organic compound feed (gestation diet) and either 0, 2, 4 and 6 kg fresh grass-clover per day.

Ingredients	Compound feed	Compound feed + 2 kg fresh grass-clover/d	Compound feed + 4 kg fresh grass-clover/d	Compound feed + 6 kg fresh grass-clover/d
Total ration, kg/d	2.10	3.75	5.4	7.0
Fresh grass-clover, g/kg ration	0.0	534	741	851
Compound feed, g/kg ration	1000	466	259	149
Barley	330	154	85.5	49.2
Rye	200	93.2	51.8	29.8
Oat	150	69.9	38.9	22.4
Corn	50.0	23.3	13.0	7.5
Peas	50.0	23.3	13.0	7.5
Wheat bran	35.0	16.3	9.1	5.2
Oat bran	50.0	23.3	13.0	7.5
Dried grass-clover meal	20.0	9.3	5.2	3.0
Soybean cake	47.0	21.9	12.2	7.0
Rapeseed cake	43.0	20.0	11.1	6.4
Calcium carbonate	14.0	6.5	3.6	2.1
Sodium chloride	4.4	2.1	1.1	0.7
Monocalciumphosphate	6.1	2.8	1.6	0.9
Vitamin and mineral mixture[1]	1.1	0.5	0.3	0.2

[1] Pr kg: 8,000 IU vitamin A; 800 IU 25-hydroxy vitamin D; 54,600 mg DL-alphatocoferol; 2,000 mg vitamin B1; 5,000 mg vitamin B2; 3,000 mg vitamin B6; 20.0 mg vitamin B12; 2,000 vitamin K3; 15,000 mg D-pantothenic acid; 20,000 mg niacin; 400 Biotin; 1,500 mg folic acid; 80,000 mg iron (FeSo4); 15,000 mg copper (CuSO4); 40,000 mg manganese (MnO); 2,000 mg iodine (Ca(IO3)2); 100,000 mg zink (ZnO); 300 mg selenium (Na2SeO3).

Table 2
Analysed composition of fresh grass-clover and basal gestation diet (0 kg grass-clover ration) and calculated chemical composition of the three rations containing fresh grass-clover.

Chemical composition, g/kg DM	Fresh grass-clover[1]	Compound feed[1]	Compound feed + 2 kg fresh grass/d[2]	Compound feed + 4 kg fresh grass/d[2]	Compound feed + 6 kg fresh grass/d[2]
Dry matter, g/kg feed	163	877	496	348	270
Protein (Nx6.25)	284	129	156	183	209
Fat	-	38.0	-	-	-
Starch	17.9	482	401	321	243
Cellulose	121	49.0	61.6	73.9	85.9
Total NSP	282	176	194	212	230
Insoluble NSP	212	148	159	170	181
Klason lignin	77.5	57.0	60.6	64.1	67.6
Ash	131	48.0	62.7	77.0	90.9
Dietary fibre	359	233	255	276	298
Gross energy (MJ/kg DM)	18.5	17.9	18.0	18.1	18.2
Amino acids, g/kg DM					
Lysine	15.57	6.32	7.94	9.53	11.08
Methionine	4.47	2.00	2.43	2.85	3.27
Cysteine	2.56	2.68	2.66	2.64	2.62
Valine	15.86	6.18	7.88	9.54	11.17

[1] Analysed chemical composition. This composition represent 0 kg/d of grass intake.

[2] Calculated from the analyses and intake of grass and compound feed (assuming no feed residues).

Table 3

Intake and utilisation of energy, urine and feces production in dry sows fed increasing amounts of freshly cut grass-clover and isoenergetic rations.

	Daily fresh grass supply, kg					P-Value		
	0	2	4	6	SEM	ANOVA	Linear	Quadratic
No. of sows	4	3	3	4				
Start body weight, kg	275	249	252	255	20	0.73	0.53	0.45
Body weight change, kg	2.6	2.1	0.3	2.9	1.9	0.74	0.91	0.37
Total GE intake, MJ/d	33.3^{b}	33.5^{ab}	34.3^{a}	34.1^{ab}	0.3	0.04	0.01	0.46
Total DE intake, MJ/d	26.7^{a}	26.3^{a}	25.7^{ab}	23.0^{b}	0.9	0.10	0.02	0.27
Total ME intake, MJ/d	25.7^{a}	25.3^{a}	24.7^{ab}	22.1^{b}	1.0	0.10	0.02	0.28
DM intake compound feed, kg/d	1.86^{a}	1.55^{b}	1.24^{c}	0.88^{d}	0.08	<0.001	<0.001	0.43
DM intake grass, kg/d	0.0^{d}	0.31^{c}	0.66^{b}	0.99^{a}	0.01	<0.001	<0.001	0.38
DIG DM intake from grass kg/d	0^{d}	0.21^{c}	0.45^{b}	0.71^{a}	0.008	<0.001	<0.001	0.03
GE intake from grass, MJ/d	0^{d}	5.7^{c}	12.1^{b}	18.3^{a}	0.24	<0.001	<0.001	0.41
DE intake from grass, MJ/d	0^{d}	4.0^{c}	8.5^{b}	12.3^{a}	0.16	<0.001	<0.001	0.01
ME intake from grass, MJ/d	0^{d}	3.8^{c}	8.2^{b}	11.8^{a}	0.23	<0.001	<0.001	0.67
Water intake, l/day	18.9^{ab}	23.4^{a}	9.11^{b}	14.3^{ab}	3.3	0.05	0.05	0.91
Urine, kg/day	7.61^{ab}	5.17^{b}	5.69^{b}	10.31^{a}	1.13	0.04	0.11	0.01
Feces, g DM/day	396^{b}	455^{ab}	447^{ab}	578^{a}	330	0.21	0.26	0.04

$^{a–d}$ = Least squares means with different superscript letters differ significantly ($P < 0.05$)

Table 4

Apparent total tract digestibility (ATTD) of dry matter (DM), organic matter (OM), nitrogen and energy of dry sows fed varying amounts of freshly cut grass-clover and isoenergetic rations.

	Daily fresh grass supply, kg					P-Value		
ATTD, %	0	2	4	6	SEM	ANOVA	Linear	Quadratic
DM (total ration)	80.8	78.0	75.5	74.7	1.7	0.09	0.02	0.59
OM (total ration)	79.8^{a}	76.5^{ab}	73.5^{b}	72.6^{b}	2.0	0.05	0.008	0.53
Nitrogen (total ration)	81.0	79.0	77.64	78.6	1.2	0.47	0.24	0.34
Energy (total ration)	80.4^{a}	78.3^{ab}	74.5^{b}	73.6^{b}	2.0	0.05	0.008	0.77

[a,b] Least squares treatment means with different superscript letters differ significantly ($P < 0.05$)

[1] Calculated value (Regression method)

[2] Calculated value (Differential method)

Table 5

Plasma metabolites in dry sows fed varying amounts of fresh grass-clover and isoenergetic rations.

Plasma metabolites	Daily fresh grass supply, kg					P-Value		
	0	2	4	6	SEM	ANOVA	Linear	Quadratic
Urea, mM	2.64^{d}	4.05^{abc}	3.59^{ab}	4.39^{a}	0.38	0.04	0.02	0.42
Glukose, mM	4.52	4.45	4.27	4.16	0.15	0.39	0.11	0.91
Lactate, mM	2.03	1.26	1.35	1.89	0.47	0.55	0.87	0.18
Creatinine, µM	159	143	148	125	12.1	0.26	0.26	0.02
NEFA, µM	81.8	33.5	37.4	84.8	27.0	0.39	0.92	0.09
Triglycerides, µM	222	251	298	285	27	0.20	0.06	0.44
Pipecolic acid, AU[1]	28.3^{d}	206.2^{c}	435.8^{b}	600.0^{a}	31.0	<0.001	<0.001	0.83
Bisnorbiotin, AU[1]	0.0^{d}	12.1^{c}	21.9^{b}	38.8^{a}	2.3	<0.001	<0.001	0.88

[1] Arbitrary Units x 1,000

[a–d] Least squares treatment means with different superscript letters differ significantly ($P < 0.05$)

Table 6

Nitrogen balance and urinary concentrations of creatinine and urea in dry sows fed varying amounts of fresh grass-clover and isoenergetic rations.

	Daily fresh grass supply, Kg					P-Value		
	0	2	4	6	SEM	ANOVA	Linear	Quadratic
N intake, g/d	43.6^{d}	50.3^{c}	58.5^{b}	65.2^{a}	3.80	<0.001	<0.001	0.0003
N in feces, g/d	8.3^{c}	10.4^{bc}	12.7^{ab}	16.8^{a}	1.53	0.003	0.003	0.49
N in urine, g/d	27.3^{bc}	33.0^{bc}	34.1^{b}	48.4^{a}	2.60	<0.001	0.001	0.49
N retention, g/d	6.5	6.9	9.7	0.3	2.73	0.47	0.46	0.23
Creatinine in urine, mM	8.02^{a}	10.04^{a}	9.41^{a}	3.43^{b}	1.46	0.05	0.06	0.024
Urea in urine, mM	75.3	142.0	130.4	69.3	25.8	0.18	0.80	0.041
N retention, % of intake	14.9	13.9	16.7	2.8	6.19	0.43	0.24	0.30

$^{a–d}$ Least squares treatment means with different superscript letters differ significantly ($P < 0.05$)

Figure 1

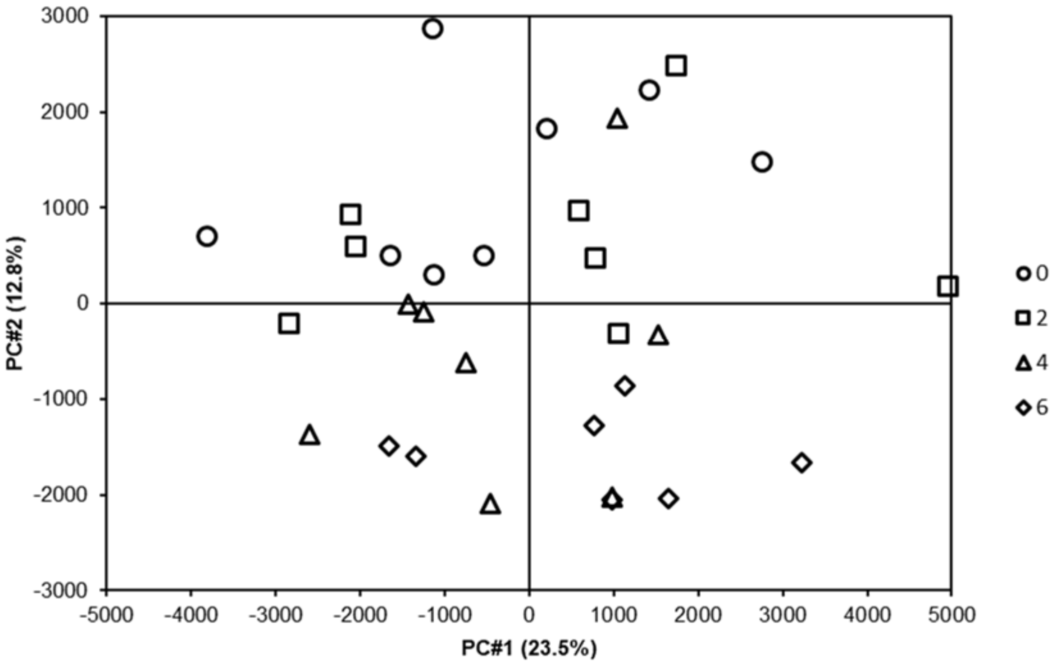

Figure 2

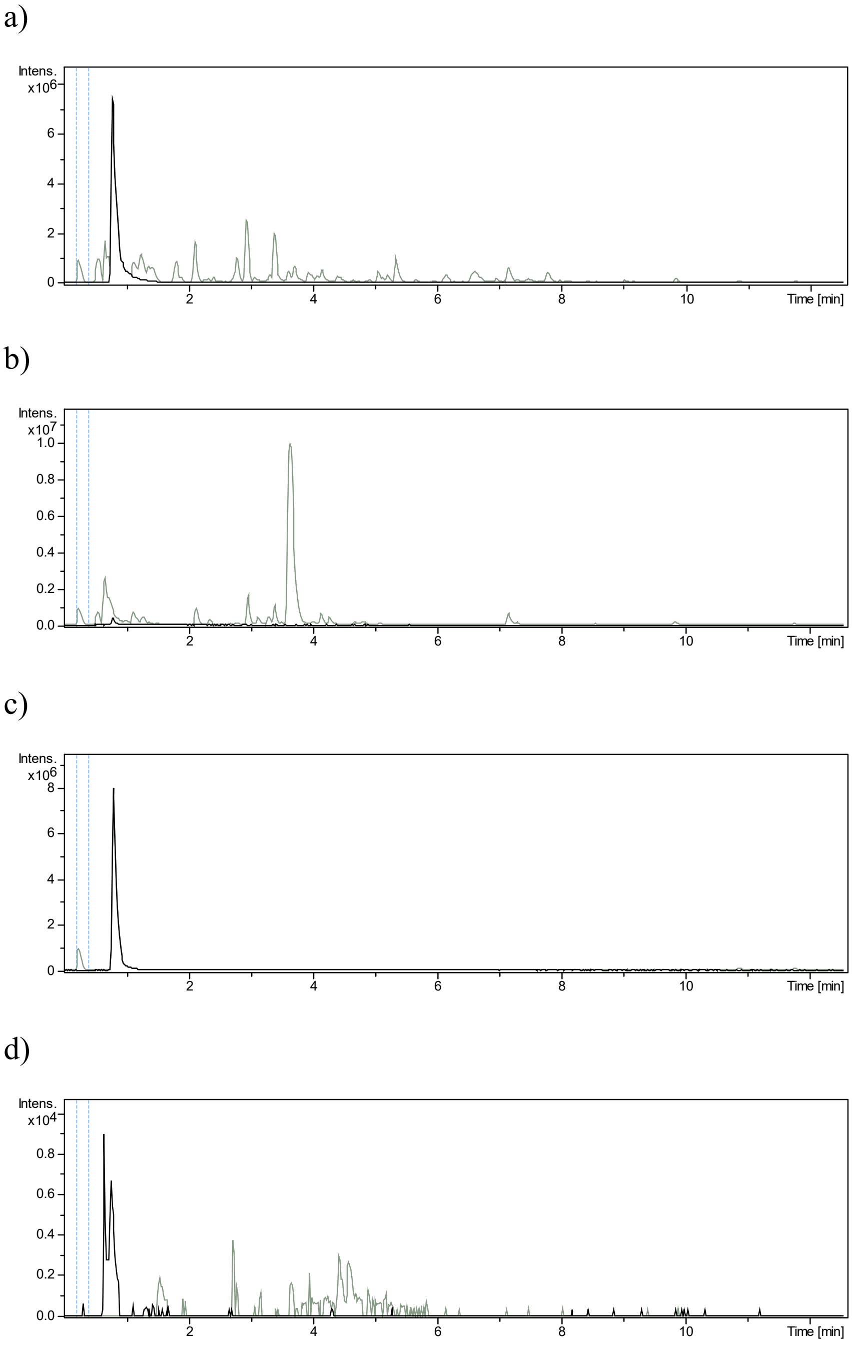
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Figure 3

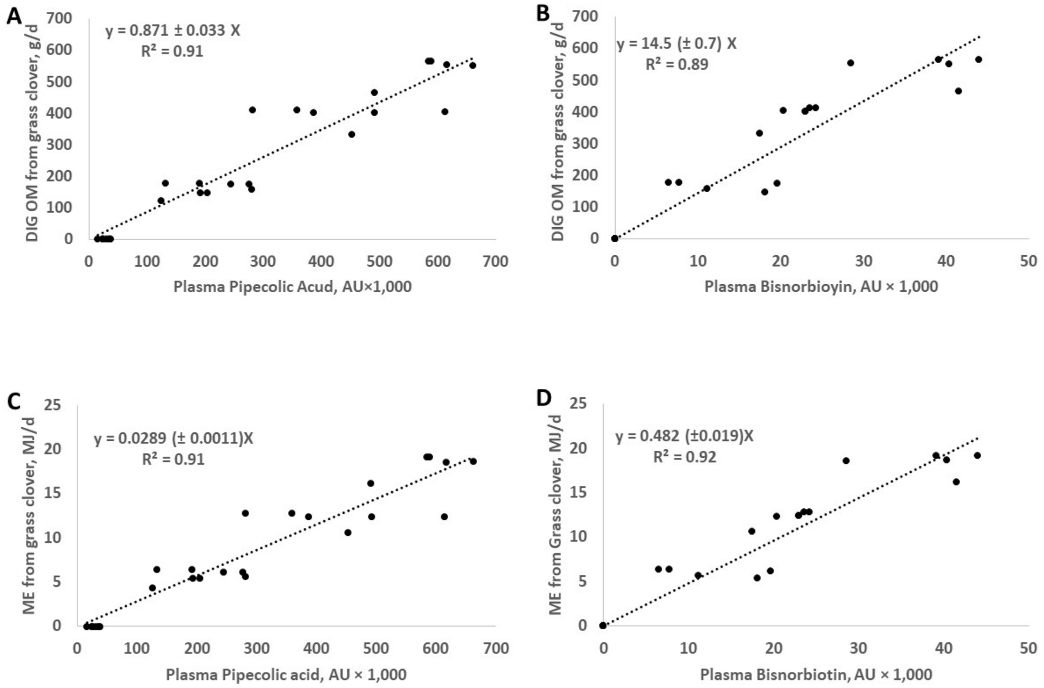

Figure legends

Figure 1. PCA scores plot showing clustering of the sow plasma metabolome by amount of grass consumed (0, 2, 4, or 6 kg of grass per day) using an unsupervised method in positive ionisation mode. The variances accounted for by the principle components are shown on the axes.

Figure 2. Representative base peak chromatograms (grey) with extracted ion chromatograms of m/z 130.0862 (black) of a) grass-clover (week 3) b) compound feed, and c) pipecolic acid (Sigma P2519) and d) extracted ion chromatogram of m/z 217.0686, tentatively identified as bisnorbiotin, in the compound feed (black) and grass-clover (grey) using LC/ESI-QTOFMS in positive ionisation mode

Figure 3. Apparent total tract digestible organic matter (OM) and metabolisable energy (ME) intake from fresh grass-clover/d as a function of plasma pipecolic acid or plasma bisnorbiotin in dry sows fed increasing fresh grass-clover and decreasing compound feed.

MANUSCRIPT II

Effect of reduced dietary protein level on energy metabolism, sow body composition and metabolites in plasma, milk and urine from gestating and lactating organic sows during temperate winter conditions

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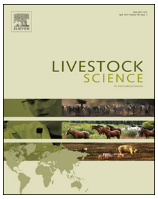

Effect of reduced dietary protein level on energy metabolism, sow body composition and metabolites in plasma, milk and urine from gestating and lactating organic sows during temperate winter conditions

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ABSTRACT

Energy spent on thermoregulation and the opportunity for increased locomotive activity increases the energy requirements of outdoor relative to indoor housed sows, whilst their protein requirement most likely is comparable on a daily basis. The purpose of this study was to quantify the energy needed for maintenance, maternal retention, milk production, thermoregulation and increased locomotive activity in organic sows.

A total of 47 gilts (Landrace x Yorkshire; 190 kg at insemination) were reared outdoor under organic conditions for five months during winter. To study dietary effects of protein, gilts were fed one of two iso-energetic compound feeds, where dietary protein differed by 12%. Gilts had ad libitum access to grass clover silage and were fed similar amounts of metabolisable energy (ME) from compound feed equivalent to the energy recommendations for indoor sows + 15% in both groups.

Collection of plasma and urine was performed on d60 and d100 of gestation and plasma, urine and milk was collected on d5, d20 and d40 of lactation. On all collection days, sows and piglets (n = 635) were weighed individually, sows were back fat scanned and heartrate and locomotive activity was registered with a tracking system. Sow body composition was estimated using the deuterium dilution technique.

Live weight and back fat thickness were not affected by the dietary protein level, neither was the number of total born, still born, piglet birth weight or piglet weight gain until weaning at seven weeks (14.5 kg).

There was no effect of protein level on locomotive activity. Milk yield peaked with 12.9 kg/d around d20. In total, 58% of the gross energy intake was associated with milk production at d20 including heat. Milk energy output was 69 MJ ME/d at peak lactation at d20. Sows fed the low protein compound feed had a lower milk yield from d20 to d40 as compared with control fed sows (8.0 vs.10.3 kg/d; $P < 0.05$).

In conclusion, the daily feed intake was clearly insufficient in early lactation, and sows lost > 1 kg of body fat/d from d5-d20. The daily protein- and amino acid requirements were met during pregnancy, also when sows were fed the low protein compound feed, but the low protein diet supplied insufficient standardised ileal digestible lysine during lactation and this compromised the milk production. The total energy requirement of high yielding first parity outdoor sows during a mild winter was found to be ~ 68 MJ ME/d in gestation and ~153 MJ ME/d at peak lactation.

1. Introduction

Access to pasture in organic livestock farming comply well with the organic principles of allowing animals to perform natural behavior and with consumer expectations. However, when intensively managed as e.g. in Northern Europe, pasture systems are characterized by high risk of nitrogen (N) losses in terms of nitrate leaching (Manevski et al., 2018), ammonia volatilization (Sommer et al., 2001) and denitrification (Petersen et al., 2001). These losses contribute to eutrophication, acidification and global warming, and compromise the organic principles of efficient nutrient utilization. Organic sows have a 34% higher daily feed consumption than indoor sows (Hansen, 2018), and it is not allowed to balance the amino acid pattern with crystalline amino acids. In order to maintain amino acid supply, organic pig diets therefore must contain increased crude protein, which increases nitrogen excretion into the environment. Moreover, organic sows on

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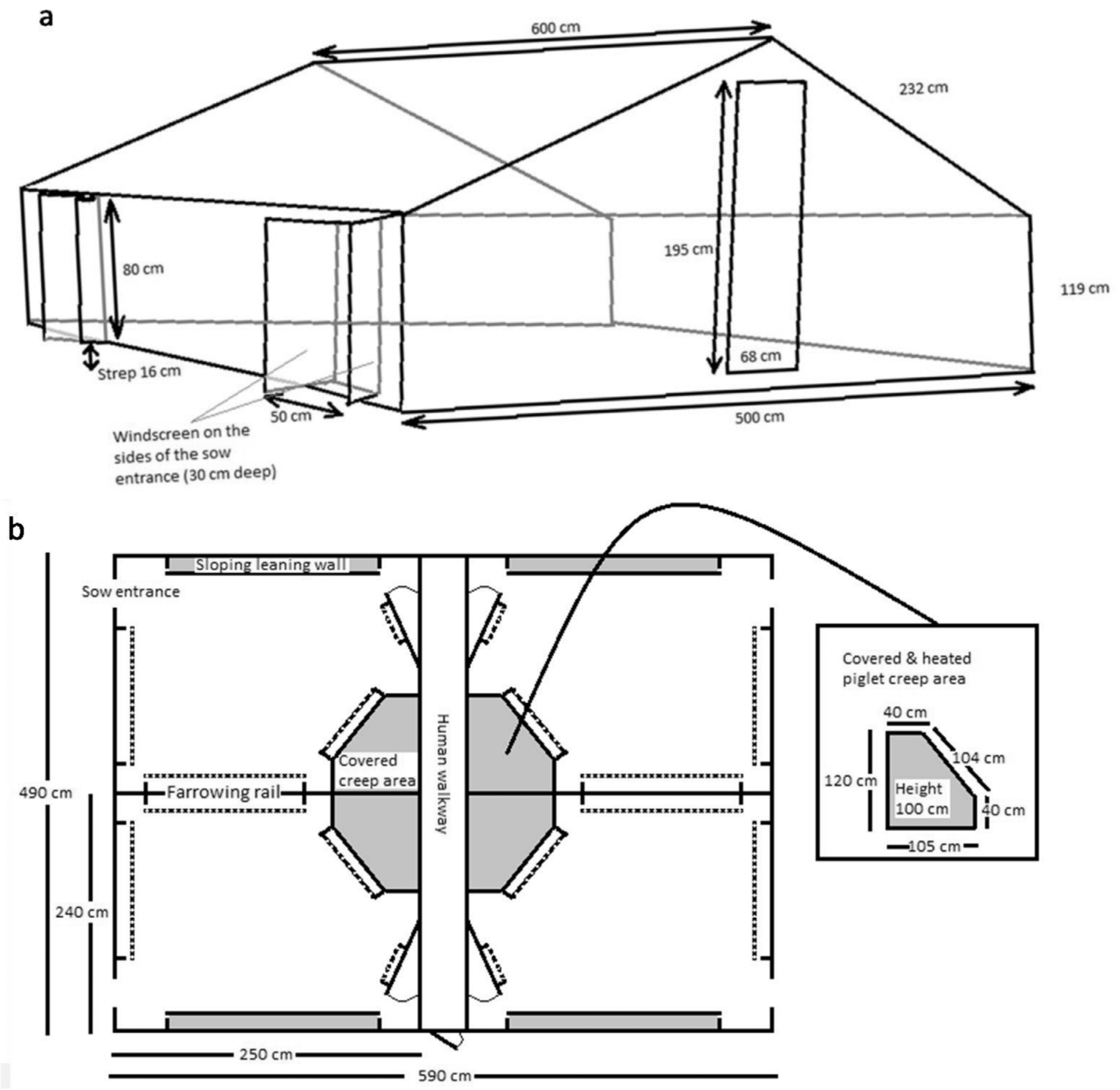

Figure 1. Sketch of the communual hut (Center for Outdoor Livestock Production, Marsvej 43, DK-8960 Randers, Denmark). Seen from the outside (panel a) and from the inside (panel b). Each hut had room for four individual housed sows. Each piglet creep area was heated with an eHEAT PLUS heater (Orbital A/S, DK-6900 Skjern). (Schild et al., 2019).

pasture may also consume protein from silage and grass. Excess dietary protein reduces feed efficiency (Pedersen et al., 2019), because it is costly to synthesize urea and this energy is lost through urine. As there is no set of nutrient recommendations for organic sows, the farmers have to adapt those from conventional sows - even though the energy requirements and feed intake differ substantially between these production systems. Danish nutrient recommendations are expressed relative to dietary energy (Tybirk et al., 2016). However, the protein-to-energy ratio formulated for conventional sows is most likely not optimal for organic production, since organic sows in pasture systems live under varying weather and temperature conditions, spend energy on thermoregulation, and have the opportunity for increased locomotory activity as compared with conventional indoor housed pigs. These aspects increase the energy requirements of outdoor pigs, whilst their protein requirement most likely is comparable on a daily basis with that of indoor sows (Close and Poornan, 1993; Jakobsen and Hermansen, 2001). The theoretical requirements for protein and energy in organic pigs have been calculated in several studies (Close and Poornan, 1993; Fernandez et al., 2006; Jakobsen and Danielsen, 2006) and the additional energy requirement of outdoor pigs compared to indoor has been estimated to be approximately 15% under Northern European conditions (Edwards, 2003), but the extra energy requirement has not been empirically quantified.

It is of major relevance to tailor organic sow feed to the organic conditions to supply energy and protein in accordance with the animal requirements and avoid excessive supply. The aim of this study was to contribute to a better understanding of the energy and protein requirements of organic sows with pasture access by quantifying energy needed for heat (maintenance, maternal retention, foetal growth and milk production), thermoregulation and increased locomotory activity.

We hypothesized, that increasing the daily energy supply by 15% and lowering the protein content in the diet by 13% below the recommended level (for indoor sows) would improve energy utilization without compromising sow productivity and at the same time reduce the environmental load form the production and the need for bought in organic protein.

2. Methodology

The animal experimental procedures were carried out in accordance with the Danish Ministry of Justice, Law no. 253/08.03.203 concerning animal experiments and care and license issued by the Danish Animal Experimental Inspectorate, Ministry of Food, Agriculture and Fisheries, the Danish Veterinary and Food Administration. The animal

Table 1
Ingredients and expected dietary contents in protein restricted mixture, gestation and lactation diets[1]

Ingredients, g/kg	Protein restricted mixture	Gestation diet (Control)	Lactation Diet (Control)
Wheat			189
Barley	770	330	300
Rye		200	100
Oat	200	150	70
Corn		50	25
Peas		50	50
Wheatbran		35	79
Oatbran		50	
Dried grass meal		20	
Soybeancake		47	107
Rapeseedcake		43	42
Fish meal			10
Calcium carbonate	15	14	12.5
Sodum chloride	5	4.4	4.9
Monocalciumphosphate	9	6.1	7.7
Vitamin and mineral mixture[2]	1	1.1	3.3
ME, MJ/kg[3]	12.38	12.26	12.77
Crude protein, g/kg	81	110	144
Lysine, g/kg	3.3	5.36	7.29
SID Lysine g/kg[3]	2.23	4.15	5.95

[1] Low protein gestation diet (compound feed) were offered by mixing 30% of the protein restricted mixture and 70% of gestation diet for sows during pregnancy until d 108. Low protein lactation diet (compound feed) were offered by mixing 30% of the protein restricted mixture and 70% of lactation diet for sows from day 109 in pregnancy until weaning at d 49.

[2] Pr kg: 8,000 IU vitamin A; 800 IU 25-hydroxy vitamin D; 54,600 mg DL-alphatocoferol; 2,000 mg vitamin B1; 5,000 mg vitamin B2; 3,000 mg vitamin B6; 20.0 mg vitamin B12; 2,000 vitamin K3; 15,000 mg D-pantothenic acid; 20,000 mg niacin; 400 Biotin; 1,500 mg folic acid; 80,000 mg iron (FeSo4); 15,000 mg copper (CuSO4); 40,000 mg manganese (MnO); 2,000 mg iodine (Ca (IO3)2); 100,000 mg zink (ZnO); 300 mg selenium (Na2SeO3).

[3] The two dietary strategies were formulated to be isoenergetic based on the Danish feed evaluation system (Danish Feed Units) which is a potential physiological energy system closely related to the NE system (Patience, 2012). ME refer to metabolisable energy, and SID lysine refer to standardised ileal digestible lysine.

experiments comply with the ARRIVE guidelines and were performed in accordance with the legislation for the protection of animals used for scientific purposes (EU Directive 2010/63/EU for animal experiments). Rearing, housing and sampling, were in compliance with Danish laws for human care and use of animals in research (Animal Experimental permit No. 2013-15-2934-00961).

Forty-seven gilts thirty-two weeks of age and weight 197 kg (SD = 3.7 kg) were inseminated twice in the second oestrus, with semen from three known Danbred Duroc boars. Twenty-two gilts were DanBred crossbred LY-gilts from a Danish SPF herd and the remaining twenty-five gilts were Topigs Norsvin L x Large White (TN70) from a Norwegian herd (impact of genetic breed will be reported elsewhere). Insemination was done from the beginning of august to the end of October 2016. The gilts were randomly assigned to one of two dietary protein strategies consisting of a standard organic feeding regimen (**Control, n = 24**) or a low dietary protein compound feed (**Low protein, n = 23**). Compound feeds were aimed to be iso-energetic, and Control and Low protein gestation compound feeds contained 12.8 and 12.9 MJ metabolisable (ME) per kg feed, respectively. Control and Low protein lactation compound feeds contained 12.7 and 12.8 MJ ME/kg, respectively. Control gestation- and lactation compound feed contained 129 g and 148 g crude protein per kg DM. Low protein gestation- and lactation compound feed contained 114g and 130 g crude protein/kg DM, respectively.

2.1. *Housing and rearing conditions*

The gilts were reared under organic conditions outdoor in the winter of 2016-2017 at Aarhus University, Denmark. Sows began farrowing in the end of November 2016 until medio February 2017. Thus, the experimental data obtained from d 60 of gestation to d 49 of lactation was collected in the period from early October 2016 to late March 2017. Gilts were reared in dietary groups in four 40 m × 100 m paddocks from day 25 of gestation. Two large gestation huts (3 × 6 m) were located in each paddock. The gilts were moved to individual farrowing paddocks (18 × 25 m) ten days prior to expected farrowing of the first sow. All gilts had access to a farrowing hut. Twelve gilts per batch were housed in a prototype communual farrowing hut (Figure 1; Schild et al., 2019). Each prototype had room for four individually housed sows. Each of the four compartments measured 2.4 m × 2.5m and included a heated piglet creep area and an insulating rubber mat in the bottom of the creep. The remaining gilts were housed in A frame farrowing huts (L:220 cm, W_{bottom}:180 cm, W_{top}:105 cm, H: 105 cm). All huts had a ventilation opening in the back (measuring 33 × 16 cm). These were opened depending on outdoor temperature. Winter strips were placed at the entrance to each farrowing hut to control air circulation into the hut. A roller was placed in the entrance of all huts between farrowing and day 10 of lactation to prevent piglets from leaving the hut. All huts were supplied with a bedding of chopped barley straw. In autumn and spring approximately 10 kg/m^2 and in winter 13 kg/m^2.

The pasture area was sown in spring 2016 with two commercial grass clover mixes (ForageMax55 and ForageMax56, DLF Trifolium, Roskilde, Denmark). In the gestation paddocks it consisted of 10% Trifolium Repens (white clover, Rivendel), 50% Lolium Perenne (perennial ryegrass, Humbi 1), 15% Lolium Perenne (perennial ryegrass, Masai) and 25% Festuca Rubra (red fesque, Gandolin). The grass clover in the farrowing paddocs was 10% Trifolium Repens (white clover, Rivendel), 30% Lolium Perenne (perennial ryegrass, Humbi 1) and 60% Festuca Rubra (red fesque, Gandolin). Health conditions were monitored daily and if necessary, animals were treated in compliance with normal procedures. Animal health was monitored by the herd veterinarian.

2.2. *Diets and feeding*

Organic gestation and lactation compound feed based on barley, rye, oat and rapeseed cake were formulated to ensure the supply of macronutrients recommended for Danish indoor housed gestating and lactating sows, respectively (Tybirk et al., 2016). These compound feeds were fed to control sows. In addition, a protein restricted mixture based on barley and oats was formulated, to dilute the protein content of the control compound feed. A mix of the control and the protein restricted mixture was fed to the low protein sows (30% protein re-stricted mixture and 70% control compound feed). Ingredients and chemical compositions of the experimental diets are shown in Tables 1 and 2. The recommendation for lysine was not fully reached in the lactation diets, but undersupply was accepted to avoid a very high crude protein content. To meet the extra demand for thermoregulation and locomotory activity, both treatment groups were fed 15% more energy than the recommended feeding curve applied for indoor sows from the Danish Pig Research Centre (Figure 2). Compound feed was manufactured by Vestjyllands Andel (Videbæk, Denmark) eight times throughout the study with approximately 8 week intervals. From each batch, 5 kg samples were taken of each compound feed during the production process. Each sample was split into subsamples using a 32-slot riffle sample divider. In total, two subsamples per diet were analyzed in duplicates at a commercial feed testing laboratorry (Eurofins Steins Laboratory A/S, Vejen, Denmark) following the European Commission Directives [EC] 64/1998 and [EC] 152/2009. In gestation, gilts were daily fed two equally sized portions with an eight hour interval

Table 2
Chemical analysis of gestation and lactation compound feed and grass clover silage.

	Gestation	Lactation			Grass clover silage
	Control	Low protein	Control	Low protein	
Dry matter, g/kg feed	877	875	864	865	292
Chemical composition, g/kg					
Crude protein (N*6.25)	113	100	128	112	41
Fat	33	33	35	33	1.0
Starch	403	424	393	417	
Cellulose	61	57	38	41	
Total non starch polysaccharides	175	170	140	145	
Insoluble non starch polysaccharides	134	132	104	111	
Klason lignin	47	44	36	36	
Ash	42	39	45	42	34
Dietary fibre	223	214	176	182	
Gross energy (MJ/kg)	16.17	16.08	15.97	15.92	
FUsow/kg[1]	0.99	1.0	0.99	0.98	0.60
ME (MJ/kg)[2]	12.3	12.2	12.8	12.7	6.44
Calcium, g/kg	7.8	7.4	8.3	7.8	7.2
Phosphor, g/kg	5.0	4.7	6.0	5.4	3.3
Amino acids, g/kg					
Lysine	6.32	4.61	6.80	6.13	1.83
Methionine	2.00	1.53	2.11	2.03	0.62
Cysteine	2.68	2.08	2.45	2.42	0.25
Threonine	4.73	3.57	5.12	4.67	1.70
Isoleucine	4.77	3.59	5.15	4.77	1.71
Leucine	9.21	6.97	9.68	9.01	3.03
Histidine	3.08	2.29	3.25	3.01	0.56
Phenylalanine	5.84	4.49	6.32	5.95	1.77
Valine	6.18	4.79	6.46	6.05	2.26
Alanine	5.92	4.44	6.04	5.69	3.23
Arginine	8.00	5.71	8.36	7.64	0.93
Asparaginacid	11.01	7.93	11.75	10.54	3.06
Glutamineacid	23.83	18.85	26.20	24.95	3.40
Proline	9.10	7.47	9.50	9.32	3.07
Serine	6.09	4.50	6.51	5.95	1.65
Glycine	5.95	4.49	6.14	5.77	1.98

[1] Danish Feed Units for sows according to the Danish energy evaluation system, which is closely related to the NE system (Patience, 2012).

[2] Content of metabolisable energy (ME) was calculated from feed units for gestating and lactating sows according to Theil et al. (2020).

(morning and afternoon). Individual feed residues were weighed 30 minutes after each meal. Diets were supplied individually in stainless steel feeding stalls during gestation. In lactation, gilts were individually fed in covered feeders protecting the feed from rain, birds and to some extends also the piglets (Sostub crip, Domino, Tørring, Denmark) once a day at 10.00 am. Piglets were offered a supplemental commercial weaning feed from 14 days of age outside the paddocks, where the sows could not reach it. The intake of weaning feed was not measured. Feed residues were collected from individual sows on a weekly basis. All animals had ad libitum access to grass clover silage and water. Fresh silage was provided in a hayrack (gestating paddocks) or in troughs (lactation paddocks) every second day, and leftovers were removed. The voluntary silage intake was low but unknown. Silage was not chopped and samples were collected every two weeks, pooled and stored at -20°C until analysis.

2.3. Recordings and sampling of gilts

Weighing, backfat scanning, application of locomotive activity gauges, D_20 enrichment and blood samplings of gilts are described below and were performed on day 60 and 100 in pregnancy and day 5, 20 and 40 in lactation. Milk sampling was performed on day 5, 20 and 40 in lactation.

Sows were caught before sunrise in the huts. Piglets were removed from the sow for approximately 30 minutes and individually weighed. Sows were weighed on a walk-in scale. Backfat was measured using a SonoGrader ultrasound scanner on the right side in the P2 position, 65 mm from the midline at the last rib. The sow were restrained with a snout brake and milk samples were collected from a standing position. To induce milk letdown, sows received an intravenous injection of 2 mL oxytocin (10 IU/ml; Leopharma, Ballerup, Denmark) via an obtional ear vein. A total of 45-50 mL milk was manually obtained from three to five teats of each sow. Milk samples were filtered through gauze and stored at –20°C until further analysis.

Locomotory activity gauges were tightened around the gilts belly (Polar Team Pro GPS tracking system, Polar, Ballerup, Denmark) to record the distance covered by the sow in the paddock and to register heart rate (Figure 3). Data was recorded during the daytime during a period of nine to twelve hours depending on battery time. Walking was used as a common term for walking/running/sprinting/stamping as the sows showed primarily slender walking.

On each sampling day, blood was sampled by jugular vein puncture in 10 ml Na – heparinized tubes (Greiner BioOne GmbH, Kremsmünster, Austria) from sows in a standing position restrained with a wire snare. Samples were stored on ice until centrifugation (3000 rpm at -4°C for 12 min). Plasma was immediately harvested and stored in 1.5 ml micro centrifuge tubes at -20°C and -80°C until analysis. Following blood sampling, gilts were enriched with deuterium (D_2O; 0.2 g 10% solution administered per kg live weight) in the neck (I.M.) using a 18G needle. A urine sample was collected the day after D_2O enrichment. The huts were closed before sunrise to avoid the sows from urinating. When sows were allowed to leave the hut (at sun rise), a urine sample was collected from the first voluntary urination by a trained staff member with a 200 ml collection pot directly from the sow. The urine sample was taken in the middle of the excreation, and it did not seem to bother the sows, as they normally did a full emptying of the bladder with several liters of urine. Most sows urinated within two hours after sunrise. The pH in urine was measured using a pH-meter and subsamples were stored at –20ºC until further analysis. The total D_2O space was estimated based on the D_2O concentration in reference blood, the D_2O concentration in urine after enrichment and the backfat and BW of the sow as described by Theil et al. (2002). Based on the measured D_2O space, the total body pools of protein, fat and ash were calculated according to (Rozeboom et al., 1994).

All farrowings were monitered by use of cameras *IPCHDBW4100EP-0360B, Dahua Technology Co., Broadway, UK. Individual cameras had wide angle lenses and were placed in every A frame hut or above each pen in the prototype communal huts, so sows were visible when they were inside their pen/huts. Recordings were saved digitally and analysed using S/VIDIA Client MegaPixel *M. Shafro and Co., Riga, Latvia.

The video recordings provided exact information on the number of stillborn, liveborn, crushed piglets and the exact time of birth.

All piglets were weighed individually and ear tagged the day after birth. Piglets that were removed from the litter during the experiment were recorded and accounted for, as well as piglets that voluntarily shifted from one sow to another due to the free system conditions. Piglets were individually weighed again on day 5, 20, and 40 in lactation and litter weight gain and litter size were used for estimation of milk yield as described by (Hansen et al., 2012). If sows gave birth to a surplus of piglets relative to the number of functional teats, litter equalisation was done once within three days after farrowing. Piglets were always added to an evenly aged or younger litter. Dead piglets were collected once a day and date of death and sow number was recorded. Piglets weighing less than 700 g. were considered non-viable and euthanized by blunt force trauma. At five days of age, male piglets were castrated.

The outdoor temperature was measured every hour throughout the study by a weather station placed in the middle of the gestation field.

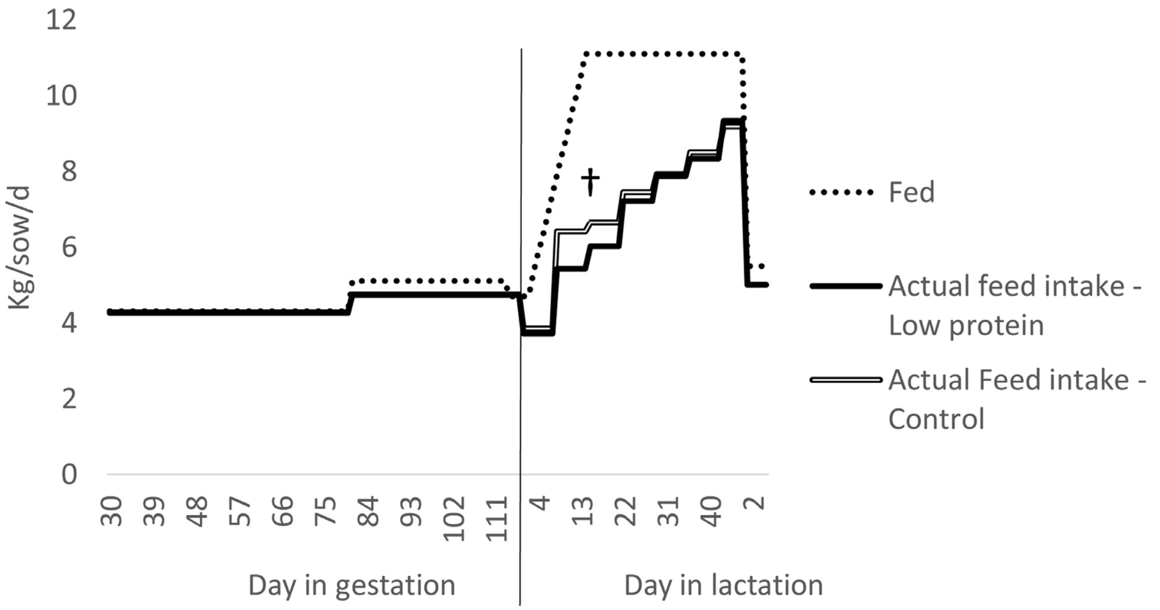

Figure 2. Feeding curve and actual feed intake in kg/d in gestation and lactation. The feeding curve is based on the recommended daily energy intake for indoor sows plus additional 15% feed (SEGES, 2016). Diets were fed to sows from the day after insemination (day 1 in gestation) until weaning (days 49 ± 3). †Indicate a statistical difference in compound feed intake between sows on Control and Low protein strategy in the period d5-d20 (P = 0.08).

One sow was removed from the experiment due to a diagnostic of lameness shortly after farrowing. Another two sows were excluded as they were not pregnant.

3. Analytical methods

Apart from amino acid analyses, chemical analyses of compound feed, silage, urine, milk and plasma were performed in duplicate. The DM content of all feed samples was determined by oven drying at 103°C. Ash was determined by oven drying at 525 °C for six hours.

Compound feed, grass and silage gross energy (GE) was determined with a bomb calorimeter (Parr 6300 Instrument Company, Moline, Illinois, USA). Starch and non-starch polysaccharides (NSP) were analyzed as described by (Knudsen, 1997).

The crude protein content was calculated as nitrogen × 6.25 as reported by (Eggum, 1970). The nitrogen content of urine was determined by the modified Kjeldahl method (Method 984.13; AOAC Int, 2000) using a KjeltecTM 2400 (Foss, Hillerød, Denmark)

Amino acids (AA) were analyzed in experimental diets, grass clover and grass clover silage samples following hydrolyzation for 23 hours at 110°C with (Cys and Met) or without (Arg, His, Ile, Leu, Lys, Phe, Tyr, Val) performic acid oxidation, and AA were separated by ion exchange chromatography and quantified by spectro-photometric detection after ninhydrin reaction.

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

The chemical composition of milk for DM content, protein, casein, lactose, and fat was analyzed in triplicate through infrared spectroscopy using a Milkoscan 4000 instrument (Foss Milkoscan, Hillerød, Denmark).

4. Calculations and statistical analyses

Milk yield was predicted based on average litter weight gain and litter size in the two periods d1 to d20 and d20 to d40 by use of a mathematical model developed to quantify milk yield of conventional sows (Hansen et al., 2012). The energy concentration in milk was calculated based on energy values (39.8 kJ/g fat, 23.9 kJ/g protein, and 16.5 kJ/g lactose; Weast et al., 1984). The output of energy in milk as the product of milk yield multiplied by energy concentration.

The 24-hour heart rate was estimated as an average of the recorded heart rates during daytime. Daily distance covered between sunrise and sunset was estimated using the recorded distance adjusted for the ratio between time from sunrise to sunset and the period of actual recordings.

Body pools of protein and fat were estimated from live weight, D_2O space and BF measurements according to the model developed by (Rozeboom et al., 1994) for LY gilts as:

$$\text{Protein pool (kg)} = 1.3 + 0.103 \times \text{BW} + 0.092 \times \text{D}_2\text{O space} - 0.108 \times \text{BF}$$

$$\text{Fatpool (kg)} = -7.7 + 0.649 \times \text{BW} - 0.610 \times \text{D}_2\text{O space} + 0.299 \times \text{BF}$$

Total heat production (HE) was estimated from the 24 hour-mean heart rate using the following equations (Krogh et al., 2018)

$$\text{Gestation:HE, MJ/d} = 0.323\text{MJ/bpm/d} \times \text{Heart rate, bpm} - 2.4\text{MJ/d}$$

$$\text{Lactation:HE, MJ/d} = 0.118\text{MJ/bpm/d} \times \text{Heart rate} + 26.7\text{MJ/d}$$

The HE for maintenance was estimated as 0.459 MJ/$kg^{0.75}$ × metabolic live weight for pregnant sows (Theil et al., 2004) and 0.482 MJ/$kg^{0.75}$ × metabolic live weight for lactating sows (Theil et al., 2002).

The HE associated with milk production was estimated from the estimated energy output in milk as: HE milk, MJ/d = Milk energy output MJ/d /0.78 – Milk energy output MJ/d.

In gestation, HE for retention was calculated as: HE retention, KJ/d = [(daily protein gain, g/d × 23.9 KJ/g)/0.60) - (daily protein gain, g/d × 23.9 KJ/g)] + [(daily fat gain, g/d × 39.8 KJ/g)/0.80) - (daily fat gain, g/d × 39.8 KJ/g)] (Theil et al., 2020) .

Retained energy in gestation was calculated as $RE = RE_{protein} + RE_{fat}$

Figure 3. Illustration of method for estimation of sow locomotory activity with the Polar Team Pro equipment. The activity gauges were wrapped around the sows belly and gel for ultrasound gestation scan was used to create permanent contact with the device. The system is originally developed for football players and provides heartrate, an activity map and covered distance in different predefined velocity zones.

$$RE_{protein}, KJ/d = (\text{Difference in protein pool between end and start, g}) / \text{period, days} \times 23.9 KJ/g$$

$$RE_{fat}, KJ/d = (\text{Difference in fat pool between end and start, g}) / \text{period, days} \times 39.8 KJ/g$$

Retained energy could not be distributed into foetal and maternal growth during pregnancy using this technique.

On the basis of (Close and Poornan, 1993), the ME expenditure for locomotive activity is calculated as: ME locomotive, KJ ME/d = (7 kJ/kg body weight/d × sow body weight, kg × covered daily distance, km)/0.8, assuming a net efficiency of energy utilization of 80%.

In lactation, ME supplied from fat mobilization was calculated as; daily fat gain, g × 39.8 KJ/g

Energy for thermoregulation is calculated as 18.8 kJ ME/kg metabolic body weight/d/24 h average air temperature 30 cm above the ground in°C under 18°C (Close and Poornan, 1993) (Verhagen et al., 1986)),

Sow weight, backfat, heart rate, daily distance, urine and plasma metabolites recorded repeatedly (60 and 100 in gestation and on day 5, 20, and 40 in lactation) were analyzed using the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + {}_k + t_l + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the observed trait, μ is the overall mean of the observations, α_i is the main effect of the dietary regimen (i= control, low protein), β_j is the effect of day in gestation or day in lactation (j= 60, 100, 5, 20 or 40), $(\alpha\beta)_{ij}$ is the interaction between diet and day in gestation or day in lactation, γ_k is the fixed effect of breed (k = DanBred, Topigs Norsvin), t_l is the random effect of gilt (k= 1, 2, 3, …, 47) to account for repeated measurements within sow and ε_{ijkl} is the residual random components.

Litter size, piglet weight and milk chemical composition were analyzed by the same model, but with only day 5, 20 and 40 in lactation being available for these parameters. Similarly, compound feed intake, lysine intake, ME from compound feed, liveweight gain, backfat gain and protein gain were analyzed with a similar model except days were replaced by four periods within the reproductive stage (1: d60-d100, 2: d100-d5, 3: d5-d20 and 4: d20-d40).

Statistical analyses were performed using the MIXED procedure of the Statistical Analysis System (SAS; version 9.4), Significant values were considered if $P < 0.05$ and tendencies were accepted at $P \leq 0.12$.

5. Results

5.1. Energy and protein intake

The analyzed protein content of the low protein gestation and lactation compound feeds were 12% lower than the control compound feed.

The daily feed intake followed the supply in gestation, but sows consumed only 75% of the supplied feed on a daily basis in early and peak lactation (Table 3). The ME intake was similar in the two groups in gestation, but between day 5 and 20 in lactation, there was a tendency to a greater feed intake in the control group (6.40 kg/d vs. 5.6 kg/d SEM 0.39, P= 0.08). The control gilts received 7 g more standardised ileal digestible (SID) lysine per day than the low protein group in this period (34.5 g/d vs. 27.2g/d, P=0.04; Figure 4). There was no effect of reduced dietary protein level on mean age at farrowing (342 and 339 days for low protein and control treatment, respectively; P = 0.65) or on live born, birthweight, piglet daily gain, litter weight or piglet mortality (Table 4).

There was an interaction between dietary treatment and reproductive stage on sow live weight loss due a greater live weight loss d 5-20 for low protein sows as compared with control fed sows. Back fat thickness was not affected by the dietary protein level. In lactation, daily ME intake increased from 66.4 MJ/d on day 5-20 to 85.5 MJ/d in the period from d20 to d40 (P=0.001). The SID lysine intake increased from 31.2 g/d in early lactation to 40.3 g/d in late lactation (P=0.001). In the period from d60 to d100 in gestation, sows gained 40.2 kg and 1.6 mm backfat. From d101 in gestation to d40 of lactation the sows lost 53.7 kg live weight (including conceptus) and 6.9 mm back fat. In late gestation, the gilts weighed on average 242 kg and had a mean backfat thickness of 19.3 mm. After 40 days of lactation, the sows in both groups had a mean backfat thickness of 13 mm and a mean live weight of 190 kg.

The total heat production estimated from heart rate increased from 30 MJ/d on day 60 in gestation to 39 MJ/d in early lactation and peaked at 42 MJ/d at d 40 of lactation (P=0.001). There was an increase in ME for maintenance from late gestation to early lactation (26.1 MJ/d vs. 28.2 MJ/d; P=0.002). The maintenance requirements dropped slightly during lactation and amounted to 27.5 MJ/d on day 20 and 26.1 MJ/d on day 40 of lactation. Heat production associated with milk production was 11.4 MJ/d in early lactation and peaked on day 20 at 17.7 MJ/d, whereafter it decreased to 11.8 MJ/d on day 40 (P=0.001).

5.2. Locomotive activity

Sows daily distance travelled was 2.78 ± 0.32 km/d in mid gestation and 2.46 ± 0.30 km/d in late gestation. On d5 in lactation, the daily distance travelled was 0.64 ± 0.12 km/d, while it was 1.54 ± 0.23 km/d and 1.68 ± 0.58 km/d on day d20 and d40 in lactation, respectively. The estimated daily energy expenditure for locomotive activity based on the travelled distances did not depend on protein level (P>0.05).

5.3. Plasma and urine

There was no overall effect of dietary protein level on plasma glucose, urea or creatinine (Table 5). Plasma lactate was higher in control sows than in protein restricted sows on d100 in gestation (P=0.02) and there was a similar tendency on d5 (P=0.08) and d40 (P=0.07) of lactation (Figure 5).

There was an interaction between plasma urea and the reproductive stage (P=0.11). On d100 in gestation, the control group had 3.1 mM plasma urea and the low protein group had 2.7 mM plasma urea

Table 3

Compound feed supply, energy- and SID lysine intake and retention/mobilisation of body pools in organic 1st parity sows fed either control og low protein strategy. Control gestation- and lactation compound feed contained 129 g and 148 g crude protein per kg DM. Low protein gestation- and lactation compound feed contained 114g and 130 g crude protein/kg DM, respectively.

	Reproductive stage, d[1]					Protein level			P-value		
	60-100	100-5	5-20	20-40	SEM	Control	Low	SEM	Stage	Protein level	Protein × stage
Compound feed supply, kg/d	4.5^{d}	4.9^{c}	8.6^{b}	9.9^{a}	0.08	7.0	6.9	0.12	<0.001	0.62	0.04
Compound feed intake, kg/d	4.5^{c}	4.2^{d}	6.0^{b}	7.8^{a}	0.39	5.7	5.5	0.53	<0.001	0.84	0.08
ME intake from compound feed, MJ/d[2]	54.47^{c}	53.00^{c}	66.38^{b}	85.56^{a}	4.61	65.64	64.17	6.29	<0.001	0.89	0.13
SID Lysine intake compound feed, g/d[2]	21.1^{c}	18.9^{c}	30.9^{b}	40.1^{a}	2.17	30.2	25.3	3.0	<0.001	0.37	0.04
Liveweight gain, kg	40.2^{a}	-15.7^{b}	-21.5^{c}	-16.5^{b}	3.1	-3.8	-2.6	3.86	<0.001	0.85	0.02
Backfat gain, mm	1.63^{a}	-0.98^{b}	-3.26^{c}	-2.63^{c}	0.52	-1.50	-1.12	0.43	<0.001	0.61	0.63
Protein gain, g/d	161^{a}	-210^{b}	-109^{b}	-126^{b}	34	-82	-59	24	<0.001	0.49	0.90
Fat gain, g/d	242^{a}	-339^{bc}	-1036^{c}	-120^{ab}	2.2	-4.62	-3.45	2.20	<0.001	0.71	0.90

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

[1] 60-100 covers day 60 to day100 in gestation. 100-5 covers day 100 in gestation to day 5 in lactation. 5-20 covers day5 to day 20 in lactation and 20-40 covers day 20 to day 40 in lactation.

[2] ME refer to metabolisable energy, and SID lysine refer to standardised ileal digestible lysine.

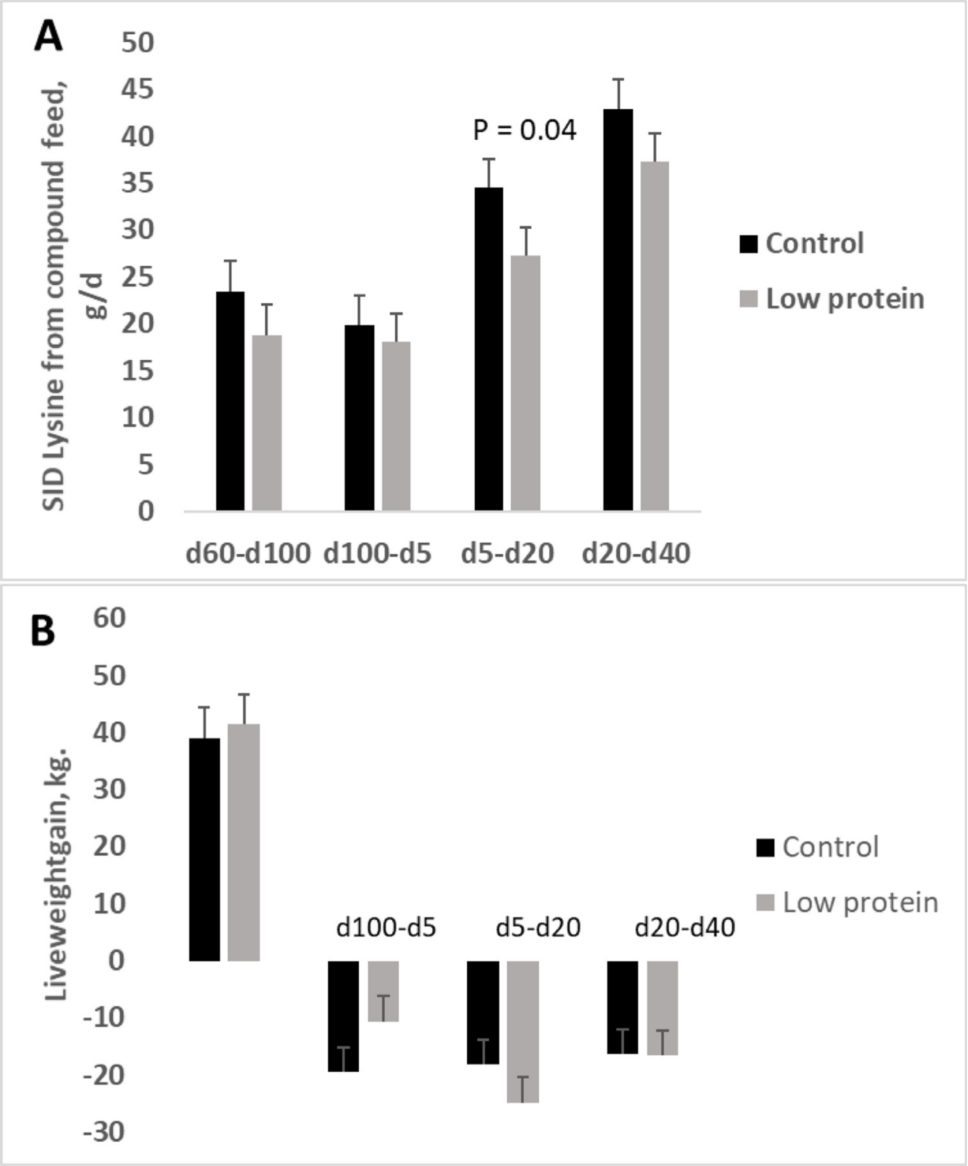

Figure 4. Standardised ileal digestible (SID) lysine intake (panel A) and live weight gain (panel B) in sows fed iso-energetic organic diets differing in proportion of protein in gestation, and early, mid and late lactation. Error bars indicate the SEM

($P = 0.03$) and there was a similar tendency on d5 in lactation ($P = 0.06$). Plasma triglyceride (TG) was higher in the low protein group (0.41 mM vs. 0.39mM, $P = 0.02$), and there was a tendency to higher plasma NEFA in the low protein group (846 µM vs. 659 µM; $P = 0.09$). There was no effect of protein level on urea in urine in this experiment.

5.4. Milk

Interactions between dietary protein and day in lactation was found for milk yield and milk energy output. On d40 the control group had a 29% higher milk yield ($P = 0.05$) and secreted more milk energy (P = 0.03), than the low protein group (Table 6; Figure 6). Except for milk fat, milk macro chemical composition was not affected by protein level (P > 0.05). On d20 in lactation, there was 15% more fat in the milk from the sows fed the low protein compound feed as compared with the control group ($P = 0.02$; Figure 7), and there was also a tendency to a greater energy output in the milk from this group on d20 ($P = 0.08$). There was a tendency to reduced milk casein in the low protein group (4.00 % vs. 4.16%; $P = 0.08$).

Days in lactation affected milk yield and milk composition. Milk yield was 8.04 kg/d on d5, 12.5 kg/d on d20 and 9.3 kg/d on d40 ($P < 0.001$). Energy output in milk was 49 MJ/d on d5, 69 MJ/d on d20 and 50 MJ/d on d40 ($P < 0.001$). Milk DM decreased from 21.4% on d5 to 18.1% on d40 ($P < 0.001$). Milk protein was higher and milk lactose lower on d5 compared to d20 and d40 ($P < 0.001$). Milk fat concentration was higher in early and mid lactation as compared with late lactation ($P = 0.004$).

5.5. Energy intake and energy expenditure

The overall energy metabolism, i.e. intake and expenditure of energy (ME requirement), using a factorial approach is shown in Figure 8. The intake of energy accounted for most, but not all energy required during gestation. In lactation, the energy intake was substantially lower than the ME requirement, especially at d 5 and 20 of lactation, and therefore sows had a substantial energy mobilization in early and peak lactation.

Table 4

Body pools, energy expenditure and reproductive performance in 1st parity organic sows fed iso-energetic 100% organic compound feed differing in level of protein.

	Reproductive stage[1]						Protein level			P-value		
	60	100	5	20	40	SEM	Control	Low	SEM	Stage	Protein	Protein × stage
Sow weight, kg[2]	215^{bc}	242^{a}	227^{b}	206^{c}	190^{d}	4.17	215	217	4.86	$<$0.001	0.76	0.84
Water pool, kg	119.6^{b}	137.6^{a}	126.0^{b}	125.8^{b}	117.3^{b}	3.51	127.9^{a}	122.7^{b}	2.22	$<$0.001	0.02	0.44
Protein pool, kg	36.4^{bc}	42.2^{a}	38.5^{b}	36.6^{bc}	34.4^{c}	1.20	38.0	37.2	0.86	$<$0.001	0.16	0.09
Fat pool, kg	44.8^{ab}	53.6^{a}	46.0^{ab}	28.9^{c}	28.0^{c}	2.40	38.8	41.7	1.68	$<$0.001	0.24	0.16
Ash pool, kg.	7.7^{b}	8.9^{a}	8.2^{b}	8.4^{ab}	7.9^{b}	0.21	8.4^{a}	8.0^{b}	0.12	$<$0.001	0.04	0.71
Back fat, mm	18.7^{a}	19.3^{a}	18.4^{a}	14.9^{b}	12.6^{c}	0.84	16.3	17.3	0.96	$<$0.001	0.55	0.82
Heartrate, bpm[3]	96^{c}	102^{b}	101^{b}	114^{a}	119^{a}	1.70	101.3	104.8	2.38	$<$.0001	0.37	0.42
Daily distance, Km	2.78^{a}	2.46^{ab}	0.64^{d}	1.54^{c}	1.68^{bc}	0.36	1.71	1.54	0.32	$<$0.001	0.80	0.57
Locomotive activity, MJ ME/d	4.3^{a}	4.4^{a}	0.8^{c}	2.1^{b}	2.5^{b}	0.47	2.7	2.9	0.38	$<$0.001	0.46	0.78
Thermoregulation, MJ ME/d	20.6^{b}	20.7^{b}	22.8^{a}	13.8^{c}	13.0^{c}	0.32	19.5	19.5	0.24	$<$0.001	0.92	0.94
Heat Production, MJ ME/d	29.9^{d}	32.3^{c}	38.9^{b}	40.2^{b}	42.0^{a}	0.50	36.7	36.6	0.26	$<$0.001	0.87	0.11
HE Maintenance, MJ ME/d[4]	26.0^{b}	26.2^{b}	28.2^{a}	27.5^{ab}	26.1^{b}	0.41	27.2	26.9	0.33	0.002	0.86	0.89
HE Milk production, MJ ME/d[4]			11.4^{b}	17.7^{a}	11.8^{b}	0.47	13.5	13.8	0.39	$<$0.001	0.56	0.68
Piglets, no/sow[5]			12.0	11.1	10.8	0.45	11.6	11.0	0.36	0.13	0.24	0.53
Piglet weight, kg[6,7]			1.62^{c}	6.2^{b}	11.3^{a}	0.24	6.5	6.3	0.25	$<$0.001	0.22	0.22
Litter weight, kg			18.8^{c}	66.9^{b}	118^{a}	3.46	69.7	66.2	3.56	$<$0.001	0.79	0.79

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

[1] Day 60 and 100 in gestation and day 5, 20 and 40 in lactation.

[2] Sows weighed 196 kg at d30 in the Control group and 199 kg in the Low protein group (P = 0.89). Back fat at d30 in the two groups were 17 mm. and 18 mm. respectively (P = 0.55).

[3] Average heart rate recorded during daytime (10 h and 23 minutes; minimum 9h; max 13 h 54 min). Average daytime 9 hours and 5 minutes from November-April.

[4] HE refer to heat energy (heat production)

[5] Liveborn piglets/litter were 14.1 in the Control group and 13.0 in the Low protein group (P = 0.28). Still born piglets/litter were 2.6 in the Control group and 1.6 in the Low protein group (P = 0.66).

[6] Piglet birth weights at day 0 were 1284 g in the Control group and 1332 g in the Low protein group (P = 0.99).

[7] Piglet weaning weights at day 49 were 14.9 kg in the Control group and 14.2 kg in the Low protein group (P = 0.34).

6. Discussion

6.1. Energy supply, retention during pregnancy and mobilization during lactation

Energy requirement for maternal growth including reproductive organs and fetuses during gestation was 12.5 MJ ME/d retained energy and 4.6 MJ ME/d for heat production associated with protein and fat retention. The sows gained 40.2 kg from d60 to d100 in pregnancy, hence the energy requirement per kg gain was 17.0 MJ ME/kg gain (including conceptus). This complies well with NRC (2012), which estimates the requirement for maternal gain to be 19.8 MJ ME/kg gain.

The sows gained backfat from d 60 to 100 and had 19 mm backfat on day 100 of gestation, which is regarded optimal for sow productivity, while they lost on average 5.8 mm back fat and 38 kg of live weight from d5 to d40 in lactation, which corresponds 17% of total body weight. Earlier investigations reported that commercial indoor sows lost 8%, 9% and 9% from d3 to d28 of lactation in three indoor trials with LY multiparous sows (Vadmand et al., 2015), and organic sows with more than 10 piglets may lose 24-30 kg or 10%-11% during 6 weeks of lactation (Weissensteiner et al., 2018).

This shows, that the sows in the current study were indeed challenged with insufficient energy intake, especially during early lactation (d5-d20), where they mobilized more than 1 kg of body fat per day. Indoor conventional sows with an average milk production mobilize 664 g/d from d3-28 (Pedersen et al., 2016) and high yielding sows mobilize 732 g/d on day 4-18 in lactation (Pedersen et al., 2019). As a consequence of the high fat mobilization, the sows in this trial had on average 12.6 mm back fat at weaning. This is in accordance with (Kongsted and Hermansen, 2009), who found the average back fat at weaning in 573 organic sows under Danish conditions to range from 10.5 mm to17.3 mm with a mean of 13 mm. and that the probability of a successful reproduction after weaning tended to decrease with decreasing back fat thickness for first parity sows.

A high NEFA concentration is indicative of mobilization of fat from the body and it was found to peak in early lactation and decline as lactation progressed. Sows mobilized substantial amounts of energy in early lactation, which is in agreement with (Strathe et al., 2020;

Table 5

Urine and plasma metabolites in first parity sows fed iso-energetic organic compound feed differing in level of protein

	Reproductive stage[1]						Protein level			P-value		
	60	100	5	20	40	SEM	Control	Low	SEM	Stage	Protein	Stage × Protein level
Urine												
Urea, mM	417^{a}	393^{a}	350^{a}	224^{b}	207^{c}	2.87	321	316	1.85	$<$0.001	0.85	0.72
Plasma												
Glucose, mM	4.45^{ab}	4.22^{bc}	5.02^{a}	4.10^{bc}	3.57^{c}	0.14	4.34	4.21	0.20	$<$0.001	0.99	0.13
Urea, mM	2.68^{b}	2.90^{b}	3.16^{ab}	3.44^{a}	3.27^{ab}	0.80	3.17	2.99	0.11	0.003	0.99	0.11
Lactate, mM	4.34^{a}	3.75^{ab}	2.57^{bc}	3.00^{abc}	2.23^{c}	0.36	3.47	2.89	0.32	$<$0.001	0.08	0.01
TG, mM[2]	0.35	0.53	0.46	0.49	0.31	0.03	0.39^{b}	0.41^{a}	0.017	$<$0.001	0.02	0.67
Creatinine, μM	105^{c}	110^{bc}	132^{a}	123^{ab}	106^{c}	6.27	113	117	7.90	$<$0.001	0.73	0.74
NEFA, μM[2]	366^{bc}	205^{c}	1317^{a}	1187^{a}	687^{b}	11.3	659	846	7.31	$<$0.001	0.09	0.89

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

[1] Day 60 and 100 in gestation and day 5, 20 and 40 in lactation

[2] TG refer to triglycerides, NEFA refer to non-esterified fatty acids

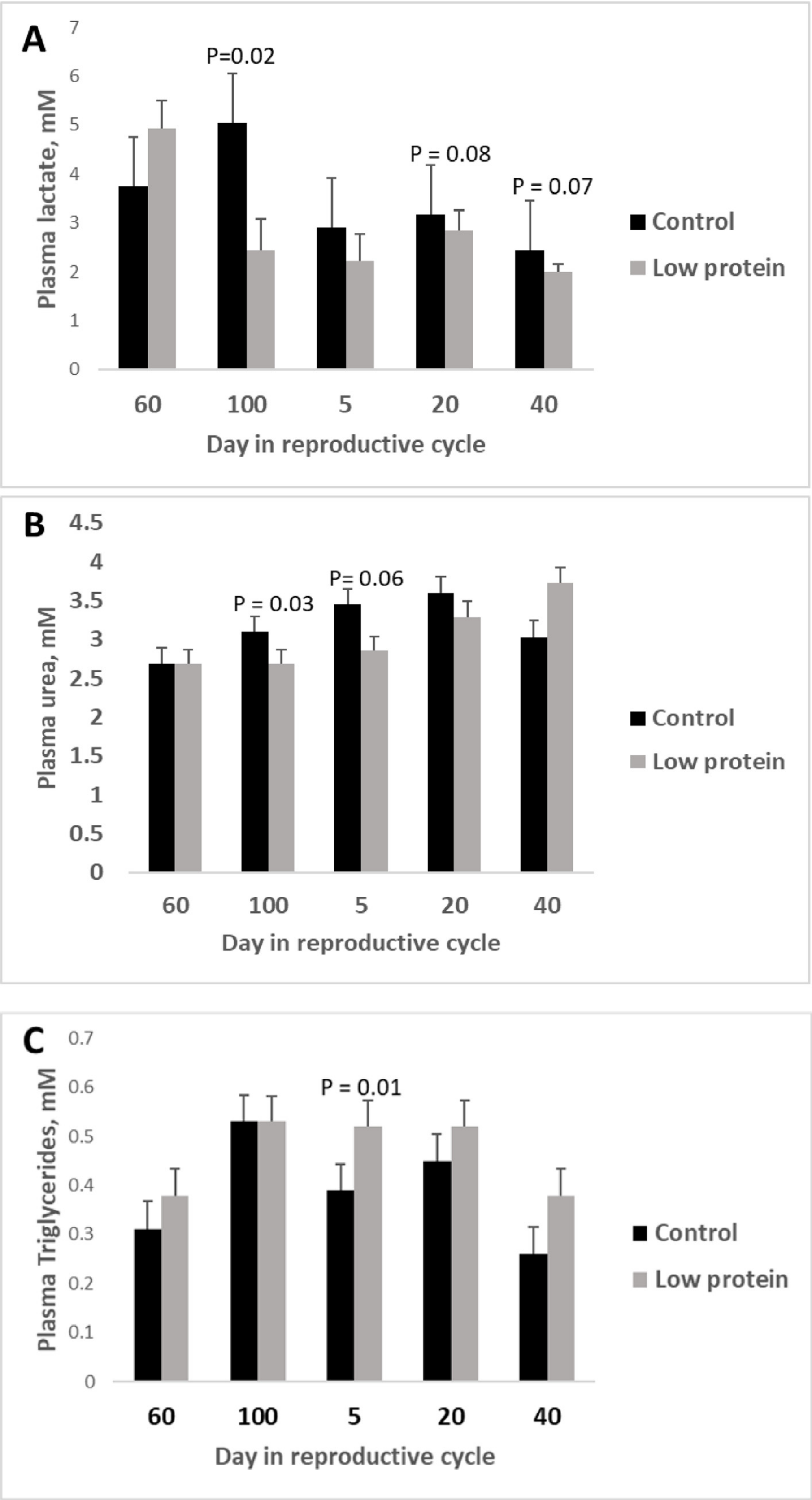

Figure 5. Plasma lactate (panel A), plasma urea (Panel B) and plasma triglycerides (TG; panel C) day 60 and 100 in gestation and day 5, 20 and 40 in lactation in sows fed iso-energetic organic compound feed differing in proportion of protein. Error bars indicate the SEM.

Table 6
Milk composition in first parity sows fed iso-energetic organic compound feed differing in level of protein

	DIM				Protein level			P-value		
	5	20	40	SEM	Control	Low	SEM	DIM	Protein	DIM × protein
Milk yield[1], Kg/d	8.04^{b}	12.52^{a}	9.26^{b}	0.45	10.28	9.59	0.36	<0.001	0.19	0.03
Milk output[2], MJ/d	49.01^{b}	68.89^{a}	49.61^{b}	3.46	56.64	55.04	3.35	<0.001	0.73	<0.001
DM, %[3]	21.37^{a}	18.58^{b}	18.10^{b}	0.34	19.26	19.44	0.32	<0.001	0.71	0.17
Protein, %	5.65^{a}	4.92^{b}	5.23^{b}	0.10	5.27	5.26	0.09	<0.001	0.89	0.23
Lactose, %	4.72^{b}	4.97^{a}	4.97^{a}	0.04	4.92	4.87	0.04	<0.001	0.22	0.08
Fat, %	10.41^{a}	8.76^{a}	7.68^{b}	0.52	8.60	9.30	0.43	0.004	0.25	0.008
Casein, %	4.24^{a}	3.95^{b}	4.06^{ab}	0.065	4.16	4.00	0.062	0.04	0.08	0.45

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

[1] Milk yield calculated as in (Hansen et al., 2012)

[2] Energy in milk calculated as in (Weast, 1984)

[3] DM refer to dry matter

Hojgaard et al., 2019a; Pedersen et al., 2019). The sows in this experiment were considerably undersupplied in early and mid lactation, whereas the energy intake matched fairly well the energy requirement in late lactation, where milk energy output was reduced. The mobilization pattern as evaluated by changes in live weight, back fat and protein and fat pools were not affected by the dietary treatment, but a tendency towards an interaction between treatment and reproductive stage appeared on changes in live weight and protein pool. These changes indicate that sows fed the low protein diet during lactation had to mobilise more protein from the body to counterbalance the insufficient protein supply from the diet. The tendency towards an interaction between treatment and reproductive stage on plasma triglycerides seemed not to be related with different mobilization pattern. In stead, this merely was a consequence of a greater milk yield in control fed sows, which in turn drained the plasma pool of triglycerides more than in low protein sows.

Milk production was the major determinant of energy requirement in lactation, as 58% of total energy requirement was associated with milk production at d20, where milk energy output was 69 MJ/d. In early and mid lactation, the sows had a total energy requirement of approximately 154 MJ ME/d. To fully meet this demand and avoid fat mobilization, the sows energy intake should have been an additional 41 MJ ME/d in early lactation and 23 MJ ME extra per day in mid lactation, which corresponds to 3.4 and 2.0 kg extra feed per day. However, sows had substantial feed residues during lactation, although the feed intake approached the feed supply as lactation progressed. This feed intake pattern strongly suggests, that the voluntary feed intake was limited by the gastric capacity in the young lactating sows. The sows had access to pasture from d 30 of gestation (when pregnancy was confirmed with ultrasound scanning), but one month was evidently not sufficient time for these young sows to adapt to a situation where dietary energy density is low and the gastric capacity a clear limiting factor for the energy intake.

6.2. Energy for thermoregulation

Close and Poornan (1993) stated, that an outdoor 240 kg sow walking 1 km/day would use 5.2 MJ ME/d for locomotive activity and 2.1 MJ ME/d for thermoregulation at 15°C and hence require an additional 7.3 MJ ME/day compared to an indoor sow of the same size. Marotta (2003) showed, that outdoor sows need 14-25% more digestible energy per day in autumn/winter as compared with indoor sows during gestation and lactation, and that there was a total daily requirement of 87 MJ DE in autumn/winter (equivalent to 84 MJ ME) in

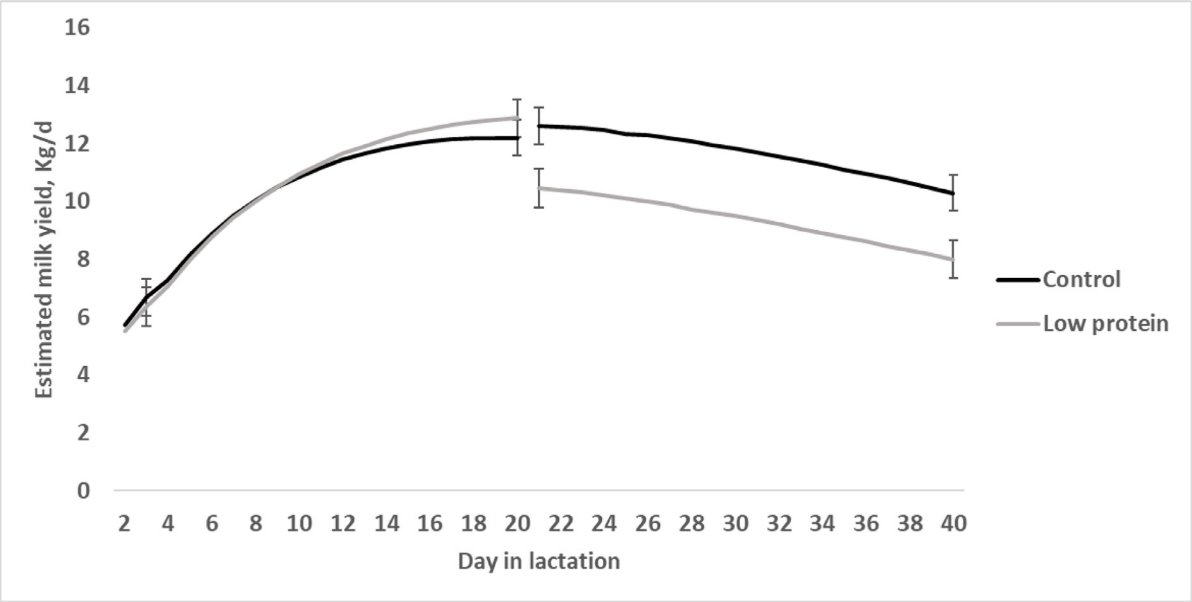

Figure 6. Estimated milk yield day 2 to day 40 in sows fed iso-energetic organic compound feed differing in proportion of protein, based on litter size and piglet weight gain in the two periods d1 to d20 (3.19 kg/d) and d20 to d40 (2.69 kg/d) ($P = 0.004$). The control group had an average daily litter gain of 2.95 kg/d and the low protein group had an average daily litter gain of 2.93 kg/d ($P = 0.25$). On day 20 the low protein group had 12.8 active glands and the control group 11.7 active glands ($P = 0.19$). On day 40 the low protein group had 12.0 and the control group had 11.3 functional teats ($P = 0.30$). Error bars indicate the SEM.

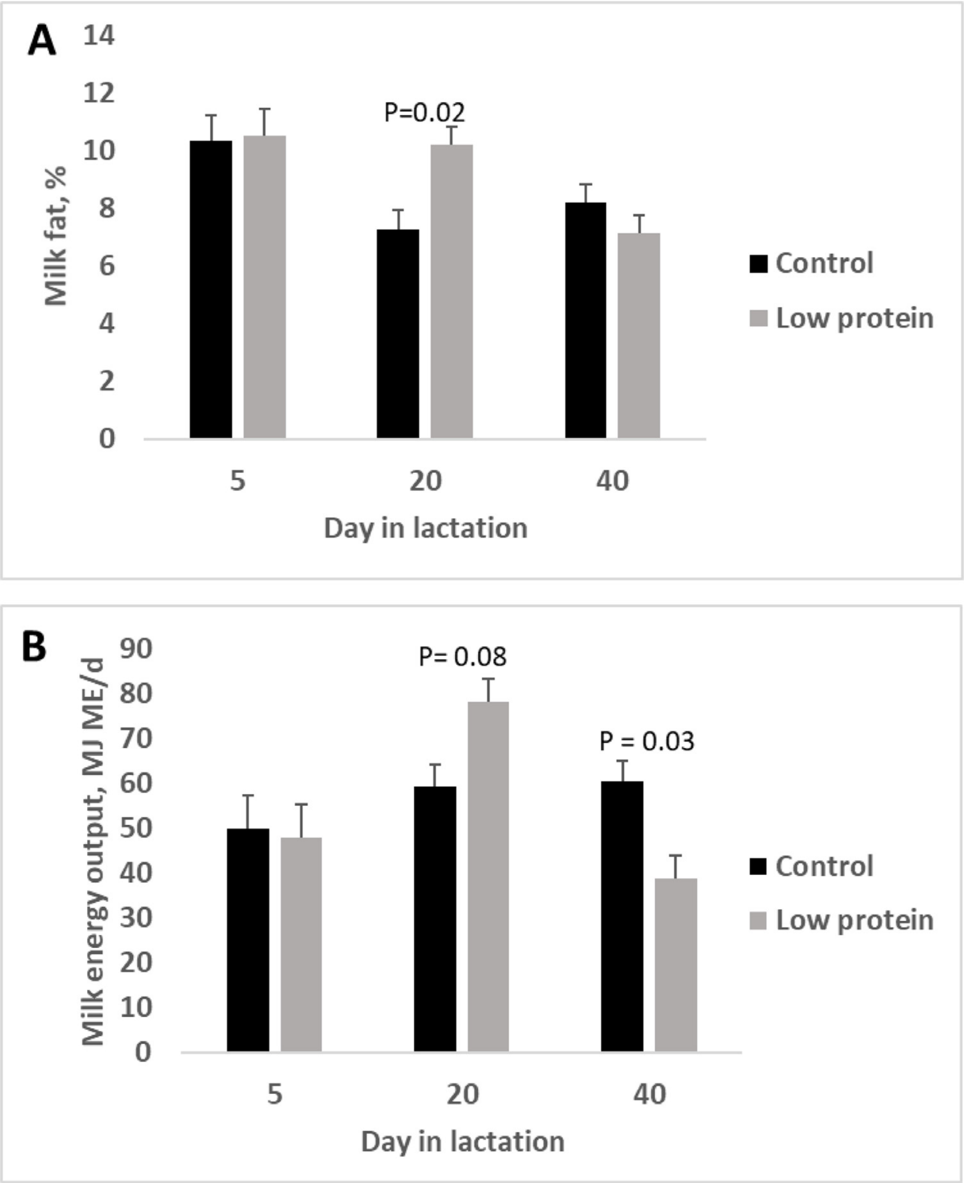

Figure 7. Milk fat % (panel A) and milk energy content (panel B) day 5, 20 and 40 in lactation in sows fed iso-energetic organic diets differing in proportion of protein. Error bars indicate the SEM.

South America with an average winter temperature of 5°C. The average air temperature in this experiment was 3°C, ambient temperature 30 cm above ground level was 4.5°C. Usually, sows' thermo-neutral zone is 21°C and the lower critical temperature is 18 at a high daily feed intake (Verhagen et al., 1986). Below the lower critical temperature, the average daily energy requirement for cold thermogenesis has been proposed to be an extra 18.8 kJ ME/kg metabolic body weight/d/°C (Close and Poornan, 1993). Thus, a 225 kg sow in our experiment would spend approximately 16 MJ ME/d on thermoregulation, but the energy requirement surely also depends on weather conditions like rainfall, snowfall, humidity, radiation and wind speed. In this experiment, there was an average wind speed of 4.14 m/s, 0.98 mm rain or snow/d and an average atmospheric pressure of 1007 mbar, but these factors were not accounted for in the calculation.

6.3. Energy for locomotive activity

The heat generated during locomotive activity and milk production may be considered to meet some of the extra thermoregulatory heat needed to compensate for the cold environment. On average, sows had a mean distance of travelling amounting to 2.2 km/d in gestation and 1.6 km/d in lactation from day 20 and onwards. In early lactation, their distance of travelling was really short as they walked mainly to defecate, urinate, eat and drink and they walked only 0.64 km/d in that period. The energy demand for locomotive activity in outdoor pigs has not previously been well quantified, and estimates of this activity in previous experiments with outdoor sows are therefore largely speculation. Our results are, however, in line with (Buckner, 1996), who showed pedometer values measured in one herd indicating a range of 0.1-3.1 km/d. Energy expenditures of 7 kJ/kg body weight/km have been suggested (Close and Poornan, 1993) and Edwards (2003) suggests a requirement of 10.1-11.3 kJ/kg metabolic live weight for each km. On this basis, a 225 kg pregnant sow walking 2.2 km/d would dissipate an extra 3.4 MJ heat/d and, assuming a net efficiency of energy utilization of 0.8, would require an additional 4.3 MJ ME/d from feed to compensate for locomotive activity. But it should be noted that the level will depend on other factors like age, distribution of pasture vegetation and other resources in the paddock, reproductive stage, and

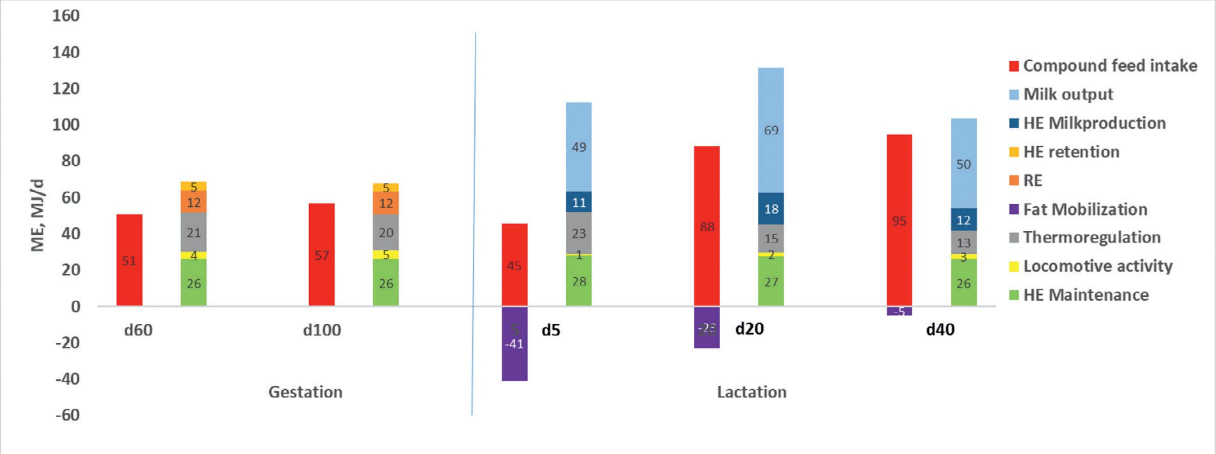

Figure 8. Daily energy intake, energy expenditure (metabolisable energy requirement) on day 60 and 100 in gestation and day 5, 20 and 40 in lactation in first parity sows fed organic diets in winter. 24h average temperatures were -1.3°C (d60), 0.05°C (d100), -3.2°C(d5), 4.38°C(d20) and 6.2°C (d40). In lactation, energy mobilisation is regarded as an input to the sow to meet the high demand of nutrients for milk production. The energy input (concentrate intake + energy mobilization) accounted for on average 84% of the total energy output in this model. The remaining 16% of the energy, which is not accounted for, most likely originated from fresh grass clover and grass clover silage intake

climate.

6.4. Protein supply

Organic protein is a limited and hence expensive resource and it is of great relevance for organic pig farmers to reduce protein oversupply. No negative effects of lowering the protein content of the diet by 12% was observed during gestation, as the low protein- and control sows had a similar daily gain from d60-d100 and litter size at birth was unaffected. The high plasma urea in control sows in late pregnancy indicates that sows fed the control diet most likely oxidised excess protein, indicating that they were oversupplied with dietary protein. During mid and late gestation, sows fed the control diet consumed at least 20 g SID lysine per day, whereas sows fed the low protein diet consumed 18.4 g/d. These data indicate that the lysine requirement was met for both dietary groups as the lysine requirement increases with progress of gestation and peaks at 17.4 g/d in late gestation (Samuel et al., 2012). From d5-20 in lactation, the control sows in-gested 43 g SID lysine/d from compound feed, whereas the low protein sows ingested only 34 g SID lysine/d. The daily amounts consumed were well below our intentions and was due to the limited gastric ca-pacity, as discussed earlier. In the period from d20 to d40 in lactation, the two groups ingested 53 and 47 g SID lysine/d, respectively. Lysine is the first limiting amino acid and at peak lactation, 95% of the daily SID lysine requirement is needed for milk production (Feyera and Theil, 2017). Close and Pornan (1993) states that outdoor sows have a lysine requirement of 36-50 g/day in lactation. High yielding indoor sows have a lysine requirement of 68 to 70 g SID lysine/d at peak lactation (Gourley et al., 2017; Hojgaard et al., 2019b), and sows in the present study had a high productivity as evaluated by their litter weight gain. The low protein sows were supplied with only 47 g SID lysine/d in lactation and consequently milk yield was compromised in mid and late lactation but milk yield was not compromised in early lactation where increased protein mobilization could counterbalance the insufficient dietary supply.

The sows in this trial probably did ingest some protein (and lysine) via grass and silage. Grass cover in the paddocks was very limited but sows had ad libitum access to grass clover silage. Unfortunately, this was not quantified in the present experiment but sows did only consume minor amounts of the silage (M. Eskildsen, personal observation).

Low protein in the diet reduced urea in plasma around parturition and less urea was also observed in milk on day 5 of lactation. Urea in plasma and milk are both indicators of protein quality and quantity and can be used as indicators of oxidation of excess protein for lactating sows. However, a high plasma urea may also indicate an imbalanced profile of dietary amino acids. It is not possible to conclude which of the two scenarios most likely explained the elevated urea in control sows around farrowing in this trial.

Milk yield was predicted by use of a mathematical model developed to quantify milk yield of conventional sows (Hansen et al., 2012) with extrapolation of the model to estimate the milk yield after d30. The model was not built to estimate milk yield for sows with a litter weight gain of more than 4.2 kg/d or more than 14 piglets/litter. A daily litter weight gain of more than 4.2 kg/d was frequently observed in individual sows, but that was the maximal allowed input to the model, so the actual milk yield is most likely underestimated. However, part of the litter weight gain also originate from piglets ingesting sow feed, mainly in the period from d20 and onwards (M. Eskildsen, personal observation).

As compared to milk of conventional indoor sows, the overall DM- and energy content of milk was 20% higher. Especially the average fat content was high (8.9%), compared to indoor studies. Beyer et al.,(2007) found the average sow milk composition to be 18.1% DM, 4.9%protein and 6.8% fat and (Noblet and Etienne, 1986) found 6.9-8.0% fat in sow milk. As the energy- and lysine (protein) intake from grass and grass clover silage is unknown, it is difficult to conclude whether the supply of energy or lysine has been a limiting factor for milk produc-tion. Due to the substantial undersupply of SID lysine at peak lactation, and the energy balance which approached zero in late lactation, it seems logical to suggest that the clear drop in milk yield from d20-d40 was caused by insufficient lysine intake. Most likely, the control fed sows were also fed insufficient dietary lysine, as gilts with high milk production require approximately 49 g SID lysine per day as a mean throughout the entire lactation period (Hojgaard et al., 2019b) and this group of sows consumed on average 38.7 g SID lysine per day. How-ever, the results indicate that these sows were indeed able to counter-balance sufficiently with increased body mobilization.

6.5. Sow productivity

In the present study, the average weaned litter size was 10.8, which is less than the 11.9 piglets weaned on average in Danish commercial organic herds (Hansen, 2018). This is probably due to fact that the experimental sows were all of first parity. A weaned litter size of 10.8 is high compared to the production level in organic herds in other European countries (Prunier et al., 2014), and Weissensteiner et al. (2018)

conclude, that litters with >10 piglets is a “large litter” in organic production systems. Average piglet weight at weaning after 47 days were 14.5 kg, which is higher compared to the 13.9 kg in Danish commercial organic herds (Hansen, 2018) and there were no negative effects of 12% reduced protein level on piglet performance or the return to oestrus interval (4.57d vs 3.84 d in control and Low protein sows respectively; SEM = 0.46, P = 0.47).

Jakobsen and Danielsen (2006) conclude, that outdoor sows have approximately 5% higher total energy requirement than indoor housed sows throughout the year, whereas Close and Pornan (1993) found, that the energy requirements are roughly 20% higher than animals kept indoor. Based on our studies, 15% extra energy, approximately 54 MJ ME/d from compound feed in gestation seems to be sufficient for pregnant gilts during a mild winter with ad lib access to silage. If no mobilization was to occur at peak lactation, the total energy requirement was found to be 153 MJ ME/d which is equivalent to approximately 12 kg/d of feed. However, this clearly exceeded the digestive capacity of the gilts, especially during early lactation.

7. Conclusion

Based on the acquired data, we accept the stated hypothesis, that increasing the daily energy supply by 15% and lowering the protein content in the compound feed by 12 % below the recommended level (for indoor sows) improves energy utilization without compromising sow productivity. The lysine requirement of pregnant sows in the low protein group were met by the elevated daily supply of compound feed. There were no negative effects of lowering the dietary protein content by 12% during gestation on number of live born piglets, litter birth weight, sow body composition, urine or plasma metabolites in first parity sows fed iso-energetic diets during the winter period, indicating that their protein requirement was met, also. During lactation, however, sows on the low protein diet were challenged by a generally low feed intake and thereby a low intake of SID lysine, which decreased the milk yield in late lactation. Thus, it seems to be problematic to lower the protein content in the lactation diet during winter, especially in gilts with limited gastric capacity.

CRediT authorship contribution statement

M. Eskildsen: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **U. Krogh:** Methodology, Writing - review & editing. **M.T. Sørensen:** Writing - review & editing. **A.G. Kongsted:** Supervision, Writing - review & editing. **P.K. Theil:** Conceptualization, Supervision, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

All authors declare that they have no conflicts of interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2020.104088.

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MANUSCRIPT III

Grass-clover intake and effects of reduced dietary protein for organic sows during summer

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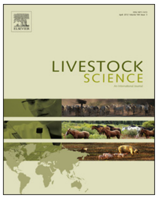

Grass clover intake and effects of reduced dietary protein for organic sows during summer

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body fat mobilization
heat production
locomotive activity
milk yield
plasma metabolites
urine

ABSTRACT

Energy spent on thermoregulation and locomotive activity may increase the energy requirements of outdoor relative to indoor housed sows, whilst their protein requirement most likely is comparable on a daily basis but lower per kg of feed. The purpose of this study was to quantify the energy and protein intake from compound feed and grazing and the energy and protein needed for maintenance, maternal retention, milk production, thermoregulation and locomotive activity in organic sows during summer, to understand how nutrition of organic sows could be improved. A total of 41 2nd parity sows (Landrace x Yorkshire; 239 kg at insemination) were reared outdoor under organic conditions for six months. Sows were fed one of two iso-energetic diets, either commercial available gestation and lactation diets (control strategy), or a 12% lower protein supply obtained by diluting the control diets with a low protein supplement. Sows had ad libitum access to a plentiful grass clover sward and were supplied similar amounts of metabolizable energy (ME) from compound feed equivalent to 10% above the energy recommended for indoor sows. Collections of plasma and urine were performed on d 60 and d 100 of gestation and plasma, urine and milk was collected on d 5, 20 and 40 of lactation. On all sample collection days, sows (and piglets; n = 671) were weighed individually, sows were back fat scanned and heart rate and locomotive activity was registered with a global positioning system (GPS) tracker. Sow body composition was estimated using a deuterium dilution technique, which allowed retention or mobilisation of protein and fat to be calculated. Grass intake was estimated via plasma pipecolic acid. Daily grass clover intake was on average 420 g DM/d during gestation, 574 g DM/d at peak lactation and 472 g DM/d on d 40 of lactation, corresponding to 2.4, 3.2 and 2.6 kg of fresh grass. There was an increased grass clover intake in the low protein group, as they consumed 14% more grass (37 g DM/d extra) than the sows fed the normal protein compound feed ($P = 0.007$). Estimated milk yield peaked at 16.3 kg/d on d20. This experiment showed no effects of dietary protein level on urinary pH, urea or creatinine and no effects on plasma glucose, urea, lactate, triglycerides, creatinine or NEFA concentrations. It was possible to reduce the protein content of organic compound feed in the summer time as grazing pregnant sows obtained 16-17% of their daily SID lysine requirement from the sward in mid and late gestation. In conclusion, the daily protein- and amino acid requirements were met by feed and grass consumption during pregnancy but not in early and at peak lactation due to insufficient feed intake. The total energy requirement of high yielding second parity outdoor sows during a normal Danish summer was found to be around 32 MJ ME/d during gestation and approximately 130 MJ ME/d at peak lactation.

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1. Introduction

Organic sows in pasture systems live under varying weather and temperature conditions. They spend extra energy on thermoregulation, a prolonged lactation period and they have the opportunity for increased locomotory activity as compared with indoor housed sows. These aspects increase the energy requirements, whilst their protein requirement most likely is comparable on a daily basis with that of indoor sows (Close and Poornan, 1993; Jakobsen and Hermansen, 2001). Outdoor organic sows consume on average 34%more feed per day than indoor sows (Hansen, 2018), and according to the organic legislations it is not allowed to use crystalline amino acids. Therefore, organic sows are fed diets with higher protein concentration than indoor sows, to ensure sufficient intake of lysine, which is re-garded being the first limiting amino acid. Moreover, in some countries, organic sows have access to pasture, where they consume protein from grass clover especially during summertime, which increases their pro-tein intake further. Excess dietary protein reduces feed efficiency (Pedersen et al., 2019a) and the protein-to-energy ratio formulated for indoor pigs is most likely not optimal for organic production. The theoretical requirements for protein and energy in organic pigs have been calculated in several studies (Close and Poornan, 1993; Fernandez et al., 2006; Jakobsen and Danielsen, 2006) and the addi-tional energy requirement of outdoor pigs compared to indoor has been estimated to be approximately 15% under Northern European condi-tions (Edwards, 2003), but the extra energy requirement has not been empirically quantified.

Based on 10% greater feed supply on a daily basis as compared with indoor housed sows, we hypothesized, that the dietary protein concentration could be lowered by 15% below the recommended level for gestating and lactating sows without compromising sow productivity. The aim of this study was to determine the energy and protein intake from grazing and to quantify the energy required for milk production and heat (thermoregulation, locomotive activity, maintenance, and heat associated with protein and fat retention and milk production) and thereby contribute to a better understanding of the energy requirements of organic sows with pasture access.

2. Methodology

The animal experimental procedures were carried out in accordance with the Danish Ministry of Justice, Law no. 253/08.03.203 concerning animal experiments and care and license issued by the Danish Animal Experimental Inspectorate, Ministry of Food, Agriculture and Fisheries, the Danish Veterinary and Food Administration. The animal experiments comply with the ARRIVA guidelines and were performed in accordance with the legislation for the protection of animals used for scientific purposes (EU Directive 2010/63/EU for animal experiments). Rearing, housing and sampling, were in compliance with Danish laws for care and use of animals in research (Animal Experimental permit No. 2013-15-2934-00961).

2.1. Housing and rearing conditions

Forty one 2nd parity Landrace × Yorkshire-sows, weight 239 kg (SD = 4.1) were inseminated twice in the first oestrus after weaning with semen from three Danbred Duroc boars with known identity. The sows were randomly assigned to one of two iso-energetic dietary treatments consisting of a standard organic feeding regimen (Control, n = 24) or a low dietary protein compound feed (Low protein, n = 19). Insemination took place indoor, where the sows were group housed and pregnancy was verified by scanning before the sows had access to pasture. Insemination took place from the end of January to the beginning of April, consequently sows began farrowing in the middle of May until the end of July. Thus, the experimental period focusing on d 60 of gestation until d 49 of lactation was performed from early April to mid September.

The sows were reared under organic conditions outdoor in the summer of 2017 at the Organic Platform at Aarhus University, Denmark. Sows had access to abundant grass clover swards in four paddocks of 4000 m^2 from day 25 of gestation, corresponding to approximately 400 m^2 per sow. The pasture area was sown in spring 2016 with two commercial grass clover mixes (ForageMax55 and ForageMax56, DLF Trifolium, Roskilde, Denmark). In the gestation paddocks it consisted of 10% Trifolium Repens (white clover, Rivendel), 50% Lolium Perenne (perennial ryegrass, Humbi 1), 15% Lolium Perenne (perennial ryegrass, Masai) and 25% Festuca Rubra (red fesque, Gandolin). The grass clover in the farrowing paddocs was 10% Trifolium Repens (white clover, Rivendel), 30% Lolium Perenne (perennial ryegrass, Humbi 1) and 60% Festuca Rubra (red fesque, Gandolin).

Two isolated 12 m^2 huts were located in each paddock in the gestation field. The sows were moved to individual 450 m^2 paddocks (18 × 25 m) ten days prior to expected farrowing of the first sow. The farrowing area was sown with 10% white clover, 30% perennial ryegrass and 60% red fescue in 2016. Twenty four sows were housed in an isolated prototype communual farrowing hut with room for four individually housed sows. Each of the four compartments measured 2.4 × 2.5 m. The remaining nineteen sows were housed in traditional A frame farrowing huts (L 2.20 m, W_{bottom} 1.80 m, W_{top} 1.05 m, H 1.05 m). All A frame huts had a ventilation opening in the back, which was opened at high outdoor temperature. A roller was placed in the entrance of all huts between farrowing and day 10 of lactation to prevent piglets from leaving the hut. All huts were supplied with a bedding of chopped barley straw: In spring approximately 10 kg/m^2 and in summer 7.5 kg/m^2.

Health conditions were monitored daily and if necessary, animals were treated in compliance with normal procedures. Animal health was monitored by the herd veterinarian.

All animals had ad libitum access to drinking water from a bowl and had access to a wallow, when day temperatures were above 15 °C.

2.2. Diets and feeding

A commercial available gestation compound feed and a lactation compound feed based on barley, rye, oat and rapeseed cake as main ingredients were used as the control treatment. Both diets were formulated to ensure, that the supply of standardized ileal digestible (SID) of other amino acids than lysine were in accordance with that recommended for Danish indoor housed gestating and lactating sows (Tybirk et al., 2016) when expressed relative to lysine. The gestation and lactation compound feed formulated for control sows were also supplied to sows on the low protein strategy, but for these sows, 30% of the daily ration was replaced by a low protein supplement. This supplement was based mainly on barley and oats and formulated to dilute the protein content of the control diet (normal protein concentration in compound feed). The lysine requirement was not met for gestation sows fed the low protein strategy and not met for both dietary groups during lactation. Sows fed the control diets were supplied 100 and 78 % of the recommended lysine intake during gestation and lactation, respectively, whereas sows fed the low protein diets were supplied 90% and 64% of the recommended lysine intake during gestation and lactation, respectively. The undersupply was accepted to avoid a very high supply of crude protein in both dietary strategies. Ingredients and chemical compositions of the experimental diets are shown in Table 1 and 2. Both treatment groups were supplied with 10% more energy than recommended for indoor sows (Tybirk et al., 2016) to meet the extra demand for thermoregulation and locomotory activity under outdoor conditions. Compound feed was manufactured at a commercial feed factory (Vestjyllands Andel, Videbæk, Denmark) four times throughout the study with approximately eight week intervals.

Pregnant sows were daily fed two equally sized portions at 7.00 am

Table 1
Ingredients of experimental compound feed. The two dietary strategies were formulated to be isoenergetic based on the Danish feed evaluation system (Danish Feed Units) which is a potential physiological energy system closely related to the NE system (Patience, 2012).

	Gestation[1]		Lactation[2]	
Ingredients, g/kg	Normal protein	Low Protein	Normal protein	Low protein
Barley	330	462	300	441
Rye	200	140	100	70
Oat	150	165	70	109
Corn	50	35	25	18
Peas	50	35	50	35
Wheatbran	35	25	79	55
Oatbran	50	35		
Dried grass meal	20	14		
Soybeancake	47	33	107	75
Rapeseedcake	43	30	42	29
Fish meal			10	7
Calcium carbonate	14.0	14.3	12.5	13.3
Sodum chloride	4.4	4.4	4.9	4.8
Monocalciumphosphate	6.1	6.8	7.7	7.9
Vitamin and mineral mixture[3]	1.1	1.1	3.3	2.6
ME MJ/kg	11.5	11.5	12.1	11.9
FU_{sow}	0.95	0.95	0.99	0.98
Std. Dig. Crude protein, g/kg	84	76	115	97
Lysine, g/kg	5.3	4.8	7.3	6.1
SID[3] Lysine, g/kg	4.2	3.5	5.9	4.8

[1] Gestation compound feeds were offered during pregnancy until d 108.

[2] Lactation compound feeds were offered from day 109 in pregnancy until weaning at d 49.

[3] Pr kg: 8,000 IU vitamin A; 800 IU 25-hydroxy vitamin D; 54,600 mg DL-alphatocoferol; 2,000 mg vitamin B1; 5,000 mg vitamin B2; 3,000 mg vitamin B6; 20.0 mg vitamin B12; 2,000 vitamin K3; 15,000 mg D-pantothenic acid; 20,000 mg niacin; 400 Biotin; 1,500 mg folic acid; 80,000 mg iron (FeSo4); 15,000 mg copper (CuSO4); 40,000 mg manganese (MnO); 2,000 mg iodine (Ca (IO3)2); 100,000 mg zink (ZnO); 300 mg selenium (Na2SeO3).

and 15.00 pm. Compound feed was supplied to individual sows in stainless steel feeding stalls and individual feed residues were weighed 30 minutes after each meal. Lactating sows were individually fed once a day at 10.00 am in covered troughs (Sostub crip, Domino, Tørring, Denmark) protecting the feed from rain, piglets and birds. Piglets were offered a supplemental commercial weaning feed from 14 days of age outside the paddocks, where the sows could not reach it. The intake of weaning feed was not measured. Feed residues were weighed from individual sows on a weekly basis in lactation. Five kg samples were taken of each compound feed from each batch, during the production process. Each compound feed sample was split into subsamples using a 32-slot riffle sample divider. In total, two subsamples per diet were analyzed for crude protein and amino acids in duplicates at a commercial laboratory following the European Commission Directives [EC] 64/1998 and [EC] 152/2009 (Eurofins Steins Laboratory A/S, Vejen, Denmark), respectively. Grass samples were collected every two weeks, pooled and stored at -20°C until analysis.

2.3. Recordings and sampling of sows and piglets

Sampling and measurements began at sunrise and were performed on day 60 and 100 in gestation and day 5, 20 and 40 in lactation. Sows were caught before sunrise in the huts. Piglets were removed from the sow for approximately 30 minutes and individually weighed.. Sows were weighed on a walk-in scale and back fat was measured using a SonoGrader ultrasound scanner on the right side 5 mm from the midline at the last rib.

Locomotory activity gauges were tightened around the sows´ bellies to record the distance covered and to register heart rate (Polar Team Pro GPS tracker system, Polar, Ballerup, Denmark).

Sows were held with a snare restraint around the snout and blood was collected by jugular vein puncture in 10 ml Na – heparinized tubes (Greiner BioOne GmbH, Kremsmünster, Austria). Blood samples were stored on ice until centrifugation (3000 rpm at -4°C for 12 min). Plasma was immediately harvested and stored in 1.5 ml micro centrifuge tubes at -20°C and -80°C until analysis. Following blood sampling, sows were enriched with deuterium oxide (D_2O; 0.0425 g 40% solution administered per kg live weight) in the neck (IM) using a 18G needle. A spontaneous urine sample was collected from sows at sunrise on the day after D_2O enrichment. The urine sample was collected from the first voluntary urination by a trained staff member with a 200 ml collection pot directly from the sow. The urine sample was taken in the middle of the excretion, and it did not seem to bother the sows, as they normally did a full emptying of the bladder with several liters of urine. Most sows urinated within two hours after sunrise. The pH in urine was measured using a pH-meter and subsamples of urine were stored at -20°C until further analysis.

All farrowings were recorded by video cameras to provide exact information on the number of stillborn and live born piglets (IPCHDBW4100EP-0360B, Dahua Technology Co., Broadway, UK). Individual cameras hang in every A frame hut and over every pen in the prototype communual huts and they were fitted with wide angle lenses, so all animals were visible at all times. Recordings were saved digitally and analysed using S/VIDIA Client MegaPixel *M. Shafro and Co., Riga, Latvia)

Piglets weighing less than 700 g were considered non-viable and euthanized by blunt force trauma.

During lactation (d5, d20 and d40 in lactation) a total of 45-50 mL milk was manually obtained from three to five teats of each sow from a standing position with a wire snare around the snout. To induce milk letdown, sows received an intravenous injection of 0.3 mL oxytocin via an ear vein (10 IU/ml; Leopharma, Ballerup, Denmark). Milk samples were filtered through gauze to remove dust and debris and stored at –20°C until analysis.

Piglets were ear tagged on d1. If sows gave birth to a surplus of piglets relative to the number of functional teats, litter equalisation was done within three days after farrowing and cross fostering was performed within treatments. At five days of age, male piglets were castrated. Piglets that voluntarily shifted from one sow to another due to the free-range conditions were ascribed to the sow, where they were found on the specific sample collection day. Piglets were individually weighed on day 1, 5, 20, 40 and at weaning on d 49 and litter weight gain and litter size were used for estimation of milk yield as described by (Hansen et al., 2012). Dead piglets were collected on a daily basis and date of death and sow number was recorded, and the actual litter size was used as input to estimate the sow milk yield.

The outdoor temperature was measured every hour throughout the study by weather stations placed in the center of the gestation and lactation paddocks.

3. Chemical analyses

Compound feed and grass clover gross energy (GE) was determined with a bomb calorimeter (Parr 6300 Instrument Company, Moline, Illinois, USA). Apart from amino acid analyses, chemical analyses of compound feed, grass clover, urine, and plasma were performed in duplicate. The DM content of compound feed and grass clover samples was determined by oven drying at 103°C. Ash was determined by oven drying at 525 °C for six hours. Starch and non-starch polysaccharides (NSP) were analyzed as described by (Knudsen, 1997). Grass intake was estimated on the basis of plasma pipecolic acid as described by Eskildsen et al., (2020a).

The crude protein content was calculated as nitrogen × 6.25 as reported by (Eggum, 1970). The nitrogen content of urine was determined by the modified Kjeldahl method (Method 984.13; AOAC Int, 2000) using a KjeltecTM 2400 (Foss, Hillerød, Denmark)

Table 2
Chemical analysis of gestation and lactation compound feed and fresh clover grass.

	Gestation		Lactation		Clover grass[1]					
	Normal protein	**Low protein**	**Normal protein**	**Low protein**	**April**	**May**	**June**	**July**	**August**	**September**
Dry matter, g/kg	877	875	864	865	223	153	174	138	165	200
GE, MJ/kg DM	18.4	18.4	18.5	18.4						
FU_{sow}/100 kg	99.8	101.4	99.5	99.1						
ME, MJ/kg DM[2]	12.1	12.3	12.2	12.0						
Chemical composition, g/kg										
Crude protein	128	114	148	130	182	236	206	194	198	190
Fat	39	38	41	38	36	44	32	37	43	38
Starch	525	555	527	557						
Cellulose[3]	79	74	51	54						
Soluble NSP[4]	53	49	49	46						
Insoluble NSP[5]	175	173	139	148						
Klason lignin	62	57	49	49						
Ash	48	44	52	49	81	125	114	97	132	242
Dietary fibre[6]	290	280	236	243						
Calcium, g/kg	7.8	7.4	8,3	7.8	7.9	12.7	8.8	7.6	6.6	4.8
Phosphor, g/kg	5.0	4.7	6.0	5.4	3.4	3.5	3.4	3.7	3.7	4.1
Amino acids, g/kg										
Lysine	6.3	4.6	6.8	6.1	11.0	10.3	12.0	11.0	11.9	9.6
Methionine	2.0	1.5	2.1	2.0	3.2	3.1	3.4	3.3	3.5	2.9
Cysteine	2.7	2.1	2.5	2.4	1.9	1.6	2.0	1.8	2.0	1.9
Threonine	4.7	3.6	5.1	4.7	8.0	7.8	8.8	8.3	9.1	7.5
Isoleucine	4.8	3.6	5.2	4.8	7.1	6.8	8.1	7.5	8.2	6.5
Leucine	9.2	7.0	9.7	9.0	14.2	13.8	15.5	14.5	15.9	12.7
Histidine	3.1	2.3	3.3	3.0	3.5	3.4	4.1	3.9	4.0	3.3
Phenylalanine	5.8	4.5	6.3	6.0	9.0	8.9	9.9	9.6	10.3	8.5
Tyrosine (calculated)	4.6	3.4	5.0	4.6	6.6	6.6	7.4	7.4	7.1	6.2
Valine	6.2	4.8	6.5	6.1	9.4	9.0	10.6	9,6	10.7	8.5
Alanine	5.9	4.4	6.0	5.7	11.0	10.5	11.6	11.2	12.6	10.6
Arginine	8.0	5.7	8.4	7.6	9.1	9.2	10.1	9.6	10.4	8.7
Asparaginacid	11.0	7.9	11.8	10.5	19.7	17.8	24.0	19.3	20.7	18.0
Glutamineacid	23.8	18.9	26.2	25.0	19.1	18.0	20.9	19.1	21.7	18.2
Proline	9.1	7.5	9.5	9.3	8.2	8.3	9.5	8.8	9.2	7.7
Serine	6.1	4.5	6.5	6.0	7.9	7.5	8.8	8.4	8.9	7.6
Glycine	6.0	4.5	6.1	5.8	9.3	8.9	10.0	9.5	10.6	8.7

[1] All grass clover analysis are on a DM basis

[2] 1 FU_{preg} ≈ 12.1 MJ ME and 1 FU_{lact} ≈ 12.3 MJ ME (Kjeldsen, 2019)

[3] Cellulose determined as the difference in NSP glucose residues after hydrolysis with 12 and 2 M H2SO4 respectively

[4] Soluble NSP determined as difference between NSP and insoluble NSP

[5] Insoluble NSP determined by summation of measured sugar residues of the insoluble NSP fraction

[6] Dietary fiber calculated as the sum of NSP and lignin

Amino acids were analyzed in experimental diets and grass clover samples following hydrolyzation for 23 hours at 110°C with (Cys and Met) or without (Arg, His, Ile, Leu, Lys, Phe, Tyr, Thr, Val) performic acid oxidation, and AA were separated by ion exchange chromatography and quantified by spectro-photometric detection after ninhydrin reaction.

The plasma concentrations of glucose, lactate, triglycerides and urea in plasma and urinary concentrations of urea and creatinine were analyzed according to standard procedures (Siemens Diagnostics Clinical Methods for ADVIA 1650) on an auto analyzer (ADVIA 1650 Chemistry System, Siemens Medical Solution, Tarrytown, NY). Plasma content of NEFA was determined using the Wako, NEFA C ACS-ACOD assay method (Wako Chemicals GmbH, Neuss, Germany).

The chemical composition of milk for DM content, protein, casein, lactose, and fat was analyzed in triplicate through infrared spectroscopy using a Milkoscan 4000 instrument (Foss Milkoscan, Hillerød, Denmark).

4. Calculations and statistical analyses

The total D_2O space was estimated based on the D_2O concentrations in body fluids (derived from plasma concentration prior to enrichment and urinary concentration after enrichment) and the back fat and BW of the sow as described by Theil et al. (2002). Based on the measured D_2O space, the total body pools of protein and fat were estimated from live weight, D_2O space and back fat (BF) measurements according to (Rozeboom et al., 1994) for Landrace-Yorkshire sows as:

$$\text{Protein pool (kg)} = 1.3 + 0.103 \times \text{BW} + 0.092 \times D_2O\,\text{space} - 0.108 \times \text{BF}$$

$$\text{Fat pool (kg)} = -7.7 + 0.649 \times \text{BW} - 0.610 \times D_2O\,\text{space} + 0.299 \times \text{BF}$$

Retained energy in gestation and late lactation was calculated as RE = $RE_{protein}$ + RE_{fat}, where $RE_{protein}$, KJ/d = (final protein pool – initial protein pool, g) × 23.9 KJ/g /number of days between initial and final pools and RE_{fat}, KJ/d = (final fat pool – initial fat pool, g) × 39.8 KJ/g/number of days between initial and final pools. Retained energy could not be separated into foetal and maternal growth during pregnancy using this technique. Heat energy (HE) for retention was calculated as: HE retention, MJ/d = (((daily protein gain, g/d × 23.9 KJ/g)/0.60) - (daily protein gain, g/d × 23.9 KJ/g) + ((daily fat gain, g/d × 39.8 KJ/g)/0.80) - (daily fat gain, g/d × 39.8 KJ/g))/1000 according to (Theil et al., 2020).

In lactation, the energy retention was negative, and $HE_{retention}$ was calculated as $RE_{Fat+protein}$ - ($RE_{Fat+protein,}$:0.88), assuming an energy efficiency of 0.88 for utilization of mobilized ME for milk production (Dourmad, 1996). $HE_{maintenance}$ was estimated as 0.459 MJ/$kg^{0.75}$ × metabolic live weight for pregnant sows (Theil et al., 2004) and 0.482 MJ/$kg^{0.75}$ × metabolic live weight for lactating sows (Theil et al., 2002).

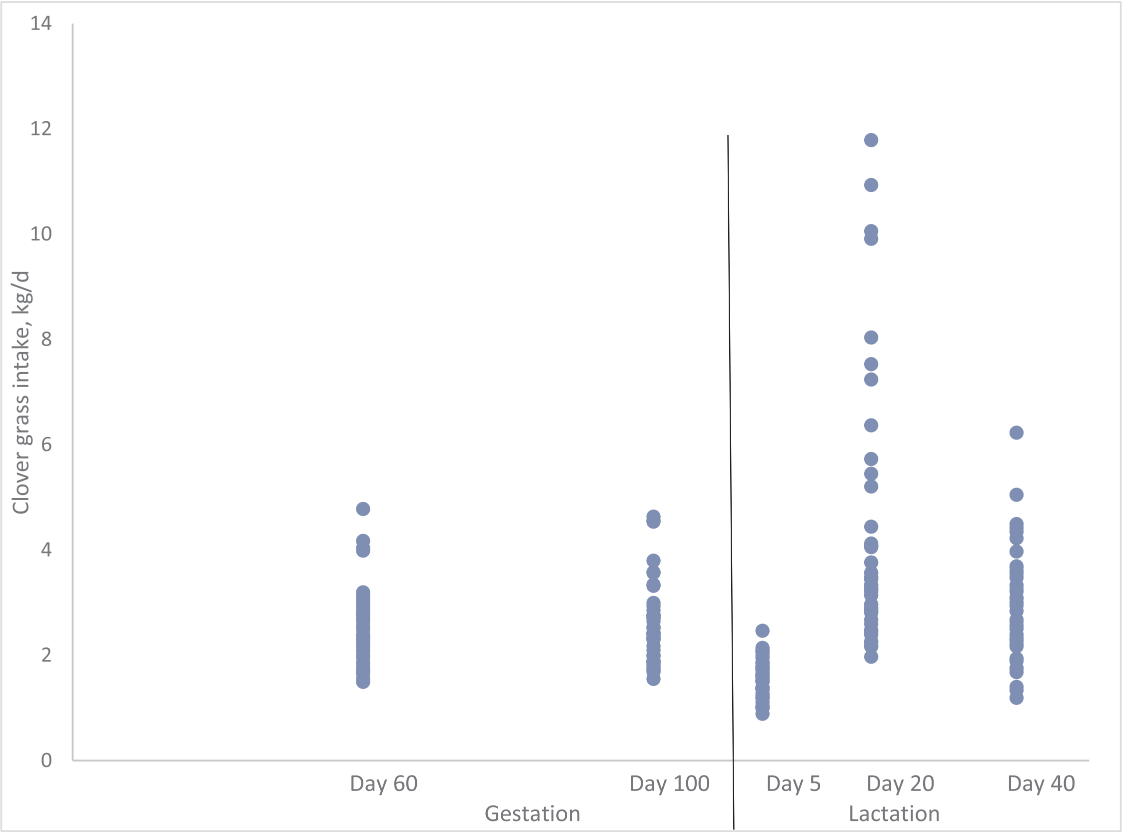

Figure 1. Individual clover grass intake in kg/d in 43 2^{nd} parity organic sows with access to pasture.

The GPS data and heart rate was recorded during the daytime during a period of 9 to 12 h depending on battery capacity. Daily distance covered between sunrise and sunset was estimated using the recorded distance adjusted for the ratio between time from sunrise to sunset and the period of actual recordings. Locomotive activity was used as a common term for walking/running/sprinting/stamping, as the sows showed primarily slender walking. On the basis of (Close and Poornan, 1993), the ME expenditure for locomotive activity was calculated as:

ME locomotive, MJ ME/d = ((7 kJ/kg body weight/d × sow body weight, kg × covered daily distance, km)/0.8)/1000, assuming a net efficiency of energy utilization of 80%.

Energy for thermoregulation was calculated as 18.8 kJ ME/kg metabolic body weight/d/24 h average air temperature 30 cm above the ground in°C below 18°C (Close and Poornan, 1993) (Verhagen et al., 1986)).

Heat produced due to retention was calculated by assuming that energy and fat was retained with an efficiency of 60% and 80%, respectively.

The total HE calculated factorially was done with the following equation in gestation: $HE_{factorial}$ = HE for maintenance + HE for locomotory activity + HE for thermoregulation + HE for retention.

Milk yield was predicted based on average litter weight gain and litter size in the two periods d 5 to 20 and d 20 to 40 by use of a mathematical model developed to quantify milk yield of conventional sows (Hansen et al., 2012). The energy concentration in milk was calculated based on energy values (39.8 kJ/g fat, 23.9 kJ/g protein, and 16.5 kJ/g lactose; Weast, 1984). The output of energy in milk was calculated as the product of milk yield multiplied by energy concentration.

The HE associated with milk production was estimated from the estimated energy output in milk as: HE milk, MJ/d = (Milk energy output MJ/d /0.78) – Milk energy output MJ/d.

The total HE in lactation was calculated as follows: Total $HE_{factorial}$, MJ/d = HE for maintenance + HE for locomotory activity + HE associated with milk production, whereas HE for thermoregulation was set to zero during summer because lactating sows produce huge amounts of water and most likely due not need to oxidise extra heat to keep a constant bldy temperature.

Moreover, the total heat production was also estimated from the 24 hour-mean heart rate using the following equations (Krogh et al., 2018)

Gestation: HE, MJ/d = 0.323 MJ/bpm × Heart rate, bpm – 2 .4 MJ/d

Lactation: HE, MJ/d = 0.118 MJ/bpm × Heart rate + 26.7 MJ/d39,

In lactation, ME supplied from fat mobilization was calculated as g daily fat gain × 39.8 KJ/g. ME supplied from protein mobilization was calculated as g daily protein gain × 23.9 KJ/g.

The response parameters sow weight, back fat, heart rate, daily walking distance, urine and plasma metabolites recorded repeatedly (60 and 100 in gestation and on day 5, 20, and 40 in lactation) were analyzed using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + t_k + \varepsilon_{ijk},$$

where Y_{ijk} is the observed trait, μ is the overall mean of the observations, α_i is the main effect of the dietary regimen (i= normal, low protein diet), β_j is the effect of reproductive stage (day in gestation or day in lactation (j= 60, 100, 5, 20 or 40), $(\alpha\beta)_{ij}$ is the interaction between diet and reproductive stage, t_k is the random effect of sow (k= 1, 2, 3, …, 41) to account for repeated measurements within sow and ε_{ijk} is the residual random components.

Litter size, piglet weight and milk chemical composition were analyzed by the same model, but with only day 5, 20 and 40 in lactation being available for these parameters. Similarly, compound feed intake, SID lysine intake, ME from compound feed, live weight gain, back fat and protein changes were analyzed with a similar model as described

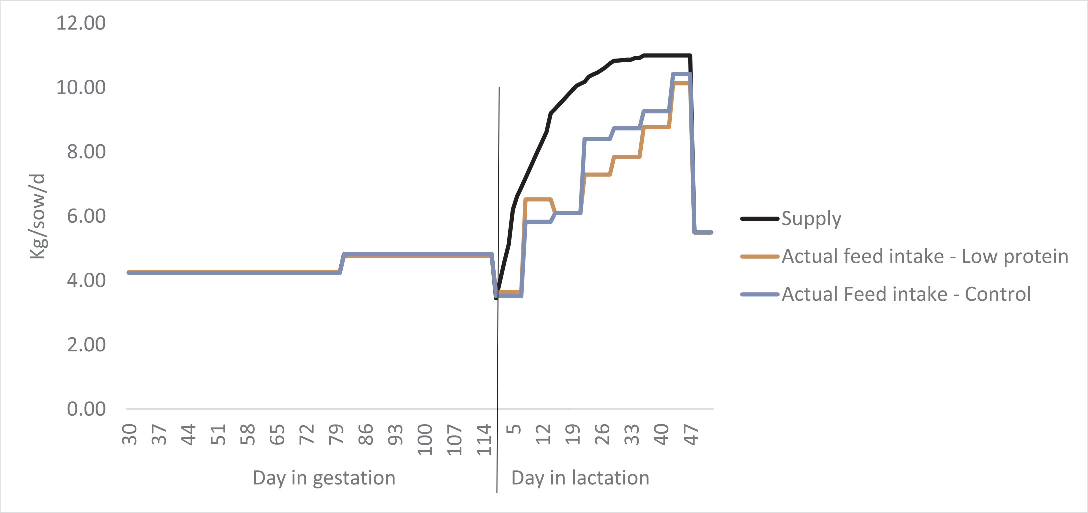

Figure 2. Feeding curve and actual intake of compound feed in kg/d in gestation and lactation. The feeding curve is based on the recommended daily energy intake for indoor sows + 10% (SEGES, 2016).

above except days were replaced by four periods within the reproductive stages (1: d 60-100, 2: d 100-5, 3: d 5-20 and 4: d 20-40).

Statistical analyses were performed using the MIXED procedure of the Statistical Analysis System (SAS; version 9.4), Significant values were considered if $P < 0.05$ and tendencies were accepted at $P \leq 0.10$.

5. Results

The experiment was executed from primo April to medio September 2017. Average sunrise was at 05:26, sunset at 21:19, and average day length was 15 h and 53 min. There was 1.9 mm/d of rain on average and the mean air temperature was 13.5 C during the day and 5.2 during the night. Average wind speed was 4.3 m/s. A huge individual variation in grass intake was observed (Figure 1).

The analyzed protein in the low protein gestation and lactation compound feeds were 12% lower than in the normal protein compound feed (Table 2) which was in accordance with the experimental design. The daily feed intake followed the feed supply throughout gestation, whereas sows only consumed 68% of the supplied feed on average in the period from d5-d20 in lactation (Figure 2).

In the period from d60 to d100 in gestation, sows gained on average 41 kg and 2.9 mm back fat. From d100 in gestation to d40 of lactation, sows lost on average 58 kg live weight (including conceptus and uterine fluids) and 6.4 mm of back fat. In late gestation, the sows weighed on average 303 kg and had a mean back fat thickness of 20.5 mm when transferred to the farrowing paddocks. Energy for retention in the period d60 – d100 was 37 MJ ME/d

After 40 days of lactation, the sows in both groups had a mean back fat thickness of 14.1 mm and a mean live weight of 246 kg. Sows had a daily fat mobilization of 1383 g/d from day 5 to day 20 and they mobilized 275 g/d from day 20 to day 40 in lactation. Based on the D_2O dilution technique, sows appeared to be undersupplied with an average of 60 MJ ME/d in the period from d5 to d20 of lactation (Table 3). On d40, energy intake matched energy output and the balance was +1.4 MJ ME/d. An interactions between diet and reproductive stage was found for protein retention/mobilisation using the D_2O dilution technique revealing that protein balance was not affected by low protein during gestation but sows fed low protein mobilized more protein from the body during lactation (Figure 3). Also an interaction was found for fat retention/mobilisation, showing that low protein fed sows retained more fat except around parturition, where these sows mobilized more fat than control sows.

5.1. Energy and protein intake

There was a tendency to increased daily ME intake from compound feed in the low protein group (68.3 MJ ME/D vs 64.7 MJ ME/d; $P = 0.10$;Table 4) and the low protein group had a higher daily grass clover intake than the control group (2.60 kg/d vs. 2.29 kg/d; $P = 0.007$; Table 5).

Heat production was lower when estimated by heart rate as compared with estimation using the factorial approach (Figure 4). In gestation, the energy requirement was 30 MJ ME/d and 34 MJ ME/d on d60 and d100. The total daily energy intake from compound feed plus grass clover amounted 58 MJ ME/d and 64 MJ ME/d on d60 and d100 in gestation, respectively. The SID lysine contribution from grass clover was 3.36 g/d and 3.40 g/d on d60 and d100, respectively, and the total SID lysine intake amounted to 21.4 g/d and 23.6 g/d in mid and late gestation, respectively. The estimated SID lysine balance was positive in gestation and amounted to 7.92 g/d and 4.71 g/d on d60 and d100 in pregnancy, respectively.

In lactation, the estimated energy requirement was 99 MJ ME/d on d5. This peaked at 130 MJ ME/d on d20 and declined to 95 MJ ME/d on d40. The lactating sows mobilized 44 MJ ME/d on d5 and 48 MJ ME/d at peak lactation (d 20). The total daily ME intake from compound feed and grass clover increased from 49 MJ/d on d5 to 103 MJ/d on d40 ($P = 0.001$). In lactation, the estimated requirement for SID lysine was 50 g/d on d5, 70 g/d at peak lactation and declined again to 52 g/d on d40 of lactation. The total SID lysine intake from compound feed and grass increased from 21 g/d in early lactation to 47 g/d in late lactation ($P < 0.001$). A pronounced deficit of SID lysine was observed at d 5 (-26 g/d) and at d 20 (-20 g/d), whereas no deficit was observed at d 40 of lactation.

There was no effect of reduced dietary protein level on sow weight, body pools, back fat, number of live born, birthweight, piglet daily gain, litter weight or piglet mortality. The total daily heat production estimated from heart rate increased from 28 MJ ME/d on day 60 in gestation to 39 MJ/d in early lactation and peaked at 40 MJ ME/d at d 40 of lactation ($P < 0.001$).

Sows daily distance was 2.47 ± 0.14 km/d in mid gestation and 1.71 ± 0.14 km/d in late gestation. On d5 in lactation, daily distance was 0.82 ± 0.13 km/d while daily distance was 1.44 ± 0.14 km/d and 1.64 ± 0.14 km/d on day d20 and d40 in lactation, respectively. The estimated daily energy expenditure for locomotory activity ranged within 1.9 MJ ME/d at peak lactation to 5.3 MJ ME/d on d60 in gestation and did not depend on protein level.

Table 3
Changes in body composition of 2nd parity sows fed 100% organic compound feed differing in proportion of protein.

	Reproductive stage, d					Protein level			P-value		
	60-100[1]	100-5	5-20	20-40	SEM	Control	Low	SEM	Stage	Protein level	Protein × stage
Live weight gain, kg	40.55^{a}	-20.41^{c}	-25.83^{c}	-11.71^{b}	1.70	-3.47	-5.22	1.25	<0.001	0.31	0.39
Backfat gain, mm	2.89^{a}	1.37^{a}	-4.12^{b}	-3.43^{b}	0.63	-0.46	-1.17	0.45	<0.001	0.26	0.48
Protein gain, g/d.	96^{a}	-169^{b}	-127^{b}	63^{a}	47	-8	-60	53	<0.001	0.55	0.05
Fat gain, g/d.	692^{a}	-223^{b}	-1383^{c}	-275^{b}	160	-342	-252	137	<0.001	0.69	0.04
$RE_{fat+protein,}$ MJ ME/d	30.8^{a}	-12.5^{b}	-60.2^{c}	-14.0^{b}	4.17	-14.7	-13.3	6.20	<0.001	0.89	0.22
$HE_{retention}$[2]	6.27										

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

[1] 60-100 covers day 60 to day100 in gestation. 100-5 covers day 100 in gestation to day 5 in lactation. 5-20 covers day5 to day 20 in lactation and 20-40 covers day 20 to day 40 in lactation.

[2] Heat energy (HE) for retention in gestation was calculated as: HE retention, MJ/d = (((daily protein gain, g/d × 23.9 KJ/g)/0.60) - (daily protein gain, g/d × 23.9 KJ/g) + ((daily fat gain, g/d × 39.8 KJ/g)/0.80) - (daily fat gain, g/d × 39.8 KJ/g))/1000 according to (Theil, 2020).

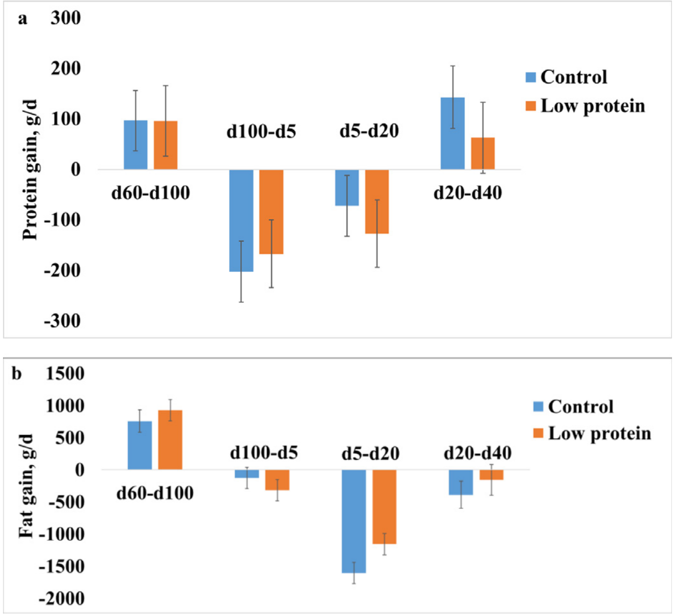

Figure 3. Retention of protein (panel a) and fat (panel b) in sows fed either a control or a low protein diet (negative retention represent body mobilisation.

When adding energy from fat mobilization with the sum of energy intake from grass clover and compound feed, the energy output estimated factorially was 5 to 13% greater than the total energy input in this model (Figure 5).

5.2. Plasma and urine

There was no overall effect of dietary protein strategy on plasma glucose, urea, lactate, triglycerides, creatinine or NEFA (Table 6). Similarly, no evidence for differences due to dietary strategies was observed for urinary urea, creatinine or pH in this experiment. Plasma glucose decreased from 4.62 mM on d5 in lactation to 4.11 mM on d20 and 3.96 mM on d40 in lactation ($P<0.001$). Plasma creatinine decreased with progress of lactation from 145 µM on d5 to 130 µM on d20 and 123 µM on d40 ($P<0.001$). In lactation, plasma NEFA increased from 871 µM in early lactation to 1454 µM at peak lactation, where after is decreased to 837 µM on d40 ($P< 0.001$).

5.3. Milk

Milk macro chemical composition was not affected by the dietary strategies, except that milk casein content was lower in sows fed the low protein strategy (3.99 % vs 4.23 %; $P = 0.002$; Table 7). Milk yield, energy output in milk and milk composition changed with progress of lactation. Milk yield was on average 9.7 kg/d on d5, 15.7 kg/d on d20 and 11.8 kg/d on d40 ($P<0.001$; Figure 6). Energy output in milk was 51 MJ/d on d5, 77 MJ/d on d20 and 55 MJ/d on d40 ($P<0.001$). Milk DM decreased from 19.1 % on d5 to 18.4% on d20 and 17.8 % on d40 ($P<0.001$). Milk protein was lower on d20 compared to d5 and d40 ($P<0.001$). Milk fat concentration was higher in early as compared with late lactation (8.17% vs 6.98%; $P=0.02$).

6. Discussion

6.1. Energy supply and mobilization

Sows on the low protein strategy consumed 8% more DM from grass clover and had a tendency to ingest more compound feed to sows fed the control diet. This might explain why there were no effects of 12% reduced protein concentration in the compound feed on body composition, plasma parameters and nearly all other measured performance traits of sows and piglets in this study. Apart from grass clover intake, dietary protein only affected milk casein content, which was lower in the low protein group. This is in accordance with recent dose-response studies with dietary protein (Pedersen et al., 2019b; Hojgaard et al., 2019b). In line with the response on casein, there was a tendency to a lower protein content (4.98% vs. 5.38%; $P = 0.12$) and lower fat content (6.96% vs. 8.27%; $P = 0.12$) in milk in the low protein group, and especially the change in milk fat could indicate that the protein supply was insufficient in the low protein group. However, milk casein and milk protein concentration continue to increase if sows are fed excessive amounts of dietary protein, so this response does not necessarily support undersupply of dietary protein (Højgaard et al, 2019a) and 4.9% protein in milk is regarded optimal for piglet growth as a mean for the entire lactation period (Hojgaard et al., 2020), which was also observed in the low protein group.

The analyzed energy content in the lactation compound feed was 12.2 and 12.0 MJ ME/kg with 148 g/kg and 130 g/kg of crude protein in the two dietary groups, respectively. The energy density and protein content in both lactation diets were lower than recommended in organic summer lactation diets byShurson et al a. 2012), who suggests an energy content of 12.8-13.9 MJ ME/kg and 179-226 g/kg of crude protein.

The sows in this study had 20.5 mm of back fat on day 100 of gestation, which is regarded optimal for sow productivity, and feed intake followed the supplied amounts throughout pregnancy. However, the daily compound feed intake was only two thirds of the supplied amount in early and peak lactation in both dietary groups, which resulted in insufficient energy intake in early and peak lactation. As a consequence of the insufficient energy intake, the sows had a considerable live weight loss and a huge daily fat mobilization (> 1300 g/d of body fat) at peak lactation. In comparison, indoor conventional sows mobilize 664 g/d of fat from d3-28 (Pedersen et al., 2016) and the fat

Table 4

Body pools, heat production and reproductive performance in 2nd parity sows fed iso-energetic organic compound feed differing in proportion of protein

	Reproductive stage[1]						Proteinlevel			P-values		
	60	100	5	20	40	SEM	Control	Low	SEM	Stage	Protein	Protein × stage
Sow weight, Kg.[2]	262.6^{c}	303.2^{a}	283.1^{b}	257.3^{cd}	245.4^{d}	4.37	271.0	269.8	3.52	<0.001	0.82	0.95
Compound feed intake, kg/d	4.28	4.76	3.64	6.00	8.60	0.19	5.39	5.53	0.12	<0.001	0.37	0.93
Waterpool, Kg.	156.1^{a}	157.0^{a}	145.4^{b}	146.0^{abc}	140.0^{c}	4.21	148.9	148.9	5.00	<0.001	0.99	0.86
Proteinpool, Kg.	45.8^{b}	49.7^{a}	45.6^{b}	43.9^{bc}	40.8^{c}	0.89	45.3	45.3	0.99	<0.001	0.90	0.97
Fatpool, Kg.	42.7^{b}	66.7^{a}	66.1^{a}	44.4^{b}	34.1^{b}	3.99	51.5	50.0	4.70	<0.001	0.84	0.84
Ashpool, Kg.	10.5^{a}	10.3^{a}	9.3^{b}	9.7^{ab}	9.6^{ab}	0.47	9.85	9.94	0.51	<0.001	0.61	0.92
Backfat, mm[3].	17.6^{b}	20.5^{a}	22.0^{a}	17.3^{b}	14.1^{c}	1.19	18.4	18.2	1.51	<0.001	0.92	0.15
Heartrate, bpm[4]	96^{c}	103^{b}	102^{bc}	115^{a}	120^{a}	2.42	107	107	2.70	<0.001	0.97	0.36
Daily distance, m	2466^{a}	1709^{b}	819^{c}	1445^{b}	1642^{b}	161	1713	1505	179	<0.001	0.49	0.26
Heatproduction												
$\text{HE}_{\text{Maintenance}}$, MJ ME/d	30.0^{bc}	33.4^{a}	33.2^{a}	30.9^{b}	29.8^{c}	0.38	31.5	31.4	0.30	<0.001	0.78	0.95
$\text{HE}_{\text{Locomotory activity}}$, MJ ME/d	5.30^{a}	3.88^{b}	1.92^{c}	3.19^{b}	3.31^{b}	0.33	3.70	3.35	0.32	<0.001	0.53	0.71
$\text{HE}_{\text{Thermoregulation}}$, MJ ME/d[5]	10.8^{a}	6.4^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.05	3.4	3.4	0.03	<0.001	0.58	0.99
$\text{HE}_{\text{Milkproduction}}$, MJ ME/d			13.7^{b}	22.1^{a}	11.5^{c}	0.48	15.1	16.5	0.46	<0.001	0.15	0.16
$\text{HE}_{\text{Factorial,}}$ MJ ME/d	46.4^{bc}	43.3^{c}	48.8^{b}	56.1^{a}	43.2^{c}	0.89	47.5	47.6	0.59	<0.001	0.81	0.68
Total heat production, MJ ME/d	28.4^{d}	30.4^{c}	38.6^{b}	40.3^{a}	40.9^{a}	0.44	34.7	34.2	0.47	<0.001	0.51	0.36
Piglet performance												
Piglets, no/sow[6]			15.6^{a}	13.0^{b}	12.7^{b}	0.29	13.9	13.7	0.24	<0.001	0.55	0.96
Piglet weight, kg[7,8]			1.75^{c}	6.20^{b}	11.5^{a}	0.19	6.43	6.54	0.21	<0.001	0.75	0.83
Litter weight, Kg			27.0^{c}	78.7^{b}	144.2^{a}	2.5	81.5	85.1	2.4	<0.001	0.41	0.64

a-dWithin a row, values without common superscript letters, differ ($P < 0.05$)

[1] Day 60 and 100 in gestation and day 5, 20 and 40 in lactation.

[2] Sows weighed 189 kg at weaning of the previous litter in the control group and 191 kg in the low protein group ($P=0.89$).

[3] Back fat at insemination were 12.3 mm in the control group and 12.9 mm in the Low protein group ($P=0.92$). Back fat at weaning were 13.9 mm and 11.5 mm in the two groups respectively ($P=0.99$).

[4] Average heart rate recorded during daytime (10 h and 27 minutes; minimum 9h; max 12 h 2 min).

[5] $\text{HE}_{\text{termoregulation}}$ in lactation is considered to be 0 as lactating sows have a very high heat production and most likely do not oxidise additional feed to maintain a constant body temperature during lactation in the summer period.

[6] Liveborn piglets/litter were 16.1 in the control group and 17.4 in the low protein group ($P=0.32$). Still born piglets/litter were 2.58 in the control group and 1.82 in the low protein group ($P=0.18$).

[7] Piglet birth weights were 1498 g in the control group and 1514 g in the Low protein group ($P=0.99$).

[8] Piglet weaning weights at d49 were 15.6 kg in the control group and 16.2 kg. in the Low protein group ($P=0.79$).

mobilization was 732 g/d on day 4-18 in high-yielding indoor sows (Pedersen et al., 2019a). The sows lost on average 7.9 mm back fat and 37.7 kg of live weight from d5 to d40 of lactation in the present study, which is substantially greater than normally observed for indoor sows. Earlier investigations reported that commercial indoor sows lost 21, 22 and 23 kg during a 4 week lactation period in three trials (Vadmand et al., 2015), and organic sows with more than 10 piglets may lose 24-30 kg in 6 weeks lactation (Weissensteiner et al., 2018). The sows in

Table 5

Fresh grass clover intake and intake of compound feed in 2nd parity sows fed 100% organic compound feed differing in proportion of protein.

	Reproductive stage[1]						Proteinlevel			P-values		
	60	100	5	20	40	SEM	Control	Low	SEM	Stage	Protein	Protein × stage
Compound intake, kg/sow/d	4.28^{c}	4.76^{c}	3.64^{d}	5.99^{b}	8.60^{a}	0.16	5.38	5.53	0.12	<0.001	0.36	0.93
ME intake, compound feed MJ/d	52.5^{c}	58.3^{c}	45.5^{d}	74.1^{b}	102.3^{a}	2.31	64.7	68.3	1.59	<0.001	0.10	0.93
Grass clover intake, kg/d	2.45^{b}	2.44^{b}	1.55^{c}	3.16^{a}	2.62^{b}	0.13	2.29^{b}	2.60^{a}	0.78	<0.001	0.007	0.83
Grass intake, g DM/d	428^{b}	409^{b}	225^{c}	574^{a}	472^{b}	0.02	403^{b}	440^{a}	0.01	<0.001	0.04	0.74
ME intake, grass clover, MJ/d	5.49^{b}	5.11^{b}	2.96^{c}	7.55^{a}	6.27^{b}	0.28	5.31	5.67	0.17	<0.001	0.17	0.99
Total ME intake, MJ/d	58.3^{c}	64.2^{c}	48.8^{d}	84.0^{b}	103.4^{a}	2.74	74.0	69.5	1.87	<0.001	0.07	0.58
SID lysine intake, compound feed, g/d	18.06^{d}	20.14^{c}	18.44^{d}	30.2^{b}	43.09^{a}	0.82	27.52^{a}	24.33^{b}	0.54	<0.001	<0.001	0.05
SID lysine intake grass clover, g/d	3.36^{b}	3.40^{b}	2.11^{c}	4.51^{a}	3.70^{b}	0.16	3.27	3.54	0.11	<0.001	0.08	0.97
Total SID lysine intake, g/d[2]	21.4^{c}	23.6^{c}	20.9^{c}	34.1^{b}	46.7^{a}	0.92	31.0^{a}	27.6^{b}	0.61	<0.001	<0.001	0.10
Energy requirement, MJ ME/d[3]	30.4^{c}	34.0^{c}	98.6^{b}	129.6^{a}	95.0^{b}	2.70	76.3	78.8	2.18	<0.001	0.50	0.75
SID lysine requirement, g/d[4,5]	13.4^{c}	18.8^{c}	49.5^{b}	69.7^{a}	51.9^{b}	2.69	39.2	41.8	1.89	<0.001	0.31	0.86
ME balance, MJ/d[6]	14.9^{a}	23.6^{a}	-43.6^{b}	-48.1^{b}	1.40^{a}	6.31	-8.62	-12.1	5.49	<0.001	0.69	0.06
SID lysine balance, g/d	7.92^{a}	4.71^{a}	-26.2^{b}	-20.2^{b}	3.81^{a}	3.18	-4.51	-7.45	2.49	<0.001	0.48	0.18

a-d Within a row, values without common superscript letters, differ ($P < 0.05$)

[1] Day 60 and 100 in gestation and day 5, 20 and 40 in lactation.

[2] Digestibility of lysine from grass clover is set to 71.4% based on (Eskildsen, et al., 2020).

[3] Energy Requirement is calculated as $\text{ME}_{\text{Requirement}} = \text{HE}_{\text{factorial}} + \text{Milk energy output}$

[4] SID lysine requirement in gestation is based on (Samuel, 2012)

[5] SID lysine requirement in lactation is calculated as 2.5 + (milk protein output (g/d) × 0.071)/0.80 (NRC, 2012; (Feyera and Theil, 2017)

[6] $\text{ME}_{\text{balance}}$ is energy for retention/mobilization

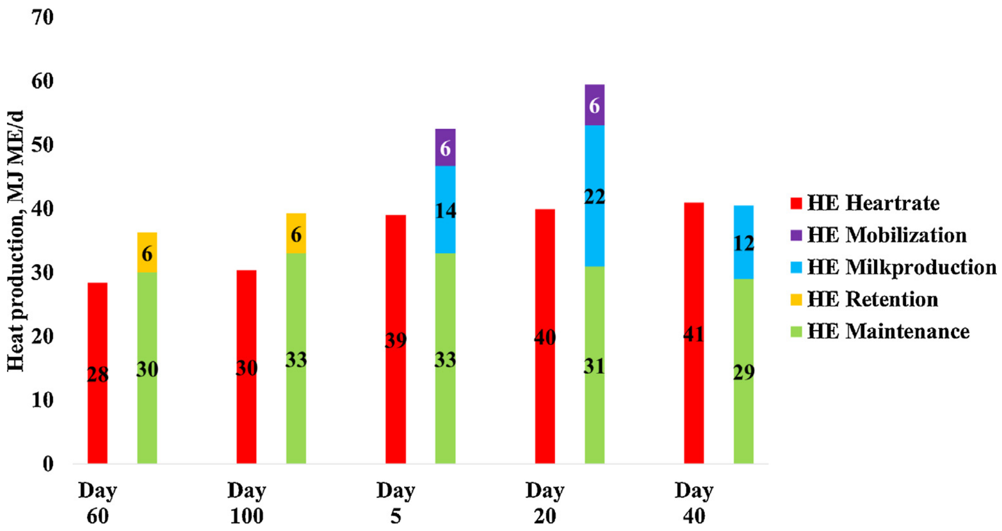

Figure 4. Heat production estimated using recorded heart rate or estimated using a factorial approach in sows fed either a control or a low protein diet.

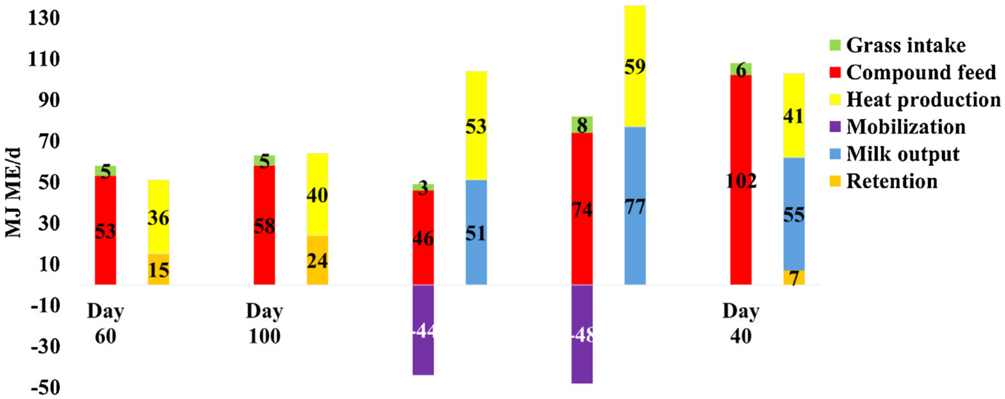

Figure 5. Estimated daily energy input and output on day 60 and 100 in gestation and day 5, 20 and 40 in lactation in 2nd parity sows fed organic compound feeds in summer. Heat production was estimated using a factorial approach. However, energy required for thermoregulation was not included as sows produce substantial amounts of extra heat due to energy retention during pregnancy and due to milk production during lactation. Moreover, energy produced due to locomotive activity was not included as minimal activity is part of the maintenance concept, and the physical activity level of outdoor sows are really low.

this trial had an average back fat thickness of 12.9 mm at weaning on d49. This is in accordance with Kongsted and Hermansen (2009), who also found the average back fat at weaning of organic sows to be 13 mm. This is an acceptable body condition at weaning as it is possible to gain 3-6 mm of back fat in the following gestation period and thereby achieve 16 to 19 mm of backfat prior to the next lactation period (Sørensen and Krogsdahl, 2018).

The concentration of NEFA in plasma also suggests, that the sows had a very high body fat mobilization. The plasma NEFA concentrations were generally higher as compared with indoor sows (Le Cozler et al., 1999; Hansen et al., 2012), suggesting that organic sows are clearly challenged with insufficient energy intake in early and peak lactation. While sows were undersupplied with energy and SID lysine in early and peak lactation, their energy intake matched fairly well their energy requirement in late lactation, where the energy output in milk and heat production associated with milk production declined from 99 MJ ME/d to 67 MJ ME/d. The markedly lower energy intake as compared with their energy supply in early lactation indicate that the appetite of the sows or, alternatively, the gastric capacity, was a limiting factor for the energy intake, which in turn caused a clear negative energy balance. The fairly low feed intake could at least partly be ascribed to the fact, that only young sows were studied in the present experiment. It is not

Table 6
Urine and plasma metabolites in 2nd parity sows with ad lib. access to clover grass and fed iso-energetic organic compound feed differing in proportion of protein

	Reproductive stage[1]						Protein level			P-values		
	60	100	5	20	40	SEM	Control	Low	SEM	Stage	Protein	Stage × Protein level
Urine												
pH	6.99^{c}	6.81^{c}	7.46^{ab}	7.66^{a}	7.35^{b}	0.08	7.28	7.23	0.07	< 0.001	0.67	0.51
Urea, mM	236^{a}	227^{a}	122^{b}	166^{b}	152^{b}	15.6	180	181	13.8	< 0.001	0.97	0.29
Creatinine	15306^{ab}	18009^{a}	11799^{bc}	12688^{b}	8407^{c}	1516	12843	13641	1697	< 0.001	0.77	0.66
Plasma												
Glucose, mM	4.27^{ab}	4.31^{ab}	4.62^{a}	4.11^{b}	3.96^{b}	0.11	4.25	4.26	0.06	< 0.001	0.89	0.85
Urea, mM	3.28^{ab}	3.08^{b}	3.01^{b}	3.51^{a}	3.53^{a}	0.13	3.27	3.29	0.13	< 0.001	0.93	0.87
Lactate, mM	2.10^{a}	2.22^{a}	1.82^{ab}	1.54^{b}	1.76^{ab}	0.17	2.03	1.75	0.18	0.005	0.39	0.71
TG, mM	0.35^{c}	0.48^{ab}	0.39^{bc}	0.51^{a}	0.39^{bc}	0.02	0.42	0.42	0.02	< 0.001	0.98	0.17
Creatinine, µM	137^{bc}	153^{a}	145^{ab}	130^{cd}	123^{d}	4.55	137	139	5.23	< 0.001	0.78	0.63
NEFA, µM	53^{c}	181^{c}	871^{b}	1454^{a}	837^{b}	84	678	680	52	< 0.001	0.99	0.99

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

[1] Day 60 and 100 in gestation and day 5, 20 and 40 in lactation

Table 7
Milk composition in 2nd parity sows fed iso-energetic organic compound feed differing in proportion of protein

	DIM				Protein Level			P-values		
	5	20	40	SEM	Control	Low	SEM	DIM	Protein	DIM × protein
Milk yield[1], Kg/d	9.67^{c}	15.69^{a}	11.77^{b}	0.62	11.61	13.14	0.71	$<$0.001	0.27	0.65
Milk output[2], MJ ME/d	51.0^{b}	76.6^{a}	55.0^{b}	3.4	58.4	63.3	2.9	$<$0.001	0.22	0.89
DM, %	19.06^{a}	18.36^{ab}	17.80^{b}	0.38	19.16	17.65	0.39	0.02	0.11	0.89
Protein, %	5.43^{a}	4.83^{b}	5.28^{a}	0.11	5.38	4.98	0.08	$<$0.001	0.12	0.57
Lactose, %	4.81^{b}	4.93^{ab}	5.02^{a}	0.04	4.88	4.96	0.03	$<$0.001	0.24	0.93
Fat, %	8.17^{a}	7.70^{ab}	6.98^{b}	0.35	8.27	6.96	0.35	0.02	0.12	0.88
Casein, %	4.17	3.99	4.15	0.06	4.23^{a}	3.99^{b}	0.06	0.09	0.002	0.35

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

[1] Milk yield calculated as in (Hansen et al., 2012)

[2] Energy in milk calculated as in (Weast, 1984)

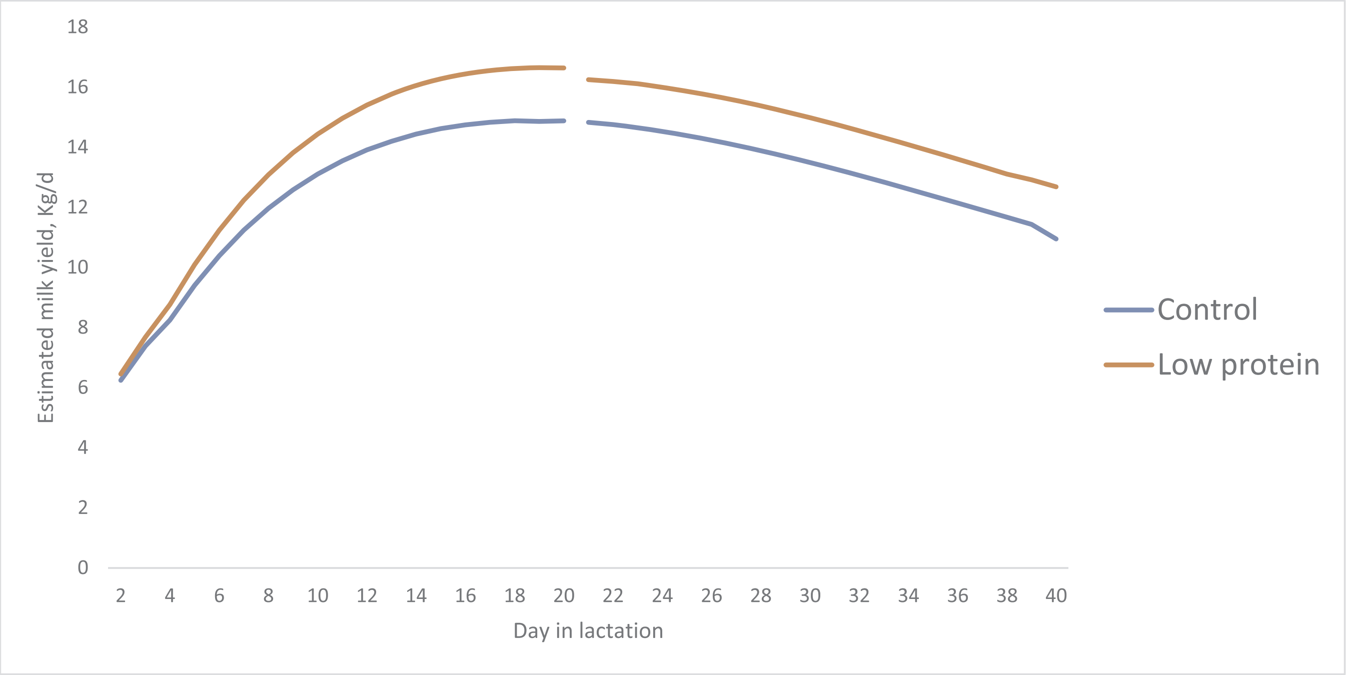

Figure 6. Estimated milk yield d2 to d40 in 2nd parity sows fed iso-energetic organic compound feed differing in proportion of protein. The milk yield is based on litter size and piglet weight gain in the two periods d1 to d20 and d20 to d40. The control group had an average daily litter gain of 2.43 kg/d and the low protein group had an average daily litter gain of 2.56 kg/d ($P = 0.42$).

likely that the low feed intake is due to heat stress, as the average temperature during the experimental period in 2017 was 13.5°C and the number of hours with sunshine was the lowest in Denmark in 17 years.

At peak lactation, the sows had a total daily energy requirement of 130 MJ ME/d. To fully meet this demand and avoid fat mobilization, the energy intake should have been an additional 48 MJ ME/d at peak lactation, which corresponds to 4 kg extra feed per day equivalent to a total of approximately 10 kg/d of compound feed. However, sows had substantial feed residues in early lactation whereas the feed intake approached the feed supply as lactation progressed, which indicates that a gastric capacity was the limiting factor during early and peak lactation. The feed intake corresponded to levels found in other outdoor experiments with 2nd parity sows. Kongsted et al., (2011) reported a daily intake of 5 kg/d on d10 and approximately 7 kg/d on d20, but they reported a body weight loss of only 470 g/d in lactation, most likely because the litter size in that study was lower and amino acid recommendations in Denmark has been improved substantially since 2013, whereby sows now produce more milk (Hojgaard, 2020).

6.2. Energy output and energy requirement

Energy requirement for maternal growth (including reproductive organs, fetuses and HE associated with these traits) during gestation was 20 MJ ME/d, which is clearly more than for indoor sows (Theil et al., 2002). The discrepancy may be linked to restoration of body condition, which normally is done in early gestation but must be prolonged for organic sows as they loose more body fat than indoor sows. The sows gained 40.6 kg from d60 to d100 in pregnancy, hence the energy requirement per kg gain was 20.3 MJ ME/kg gain (including conceptus). This complies well with the NRC (2012), which estimates the requirement for maternal gain to be 19.8 MJ ME/kg gain.

Thermoregulation is an aspect, where the energy requirement potentially may deviate between organic and conventionally produced sows, and of course this is most pronounced during the winter period (Eskildsen et al, 2020b). The lower critical temperature of sows is 18°C (Verhagen et al., 1986) and energy required for thermoregulation in pregnant sows in April (9.2°C) and May (13.3°C) was on average 10.8 MJ ME/d and 7.40 MJ ME/d, which is equivalent to 12-16% of the total energy output. However, the pregnant sows were fed well above their maintenance requirement and their energy retention produced considerable amounts of heat, which most likely eliminate their need for thermoregulation. During lactation, the 12-h day time temperatures were higher (13.3°C - 17.4°C), and energy spent on thermoregulation was calculated to be 1.19 ME MJ/d to 3.71 MJ ME/d. Again, most likely, sows do not require additional oxidation of nutrients to maintain a constant body temperature during lactation in the summer period, because milk production per se generate a huge amount of heat. Therefore, the calculated energy required for thermoregulation was not included in the total heat production and total energy required estimated factorially during the summer.

The energy demand for locomotive activity in outdoor sows has not previously been studied in details, and estimates of this activity has therefore largely relied on speculation. In the current study, however, locomotive activity had a minor or even negligible impact on the amount of energy required. Our results are in line with Buckner, (1996), who showed pedometer values indicating a range of 0.1-3.1 km/d. It seems that the energy expenditure for physical activity in organic pregnant sows is lower than the heat production associated with physical activity for indoor housed sows. Pregnant sows kept outdoor are standing/walking 7% of the day in summer (Buckner et al.,1998), whereas (Lambert et al., 1983) find, that indoor pregnant sows are active 18-24% of the day. The activity level has been shown to depend on the level of hunger (Edwards, 2003), and due to the ad libitum access to grass clover, outdoor sows probably feel less hungry than indoor sows. Also, indoor sows may express stereotypic behavior, which outdoor sows most likely do not perform. The energy expenditure for physical activity was approximately 5.3 MJ ME/d in mid gestation and 3.9 MJ ME/d in late gestation. In lactation, the cost due to physical activity was 3.2 MJ ME/d after d 20, which was in accordance with Close and Poornan (1993), who calculated that an outdoor ringed 240 kg sow walking 1 km/day would dissipate an additional 2.1 MJ ME/d due to locomotive activity. A minimum of physical activity is included in the maintenance concept, and since the organic sows appear to be less physical active than indoor sows (which stand up for 6 and 4 h in gestation and lactation, respectively), the calculated. HE estimated by heart rate was clearly lower than the heat production calculated factorially, and most likely the factorial approach was more reliable. Part of the reason for that is that most of the time, periods with high physical activity were deleted when recording the heart rate, as the active periods occurred shortly after the equipment was put around the sows. However, it was not possible to clarify when a high heart rate was due to stress of the sow and when it was due to elevated physical activity. The heat production was estimated too low using the heart rate, as it was even lower than the energy required for maintenance during gestation and it is well known, that feed intake above maintenance increases the heat production further. Also during lactation the heat production estimated using heart rate was not reliable, as the heat production was estimated to be almost constant using this approach, in spite of a substantial increase in milk production from d 5 to 20, which is known to generate a lot of extra heat (Feyera and Theil, 2017). The heat production estimated factorially was higher for lactating sows in the present study than that measured by Theil et al. (2004), because sows in the latter study weaned less than 10 piglets and therefore had a much lower milk production than the sows in the present study, which weaned almost 13 piglets.

Optimal nutrient supply during lactation is important because milk production is associated with a massive drainage of nutrients each day and the lactation period in organic production is at least 40 days according to EU legislation and even higher in some countries due to national industry agreements, e.g. 49 days in Denmark. In this experiment, 76% of the total energy requirement was associated with milk production at peak lactation. On d20, the sows weighed 257 kg and produced 15.7 kg milk/d for 13 piglets/litter with a daily litter gain of 3.4g/d, which corresponds levels found in high performing indoor herds (Hojgaard et al., 2019a; Hojgaard et al., 2019b). This was at a cost of 76.6 MJ ME/d for milk output and 22.1 MJ ME/d for heat associated with milk production. This is slightly higher than that reported by Close and Poornan (1993), who stated, that a 240 kg outdoor sow with 12 piglets have a calculated energy requirement of 69,6 MJ ME/day when supporting 2.4 kg/d of litter gain. As compared with milk composition of conventional indoor sows, the overall DM- and energy content was similar. Milk yield was predicted by use of a mathematical model developed to quantify milk yield of conventional sows with extrapolation of the model to organic conditions. The model was not built to estimate milk yield for more than 30 days, or for sows with a litter weight gain of more than 4.2 kg/d or more than 14 piglets/litter. A daily litter weight gain of more than 4.2 kg/d was frequently observed in some individual sows in the present study, indicating a really high milk yield, but this value is the maximal allowed input to the model, so the actual milk yield was most likely underestimated, which is supported by the greater energy mobilisation estimated with the D_2O technique as compared with the energy balance estimated factorially. However, an unknown part of the litter weight gain also originated from piglets ingesting sow feed, mainly in the period from d20 and onwards (M. Eskildsen, personal observation).

6.3. Grass intake

The daily grass intake was estimated to be 420 g DM/d in gestation, based on a prediction equation using plasma pipecolic acid concentration in sows fed a known amount of grass clover (Eskildsen et al., 2020a). By use of the N-alkane method, Sehested (1999) reported a daily DM grass intake of 2.4 ± 0.6 kg/d in June and 3.7 ± 2.1 kg/d in August of pregnant sows supplied with only 1 kg/d of compound feed. Fernandez et al. (2006) calculated, that the average daily intake of clover grass was 21 MJ ME/d based on the daily gain of pregnant sows. The average grass clover intake in the study by Fernandez et al (2006) contributed with 18% of the maintenance energy requirements. Edwards (2003) reported that the intake of grazed herbage is 2,0 kg DM/d for dry sows fed restricted compound feed and that this intake can contribute with up to 50% of the maintenance energy requirement. Likewise, Rivera Ferre et al. (2001) reported that herbage intake amounted to proportionately 50% (spring), 66% (early summer) or 49% (late summer) of the maintenance energy requirement of pregnant sows. The sows in the studies by Rivera Ferre et al. (2001) were fed 1.5 or 3.0 kg compound feed once a day, and as earlier observed in growing pigs (Danielsen et al., 2001; Kongsted et al., 2015), it would probably have been possible to increase the daily grass intake, if sows were supplied less compound feed supply during gestation than the 4.6-5.0 kg/d supplied in two meals in the current study.

The nutritional contribution made by grazing depends on the availability, nutrient composition, intake and quality (fermentability) of the grass. The grass quality decreased over the summer in this experiment as evidenced by the energy concentration in grass clover, which declined from 12.4 MJ ME/kg ultimo April to 6.7 MJ ME/kg primo September. Also, the content of calcium, phosphor and most amino acids in grass were reduced through the growing season. In the study by Rivera Ferre et al, (2001), the apparent total tract digestibility of organic matter in ryegrass varied from 79% in the spring to 47% in the summer. The voluntary daily intake of organic matter from herbage varied from 0.2 kg/d to 1.8 kg/d in spring, between 0.9 kg/d and 2.4 kg/d in early summer and between 1.3 kg/d and 4.8 kg/d in late summer.

The intake of grazed grass clover varied widely between individuals and is in accordance with previous studies (Rivera Ferre et al., 1999; Sehested et al., 1999; Rivera Ferre et al., 2001; Edwards, 2003). Especially at peak lactation, where the sows were metabolically challenged, 20% of the sows consumed more than 6 kg/d of grass clover, and some even ingested up to 11 kg/d. The reason for this remarkable individual variation is largely speculation, as the appetite of the sow is influenced by a number of animal, dietary, environmental and husbandry factors. However, grass clover intake was highly correlated to live weight, as heavy sows consumed more grass clover than lighter sows, especially in late gestation and early lactation ($P<0.001$). At peak lactation, there was a positive correlation between grass clover intake and daily distance ($P<0.001$, $R^2 = 0.41$).

The higher grass clover intake in the low protein group, could not compensate for the lower SID Lysine content in the compound feed, and therefore the control group had a 12% higher daily intake of SID lysine. The increased grass intake in the low protein group confirm and the positive lysine balance indicate, that it is possible to reduce the protein content of organic compound feed in the summer time during gestation, whereas it cannot be recommended to reduce the dietary concentration of protein fed to lactating sows.

The pregnant sows ingested approximately 3 g/d of SID lysine from grass clover alone and the total SID lysine intake was around 22 g/d in mid and late gestation. Samuel et al. (2012) suggested a requirement of 13.4 and 18.7 g/d of SID lysine in early and late gestation of second-parity sows, respectively. Hence, grass clover intake can cover 16%-23% of the daily requirement for SID lysine in sows with an energy intake from compound feed of 53-58 MJ ME/d (4-4.5 kg/d) in pregnancy, and probably more if sows were more restrictedly fed.

Dietary crude protein levels of 129-156 g/kg for pregnant organic sows has been proposed, assuming no pasture supplementation (Shurson, et al., 2012) but it seems that, the dietary crude protein content can be reduced even further than 114 g/kg DM in gestation compound feeds without impairing sow productivity, when pregnant sows have ad libitum access to a good quality grass clover sward.

In lactation, sows with high milk yield require a high SID Lysine:ME ratio. The ratio was 0.40 at peak lactation in this study, which is clearly below the ideal ratio of dietary SID lysine to ME ratio of 0.55 reported being optimal for milk production at peak lactation (Feyera and Theil, 2017). At peak lactation, 95% of the daily SID lysine requirement is used for milk production (Feyera and Theil, 2017) and Close and Poornan (1993) stated, that outdoor sows between 160-360 kg with 10-12 piglets have a daily lysine requirement of 36-50 g in lactation. Sows in the present study had a high productivity as evaluated by their number of live born (16.5 piglets/litter) and daily litter gain (3.4 kg/d), and therefore their daily SID lysine requirement was as high as 70 g/d of SID lysine at peak lactation. This level is confirmed in high yielding indoor sows, which also had SID Lysine requirement of 68 to 70 g SID lysine/d at peak lactation (Gourley et al., 2017; Hojgaard et al., 2019b). The total SID lysine intake from compound feed and grass clover in early and peak lactation amounted only to 21 and 34g/d, respectively, emphasizing that SID lysine most likely was the limiting factor for milk production. On this basis, it is not recommendable to reduce the protein content in lactation compound feed for high producing outdoor sows on pasture unless a daily compound feed intake of at least 10 kg/d can be obtained.

6.4. Sow productivity

Sows in the current study had an average total energy intake of 67 MJ ME/d. A benchmark calculation in 13 Danish organic herds with sows in pasture systems (4806 sows in total) showed an average energy consumption of 66 MJ ME/d (SEGES Økologi, 2018). In comparison, the national average in conventional indoor pig production (416.481 sows) was 48 MJ ME/d, hence the sows in this experiment consumed 40% more energy on a daily than indoor sows when including their energy intake from gass. Others have reported, that outdoor sows have approximately 5-20% higher total energy requirement than indoor housed sows throughout the year (Close and Poornan, 1993; Jakobsen and Danielsen, 2006). In the present study, the average weaned litter size was 12.9, which is one piglet more per litter than weaned on average in Danish commercial organic herds (Hansen, 2018). This is probably due to fact, that the experimental sows were all of second parity. A weaned litter size of 12.9 is very high compared to the production level in organic herds in other European countries (Prunier et al., 2014), and (Weissensteiner et al., 2018) conclude, that litters with >10 piglets is regarded being a “large litter” in organic production systems. Average piglet weight at weaning after 49 days were 15.9 kg, which is also high compared to the 13.9 kg in Danish commercial organic herds (SEGES 2017).

7. Conclusion

Organic sows fed protein 12% below the Danish indoor recommendation during the summer period performed at least as good as control sows during gestation because their protein (and lysine) requirement were met by grazing and by greater intake of compound feed as compared with the feeding curve recommended for indoor sows. The protein restricted pregnant sows showed no negative effects on number of live born piglets, litter birth weight, sow body composition or urine and plasma metabolites during the summer period. Lactating sows did not meet their protein (and lysine requirement) because they did not manage to eat more compound feed than recommended for indoor sows. There is a large individual variation in voluntary grass clover intake and the gastric capacity seems to be a limiting factor for energy and protein intake from pasture during lactation. On average, sows fed low protein compound feed consumed 14% more fresh grass clover, and numerically their milk production was greater than sows fed the control diet. There were no indications that the low protein diet compromised the productivity of the sows, neither during gestation nor during lactation.

Declaration of Competing Interest

All authors declare that they have no conflicts of interests.

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Supplementary materials

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BRIEF SUMMARY OF INCLUDED MANUSCRIPTS

MANUSCRIPT I

Impact of increasing fresh grass-clover intake on nitrogen metabolism and plasma metabolites of sows

Maria Eskildsen, Mette Skou Hedemann, Peter Kappel Theil and Jan Værum Nørgaard

Submitted to Livestock Science in November 2019. Revised in April 2020

Background:

The contribution of energy and protein from pasture is largely unknown in organic sows.

Aims:

Paper I aims to provide information to help understand the impact on nitrogen and energy metabolism from increasing clover grass intake in grazing sows. The objective was to develop a simple and fast method to assess the voluntary daily intake of grass-clover on pasture and estimate the digestibility of protein and energy of fresh grass-clover in sows.

Main results:

The paper reports two plasma metabolites that can be used to predict the clover-grass consumption of sows on pasture, as the daily intake of organic matter or metabolisable energy from fresh grass-clover was found to be highly positively correlated with plasma pipecolic acid and also with a metabolite tentatively identified as plasma bisnorbiotin.

Apparent total tract digestibility of dry matter, organic matter, nitrogen and energy estimated for 100% fresh grass-clover intake in dry sows was 64%-72%. Nitrogen intake increased linearly with increasing fresh grass-clover intake, but nitrogen deposition and nitrogen utilization were not affected by grass intake within the range investigated. There was a linear increase in plasma urea content, when grass intake increased from 0 to 6 kg per day. Plasma glucose, lactate, creatinine, NEFA, and triglycerides were not affected by increased grass-clover intake.

MANUSCRIPT II

Effect of reduced dietary protein level on energy metabolism, sow body composition and metabolites in plasma, milk and urine from gestating and lactating organic sows during temperate winter conditions

Maria Eskildsen, Uffe Krogh, Martin Sørensen, Anne Grete Kongsted, Peter Kappel TheilSubmitted to Livestock Science in March 2020. Accepted in May 2020.

Background:

Organic sows consume 30% more compound feed with the same protein-to-energy ratio as indoor sows. This will likely cause decreased protein- and energy utilization and possibly cause oversupply of dietary protein resulting in increased nitrogen leaching from organic sows compared to indoor conventional sows.

Aims:

Paper II aims to contribute to a better understanding of the nutrient requirements of organic sows and improve the energy utilization of organic compound feed. The objective was to quantify energy needed for heat (maintenance, retention and milk production), thermoregulation and locomotory activity and study the effect of a reduced protein-to-energy ratio on metabolism and productivity of 1st parity sows during winter.

Main results:

Increasing the daily energy supply by 15% and lowering the protein content in compound feed by 12 % below the recommended level for indoor sows improved energy utilization without compromising sow productivity. The daily protein- and amino acid requirements of pregnant sows in the low protein group were met in gestation. However, it seems to be problematic to lower the protein content in lactation diets fed to first parity sows in winter, as they had insufficient feed intake in early and at peak lactation.

The total energy requirement of high yielding first parity outdoor sows during a mild winter was found to be 68 MJ ME/d in gestation and 153 MJ ME/d at peak lactation.

MANUSCRIPT III

Grass-clover intake and effects of reduced dietary protein for organic sows during summer

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Background:

Danish organic sows have access to pasture, where they will consume energy and protein from grass-clover especially during summertime. This contribution is, however, unknown, and this makes it difficult to optimize the compound feed for organic sows with regards to protein and energy content.

Aims:

Paper III aims to improve the understanding of energy and protein utilization of pasture fed sows. The objective was to quantify the energy and protein intake derived from grazing and the energy needed for maintenance, maternal retention, milk production, thermoregulation and locomotive activity in 2nd parity organic sows during summer.

Main results:

It is possible to reduce the protein content of organic concentrate in the summer time as grazing pregnant sows can obtain 16-17% of their daily SID lysine requirement from the sward. The daily protein- and amino acid requirements were met during pregnancy but not in early and at peak lactation. The total energy requirement of high yielding second parity outdoor sows during a normal Danish summer was found to be around 46 MJ ME/d during gestation and approximately136 MJ ME/d at peak lactation of which grass-clover contributed with 11% and 6%, respectively.

RESULTS

Protein level

- Sows on the low protein strategy consumed more grass-clover in summer than sows on the control protein strategy (P=0.007)
- Overall there was no effect of a 12% reduction in protein level on live weight of sows, body composition, energy requirements, or the measured plasma- and urine nutrients. Neither did litter size, milk yield, milk composition, or litter average daily gain differ between dietary treatments (P≥=0.10). The only exception was the casein content of milk, which was lower in milk from sows fed the low protein strategy (P<0.001)

Season and parity effects (confounded due to the experimental design)

- Sows gained 54 kg from first to second parity (P<0.001). There were 3.3 more liveborn piglets per litter in second parity compared to first parity (P=0.005)
- A seasonal/parity effect was observed in plasma concentrations of creatinine and lactate of sows. Plasma creatinine was higher during summer than winter (P= 0.02). In contrast, plasma lactate concentrations were higher in winter than during summer (P<0.001)
- Sows produced 2.2 kg more milk per day in summer (P=0.02). In contrast, NAGase activity in milk was higher (P<0.001), and there was a tendency to more fat in milk in winter (P=0.08)
- Piglet birth weight was ≈ 200 g higher (P=0.007), and litter weight at weaning was 50 kg higher (P= 0.03) in the second parity (154 kg vs. 204 kg). Piglets had higher plasma glucose levels (P=0.005) and lowered plasma NEFA concentration (P =0.04) in summer than in winter
- The energy requirement for thermoregulation was 13.5 MJ ME/d in winter and 5.23 MJ ME/d in summer (P<0.001), when energy for thermoregulation in lactation is not set to zero during the summer period.
- The total energy requirement was 13% higher in the second parity (P<0.001)

Reproductive stage

- The reproductive stage affected almost all measured parameters in Experiment 2. Sows weighed 273 kg and had a back fat thickness of 20.1 mm on d100 of gestation. From d5 to d40 of lactation, they lost, on average, 37 kg and 6.8 mm of back fat, which was equivalent to 48% of their body fat pool or ≈ 800 g/d of body fat as a mean value for the entire lactation ($P<0.001$).
- Urea in urine was higher in gestation than in lactation ($p<0.001$). Plasma urea was lower in gestation than in lactation ($p<0.001$). The content of urea in milk increased with the progress of lactation and reached a level of 894 on d40 ($P<0.001$). In contrast, milk glucose and the activity of LDH and NAGase decreased with the progress of lactation ($P<0.001$).
- Creatinine in urine was lower on d20 and d40 in lactation than in gestation and early lactation ($P<0.001$).
- Glucose concentration in plasma peaked on d5 with 4.78 mM, whereafter, it decreased to 3.81 mM on d40 of lactation ($p<0.001$).
- Plasma NEFA was on average 188 µM in gestation, where after is increased to 1049 µM on d5 of lactation and reached 1377 µM on d20 were after the concentration decreased to 805 µM on d40 ($p<0.001$).
- Milk energy output was 49.5 MJ ME/d on d5 and peaked with 72.7 MJ ME/d on d20 of lactation, whereafter, it decreased to 52.0 MJ ME/d on d40 of lactation ($P<0.001$). The glucose level of piglet plasma also peaked with 6.70 mM at peak lactation ($P<0.001$).
- The average distance walked in mid-gestation was 2.46 km/d, and in early lactation sows walked 0.7 km/d ($P<0.001$). The energy expenditure for locomotory activity was 4.83 MJ ME/d in mid-gestation and 1.40 MJ ME/d in early lactation ($P<0.001$).
- The total ME requirement was found to be on average 29 MJ ME/d in gestation, 93 MJ ME/d in early and late lactation, and 130 MJ ME/d at peak lactation ($p<0.001$).

Compared to low grass-clover intake, sows with high grass-clover intakes had:

- Higher intake of compound feed (P<0.001; R2 = 0.27). This was especially the case in late gestation plus early- and late lactation.
- Leaner bodies as grass-clover intake was negatively correlated to fatpool (P=0.02; R2 =-0,17) and backfat (P<0.001; R2=-0.28). There was a tendency to a positive correlation between grass-clover intake and body protein pool (P = 0.09; R2 =0.11).
- Higher activity level. There were higher heart rates in sows with high grass intake (P=0.01; R2 = 0.16), and they showed indications of a longer travelled daily distance (P=0.13; R2 = 0.11).
- Higher pH in urine (P=0.05; R2 =0.13).
- Less urinary creatinine (P<0.001; R2 = -0.25) and urea in urine (P<0.001; R2 = -0.23) in gestation plus early and peak lactation in Experiment 2. This is confirmed in Experiment 1, where the content of creatinine in urine also decreased with increasing grass-clover intake (P=0.05). However, in dry sows in Experiment 1, urea content in urine had a quadratic response and increased from 0 kg/d to 2 kg/d of clover grass whereafter it decreased (P= 0.04).
- Higher plasma urea in pregnancy and lower plasma urea at peak and late lactation than sows with low grass intake in Experiment 2. In the dry sows in Experiment 1, there was a linear increase in plasma urea content, when grass-clover intake increased from 0 to 6 kg per day (P=0.02).
- Lower plasma NEFA in mid gestation and throughout lactation in Experiment 2. There was no effect of grass-clover intake on plasma NEFA in Experiment 1.
- Lower blood glucose. In Experiment 1, there is an indication of a linear decreased plasma glucose level with increasing grass-clover intake (P=0.11). This is confirmed in Experiment 2, where grass-clover intake is negatively correlated to plasma glucose in mid-gestation (P=0.003; R2 = -

0.20). The effect is, however, reversed at peak and in late lactation, where it seems that plasma glucose is positively correlated to grass-clover intake (P= 0.06; R2 = 0.14) and (P=0.01, R2 = 0.19) on d5 and d20 of lactation.

- Higher content of triglycerides in plasma in gestation, but lower in lactation. Dry sows in Experiment 1 also showed a linear tendency to increased plasma triglycerides, with increasing grass-clover intake (P=0.06).
- Lower content of DM in milk. The content of fat and protein in milk was negatively correlated to grass-clover intake in early and late lactation, but not on d20.

Breed

- The TN70 sows weighed, on average 20 kg more than the DanBred sows (P=0.001). They were leaner, as they had a 3.10 kg larger protein pool and an average backfat thickness of 16.0 mm compared to the 19.00 mm in the DanBred sows (P<0.001).
- DanBred sows had on average, 0.9 live born and 0.5 still born piglet more per litter (P<0.001; P=0.02).
- Piglet birth weight was 252 g higher and total litter weight at weaning 16 kg higher in TN70 sows (P<0.001).
- Piglet mortality was 6.5 dead piglets per litter in Danbred sows and 4.1 dead piglets per litter in TN70 sows (P=0.001), and there was no difference in the number of weaned piglets per litter.
- TN70 had higher urinary levels of creatinine (P<0.001) and plasma glucose (P=0.05). DanBred sows had higher plasma urea levels (P<0.001).
- There was a tendency to a higher daily milk yield in TN70 sows (11.5 kg/d vs. 10.9 kg/d; P=0.09). There was also a tendency to higher plasma glucose levels in piglets from the TN70 sows (P=0.06).
- There was less casein (P=0.007), less glucose (P=0.003), and more NAGase (P<0.001) in milk from the DanBred sows.
- The DanBred sows spent less energy on locomotive activity, and thermoregulation and the total energy requirement was 5% higher in TN70 sows (P=0.05).

Table 7. Body pools, heat production and reproductive performance in 1st and 2nd parity sows fed iso-energetic organic compound feed differing in proportion of protein

	Reproductive stage[1]						Season[2]			Protein level			Breed			P-values			
	d60	**d100**	**d5**	**d20**	**d40**	**SEM**	**Winter**	**Summer**	**SEM**	**Control**	**Low protein**	**SEM**	**DanBred**	**TN70**	**SEM**	**Stage**	**Season**	**Protein**	**Breed**
Live weight, kg.	238^{c}	273^{a}	255^{b}	232^{c}	218^{d}	2.89	216^{b}	270^{a}	2.83	243	243	2.85	233^{b}	253^{a}	2.27	<0.001	<0.001	0.85	<0.001
Back fat, mm.	17.5^{b}	20.1^{a}	20.2^{a}	16.3^{b}	13.4^{c}	0.78	16.8	18.3	0.95	17.3	17.7	0.87	19.0^{a}	16.0^{b}	0.70	<0.001	0.31	0.79	<0.001
Protein pool, kg.	41.5^{b}	45.8^{a}	42^{b}	40.1^{bc}	39.3^{c}	0.58	37.6^{b}	45.9^{a}	0.47	42.2	41.3	0.48	40.2^{b}	43.3^{a}	0.42	<0.001	0.009	0.32	<0.001
Fat pool, kg.	41.4^{b}	60.4^{a}	57.8^{a}	37.4^{bc}	30.0^{c}	3.05	40.4	50.5	3.50	45.3	45.5	3.49	44.2	46.7	2.62	<0.001	0.14	0.95	0.16
Heart rate, bpm.	98^{d}	104^{c}	102^{cd}	114^{b}	124^{a}	1.76	111	107	1.76	109	109	1.42	109	108	1.20	<0.001	0.12	0.88	0.49
Distance, m.	2463^{a}	1881^{b}	704^{d}	1482^{c}	1674^{bc}	158	1661	1621	191	1725	1557	187	1631	1650	138	<0.001	0.89	0.56	0.81
Compound feed intake, kg/d	4.52^{c}	4.72^{c}	3.95^{d}	6.09^{b}	8.65^{a}	0.24	5.33	5.85	0.32	5.65	5.53	0.32	5.66	5.52	0.23	<0.001	0.30	0.79	0.16
Grass-clover intake (summer), Kg/d	2.45^{b}	2.44^{b}	1.55^{c}	3.16^{a}	2.62^{b}	0.13				2.29^{b}	2.60^{a}	0.78	2.47	2.42	0.08	<0.001		0.007	0.67
MElocomotive, MJ/d	4.83^{a}	3.90^{b}	1.40^{d}	2.62^{c}	2.96^{c}	0.28	2.76	3.52	0.27	3.21	3.07	0.26	2.91^{b}	3.37^{a}	0.22	<0.001	0.10	0.74	0.01
MEthermoregulation, MJ/d	15.4^{a}	8.14^{b}	14.7^{a}	8.04^{b}	0.34^{c}	0.36	13.5^{a}	5.23^{b}	0.23	9.33	9.34	0.24	8.96^{b}	9.72^{a}	0.23	<0.001	<0.001	0.96	0.02
HEfactorial, MJ ME/d	27.6^{a}	30.9^{d}	43.1^{b}	48.1^{a}	38.9^{c}	0.39	34.7^{b}	40.8^{a}	0.36	37.4	38.0	0.36	36.7^{b}	38.7^{a}	0.30	<0.001	<0.001	0.29	<0.001
HEmaintenance, MJ ME/d	27.8^{c}	30.8^{a}	30.7^{a}	28.6^{b}	27.2^{c}	0.26	26.6^{b}	31.5^{a}	0.26	28.9	29.0	0.25	28.1^{b}	29.9^{a}	0.21	<0.001	<0.001	0.87	<0.001
Retained energy, MJ ME/d[2]	24^{a}		$-12.^{b}$	-53.9^{c}	-9.56^{b}	3.20	-14.6	-11.3	2.37	-13.4	-12.5	2.63	-10.9	-14.9	2.55	<0.001	0.46	0.83	0.24
HEretention, MJ ME/d[3]	6.48^{a}		2.03^{b}	6.5^{a}	4.26^{ab}	0.67	4.78	4.85	0.63	4.87	4.76	0.55	4.72	4.92	0.52	<0.001	0.94	0.89	0.77
MErequirement, MJ/d	27.5^{c}	31.2^{c}	94.2^{b}	130.3^{a}	91.0^{b}	2.01	68.5^{b}	77.2^{a}	1.40	72.3	70.3	1.37	70.9b	74.7a	1.38	<0.001	<0.001	0.62	0.05
Liveborn/litter, no.							13.5^{b}	16.8^{a}	0.50	15.1	15.2	0.49	16.1^{a}	15.2^{b}	0.40		0.005	0.83	<0.001
Still born/litter, no.							2.10	2.17	0.64	2.59	1.69	0.64	2.40^{a}	1.86^{b}	0.47		0.93	0.37	0.02
Piglet birthweight, g.							1320^{b}	1509^{a}	30.2	1413	1416	30.8	1289^{b}	1541^{a}	24.5		0.007	0.95	<0.001
Weaning weight, Kg							14.6	16.1	1.07	15.5	15.2	1.07	14.7^{b}	15.9^{a}	0.77		0.36	0.86	<0.001
Litter weight d49, Kg							154^{b}	204^{a}	12.2	179	179	12.3	171^{b}	187^{a}	9.01		0.03	0.97	0.002
Total dead d1-40,no							4.90	5.60	0.73	5.60	5.00	0.75	6.50^{a}	4.10^{b}	0.55		0.54	0.55	<0.001
Weaned/ litter, no.							10.9^{b}	12.6^{a}	0.29	11.7	11.9	0.30	11.7	11.9	0.26		0.009	0.65	0.63

[1] D60 and d100 is days from insemination. D5, d20 and d40 are days post partum

[2] Season is confounded with parity, as the sows in winter were 1st parity, and sows in summer were 2nd parity

[3,4] Retained energy and $HE_{retention}$ on d60 is for the period d60-d100. d5 is for the period d100-d5. D20 is for the period d5-d20. D40 is for the period d20-d40

Table 8. Urine and plasma metabolites in 1st and 2nd parity sows with ad lib. access to clover grass and fed iso-energetic organic compound feed differing in proportion of protein

	Reproductive stage[1]						**Season**[2]			**Protein level**			**Breed**			**P-values**			
	d60	d100	d5	d20	**d40**	**SEM**	**Winter**	**Summer**	**SEM**	**Control**	**Low protein**	**SEM**	**Danbred**	**TN70**	**SEM**	**Stage**	**Season**	**Protein**	**Breed**
Urine																			
Urea	323^{a}	309^{a}	230^{b}	203^{b}	186^{b}	15.1	319^{a}	181^{b}	9.72	251	249	9.60	238	262	9.66	<0.001	<0.001	0.86	0.08
Creatinine, mM	20.6^{a}	20.9^{a}	18.7^{a}	13.9^{b}	10.2^{b}	1.34	20.4^{a}	13.3^{b}	1.23	16.0	17.7	1.33	14.9^{b}	18.8^{a}	1.09	<0.001	0.03	0.41	<0.001
Plasma																			
Glucose, mM	4.34^{b}	4.27^{b}	4.78^{a}	4.11^{bc}	3.81^{c}	0.09	4.28	4.25	0.06	4.27	4.25	0.06	4.18^{b}	4.35^{a}	0.06	<0.001	0.75	0.75	0.05
Urea, mM	3.06^{b}	2.98^{b}	3.03^{a}	3.45^{a}	3.40^{a}	0.10	3.08	3.28	0.10	3.19	3.18	0.09	3.34^{a}	3.03^{b}	0.07	<0.001	0.22	0.95	<0.001
Lactate, mM	3.13^{a}	2.94^{ab}	2.63^{ab}	2.28^{ab}	2.08^{b}	0.22	3.33^{a}	1.89^{b}	0.14	2.73	2.50	0.14	2.53	2.69	0.14	0.006	<0.001	0.26	0.44
Triglyceride, mM	0.35^{b}	0.43^{ab}	0.43^{a}	0.51^{a}	0.37^{b}	0.02	0.44	0.42	0.01	0.42	0.45	0.01	0.45	0.42	0.01	<0.001	0.50	0.30	0.15
Creatinine, µM	123^{b}	135^{a}	137^{a}	124^{b}	114^{c}	3.36	115^{b}	138^{a}	3.06	125	128	3.63	126	127	2.84	<0.001	0.02	0.61	0.69
NEFA, µM	169^{c}	207^{c}	1049^{b}	1377^{a}	805^{b}	71.8	756	686	48.2	687	756	46.4	736	707	47.1	<0.001	0.37	0.35	0.65

[a, b,c] Within a row and within a main effect; means with different superscripts differ (P<0.05)

[1] D60 and d100 is days from insemination. D5, d20 and d40 are days post partum

[2] Season is confounded with parity, as the sows in winter were 1st parity, and sows in summer were 2nd parity.

Table 9. Effects of feeding lactating 1st and 2nd organic parity sows on pasture either control or low dietary protein diet on milk composition and piglet plasma during winter and summer

	Day in lactation				Season[1]			Protein level			Breed			P-values			
	5	20	40	SEM	Winter	Summer	SEM	Control	Low protein	SEM	DanBred	TN70	SEM	Stage	Season	Protein	Breed
Milk																	
Yield, Kg/d	8.9^{c}	14.1^{a}	10.7^{b}	0.45	10.1^{b}	12.3^{a}	0.50	11.1	11.3	0.50	10.9	11.5	0.41	<0.001	0.02	0.74	0.09
Energy output, MJ ME/d	49.5^{b}	72.7^{a}	52.0^{b}	3.0	55.5	60.7	2.20	57.3	58.9	2.30	56.2	59.9	2.13	<0.001	0.09	0.62	0.23
Dry matter, %	20.0^{a}	18.7^{b}	18.1^{b}	0.38	19.5	18.4	0.35	19.2	19.5	0.35	187.8	19.1	0.30	<0.001	0.09	0.37	0.37
Fat, %	9.20^{a}	8.27^{a}	7.27^{b}	0.40	8.85	7.64	0.39	8.36	8.36	0.39	8.26	8.23	0.33	<0.001	0.08	0.72	0.91
Protein, %	5.55^{a}	4.92^{b}	5.29^{a}	0.09	5.23	5.18	0.09	5.36	5.15	0.09	5.15^{b}	5.36^{a}	0.07	<0.001	0.34	0.17	0.02
Casein	4.20^{a}	3.97^{b}	4.11^{ab}	0.04	4.07	4.12	0.03	4.19^{a}	3.99^{b}	0.03	4.01^{b}	4.17^{a}	0.03	0.006	0.51	<0.001	0.007
Lactose	4.77^{b}	4.94^{a}	4.99^{a}	0.03	4.89	4.92	0.03	4.90	4.90	0.03	4.88	4.93	0.02	<0.001	0.55	0.99	0.17
Milk metabolites																	
Glu6P[2], mM	0.21^{a}	0.09^{c}	0.14^{b}	0.007	0.16	0.13	0.007	0.15	0.15	0.007	0.15	0.14	0.01	<0.001	0.07	0.79	0.11
Urea	562^{c}	687^{b}	894^{a}	31.4	737	693	39.5	719	711	39.6	730	700	29.9	<0.001	0.47	0.90	0.19
Glucose, mM	0.19^{a}	0.12^{b}	0.07^{b}	0.01	0.12	0.14	0.01	0.12	0.12	0.01	0.10^{b}	0.16^{a}	0.01	<0.001	0.31	0.72	0.003
Uric acid, μM	24.8^{c}	30.1^{b}	44.1^{a}	2.67	36.3	29.6	2.67	33.6	32.3	2.67	31.6	34.3	2.03	<0.001	0.14	0.74	0.07
BHBA[2], μM	25.9	23.0	23.7	1.61	25.6	22.7	1.65	25.7	22.7	1.65	24.9	23.5	1.33	0.25	0.28	0.24	0.29
Isocitrate, mM	0.11^{a}	0.09^{ab}	0.088^{b}	0.004	0.098	0.097	0.004	0.097	0.099	0.004	0.098	0.098	0.004	0.01	0.90	0.71	0.84
NAGase, U/l	22.0^{a}	9.88^{b}	7.40^{c}	0.56	15.1^{a}	11.1^{b}	0.48	13.0	13.1	0.47	14.4^{a}	11.7^{b}	0.48	<0.001	<0.001	0.89	<0.001
LDH[2], U/l	4.67^{a}	2.95^{b}	3.17^{b}	0.26	3.95	3.25	0.26	3.63	3.57	0.25	3.60	3.60	0.22	<0.001	0.11	0.86	0.99
Piglet plasma																	
Creatinine	24.8^{c}	50.9^{b}	57.4^{a}	3.42	42.1	46.6	3.82	45.4	43.2	4.12	41.9	46.8	3.20	<0.001	0.48	0.72	0.12
Glucose	5.14^{b}	6.70^{a}	5.40^{b}	0.15	5.44^{b}	6.04^{a}	0.16	5.71	5.77	0.17	5.51	5.97	0.16	<0.001	0.005	0.81	0.06
Urea	2.18	2.12	2.16	0.24	1.82	2.49	0.32	2.47	1.83	0.30	2.23	2.07	0.23	0.80	0.17	0.17	0.63
NEFA	426	468	522	42.8	551^{a}	393^{b}	37.5	483	462	39.6	501	443	34.7	0.09	0.04	0.72	0.27

[a, b,c] Within a row and within a main effect; means with different superscripts differ ($P<0.05$)

[1] Season is confounded with parity, as the sows in winter were 1st parity, and sows in summer were 2nd parity.

Table 10. Correlations between grass-clover intake and selected parameters in Experiment 2.

	Sow weight	**Compound feed intake**	**Protein pool**	**Fat pool**	**Ashpool**	**Back fat**	**Heart rate**	**Distance**	**Milk Yield**
P-value	0.96	<0.001	0.09	0.02	0.03	<0.001	0.01	0.13	<0.001
r-value	0.003	0.27	0.11	-0.17	0.15	-0.28	0.16	0.11	0.29

Table 11. Correlations between grass-clover intake and selected nutrients in urine and plasma in gestation and in urine, plasma and milk in lactation

	Gestation				**Lactation**					
	d60		**d100**		**d5**		**d20**		**d40**	
	P-value	**r-value**	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**	**r-value**
Urine										
Creatinine	0.0004	-0.24	<0.001	-0.26	0.01	-0.18	0.02	-0.16	0.36	-0.07
Urea	0.01	-0.18	0.003	-0.21	0.02	-0.18	0.01	-0.18	0.90	0.01
Plasma										
Glucose	0.003	-0.20	0.53	-0.04	0.06	0.14	0.01	0.19	0.96	0.00
Urea	<.0001	0.55	0.01	0.18	0.23	-0.09	0.01	-0.27	<0.001	-0.32
Lactate	0.91	-0.01	0.02	-0.17	0.66	-0.03	0.23	-0.08	<0.001	-0.29
Triglycerides	0.02	0.16	<.0001	0.36	0.07	-0.13	0.03	-0.16	0.02	-0.16
Creatinine	0.01	0.17	0.68	0.03	0.54	-0.04	0.39	0.06	0.01	-0.18
NEFA	0.004	-0.20	0.18	-0.09	0.001	-0.25	0.04	-0.14	0.02	-0.17
Milk										
Yield					0.24	0.08	0.006	-0.19	0.24	-0.08
Protein%					0.005	-0.20	<0.001	-0.23	<0.001	-0.24
Fat%					<0.001	-0.25	0.12	-0.10	<0.001	-0.38
DM%					0.005	-0.24	0.08	-0.12	<0.001	-0.36
Urea					0.02	0.16	<0.001	-0.35	0.004	-0.20
Piglet weight					0.43	0.06	0.007	-0.18	<0.001	-0.24

DISCUSSION

100% LOCALLY PRODUCED FEED AND THE ENVIRONMENT

Foraging comprises a mixture of grazing above ground and rooting to ingest fruits, mast crops, roots, and invertebrates under ground. The latter contributions would not be detected by the method developed in Experiment 1. However, as most Danish outdoor sows, the sows in Experiment 2 were ringed, and grass-clover was probably the largest contributor to the daily nutrient intake from pasture.

Sows on the low protein strategy in Experiments 2 consumed more grass-clover, than sows fed the control compound feed, indicating that it is possible to stimulate the sows into grazing by decreasing the protein content of the compound feed. It is seen in other experiments with preference tests, that protein deprived pigs can select a diet that meets their protein requirements - and avoid excess intake at the same time (Kyriazakis, 1991). However, Experiment 1 showed that increasing grass-clover intake was associated with increased excretion of nitrogen in urine and feces, and thereby an increased risk of nitrogen leaching to the environment.

Grass-clover intake was positively correlated to the consumption of compound feed in Experiment 2, which strongly indicates that certain sows were oversupplied with protein, whereas others were undersupplied. A high feed intake in lactating sows is necessary to ensure that the nutrients required for milk production are met, and this variation in feed+grass intake might cause lower milk production in undersupplied sows. However, in Experiment 2 there was a negative correlation between milk yield and grass-clover intake at peak lactation (P=0.006; r =0-0.19), maybe because the high grazing sows had a tendency to move around more (P=0.13; r= 0.11) instead of feeding the piglets or perhaps because sows with low milk yield are more suppressed by inadequate SID lysine supply and therefore seeks to eliminate this by increased grazing.

Urea in urine was higher in gestation than in lactation in Experiment 2, which is supported by the increased content of urea in milk with the progress of lactation.

Compared to indoor sows, the level of urea in urine was generally very high in Experiment 2, indicating that organic sows are fed with more imbalanced diets. The urinary urea concentrations in the indoor sows in Experiment 1 were similar to levels found in other indoor studies. There was a tendency to a linear increase in urine production in kg/d (P=0.11) with increasing grass-clover intake in Experiment 1, which caused the total daily excretion of nitrogen to increase with increasing grass intake (P<0.001). Water intake from the water nipples decreased with increasing grass-clover intake, so the increased urine production was caused by the high water content from ingested grass-clover.

Apart from nitrogen, fresh grass-clover contains phosphorus and other minerals, and they may also pose a risk of increased leaching to the environment if supplied in excess. Based on the grass-clover intake from Experiment 2, approximately 3-8% of the (indoor) recommended daily intake of calcium and phosphorus of pregnant sows can be covered by grass-clover intake in summer, Figure 17

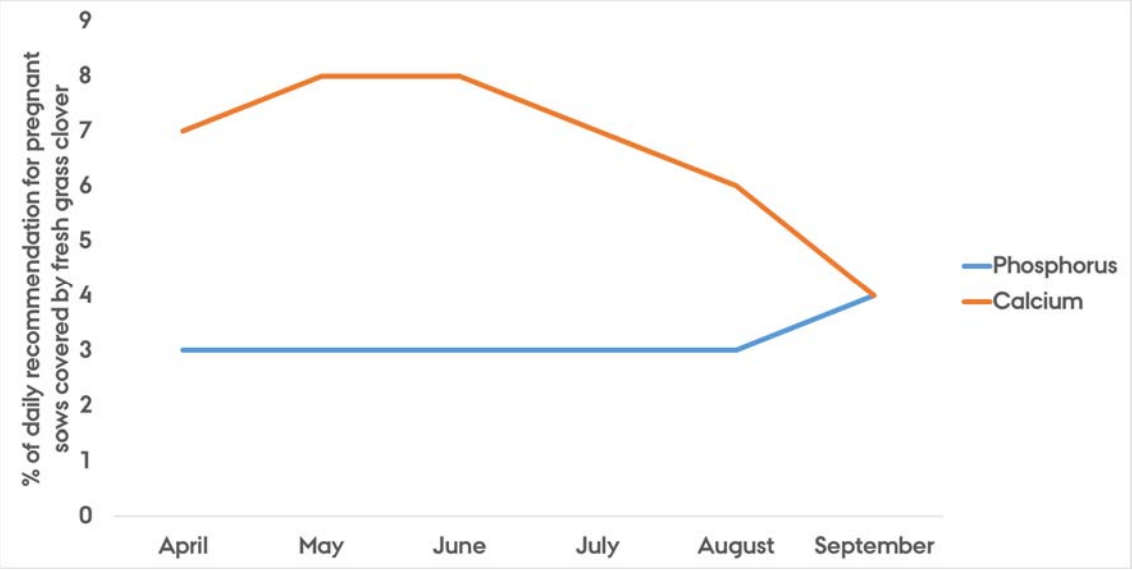

Figure 17. Percent of calcium/phosphorus requirement covered by the daily intake of grass-clover in pregnant sows in summer in Experiment 2. Requirements are based on Tybirk et al., (2019) and a daily intake of 57 MJ ME. Digestibility of P was set to 0.30 (Jongbloed et al., 2004). DM intake from grass-clover was 420 g/d in pregnancy.

There could be a similar opportunity to reduce the content of phosphorus in the compound feed for outdoor sows during gestation in summer, and thereby slightly decrease the P excretion and indirectly save farmland for organic production.

A correct feed consumption is essential to get reliable results in nutritional experiments. Danish organic sows are in general supplied with 1/3 more feed than indoor sows, but results from Experiment 2 show, that they do not have a 33% higher energy demand. Hence, there are some perspectives in reducing feed waste in organic pig production, as the waste is considerable, and also increases the discharge of nutrients to the environment (Nissen, 2019). In Experiment 2, feed waste was minimal in gestation, as individual feed residues were removed and weighed immediately after every meal, which makes the estimated feed consumption very reliable. In lactation, sows were fed in feeding troughs with a lid to minimize feed waste caused by the rooting behavior of sow and piglets and to prevent birds and rodents from ingesting feed leftovers. However, some individual feed waste (and thereof incorrect feed consumption) was inevitable as residues were only weighed back once a week during lactation, Figure 18.

Figure 18. Feed waste in Experiment 2.

UNKNOWN INTAKE OF PROTEIN AND ENERGY FROM PASTURE

In both experiments, there was a considerable individual variation in the voluntary fresh grass-clover intake, as some sows in Experiment 1 would not consume any grass at all, and some sows in Experiment 2 consumed up to 11 kg/d of fresh grass-clover. In the statistical analysis of Experiment 2, these "outliers" were removed even though the estimated grass intake probably was correct. If the single sows with a very high preference for grass intake had been included, the average grass intake had been overestimated, and the results not useful for most sows under practical conditions. By the removal of these animals, the mean fresh grass-clover intake in gestation went from 2.7 kg/d to 2.4 kg/d, and the SEM went from 334 g/d to 130 g/d.

Individual variation is also seen in other experiments with grazing sows and may be explained by various factors such as age, breed, health status, and previous experience. Environmental factors presumably also influence the individual motivation for grass intake, but temperature, humidity, etc. were more or less identical for all sows in the two individual experiments. In experiment 1, sows were of the same breed, but different age (second to sixth parity), and there was no correlation between parity or live weight and the willingness to consume grass-clover. The sows in Experiment 2 were all of the same age, i.e., second parity during summer, hence differences in age or live weight could not explain the observed individual variation in grass-clover intake.

Genetic selection for low fat percentage in pork and increased feed efficiency might lead to a selection for sows with a reduced appetite. In Experiment 1, all sows were Danish Landrace×Yorkshire. Still, in Experiment 2, approximately half the sows were Danish Landrace×Yorkshire from the Danish breeding company DanBred, and the other half was Norvegian Landrace×Yorkshire, also known as TN70 from Topigs Norsvin. The two breeds differ in many of the measured plasma, urine, milk, and performance parameters, but there was no interaction between breed and protein level. The TN70 sows was 20 kg bigger than the DanBred sows, but it was not possible to demonstrate any difference in grass-clover uptake between the two breeds in summer (DanBred=2.87 kg/d and TN70 = 2.78 kg/d; SEM = 0.37; P=0.67) or any correlation between live weight and grass-clover intake (P= 0.96). Kelly et al. (2007) compared offspring from different maternal breed types, and also found no differences in feed intake or the proportion of forages consumed between "modern" and more traditional genotypes.

In Experiment 1, the SPF health status of all sows were blue SPF+Myc+Ap6+Ap12. At the beginning of Experiment 2, SPF health status was blue SPF + Myc in the Danish sows and Blue SPF in the Norwegian sows. The official health status, however, does not include gastric ulcers or other diseases of the gastrointestinal tract who could affect grass-clover intake, other than Salmonella and Dysentery. In Experiment 1, sows originated from different commercial herds and had previously been exposed to different unknown diets. Health conditions and previous experiences can, therefore, not be rejected as a cause of the diverging grass-clover intake in the three-weeks preparation period.

Contribution of nutrients from grass-clover

In gestation, approximately 16% to 25% of the daily requirement of different digestible amino acids for pregnant sows was covered by a grass-clover intake of 2.4 kg fresh grass-clover/d throughout the growing season from April to September, Figure 19. This illustrates that it is possible to reduce the crude protein content of compound feed for grazing pregnant sows during summer.

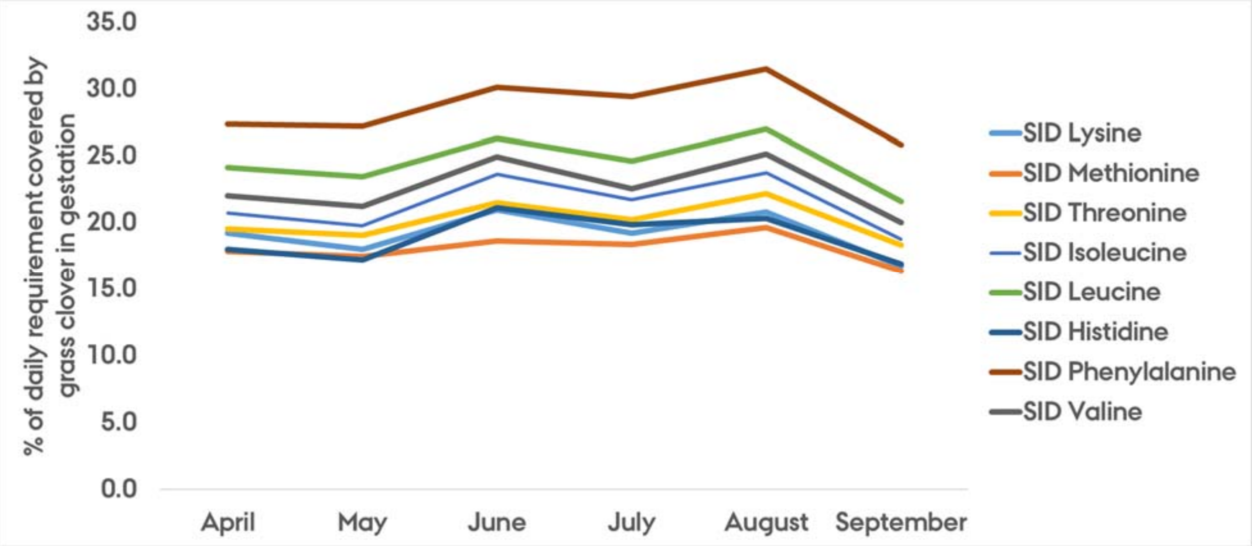

Figure 19. Percent of SID amino acid requirement covered by the daily intake of grass-clover in pregnant sows in summer in Experiment 2. Requirements are based on Tybirk et al., (2019) and a daily energy requirement of 30 MJ ME. Digestibility of amino acids was set to 68.4 % on the basis of the N-digestibility found in Experiment 1. DM intake from grass-clover was 420 g/d in pregnancy.

High yielding indoor sows have a lysine requirement of 61 to 70 g SID lysine/d at peak lactation (Gourley et al., 2017; Feyera and Theil, 2017; Hojgaard et al., 2019b). This level is also confirmed for outdoor high yielding sows, as the second parity sows at peak lactation in Experiment2 had a SID lysine requirement of 69.7 g/d and, on average, grass-clover contributed with 6% of this daily requirement. At peak lactation, a fresh grass-clover intake of 3.2 kg/d only contributed 3% - 7% of the estimated daily requirement of selected amino acids, and based on this, it can´t be recommended to rely on amino acid contribution from grass-clover in lactation, Figure 20.

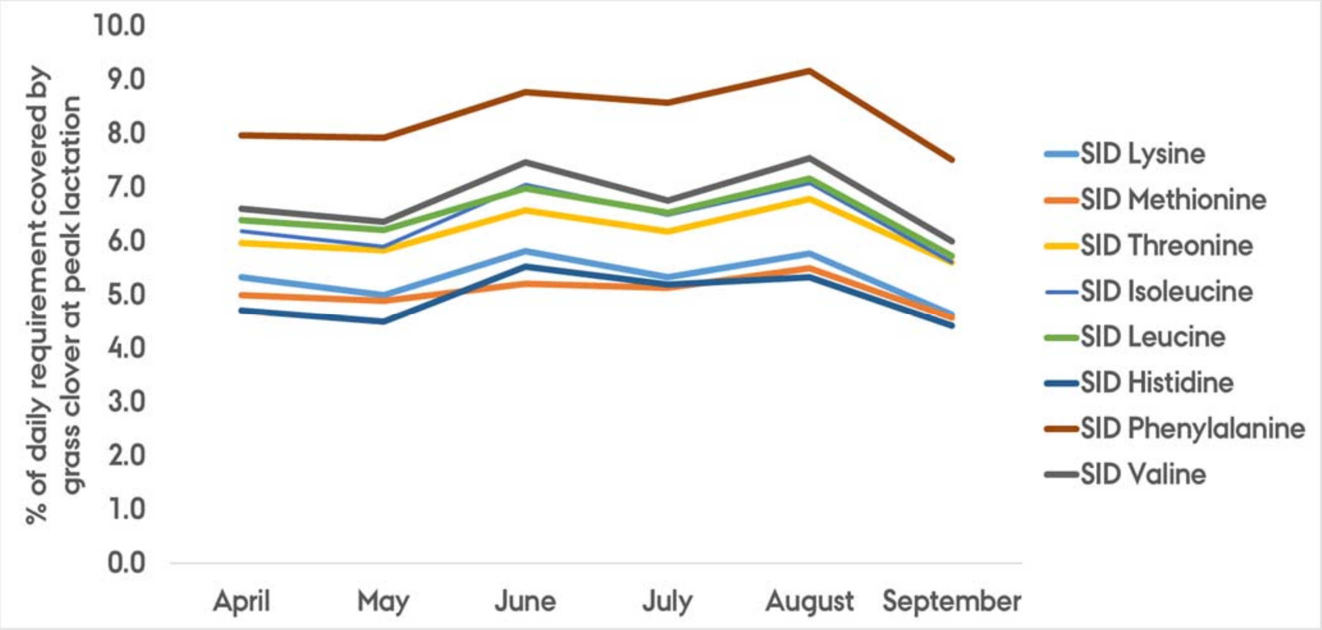

Figure 20. Percent of SID amino acid requirement covered by the daily intake of grass-clover in lactating sows in summer in Experiment 2. Requirements are based on Tybirk et al., (2019) and a daily energy requirement of 130 MJ ME. Digestibility of amino acids was set to 68.4 % on the basis of Experiment 1. DM intake from grass-clover was 574 g/d at peak lactation.

From experiments with ringed sows, it has been documented, that it is possible for sows to take up as much as 40-65% of the total energy requirements from grass-clover (Sehested et al. 2004; Fernández et al. 2006) and 50-60% of maintenance requirements (Rivera Ferre et al. 2001). This was also the case for the dry sows, who received 6 kg grass/d in Experiment 1. These sows consumed15.8 MJ ME from compound feed and, on average, 18.4 MJ ME/d from grass-clover. In Experiment 2, the average grass-clover intake was only 2.4 kg/d and to be able to motivate the sows to consume higher amounts of grass-clover under outdoor conditions voluntarily, it is probably necessary to decrease the access to compound feed – and not as in Experiment 2, where the energy supply was increased by 10% compared to indoor recommendations during summer. The 10% extra compound feed did not matter much after farrowing, as the sows did not consume more than ≈ 70% of their lactation diet. The assumption that daily grass intake corresponds to one Danish feed unit for sows (≈12.3 MJ ME/d) therefore seems somewhat exaggerated, at least if sows are young with limited gastric capacity and/or if they have access to semi ad libitum amounts of compound feed.

The contribution of energy and protein depends on the chemical composition of the grass-clover, which changes during the growing season. The fat and protein content of dry matter in Experiment 2 was more or less constant, whereas the content of highly digestible carbohydrates was high in the spring and decreased during summer, Figure 21.

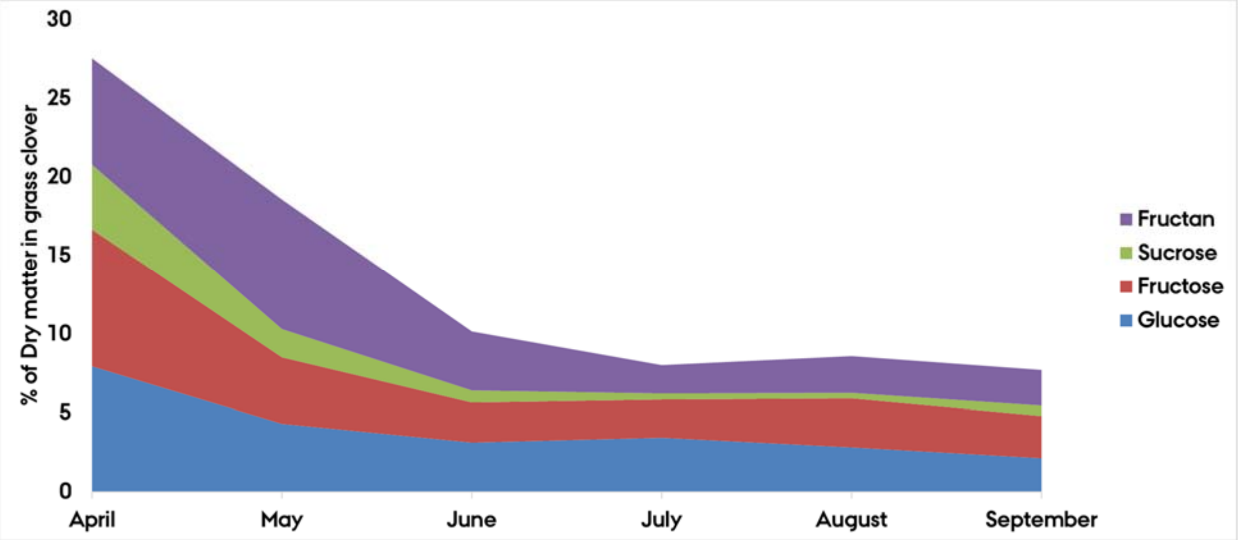

Figure 21. The content of easily digestible carbohydrates in fresh grass-clover during the growing season from April to September 2017.

Hence the content of ME per kg DM was almost reduced by 50% from April to September, Figure 22.

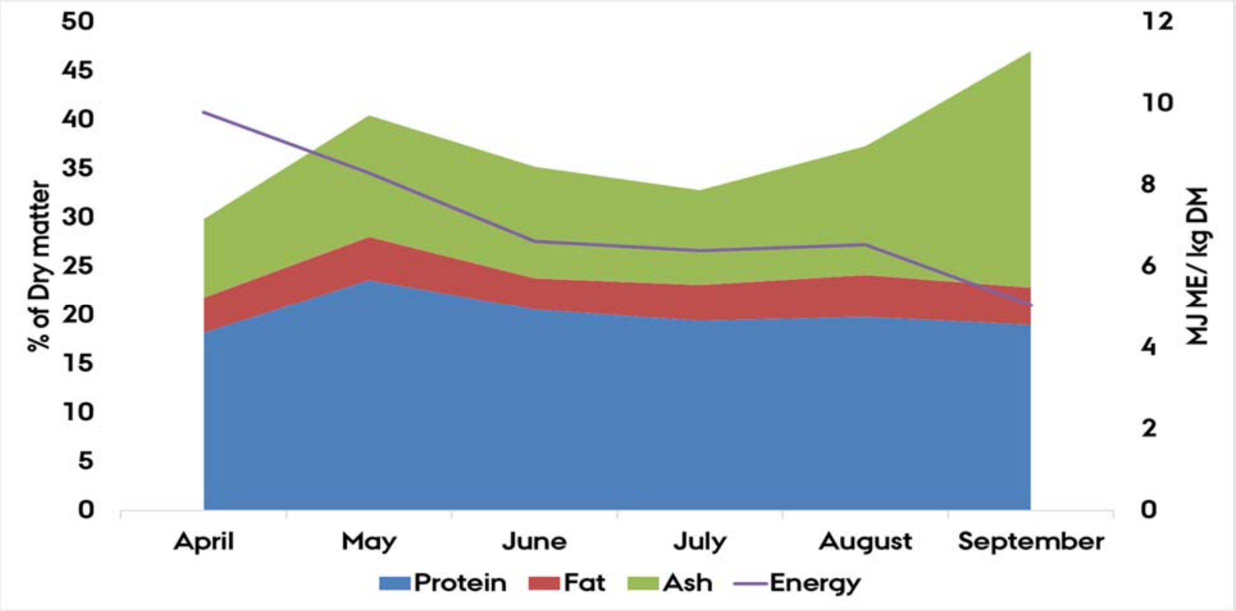

Figure 22. The content of protein, fat, ash and metabolisable energy in the fresh grass-clover from Experiment 2. Energy analyses are based on the Danish Feed Unit system.

Like for fresh grass-clover, information on the nutritive value of ensiled grass-clover for organic sows is scarce. Whittemore and Hendersen (1977) found that silage made from early grass contained 34.6 g nitrogen, 19.5 MJ gross energy, and 271 g dietary fiber/kg dry matter. Digestibility coefficients in sows were 0.73 for nitrogen, 0.60 for gross energy, 0.60 for cellulose, and 0.51 for dietary fiber. Using the N-alkane method in a small scale experiment with twelve growing pigs (50-60 kg), who had ad libitum access to a standard compound feed, herbage contributed approximately 4% to the daily organic matter consumption (Kelly et al., 2007). This is comparable with the 3% silage intake reported for growing pigs on five commercial Danish organic farms (Lauritsen, 1999). In female growing-finishing pigs, feed and energy intake was not reduced in the period from eighteen weeks until slaughter when grass silage was offered ad libitum.(Peet - Schwering, 2006). By use of a chromium marker Peet-Schwering (2010) investigated the digestibility and energy value of five qualities of grass silage in outdoor pregnant sows. The net energy intake varied between 7.39 MJ NE/d to 10.82 MJ NE/d and silage intake was improved when cut at an early stage of maturity and ensiled with a relatively low dry matter content.

Bikker (2014) found that it is possible to replace 9 MJ of energy from compound feed by ad libitum access to grass silage. The mean daily energy intake of the sows was 11 MJ NE from grass silage. However, like with fresh grass, the individual silage intake varied from less than 0.1 to 6 kg of silage dry matter per day between individuals, with a lower mean intake in young sows, maybe due to limited number of eating places.

The contribution of energy and protein from silage is low but unfortunately unknown during winter in Experiment 2. Personal observations reveal that the sows were not motivated to eat the silage from November to January, where there was still some grass-clover in the paddocks. They, and especially the piglets, only used the silage as rooting material. From February and onwards, the grass cover in the paddocks was minimal, and the sows showed a greater interest in eating the silage.

INCREASED ENERGY REQUIREMENT

An optimal supply of energy in gestation is crucial not only for the present pregnancy but also for the subsequent lactation and the consecutive reproductive cycle. The daily requirements for maintenance at thermoneutrality and moderate physic activity amounts to about 0.420-0.441 MJ ME/kg 0.75 × metabolic live weight in indoor gestating sows. (Noblet, 1987; Noblet, 1990; Noblet, 1997). This corresponds to approximately 26 MJ ME/d or approximately 2 kg of feed/d for a 240 kg sow. In indoor lactating sows, the daily requirements for maintenance at thermoneutrality is approximately 0.458-0.525 MJ ME/kg 0.75× metabolic live weight (Verstegen, 1985; Noblet, 1987; Noblet, 1990 ; Theil, 2004), which is 28-32 MJ ME/d for a 240 kg lactating sow.

In Experiment 2, sows weighed on average 216 kg in first parity and 270 kg in the 2nd parity, which gave a maintenance requirement of 26.6 and 31.5 MJ ME/d in winter and summer, respectively.

ENERGY INTAKE

The biggest challenge in Experiment 2 turned out to be insufficient feed intake in lactation, both winter and summer, and the average consumption in lactation was only 2/3 of the expected amount, Figure 23.

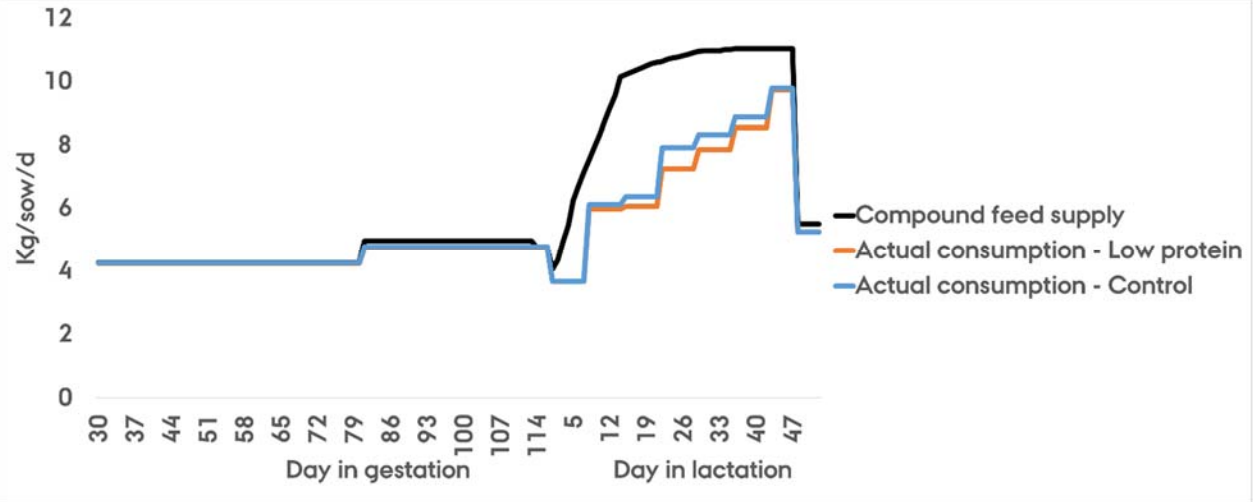

Figure 23. Feeding curve and actual intake of compound feed in kg/d in gestation and lactation throughout Experiment 2. The feeding curve is an average of the recommended daily energy intake for indoor gilts +15% in winter and for indoor sows+10% in summer (SEGES, 2016).

In the first week post partum, the average feed intake was 3.7 kg/d. In the second week 6.0 kg/d and in the third week, 6.2 kg/d. This is much lower than feed intake reported for indoor sows and in organic sows, Weissensteiner et al. (2018) report 6.1-6.6 kg/d the first two weeks postpartum. However, these sows were older (>5 parity on average), and the gastric capacity probably higher than the young sows used in Experiment 2.

The average feed intake in weeks 3-6 was 7.8 kg/d, and 8.2 kg/d in the two dietary groups and the consumption approached the amount of feed supplied daily (10.8 kg/d) with the progress of lactation. Average feed intake in mid and lactation was comparable to levels found by Weissensteiner et al. (2018), who report 7.5-7.9 kg/d in weeks 3-6 in organic sows.

Nevertheless, feed intake was inadequate in early and mid-lactation as sows mobilised massively. Feed intake might have been higher if sows had been older or if they had been born free-range and their digestive system thereby more adjusted to outdoor conditions. A third explanation could be, that the maternal instincts of the outdoor sows, kept them in their huts and away from the troughs in early lactation, while indoor sows are placed in front of the trough at all times. This is supported by the activity data from Experiment 2, where daily distance in early lactation is reduced.

A third explanation for the low feed intake in early lactation could be imbalanced diets. Pedersen et al. (2016) found that indoor second parity sows could consume 5.3 kg/d the first week, 6.9 kg/d the second week, and 7.6 kg/d the third week of lactation if fed a balanced two-component diet adjusted to individual milk production. Sows are indeed able to consume much more feed than they usually are provided, and the findings suggest that the appetite of sows may be depressed when the feed composition is not matching their nutrient requirement very well. This might be a more significant challenge for organic sows due to the ban on using crystalline amino acids and hence higher content of crude protein. The energy requirement in lactation is determined by both maintenance and milk yield, while the requirement for SID Lysine is almost exclusively defined by milk yield. In a two-component feeding regime, the sows are fed according to their individual needs, which enable a more balanced diet and a greater feed intake, Figure 24 (Pedersen et al., 2016). Two-component feeding might be a future prospect in balancing diets without crystalline amino acid supplementation for organic sows.

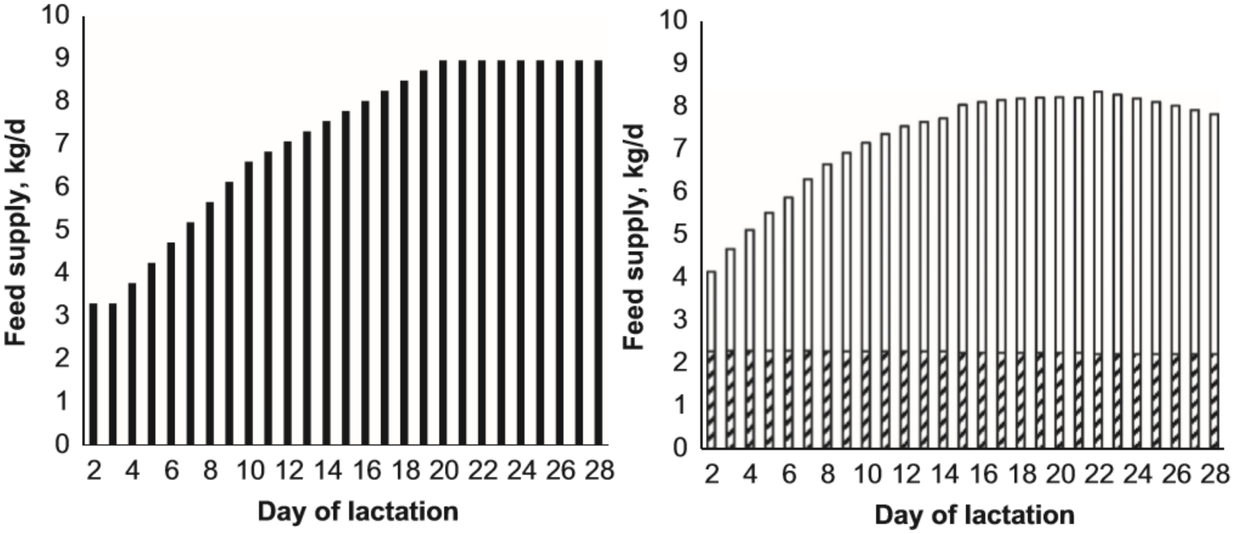

Figure 24. Lactation feeding curves with one or two components. The sows fed one compound feed were fed according to Danish recommendations, whereas sows fed the two components were fed according to their individual energy requirements for maintenance (basal diet; shaded) and individual energy requirements for milk production (lactation supplement; white bars). Adapted from Pedersen et al., (2016).

The intake of compound feed was 10% higher in the second parity. This was expected, as the average daily feed intake usually is 10-23% higher in second compared to first parity sows (Pedersen et al. 2016.) On average, grass-clover intake contributed with 12% of the SID lysine intake and 5.5 MJ ME/d or 8% of the total energy consumption during summer.

ENERGY OUTPUT

Thermoregulation

The daily energy requirement for thermoregulation was13.5 MJ ME and 5.23 MJ ME during winter and summer, respectively. This corresponds to 20% and 7% of the daily energy requirement in winter and summer. These results are in line with Rodríguez-Estévez (2010), who found an average daily energy requirement of 5.5 ± 0.14 MJ ME for thermoregulation in Iberian fattening pigs at an average temperature of 7.6 °C. This was equivalent to 21.5% of the daily amount of ingested ME.

Personal observations reveal that the sows responded behaviorally to changes in the climatic environment. Above 5°C, the ambient temperature seemed to have little effect on the shelter seeking behavior, but on rainy days, the sows were observed to be more inside the huts. High wind speed appeared to affect the behavior the most, with the sows usually choosing to avoid the windiest parts of the paddocks. Although the pigs sought shelter more below 5 C, most of the experiment was performed well under their estimated lower critical temperature. Thus most of the time, they spend outside the huts involved an additional heat loss.

In the period from 1982 to 2012, the Danish average temperature in the winter months (November-March) has been -1.8 °C, which is 3.0 °C lower than the average temperature measured during the mild winter of 2016/2017 at AU Foulum. The energy expenditure for thermoregulation for a 240 kg sow would have been 3.2 MJ ME/d or 260 g/d of compound feed extra, if Experiment 2 had been conducted during a "normal" Danish winter.

During summer, sows got access to a wallow, when daytime temperatures started to exceed 15°C, Figure 25. There were only very few days with daytime temperatures above 25°C, for which reason energy required for thermoregulation during heat stress is not addressed in this thesis.

Figure 25. Illustration of the temperature variation in Experiment 2.

Locomotive activity

The daily energy requirement for locomotive activity was 2.8 MJ ME and 3.5 MJ ME during winter and summer, respectively. This corresponded to 4% and 5% of their daily energy requirement. Locomotive activity represents 19-42% of observational time depending on the level of supplied compound feed in pigs (Andresen and Redbo 1999; Rivero et al. 2013; Kongsted et al. 2013). Sows in Experiment 2 were fed semi ad libitum amounts of compound feed, and therefore probably they were not as eager to fourage, as if they had been fed more restrictedly. The crude protein or SID lysine requirement is most likely not affected by the level of physical activity in this study.

Milk production

Milk production is highly prioritized by lactating sows, and the sows mobilise from muscle or adipose tissue if they are unable to increase feed intake at the same rate as milk production increases (Vadmand et al., 2015). Both high feed intake and a high mobilisation seems to be a prerequisite for milk production in hyper-prolific sows (Strathe et al., 2017)

Milk composition was not affected by the dietary treatment except for the casein content, which was lower in milk from sows fed the low protein compound feed. This is supported by Strathe et al. (2016), who found that casein increases linearly with increasing SID crude protein content of the diet from d17 of lactation an onwards.

Hojgaard et al. (2020) found the same response, and showed in a follow-up study that piglets are not able to convert the extra casein (and protein) in milk into growth when the milk protein exceeded 4.9% milk protein.

In Experiment 2, d20 of lactation has been used as "peak lactation," but it is possible that the actual peak occurs some days earlier. Strathe et al., 2016 found milk yield to peak on d 16-17 of indoor first and second parity sows.

Milk energy output was 56 MJ ME/d in winter and 61 MJ ME/d in summer, which is higher than reported for milk from indoor sows mainly due to an increased milk fat content in organic milk, probably caused by the high mobilisation of body fat, Figure 26.

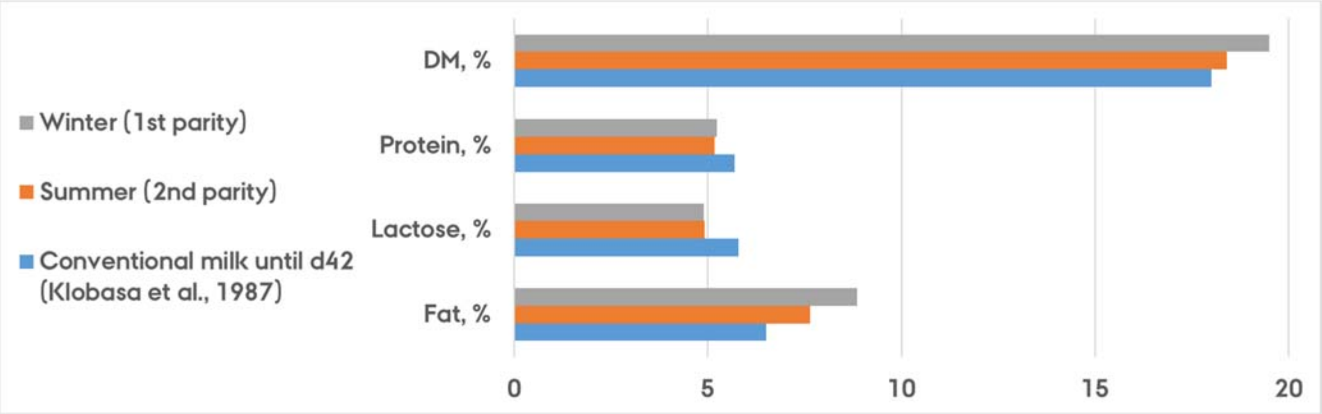

Figure 26. The composition of sow milk according to production type. Milk composition was measured on d5, d20 and d40 in Experiment 2 and at 14 points until d42 under conventional indoor conditions in the study by Klobasa et al. (1987).

Milk yield was 8.9 kg/d on d5 and 14.1 kg/d on d20, which is in line with milk yield values reported for high prolific indoor sows. The increase in milk yield from first to second parity was 22% (10.1 kg/d to 12.3 kg/d; P=0.02) and more massive than reported indoor, where Strathe et al., 2016 reported an increase in milk yield of 6% from first to second parity. Milk yield dropped numerically more for the low protein sows compared to the control sows in winter than in summer, and this was most pronounced in late lactation. It seems that the first parity sows were more challenged by insufficient SID lysine supply during winter than the second parity sows were during summer, and that both dietary treatment groups have been able to mobilise adequate body protein in the second parity to support milk production. Milk yield was positively correlated to grass intake (P<0.001; R2 = 0.28), but also to compound feed intake (data not shown), which makes it difficult to conclude, that the further increase in milk yield from winter to summer is caused solely by grass-clover intake.

Measuring milk production on free-range animals entails some challenges not present indoor. When using the prediction model to estimate milk yield, it is based on inputs of the daily gain of the litter and the litter size. Under indoor conditions, these two measurements are simple to obtain and quite reliable. In the organic production system, piglets are free to move around from sow to sow, and sometimes they do not sleep or live by their own mother. This became a problem in Experiment 2, when the gain of a specific piglet was ascribed to the sow, where it was found at sunrise. Hence, some sows gave milk to piglets, which were believed to belong to another sow, and unfortunately, the piglets did not always switch within dietary treatment group, Figure 27.

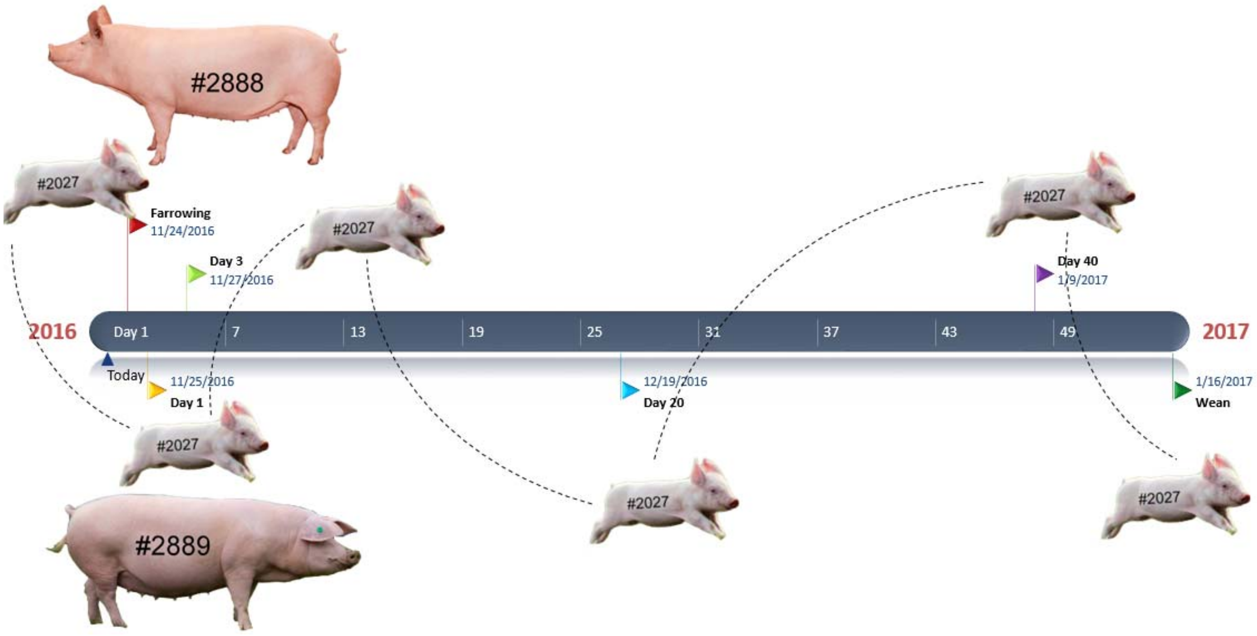

Figure 27. Example of a piglet, who switched "mother" at least five times from birth to weaning.

SOW MOBILISATION

In lactation, usually sows turn into a catabolic state and take up nutrients derived from both feed and body reserves. Body mobilisation is positive for the piglets, but might be harmful to the sow and her subsequent reproduction. Several studies have shown that sows with substantial weight loss in lactation have extended weaning to estrous intervals and an increased incidence of anestrus (Einarsson, 1993).

Many factors confirm that the sows in Experiment 2 were indeed mobilising energy from body fat reserves in lactation. First of all, there was a considerable weight and backfat loss with the progress of lactation both winter and summer. The weight loss from d5 to d40 of lactation was 16% (1057 g/d) in winter and 13% (1086 g/d) in summer, and the overall back fat loss in both seasons was on average 6.8 mm, which is approximately double as much as experienced by indoor sows (Vadmand et al., 2015).

In organic sows, Weissensteiner et al. (2018) reported total live weight losses of 678 g/d and 836 g/d in a six weeks lactation period of organic sows fed either high or low dietary protein. In comparison, Noblet and Etienne (1987) found a lactation weight loss of 1122 g/d in energy-depleted gilts on d2-d21 under indoor conditions. In well-fed animals under indoor conditions, the lactation weight loss was reported to be 10.1% in first parity and 10.3% in second parity with four weeks of lactation. (Strathe et al., 2016).

Piglet pre-weaning mortality of live-born piglets was 19% in winter and 25% in summer. Rangstrup-Christensen (2018) confirm that piglet mortality in organic herds is increased in the summertime, and the national average in organic herds was 21% while it was 14.5 % in indoor sows in 2017 (Hansen, 2019). Weissensteiner et al. (2018) also report piglet losses of 20.8%-21.9% under organic conditions.

All dead piglets within d3 were post mortem autopsied by a trained veterinarian to determine the cause of death, and there were no indications of agalactia in any of the dietary groups, as only very few piglets died from starvation compared to 10% starved piglet found in 2672 necropsied organic piglets by Rangstrup-Christensen (2018), Figure 28.

Figure 28. The primary causes of piglet mortality on d0-d3 in Experiment 2. Thirty-two litters included still born piglets and necropsies revealed that 71% of these died during farrowing. (modified from Schild et al., 2019)

Primary cause of death	Number of piglets
Still born	
During farrowing	30
Before farrowing	11
Time unknown	5
Pre-weaning mortality	
Crushed (overlain, trampled, bitten)	154
Euthanized (BW<700 g, injury)	111
Starvation, infection, malformation	14
Unknown	28

Sow reproduction seemed not to be impaired by the great weight loss observed in the first lactation period (during winter), as the weaning-to-estrus interval was 4.2 days on average and the number of total born in the second parity was 18.9 piglets/litter. This performance is in agreement with the national average under indoor conditions in 2017 (18.7 piglets per litter with 22.7% first parity sows; Hansen, 2019). The low protein strategy, therefore, did not seem to compromise the sow productivity. During lactation, sows on low protein were able to mobilise more body protein in summer to counterbalance the more moderate protein and lower lysine intake from compound feed.

It takes about five hours after enrichment for deuterium to disperse equally within the body fluids and attain an equilibrium of water inside and outside the cells. Under indoor conditions, sows would not be allowed to drink after enrichment, and the P2 sample would have been taken after five hours. Due to the outdoor conditions and animal welfare considerations, the P2 sample in Experiment 2 was taken on average twenty hours after enrichment. This adds some uncertainties to the quantified body pools of protein and fat (and hence also the estimated daily retention/mobilisation), as sows have been able to drink, give milk and urinate several times and thereby dilute the injected deuterium, causing an overestimation of the water pool. Sow mobilisation will be discussed below by using selected milk and plasma metabolites as indicators.

Milk metabolites

Milk LDH and NAGase as metabolites for detecting mastitis were not clear, as no mastitis was observed, but concentrations were higher in early lactation as reported in dairy cows. Milk Glu6P and milk BHBA decreased with the progress of lactation, which indicates a higher mobilisation or turnover of both protein and fat in early lactation.

Milk isocitrate decreased from early to late lactation. (Garnsworthy, 2006) stated that the variation in milk isocitrate is related to novo synthesis of fatty acids. Larsen (2014) reports that isocitrate is directly proportional to days in milk but inversely proportional to milk yield, which also seems to be the case in organic sows.

Plasma glucose

Physiological measures of sow mobilisation include decreased glucose concentration in plasma (Valros, 2003; Mosnier, 2010), which explains the decrease in plasma glucose with the progress of lactation in Experiment 2. Glucose is the primary substrate for lactose production in the mammary gland and after parturition glucose is prioritized for milk production, which also explains why plasma glucose levels are higher on d5 of lactation than in gestation.

Plasma glucose levels were generally low compared to levels reported indoor, and it is a comprehensive source of error that blood samples were not taken at the same time prior to feeding in Experiment 2. Plasma glucose concentration depends on time relative to feeding, and Valros et al., 2003 reported an increase in plasma glucose from 5.12 mM to 5.75 mM when comparing glucose concentrations half an hour before the morning feeding with the mean of seven samples taken every 15 minutes after the morning meal in week 1 of lactation. In week 3, the difference was even more pronounced, from 4.23 mM before feeding to 5.67 mM after feeding.

Even though the glucose levels in Experiment 2 were from sows fasted overnight, the change from pre- to postprandial concentrations were generally much lower than the levels reported in indoor fasting sows, suggesting that the access to silage in winter and grass-clover in summer contributed with a more constant nutrient absorption and increased microbial fermentation in the gut. Serena et al. (2009) find that variation in diurnal glucose concentration decreases when feeding high levels of soluble fibers compared to a low fiber diet. This is supported by a tendency to linearly reduced plasma glucose levels four hours after feeding with increasing grass-clover intake in Experiment 1 (P=0.11) and a positive correlation between grass-clover intake and blood glucose on d5 (P=0.006) and d20 (P=0.001) of lactation in Experiment 2.

Plasma urea

Urea is a product of amino acid catabolism. Still, it is also influenced by protein intake – and quality, and in other studies, an effect of increased protein level on plasma urea has been reported (Mosnier, 2010; Hojgaard, 2019). Plasma urea increased linearly with increasing grass-clover intake in Experiment 1, indicating a less optimal amino acid profile in grass-clover compared to the compound feed or that sows with a high grass intake ingested excessive amounts of protein.

Opposite to Weissensteiner et al. (2018), Experiment 2 showed no difference in plasma urea concentration of organic sows fed two levels of dietary protein. In the study by Weissensteiner et al. (2018), the protein content differed by 28 g/kg, whereas it only differed 18 g/kg in the current study, which might explain the missing effect. The crude protein contents of the low protein diet (151 g/kg) in the experiment by Weissensteiner was slightly higher than the high protein diet in Experiment 2 (148 g/kg).

Plasma urea levels in Experiment 2 were generally low compared to fasting values found in indoor animals in weeks 1 and 3 of lactation (Valros, 2003). Plasma urea measured in fed animals in Experiment 1 were similar to levels reported for indoor sows.

Plasma lactate

As seen in indoor sows, plasma lactate content decreased from gestation to lactation. Plasma lactate levels in Experiment 2 were higher than levels reported for indoor pregnant and lactating sows. Organic compound feeds generally contain more carbohydrates and less protein than conventional diets, and a minor amount may originate from a higher fermentation of starch in organic sows. Still, the most plausible explanation is probably an increased muscle activity in outdoor compared to indoor sows.

Plasma Triglycerides

Plasma triglycerides are used for milk fatty acid synthesis, and they originate mainly from the feed. In experiment 2, there was an increased plasma triglyceride level in the low protein sows (P=0.01). These sows consumed more grass-clover during summer (P=0.007), and in Experiment 1, there was a linear tendency to increased plasma triglyceride level in sows with increasing grass-clover intake (P=0.06). This could indicate that part of the fat originate from hepatic de novo synthesis from short-chain fatty acids. As expected, there was an increased level of plasma triglycerides in early and peak lactation compared to gestation and late lactation, as suggested by Mosnier (2010).

Plasma creatinine

The decreasing plasma creatinine found with progress of lactation indicates a higher rate of protein turnover in early lactation compared to d20 and d40, and might be due to the regression of the uterus (Feyera and Theil, 2017), and not necessarily because of an increased muscle breakdown after farrowing. This decrease in creatinine through lactation is also reported by others (Mosnier, 2010; Strathe et al., 2016).

There was a higher plasma creatinine level during summer, probably because the sows in second parity had 22% larger protein pools than in first parity. Creatinine levels were generally much lower than levels reported for indoor sows (Pedersen et al., 2016; Krogh et al., 2017; Strathe et al., 2017 Hojgaard, et al., 2019). This indicates that the protein turnover was somewhat reduced in the organic sows, maybe because of inadequate protein supply.

Plasma NEFA

NEFA in blood primarily originates from mobilisation of body fat, and physiological measures of fat catabolism include an increased concentration of NEFA in plasma (Valros, 2003; Mosnier, 2010). Regardless of season, plasma NEFA concentrations were low in gestation and increased rapidly at the onset of lactation with a peak on d20. The pattern of NEFA concentrations fits well with the higher mobilisation of body fat observed in the period from d5 to d20 and observed changes in back fat. Plasma NEFA levels were very high compared to levels reported for indoor animals (Revell, 1998; Valros, 2003; Strathe, 2017; Hojgaard, 2019) and similar to levels reported in organic sows in gestation but not lactation (Weissensteiner, 2018). This also supports that the sows were indeed challenged with insufficient energy intake in early and peak lactation.

When interpreting the NEFA concentrations, it is necessary to consider the time of sampling: In lactation, blood samples were taken from fasted sows > 24 h after the last feeding. In 1st parity sows, Rojkittikhun et al. (1993) observed higher NEFA concentrations before the morning feeding in lactating sows. One week after farrowing plasma NEFA values of indoor sows before feeding were, on average, 36% higher than after the morning meal. At peak lactation, preprandial plasma NEFA concentrations were 57% higher than plasma NEFA after feeding (Valros, 2003). Nevertheless, even though blood samples were drawn from animals in a fasted state, NEFA values were still very high, indicating massive mobilisation in early and peak lactation, which also visually was very clear, Figure 29.

Figure 29. Random lactating sows from Experiment 2 on d5 and d40 of lactation, respectively. Average back fat was 20.2 mm on d5 and13.4 mm. on d40.

CONCLUSION

In terms of organic pig nutrition, there is an increasing focus on grass protein in Denmark, as it can be locally and sustainably produced and, therefore preferable in organic feeding rather than imported soy. Inclusion of “green protein” from grass-clover in the diet of organic sows may reduce the need for import of soy and contribute to the closure of regional nutrient cycles. This study shows that fresh grass-clover has an energy digestibility of 68% in sows, and plasma pipecolic acid concentration was found to reflect the intake of fresh grass-clover. For pregnant sows fed semi ad libitum amounts of compound feed, the voluntary consumption of grass-clover was 0.42 kg dry matter or 5.3 MJ ME/d in summer, though with considerable individual variation. Fresh grass-clover intake contributed with 3.4 g/d and 4.5 g/d of SID lysine in gestation and at peak lactation, respectively, and grass-clover intake increased nitrogen excretion in urine and thereby also the environmental impact.

Overall, milk casein was highly correlated to protein concentration in the compound feed. Otherwise no differences were observed in the concentration of nutrients in plasma, urine, milk, or in sow productivity in terms of live-born or milk yield between the two protein levels. This may be because sows on the low protein strategy consumed 14% more fresh grass-clover than the control group in summer. The energy demand for thermoregulation corresponded to 20% and 7% of the daily energy requirement in winter and summer, whereas the energy demand for physical activity constitute 4-5% in both seasons.

An adjustment on the protein to energy ratio in organic sow diets should be considered to match the increased energy demand for thermoregulation and to reduce the negative environmental impact from organic pig production. It is suggested that organic sows need more energy (kg feed) on a daily basis but less dietary protein per kg of feed, because they ingest protein from grazing in summer and probably also from roughage in the winter. These findings contribute to a better understanding of the energy and protein requirements of organic sows with access to pasture and confirm that pregnant- but not lactating sows are able to utilize some of the nutrients in the sward.

PERSPECTIVES

It is of major relevance to tailor organic sow compound feed to the organic production conditions. If nutrients are allocated in accordance with requirements, feed efficiency will increase, and excessive supply can be reduced. This thesis provides a better understanding of the energy requirements of organic sows all year and the nutrient contributions from grass-clover in the grazing period from April to November. This will hopefully lead to an improved formulation of compound feed for organic sows, and some of the major players on the organic feed market have already modified their product portfolio based on the results from these experiments. They are now offering a summer and a winter assortment.

Several new questions within the research area of organic pig nutrition have arisen during this PhD period:

- The amount of supplied roughage is hardly known for the total herd during wintertime. Consequently, the energy and protein supply from roughage is normally not accounted for when designing compound feed for organic sows under Danish conditions. Now that that energy and protein contribution from grazing is better described for organic sows, it would be equally beneficial to uncover the intake of nutrients from roughage/silage during winter in both piglets, finishers and sows.
- Likewise, there is a great potential in identifying the contribution of vitamins and minerals from both grazing in summer and silage intake during winter, as these can be rather expensive nutrients and because the legal number of pigs per farm is tightly regulated on the bought-in amounts of, especially phosphorus. Therefore, many farmers might be able to have more sows on the same land, if the mineral intake from pasture could be deducted, when formulating the compound feed.
- Feed intake is insufficient in early and peak lactation. It would be relevant to identify causal relations between reduced feed intake and factors like health status, upbringing, or diet composition.
- It is difficult to correct for weather and other inconstant factors, but it could be interesting to examine whether the use of different beddings, insulated huts,

wallows, shade, and improved management would be able to minimize the environmental effects on the relatively high feed consumption in organic herds.

- In the same ball game, it would be useful to pinpoint the best type of feed presentation, such as pellets, rolls, or biscuits. For the practical feeding, waterproofing qualities, durability, and binding capabilities are important factors that could be examined.
- The EU directive (EC 1804/1999) includes cereal straw as a roughage, whereas this is not the case in the Danish amendment. Sows will consume straw, which also has some nutritional value. Still, for now, straw is considered a bonus rather than a part of the feeding strategy for outdoor sows, which might change if the nutritional value and intake of straw were checked or even improved.
- There is a considerable individual variation in grass-clover intake, and as far as possible, nutritional management should aim to consider individual animals or small groups rather than the whole sow herd. In that perspective, electronic sow feeding stations or other transponder technologies, which enables individual feeding of outdoor sows should be developed further.
- The nutritional value of grass-clover could undoubtedly be improved with seeding developed especially for organic pig production, and it could be interesting to examine the effect of different grass mixtures to maximize sow productivity.
- When feeding organic sows, the aim is to ensure sufficient body reserves to compensate for the adverse environmental conditions and a long lactation period. The current experiment showed very high levels of mobilisation both summer and winter, and it could be interesting to examine whether very high energy compound feed (with high fat and starch content) would be able to reduce this loss of body fat.
- When going for the 100% homegrown feed, it is relevant to investigate the nutritional value of alternative Danish feeds like grass pulp silage, potato pulp, marine protein etc. for organic pigs.

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