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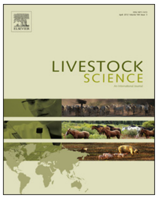

Effect of reduced dietary protein level on energy metabolism, sow body composition and metabolites in plasma, milk and urine from gestating and lactating organic sows during temperate winter conditions

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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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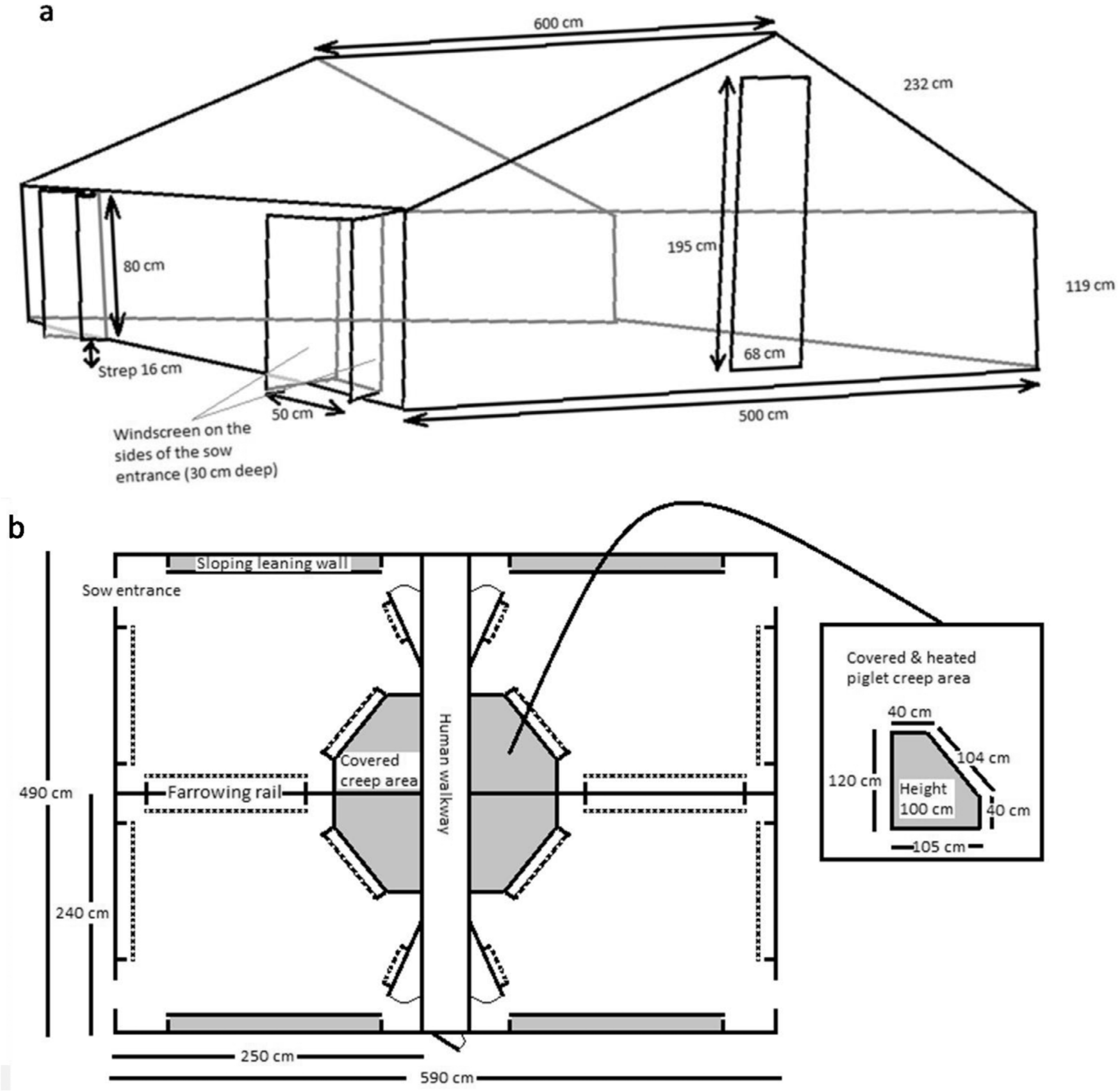

Figure 1. Sketch of the communual hut (Center for Outdoor Livestock Production, Marsvej 43, DK-8960 Randers, Denmark). Seen from the outside (panel a) and from the inside (panel b). Each hut had room for four individual housed sows. Each piglet creep area was heated with an eHEAT PLUS heater (Orbital 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 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 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 restricted 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 obional 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 –20°C 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.

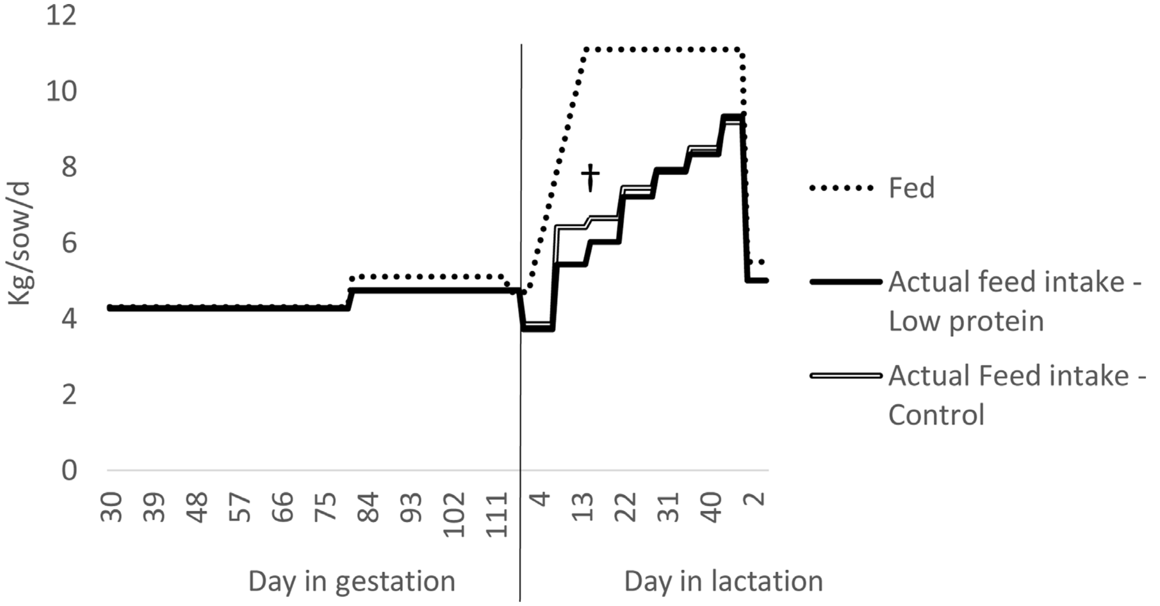

Figure 2. Feeding curve and actual feed intake in kg/d in gestation and lactation. The feeding curve is based on the recommended daily energy intake for indoor sows plus additional 15% feed (SEGES, 2016). Diets were fed to sows from the day after insemination (day 1 in gestation) until weaning (days 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:

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

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

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/$\text{kg}^{0.75}$ × metabolic live weight for pregnant sows (Theil et al., 2004) and 0.482 MJ/$\text{kg}^{0.75}$ × 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 $\text{RE}=\text{RE}_{\text{protein}}+\text{RE}_{\text{fat}}$

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2	48 %	03:14:51	2	4 %	0.01
1	1 %	00:03:45	1	1 %	0.00

- slag/min
- km/t
- Skridt/min

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.

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

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

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.

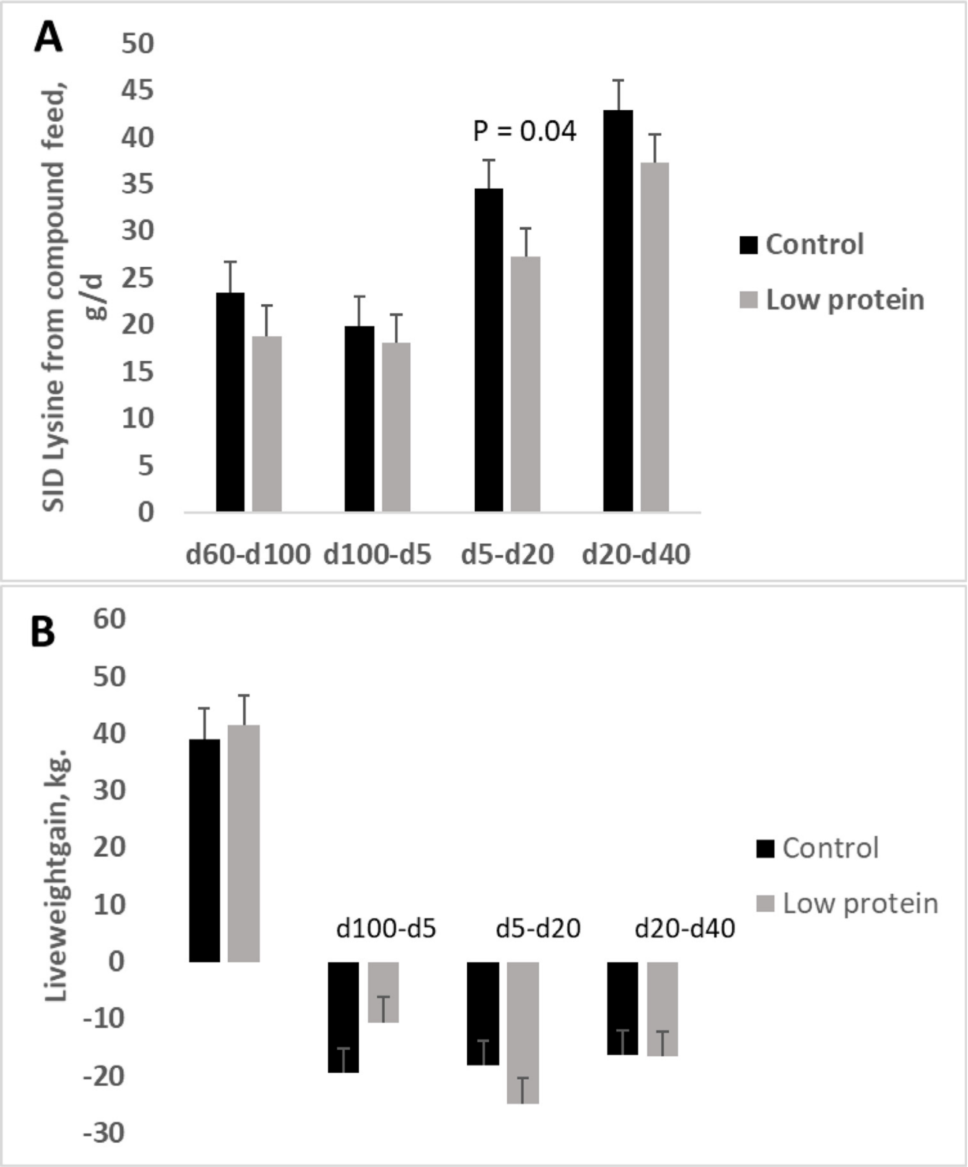

Figure 4. Standardised ileal digestible (SID) lysine intake (panel A) and live weight gain (panel B) in sows fed iso-energetic organic diets differing in proportion of protein in gestation, and early, mid and late lactation. Error bars indicate the SEM

(*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

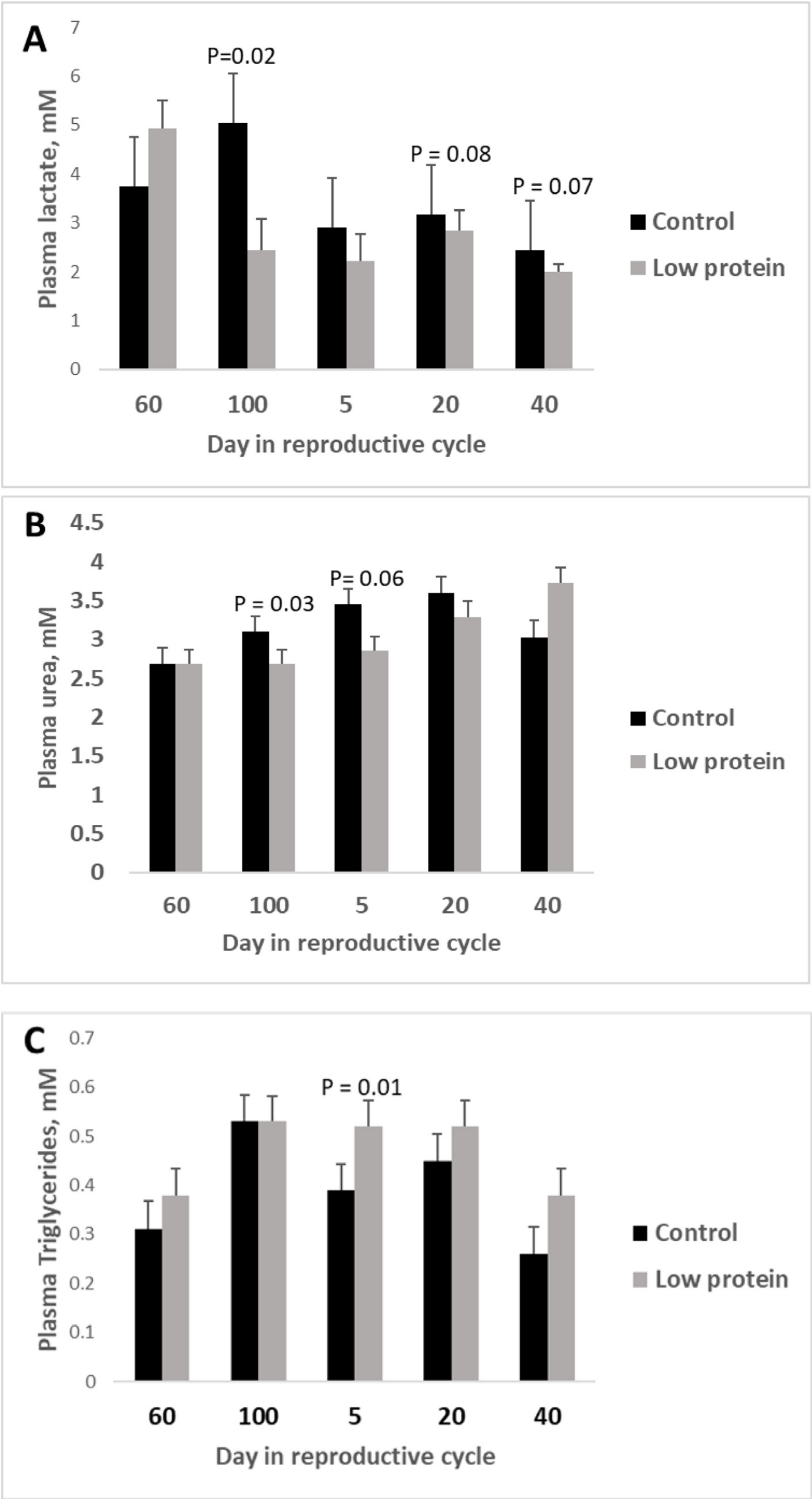

Figure 5. Plasma lactate (panel A), plasma urea (Panel B) and plasma triglycerides (TG; panel C) day 60 and 100 in gestation and day 5, 20 and 40 in lactation in sows fed iso-energetic organic compound feed differing in proportion of protein. Error bars indicate the SEM.

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

	DIM 5	20	40	SEM	Protein level Control	Low	SEM	P-value 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

$^{a\text{-}b}$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

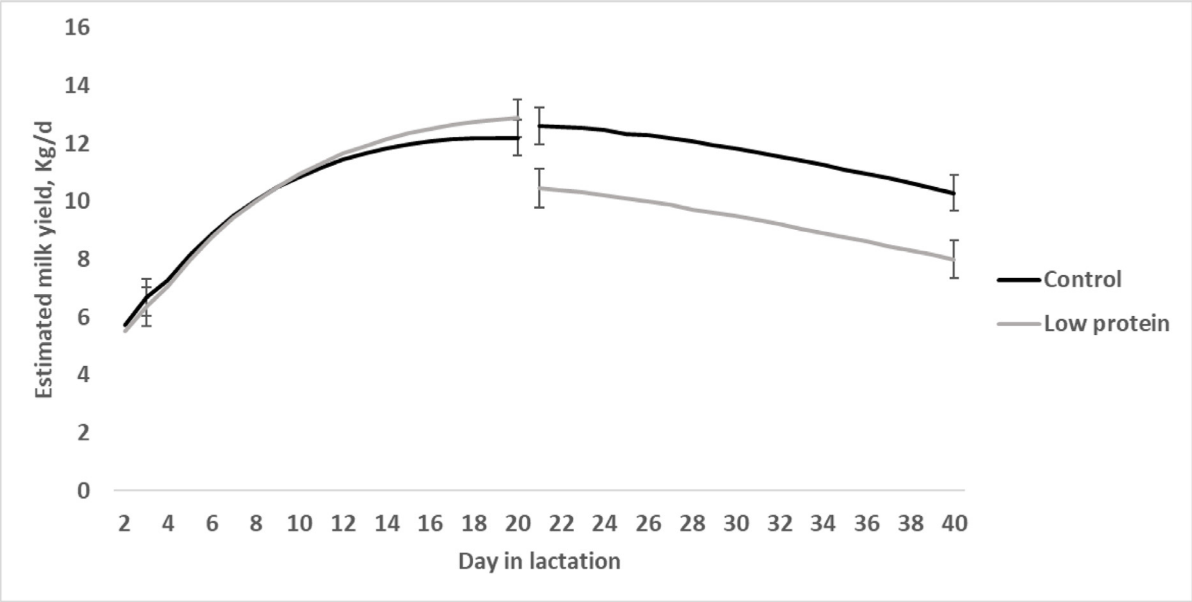

Figure 6. Estimated milk yield day 2 to day 40 in sows fed iso-energetic organic compound feed differing in proportion of protein, based on litter size and piglet weight gain in the two periods d1 to d20 (3.19 kg/d) and d20 to d40 (2.69 kg/d) ($P = 0.004$). The control group had an average daily litter 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.

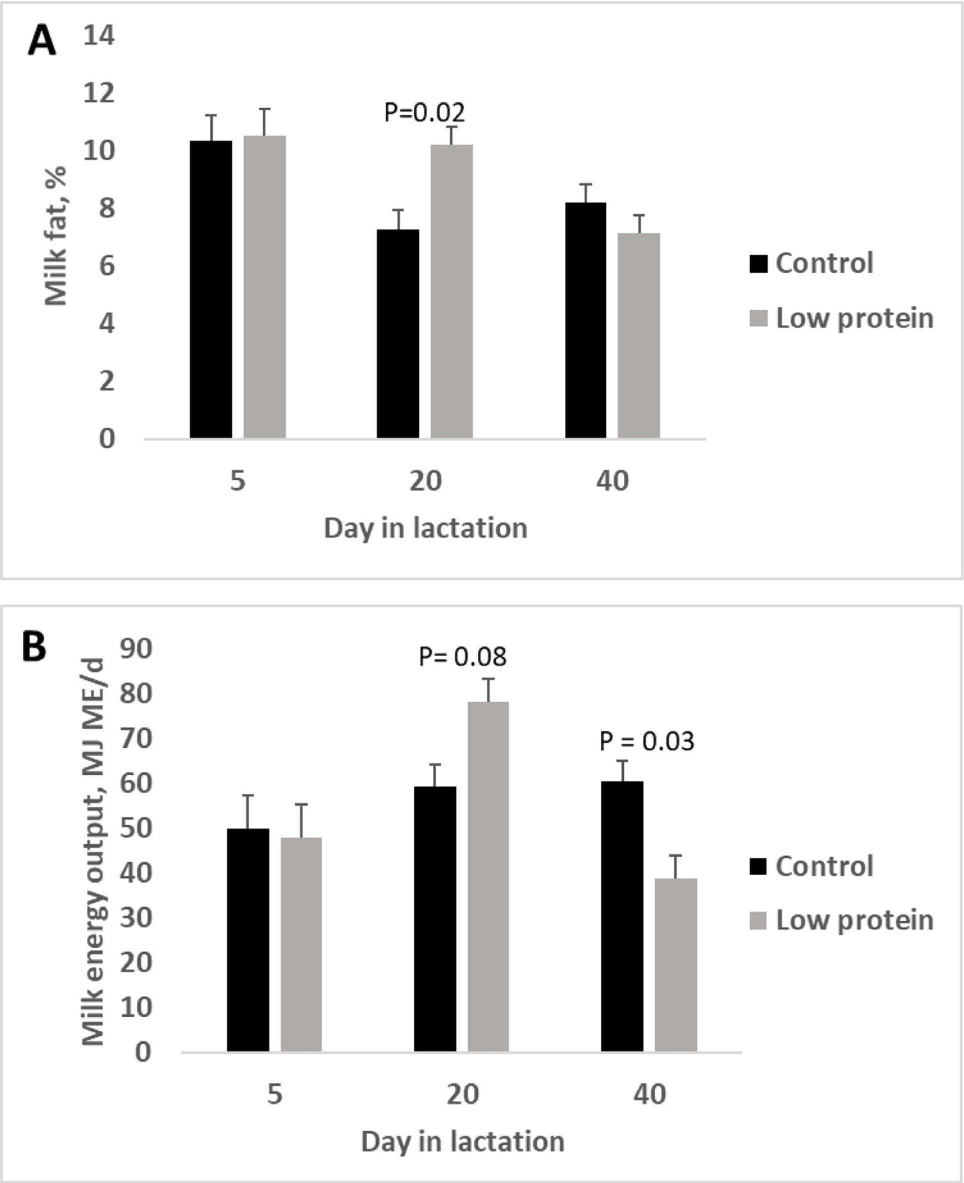

Figure 7. Milk fat % (panel A) and milk energy content (panel B) day 5, 20 and 40 in lactation in sows fed iso-energetic organic diets differing in proportion of protein. Error bars indicate the SEM.

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

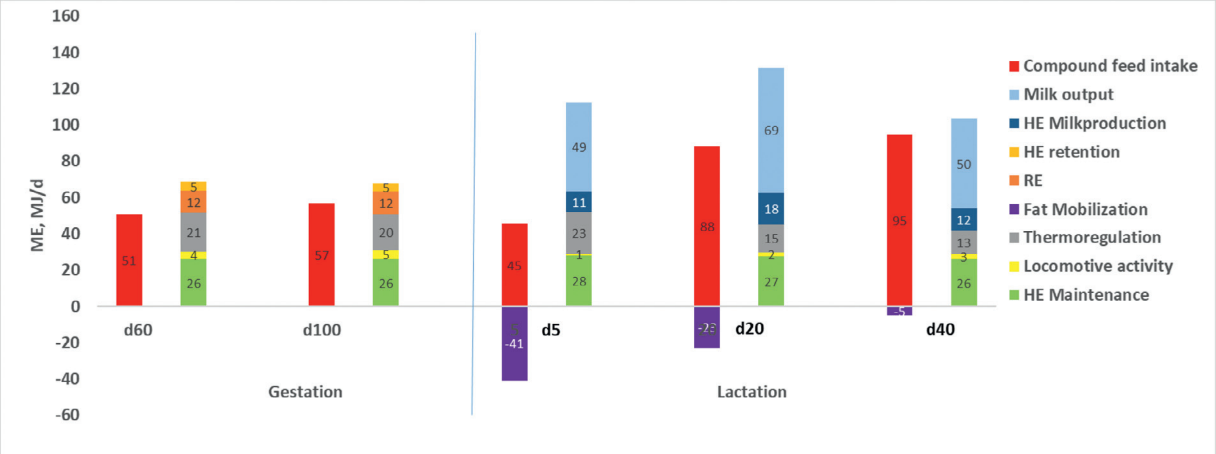

Figure 8. Daily energy intake, energy expenditure (metabolisable energy requirement) on day 60 and 100 in gestation and day 5, 20 and 40 in lactation in first parity sows fed organic diets in winter. 24h average temperatures were -1.3°C (d60), 0.05°C (d100), -3.2°C(d5), 4.38°C(d20) and 6.2°C (d40). In 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 ingested 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 capacity, 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 production. 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. However, the results indicate that these sows were indeed able to counterbalance 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

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