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

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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 regarded 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 protein 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 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.

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 communal 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).

Ingredients, g/kg	Gestation[1] Normal protein	Low Protein	Lactation[2] 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).

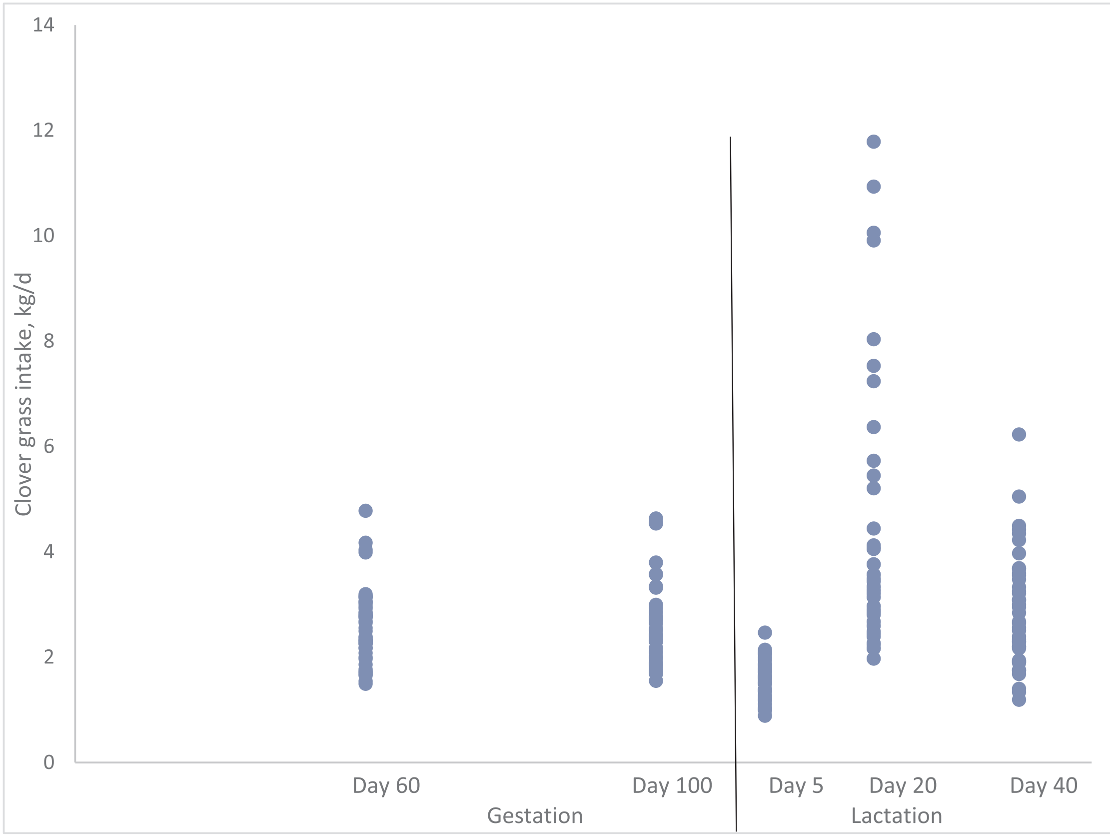

Figure 1. Individual clover grass intake in kg/d in 43 2nd parity organic sows with access to pasture.

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

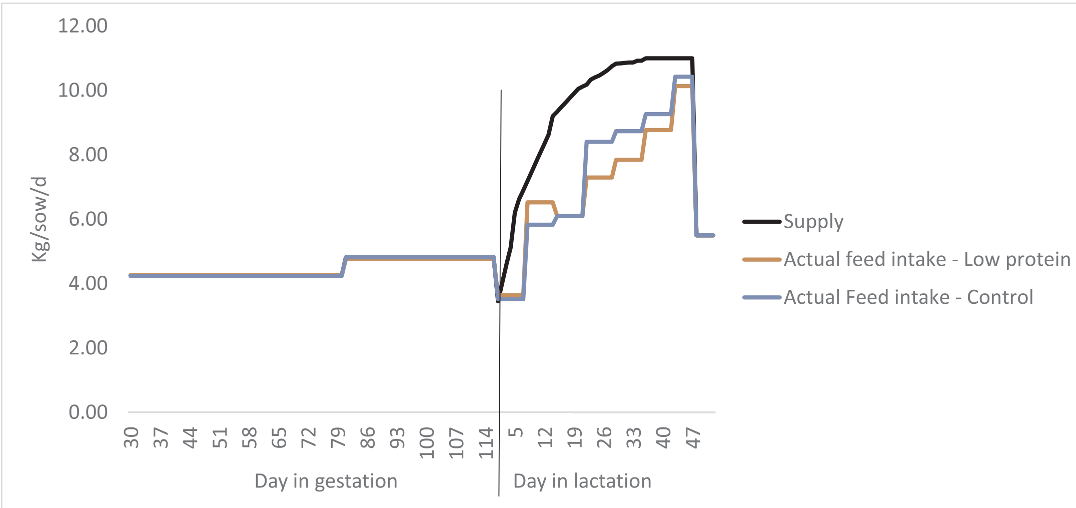

Figure 2. Feeding curve and actual intake of compound feed in kg/d in gestation and lactation. The feeding curve is based on the recommended daily energy intake for indoor sows + 10% (SEGES, 2016).

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).

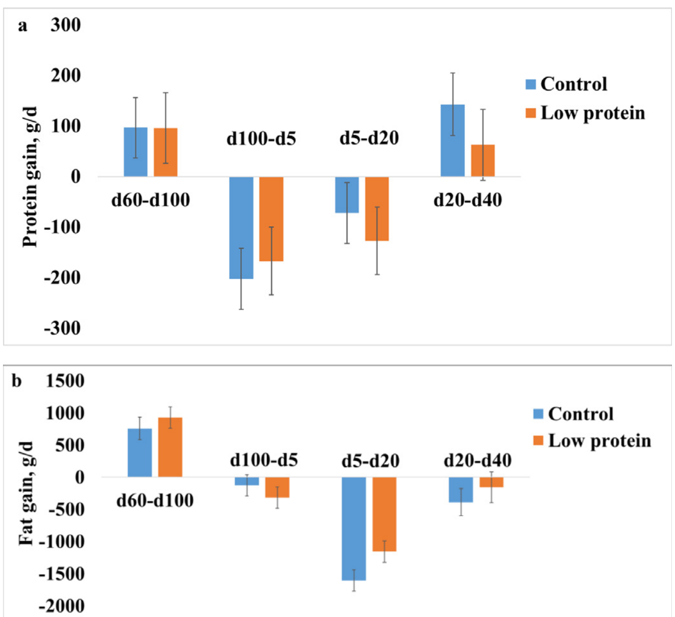

Figure 3. Retention of protein (panel a) and fat (panel b) in sows fed either a control or a low protein diet (negative retention represent body mobilisation.

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												
$HE_{Maintenance}$, 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
$HE_{Locomotory\ activity}$, 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
$HE_{Thermoregulation}$, 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
$HE_{Milkproduction}$, 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
$HE_{Factorial}$, 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] $HE_{termoregulation}$ 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 $ME_{Requirement} = HE_{factorial}$ + 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] $ME_{balance}$ is energy for retention/mobilization

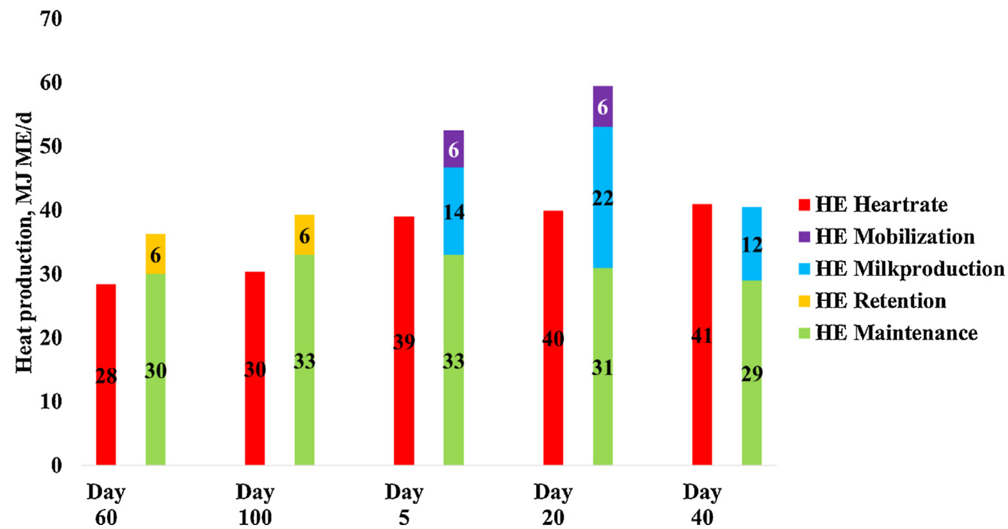

Figure 4. Heat production estimated using recorded heart rate or estimated using a factorial approach in sows fed either a control or a low protein diet.

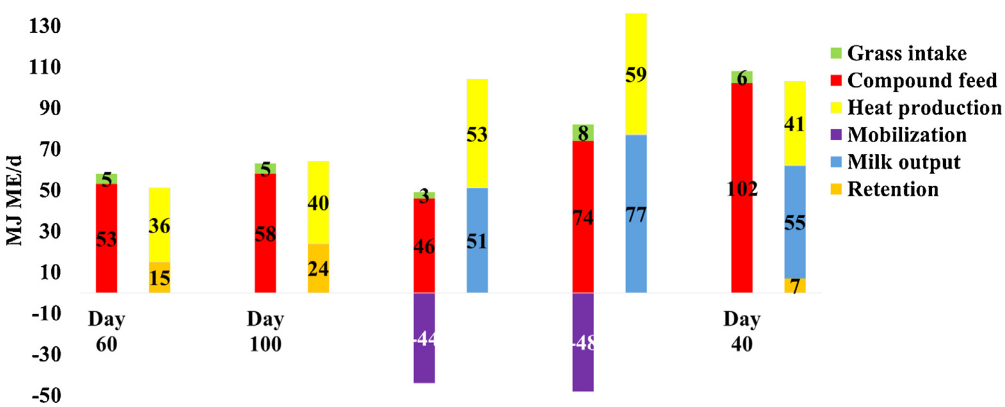

Figure 5. Estimated daily energy input and output on day 60 and 100 in gestation and day 5, 20 and 40 in lactation in 2nd parity sows fed organic compound feeds in summer. Heat production was estimated using a factorial approach. However, energy required for thermoregulation was not included as sows 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-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-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)

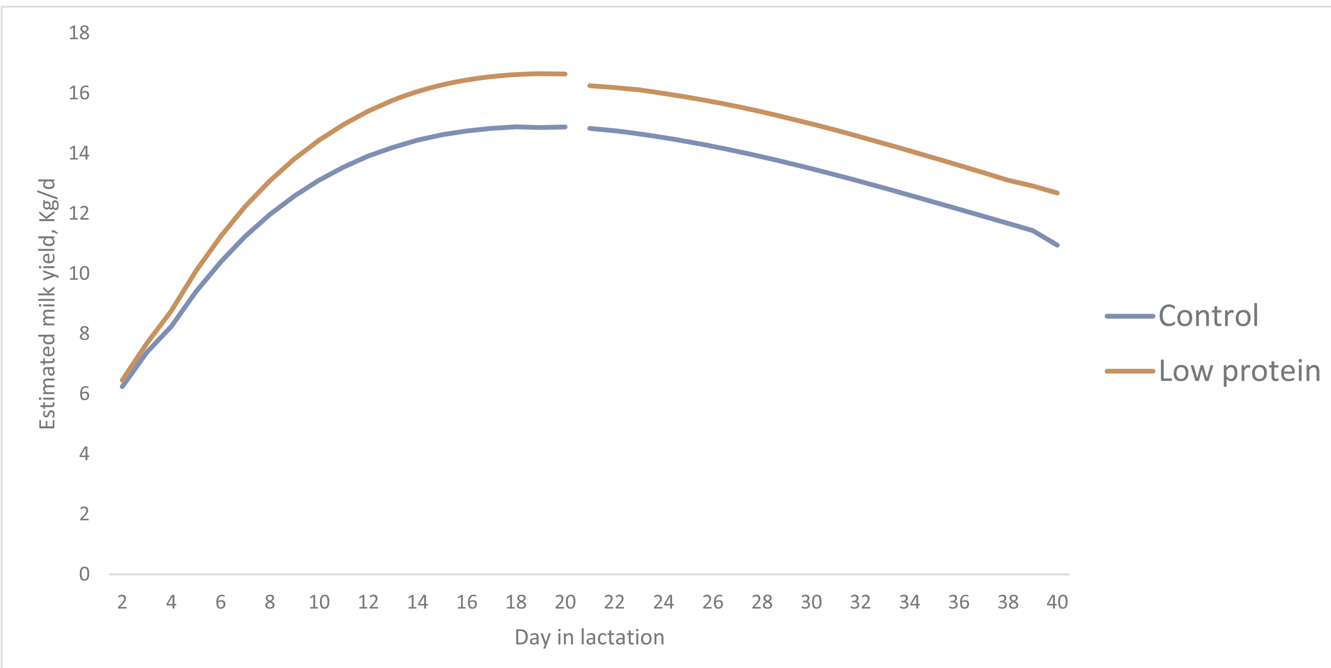

Figure 6. Estimated milk yield d2 to d40 in 2nd parity sows fed iso-energetic organic compound feed differing in proportion of protein. The milk yield is based on litter size and piglet weight gain in the two periods d1 to d20 and d20 to d40. The control group had an average daily litter gain of 2.43 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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References

AOAC, 2000. Official Methods of Analysis, 17th ed. Association of Analytical Chemist (AOAC), Gaithersburg, MD.
Buckner, L.J., 1996. Physical And Mathematical Modelling of The Energy Requirements of the Outdoor Sow. PhD Thesis. The University of Aberdeen.
Buckner, L., Edwards, S.A, Bruce, J.M., 1998. Behaviour and shelter use by outdoor sows. Appl Anim Behav Sci 57 (1-2), 69–80.
Close, W.H., Poornan, P.K., 1993. Outdoor Pigs - Their Nutrient Requirements, Appetite and Environmental Responses. Rec Adv An 175–196.
Danielsen, V., Fernandez, J.A., Jørgensen, H., 2001. Roughage for sows and finishing pigs (In Danish; Grovfoder til søer og slagtesvin). DJF report no 145.
Edwards, S.A., 2003. Intake of nutrients from pasture by pigs. Proceedings of the Nutrition Society 62 (02), 257–265.
Eggum, B.O., 1970. Blood Urea Measurement as a Technique for Assessing Protein Quality. British Journal of Nutrition 24 (4), 983–987.
Eskildsen, M., Hedemann, M.S., Theil, P.K, Nørgaard, J.V, 2020 a a. Impact of increasing fresh grass clover intake on nitrogen metabolism and plasma metabolites of sows. Under revision.
Eskildsen, M., Krogh, U., Sørensen, M.T., Kongsted, A.G., Theil, P.K, 2020 b b. 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. Livest Sci. https://doi.org/10.1016/j.livsci.2020.104088.
Fernandez, J., Danielsen, V., Søegaard, K., Damgaard, H.P., Jensen, S.K., 2006. Clover grass - grazed or ensiled, can cover half the energy requirement of pregnant sows. In danish (Kløvergræs - afgræsset eller ensileret - kan dække mindst halvdelen af drægtige søers næringsbehov). DJF report no 72.
Feyera, T., Theil, P.K., 2017. Energy and lysine requirements and balances of sows during transition and lactation: A factorial approach. Livest Sci 201, 50–57.
Gourley, K.M., Nichols, G.E, Sonderman, J.A., Spencer, Z.T., Woodworth, J.C., Tokach,

M.D., DeRouchey, J.M., Dritz, S.S., Goodband, R.D., Kitt, S.J., Stephenson, E.W., 2017. Determining the impact of increasing standardized ileal digestible lysine for primiparous and multiparous sows during lactation. Trans Anim Sci 1 (4), 426–436.
Hansen, A.V., Strathe, A.B., Kebreab, E., France, J., Theil, P.K., 2012. Predicting milk yield and composition in lactating sows: A Bayesian approach. J Anim Sci 90 (7), 2285–2298.
Hansen, C., 2018. National average productivity of Danish Pig Farms 2017. SEGES Danish Pig Research Centre.
Hojgaard, C.K., Bruun, T.S., Theil, P.K., 2019 a a. Optimal lysine in diets for high-yielding lactating saws. J Anim Sci 97 (10), 4268–4281.
Hojgaard, C.K., Bruun, T.S., Theil, P.K., 2019 b b. Optimal crude protein in diets supplemented with crystalline amino acids fed to high-yielding lactating sows. J Anim Sci 97 (8), 3399–3414.
Hojgaard, C.K., 2020. Optimal protein and lysine contents in diets for high-yielding lactating sows. PhD Thesis. The Graduate School of Science and Technology, Aarhus University, Denmark.
Jakobsen, K., Danielsen, V., 2006. Optimal fodring af søer. (Optimal feeding of sows). DJF report no 75. Danish Ministry of Food, Agriculture and Fishery.
Jakobsen, K., Hermansen, J.E., 2001. Organic farming - a challenge to nutritionists. J Anim Feed Sci 10, 29–42.
Kjeldsen, N, 2019. From feed to pig – supply and utilisation of nutrients. Textbook in physiology SEGES Danish Pig Research Center.
Knudsen, K.E.B., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim Feed Sci Tech 67 (4), 319–338.
Kongsted, A.G., Hermansen, J.E., 2009. Sow body condition at weaning and reproduction performance in organic piglet production. Acta Agric Scand a-An 59 (2), 93–103.
Kongsted, A., Claudi-Magnussen, G.C., Hermansen, J.E., Horsted, K., Andersen, B.H., 2011. Effect of breed on performance and meat quality of first parity sows in a seasonal organic rearing system. J Sci Food Agr 91 (15), 2882–2887.
Kongsted, A.G., Norgaard, J.V., Jensen, S.K., Lauridsen, C., Juul-Madsen, H.R., Norup, L.R., Engberg, R.M., Horsted, K., Hermansen, J.E., 2015. Influence of genotype and feeding strategy on pig performance, plasma concentrations of micro nutrients, immune responses and faecal microbiota composition of growing-finishing pigs in a forage-based system. Livestock Science 178, 263–271.
Krogh, U., Eskildsen, M., Sørensen, M.T, Jørgensen, H., and Theil, P.K., 2018. Heat production in free-range sows estimated by heart rate recordings. Proceedings of the 14th International Symposium on Digestive Physiology of Pigs Brisbane, Australien.
Lambert, R.J., Ellis, M., Rowlinson, P., Saville, C.A., 1983. Influence of housing feeding system on sow behavior. Anim Prod 36 532-532.
Le Cozler, Y., Beaumal, V., Neil, M., David, C., Dourmad, J.Y., 1999. Changes in the concentrations of glucose, non-esterified fatty acids, urea, insulin, cortisol and some mineral elements in the plasma of the primiparous sow before, during and after induced parturition. Reprod Nutr Dev 39 (2), 161–169.
National Research Council, 2012. Nutrient requirements of swine. 11th rev ed. Natl Acad Press, Washington DC.
Patience, J.F., 2012. Feed efficiency in swine rev. ed. Wageningen Academic, WageningenThe Netherlands.
Pedersen, T.F., Bruun, T.S., Feyera, T., Krogh, U., Theil, P.K., 2016. A two-diet feeding regime for lactating sows reduced nutrient deficiency in early lactation and improved milk yield. Livest Sci 191, 165–173.
Pedersen, T.F., Chang, C.Y., Trottier, N.L., Bruun, T.S., Theil, P.K., 2019a. Effect of dietary protein intake on energy utilization and feed efficiency of lactating sows. J Anim Sci 97 (2), 779–793.
Pedersen, T.F., Bruun, T.S., Trottier, N.L., Theil, P.K., 2019b. Nitrogen utilization of lactating sows fed increasing dietary protein. J Anim Sci 97, 3472–3486.
Prunier, A., Dippel, S., Bochicchio., D., 2014. Characteristics of organic pig farms in selected European countries and their possible influence on litter size and piglet mortality. Org. Agr. 4, 163–173.
Ferre, Rivera, M.G., Edwards, A., S., Mayes, R.W., Riddoch, I., Hovell, F.D.DeB., 1999. Grass utilisation by outdoor sows in different seasons measured by the n-alkane technique. In: Hermansen, JE, Lund, V, Thuen, E. (Eds.), Ecological Animal Husbandry in the Nordic Countries. Proceedings from NJF-seminar No 303, Horsens, Denmark 16-17 September 1999.
Rivera Ferre, M.G., Edwards, S.A., Mayes, R.W., Riddoch, I., Hovel, F.D.DeB., 2001. The effect of season and level of concentrate on the voluntary intake and digestibility of herbage by outdoor sows. Anim. Sci. 72, 501–510.
Rozeboom, D.W., Pettigrew, J.E., Moser, R.L., Cornelius, S.G., Elkandelgy, S.M., 1994. In-Vivo Estimation of Body-Composition of Mature Gilts Using Live Weight, Backfat Thickness, and Deuterium-Oxide. J Anim Sci 72 (2), 355–366.
Samuel, R.S., Moehn, S., Pencharz, P.B., Ball, R.O., 2012. Dietary lysine requirements of sows increases in late gestation. J. Anim. Sci. 90, 4896–4904.
Sehested, J, Soegaard, K., Danielsen, V., Kristensen, V.F., 1999. Mixed grazing with sows and heifers: effects on animal performance and pasture. In Ecologigal Husbandry in the Nordic countries pp 35-39 (JE. Hermansen, V. Lund E. Thuen editors). Foulum, Denmark.
SEGES økologi, 2018. National average productivity of Danish Organic Pig Farms 2017. Data is reported in a cooperation between the slaughter houses Friland A/S, Organic Pork, Tican/Tönnis and SEGES Organic Innovation.
Shurson, J., Whitney, M., Johnston, L., Koehler, B., Hadad, R., Koehler, D., 2012. Designing Feeding Programs for Natural and organic Pork Production. College of Agricultural, Food and Environmental Sciences, University of Minnesota Available at. http://albany.cce.cornell.edu/resources/Designing-Feeding-Programs-for-Natural-and-organic-Pork-Production.
Sørensen, G., Krogsdahl, J., 2018. Extra compound feed, fiber and protein does not increase birtweight or piglet survival. (In Danish; Ekstra foder, fibre eller protein øger ikke fødselsvægten eller pattegriseoverlevelsen). SEGES Pig Research Center, Report no. 1158.
Theil, P.K., Nielsen, T.T., Kristensen, N.B., Labouriau, R., Danielsen, V., Lauridsen, C., Jakobsen, K., 2002. Estimation of milk production in lactating sows by determination of deuterated water turnover in three piglets per litter. Acta Agr Scand a-An 52 (4), 221–232.
Theil, P.K., Jorgensen, H., Jakobsen, K., 2004. Energy and protein metabolism in lactating sows fed two levels of dietary fat. Livest Prod Sci 89 (2-3), 265–276.
Theil, P.K., Chwalibog, A., Jørgensen, H., 2020. Energy: metabolism, requirement, utilisations and prediction of content in diets. In Nutritional physiology of pigs (Editors: K. E. Bach Knudsen, N. J. Kjeldsen, H. D. Poulsen and B. B. Jensen), Chapter 20, Freely available at:. https://svineproduktion.dk/Services/Undervisningsmateriale2.
Tybirk, P., Sloth, N.M., Kjeldsen, N., Shooter, L., 2016. Danish Nutrient requirement standards (In Danish: Normer for næringsstoffer). 20th ed. SEGES Pig Research Centre, Axelborg, Denmark.
Vadmand, C., Krogh, U., Hansen, C.F., Theil, P.K., 2015. Impact of sow and litter characteristics on colostrum yield, time for onset of lactation, and milk yield of sows. J Anim Sci 93 (5), 2488–2500.
Verhagen, J.M.F., Verstegen, M.W.A., Geuyen, T.P.A., Kemp, B., 1986. Effect of Environmental-Temperature and Feeding Level on Heat-Production and Lower Critical-Temperature of Pregnant Sows. J Anim Physiol an N 55 (4-5), 246–256.
Weast, R.C., Astle, M.J., Beyer, W.H., 1984. CRC Handbook of Chemistry and Physics. (F. CRC Press Inc, Boca Raton editor.).
Weissensteiner, R., Baldinger, L., Hagmuller, W., Zollitsch, W., 2018. Effects of two 100% organic diets differing in proportion of home-grown components and protein concentration on performance of lactating sows. Livest Sci 214, 211–218.