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Non–Ruminant Nutrition

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

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HIGHLIGHTS

- Organic sows benefitted from being fed reduced dietary protein during winter.
- Nutrient deposition was unaffected when gestating sows were fed reduced protein.
- Gestating organic sows excretes less urea when fed reduced dietary protein.
- Low dietary protein in gestation increases feed intake, milk yield and litter gain.

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ABSTRACT

Oversupply of protein is a challenge in organic sow production. Currently, organic sow feed is composed in accordance with feeding standards of conventional sows, which do not take the higher daily feed allowance and nutritional contribution from roughage into consideration. The objective of the current study was to investigate the effect of reduced dietary protein in gestational compound feed for organic outdoor sows during winter conditions, using metabolites in plasma, urine, and milk as indicators of sows' metabolic status.

In total, 20 sows (Topigs Norsvin; TN70) were included in the experiment, lasting from d30 of gestation until weaning at d49 of lactation under outdoor conditions during winter. During gestation, sows were fed one of two isoenergetic diets containing 88 g SID CP pr. kg DM (Control) and 72 g SID CP pr. kg DM (Low protein), corresponding to 16% and 31% below the current recommendation for indoor sows. In lactation, all sows were fed a standard diet containing 125 g SID CP pr. kg DM.

Sow performance traits were not affected by dietary protein level during gestation. An interaction indicated that sows fed the control diet had 23% and 11% higher urinary urea concentrations at d60 and d100 of gestation, respectively, compared with the low protein diet. During lactation, the milk yield of sows fed low protein in gestation increased more than that of control sows ($P < 0.05$). Concurrently, the litter gain of the low protein sows was improved, and their litters were heavier at d49 compared to control sows (276 kg vs. 238 kg; $P < 0.001$).

In conclusion, organic outdoor sows benefitted from reduced dietary protein during gestation in winter conditions, as indicated by urinary urea concentration, milk yield, and litter gain.

1. Introduction

The production conditions of organic outdoor sows differ considerably from that of conventional indoor sows, and consequently, the nutrient requirements differ. Most noticeable, outdoor sows have a higher energy requirement, primarily due to a higher demand for thermoregulation during winter (Buckner, 1996; Eskildsen et al., 2020b). Under Northern European conditions, the energy requirement of outdoor sows is estimated to be 15–20% higher than indoor sows, which requires a higher feed allowance (Close and Poornan, 1993; Edwards, 2003). However, the daily protein requirement is seemingly not affected by production conditions.

Currently, organic outdoor sow feed is composed according to the indoor feeding standards, without considering the higher feed allowance

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and consequently, the outdoor sows are oversupplied with protein. Oversupply is unfavorable, as it increases the energy output in urine through urea and additional heat production (Theil et al., 2020), and which reduces feed efficiency (Pedersen et al., 2019b). On the contrary, supplying inadequate amounts of protein is also undesirable, as this will negatively affect the productivity of the sows (Hojgaard et al., 2019a).

EU legislation requires organic sows to have permanent access to roughage (Regulation (EU), 2018). This serves to improve animal welfare, as it allows for manipulation and increases satiety which reduces stereotypic behavior (Bergeron et al., 2000; Danielsen and Vestergaard, 2001), and enhances gastro–intestinal health of the sows (Lindberg, 2014; Jha et al., 2019). Roughage consumption also has a nutritional value to the sow and can contribute considerable amounts of nutrients (Fernándéz et al., 2006), and if the roughage intake is not taken into consideration, excess protein is ingested. Not only is excess protein physiologically unfavorable, but it also increases the environmental impact of organic pig production (Eriksen et al., 2002). Thus, it is highly relevant to adjust and optimize the dietary protein concentration in the compound feed to the organic outdoor production conditions to meet the requirement of organic sows and to avoid the undesired consequences of feeding excess dietary protein. In addition, it is important to consider the protein contribution from roughage and include this into the total daily ration.

Recent studies carried out by Eskildsen et al. (2020a, 2020b) showed that protein restriction during gestation is beneficial, whereas protein restriction during lactation compromises sow productivity because lysine becomes too limiting. In this study, further reduction in dietary protein was tested during gestation while all sows were fed a common lactation diet for organics sows.

It was hypothesized that sows fed low protein during gestation received adequate amounts of protein and lysine and oxidized less protein than control sows without affecting their performance. This study aims to investigate how reducing the concentration of dietary protein in organic compound feed for gestating sows affects the metabolic status and productivity of the sows during their production cycle in winter conditions, using productivity measures and metabolites in plasma, urine, and milk as indicators of metabolic status.

2. Materials and methods

The animal experiment procedures were performed according to the Danish Ministry of Justice, Law no. 474 of 15/02/2014 regarding animal experiments issued by the Danish Ministry of Environment and Food. Rearing, housing, and sampling were in coherence with Danish laws for the care and use of animals for research purposes.

2.1. Animals and housing

Twenty sows (Topigs Norsvin; TN70) of mixed parity (2nd–5th) were inseminated with commercial DanBred Duroc production semen. In gestation, the sows were randomly assigned to one of two iso–energetic diets (NE); a standard organic diet (Control, $n = 10$) or a low protein diet (LP, $n = 10$). All sows were fed the same commercial standard organic lactation diet from approximately two weeks prior to parturition and up until weaning a day 49 of lactation. Sows were supplied grass–clover silage or barley–pea whole–crop silage during the experimental period, but the impact of silage type will be published elsewhere. Sows were purchased from a Danish commercial organic herd with high productivity. Insemination was performed between October 22nd and 27th, and the sows arrived at the experimental herd at Aarhus University on November 24th after a positive scan for pregnancy. Then, the sows were reared outdoor under organic conditions during the winter 2020–21 at the Organic Platform at Aarhus University, Denmark. During gestation, sows were housed in one of two paddocks according to the type of roughage supplied. The gestation paddocks measured 4000 m^2 (80 × 50 m), and two 12 m^2 isolated gestation huts were located in each paddock. Two weeks prior to expected farrowing, sows were moved to individual paddocks measuring 480 m^2 (30 × 16 m). Two types of farrowing huts were used: Three four–compartment communal huts, where each individual section measured 2.4 × 2.5 m (Eskildsen et al., 2020b) and four two–compartment communal huts, where each induvial section measured 2.0 × 1.9 m (Fig. 1). Sows on each combination of dietary protein level x roughage type were equally distributed among the two types of huts. A heated piglet creep area was associated with each individual section. All huts were supplied with chopped straw as bedding, approximately 12,5 kg/m^2.

Gestation and lactation paddocks were sown with a commercial grass–clover mix (ForageMax 22A, DLF Trifolium, Roskilde, Denmark), consisting of 15% Trifolium Repens (white clover; Silvester and Rivendel) and 85% Lolium Perenne (perennial ryegrass; Ovambo, Masai, Humbi 1, Garbor and Bovini). Sows were carrying nose–rings to reduce rooting and keep the sward intact in accordance with Danish organic practice.

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

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

2.2. Diets and feeding

A commercially available organic compound feed, based on organic cereals, rapeseed cake, and peas, was used as a control diet during the gestation period (Table 1). The control diet was formulated to supply the recommended amount of standardized ileal digestible (SID) amino acids (AA), except lysine and crude protein (CP), for gestating sows, according to Danish feeding standards (Tybirk et al., 2020). A customized compound feed, based entirely on organic cereals, was used as low–protein diet during gestation. The control and low–protein diets were formulated to be iso-energetic using the potential physiological energy system used for sows in Denmark, which is comparable to net energy (Patience, 2012). The content of SID CP in both diets were 16% and 31%, respectively, below the Danish recommendation, which amounts to 105 g SID CP/kg DM. The gestation diets were offered from the experimental start until two weeks before expected farrowing, after which all sows were fed the same lactational diet until weaning at day 49 of lactation. The lactation diet was a standard commercially organic lactation compound feed based on organic cereals, peas, and soybeans (Table 1). The lactation diet was formulated to supply the recommended amount of SID AAs except lysine, for lactating sows, according to Danish feeding standards (Tybirk et al., 2020). All diets were formulated to fulfill the sow's requirements of vitamins and minerals at the given production stage. Roughage was supplied according to sows' appetite to ensure maximal intake and reduce residues.

The compound feeds were manufactured at a commercial feed company (Vestjyllands Andel, Vildbjerg, Denmark) and delivered twice during the study; upon arrival of the sows and before sows farrowed.

During gestation, sows were fed according to the recommendation for indoor sows fed high amounts to restore backfat, and during lactation, the feeding curve recommended for highly productive sows was used (Bruun et al., 2017; Sørensen, 2019) in early lactation, but in contrast to conventional sows, the feed supply continued to increase according to their appetite and reached a final feed intake of 11 to 17 kg/d of compound feed.

Gestating sows were fed both compound feed and silage twice per day at 9.00 AM and 2.00 PM. The feeding system used allowed individual feeding of the compound feed and after 45 min, feed leftovers were collected and weighed to calculate the realized feed intake. Silage was supplied in open troughs and leftovers were collected and weighed daily. During lactation, sows were fed compound feed and silage only

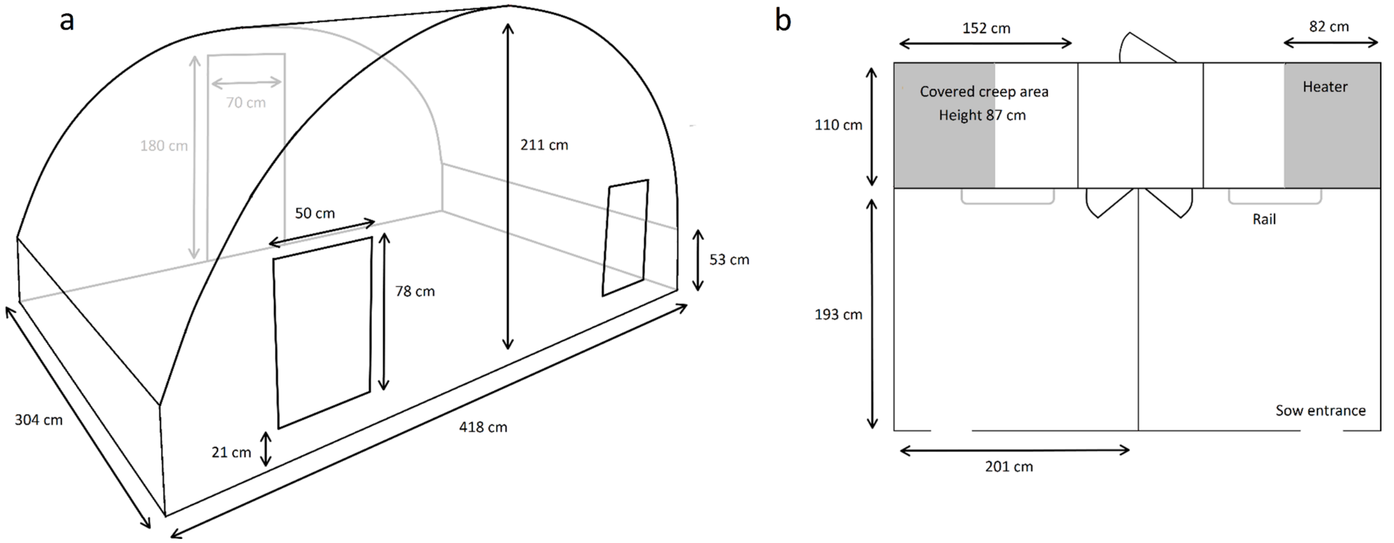

Fig. 1. Sketch of the two–compartment communal hut, seen from the outside (panel a) and the inside (panel b).

Table 1
Ingredients of experimental organic diets.

Ingredients, g/kg	Gestation[1] Control	Low protein	Lactation[2]
Barley	334	722	330
Rye	200	100	100
Oat	150	150	102
Wheat	100		250
Wheat bran	100		
Rapeseed cake	42.6		25.2
Peas	34.4		60
Starfish meal	10		20
Potato protein	3.8		20
Calcium carbonate	11.3	13.1	30
Mono calcium phosphate	6.7	8.6	20
Sodium chloride	5.1	4.8	20
Vitamin and mineral mix[3]	1.6	1.1	5.9

[1] Offered from d30 to d100 of gestation.

[2] Offered from d100 of gestation to weaning at d49 of lactation.

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

once per day at 9.00 AM. The compound feed was provided in feeders with a lid to minimize the feed loss to rodents and birds. During lactation, residues of compound feed and roughage were collected and weighed at sow level each week.

One sample of each experimental diet was taken during the study. Each feed sample was split into two subsamples and analyzed for DM, energy, CP, fat and ash content, and AA composition (Eurofins Steins Laboratory A/S, Vejen, Denmark). Five representative samples of each silage were taken throughout the experimental period and stored at –20°C until analysis. The silage samples were analyzed for DM, energy, CP, fat and ash content, and AA composition (Eurofins Steins Laboratory A/S, Vejen, Denmark). The analyzed chemical composition of the diets and roughages are presented in Table 2. The roughage samples displayed no systematic variation through the experimental period; thus, the values presented in Table 2 are simple means of the five samples.

2.3. Recording and sampling

Recordings of liveweight, backfat thickness, sample collections of blood and urine and deuterium (D_2O) enrichment were performed at d60 and d100 of gestation and d5 and d20 of the lactation period. At sunrise, sows were caught in the huts and transported to a wagon, where the experimental procedures were performed. First, the liveweight of the sows was measured on a walk–in scale, and simultaneously, the backfat thickness was measured. Backfat was measured using the digital ultrasound scanner LEAN MEATER (Baltic Korn A/S, Naestved, Denmark) in the P2 point; approximately 70 mm from either side of the spine at the last rib. These measurements were also performed at d30 of gestation and d49 of lactation. Next, sows were fixated using a snare restraint around the snout, and blood was collected from the jugular vein in a 10 ml Na–heparinized tube (Greiner BioOne GmbH, Kremsmünster, Austria) while the sow was standing. The blood samples were immediately centrifuged ($1,558 * g$ at –4°C for 12 min), and plasma was harvested into a 1.5 ml microcentrifuge tube and frozen at –20°C and –80°C until analysis. Immediately after blood sampling, sows were enriched with D_2O (0.0425 g 40% solution pr kg liveweight) intramuscularly in the neck. On days 5 and 20 of lactation, sows were injected 0.3 ml oxytocin (10 IU/ml: Leopharma, Ballerup, Denmark) into the ear vein to induce milk letdown, and sows were manually milked from random teats while standing fixated. The milk samples were filtrated through gauze and stored at –20°C until analysis.

The following day a spontaneous spot urine sample was collected: Before sunrise, the sows were caught in the huts. After sunrise, the sows were individually released, and spontaneous urine samples were collected in the middle of the excretion in a 200 ml collection pot.

On d1 of lactation, live piglets were ear–tagged. No litter equalization was performed. On days 1, 5, 20, and 49 (weaning) of lactation, the piglets were individually weighed. Male piglets were castrated on d5. Dead piglets were collected and registered daily.

2.4. Chemical analysis

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

DM content of compound feed and roughage samples was determined by oven drying at 103°C until reaching constant weight, and crude ash content was determined by oven drying at 550°C (Commission Regulation EC, 2009). Crude protein (CP) content was calculated by multiplying the nitrogen content of the sample by 6.25. Nitrogen content was determined by the international Dumas method (Hansen, 1989). The AA content in compound feed and roughage samples were analyzed

Table 2

Chemical composition of experimental diets and roughages. Crude protein and amino acids in parentheses are standardized ileal digestible (SID).

	Gestation[1]				Lactation[2]		Grass–clover silage		Barley–peawhole–crop silage	
Chemical composition	Control		Low protein							
DM, g/kg	865		866		864		394		226	
FUsow/kg DM[3]	1.17		1.17		1.23		0.65		0.63	
ME, MJ/kg DM[4]	14.33		14.30		15.30		9.83/10.06[5]		9.67/9.88[5]	
CP, g/kg DM	118	(88)	98	(72)	153	(125)	132	(63)[6]	125	(57)[6]
Fat, g/kg DM	45		37		50		37		30	
Ash, g/kg DM	54		48		58		69		68	
Amino acids, g/kg DM										
Lysine	5.23	(4.00)	3.93	(2.77)	7.48	(6.16)	5.13	(2.69)[6]	4.39	(2.12)[6]
Methionine	2.21	(1.81)	1.56	(1.29)	2.70	(2.34)	1.61		1.56	
Cystine	2.65	(1.96)	2.42	(1.77)	3.04	(2.34)	1.00		1.06	
Threonine	4.23	(3.20)	3.26	(2.37)	5.83	(4.72)	5.25		4.70	
Tryptophan	1.49	(1.11)	1.24	(0.88)						
Isoleucine	4.00	(3.09)	3.42	(2.52)	6.08	(4.86)	5.37		4.37	
Leucine	7.72	(6.19)	6.65	(5.07)	10.89	(9.10)	9.28		7.47	
Histidine	2.76	(2.21)	2.11	(1.62)	3.38	(2.62)	2.10		2.03	
Phenylalanine	5.03		4.42		6.68		5.90		4.52	
Tyrosine	3.29		3.05				3.15		1.32	
Valine	5.51	(4.26)	4.86	(3.52)	6.98	(5.69)	7.10		6.15	

[1] Offered from d30 to d100 of gestation.

[2] Offered from d100 of gestation to weaning at d49 of lactation.

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

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

[5] For gestating and lactating sows, respectively.

[6] The standardized ileal digestibility was calculated in accordance with Tybirk et al. (2006).

according to Commission Regulation EC (2009): Samples were hydrolyzed for 23 h at 110°C, with (Cys and Met) or without (His, Ile, Leu, Lys, Phe, Thr, Tyr, and Val) performic acid oxidation. Following, AAs were separated using ion-exchange chromatography and quantified by reacting with ninhydrin and photometric detection. To analyze the content of tryptophane, samples were hydrolyzed for 20 h at 110°C in an alkaline solution. The AAs were separated using liquid chromatography and quantified by fluorescent detection (Commission Regulation EC, 2009).

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

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

2.5. Calculations and statistical analysis

Milk yield (MY) at d5 and d20 was predicted according to Hansen et al. (2012), developed to quantify MY of conventional sows, using litter gain and litter size as predictors. The MY after d20 was purposedly not estimated due to piglets' intake of compound feed. Milk energy content was calculated based on the gross energy (GE) values of the constituents of milk (39.8 MJ/kg fat, 23.9 MJ/kg protein, and 16.5 MJ/kg lactose (Theil et al., 2020)). The daily energy output in milk was calculated as the energy content of milk multiplied by the daily estimated MY.

The D_2O space was calculated based on the D_2O concentration in reference blood, the D_2O concentration in urine after enrichment, and the mass and concentration of the D_2O solution used for enrichment, as described by Theil et al. (2002). Sow's body protein and fat pool were estimated according to the prediction equations of Rozeboom et al. (1994) for gilts (Yorkshire x Landrace), using liveweight, D_2O space, and backfat thickness.

Plasma and urine metabolites and estimated sow body protein and fat were analyzed using the following statistical mixed model:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + (\alpha\beta)_{ij} + \nu_m + \varepsilon_{ijklm}$$

Where Y_{ijklm} is the observed trait, μ is the overall mean of observations, α_i is the effect of dietary protein ($i =$ control or low protein), β_j is the effect of day in gestation or lactation ($j=$ d60, d100, d5 or d20), γ_k is the effect of roughage type ($k=$ grass–clover silage or barley–pea whole–crop silage), δ_l is the effect of parity of sows ($l= 2$ or >2), $(\alpha\beta)_{ij}$ is the interaction between dietary protein and day in gestation or lactation, ν_m is the random effect of sow ($m= 1, 2,\ldots, 20$) and ε_{ijklm} is the residual random components. A compound symmetry structure was used to account for the correlation between repeated measures within animal across sampling days. Milk yield and milk chemical composition were analyzed using the same model; however, only for d5 and d20 of lactation. Compound feed intake, ME intake, SID CP intake, SID lysine intake, liveweight gain, backfat gain, body protein, and fat gain were analyzed using the same model, except day was replaced by five periods; d30–60, d60–100, d100–5, d5–20 and d20–49. Litter gain was analyzed using the same model; however, day was replaced by three periods; d0–5, d5–20, and d20–49. Litter weight and litter size were analyzed using the same model, however, for d0, d5, d20, and d49 of lactation. Finally, sow liveweight and backfat were analyzed using the same model as described above, for d30, d60, and d100 of gestation and d5, d20, and d49 of lactation.

Piglet weight was analyzed using the following statistical mixed model:

$$Y_{ijklmn} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + (\alpha\beta)_{ij} + \nu_m + \tau_{mn} + \varepsilon_{ijklmn}$$

Where Y_{ijklmn} is the observed trait, μ is the overall mean of observations, α_i is the effect of dietary protein ($i =$ control or low protein), β_j is the effect of day in lactation ($j=$ d0, d5, d20 or d49), γ_k is the effect of roughage type (k= grass–clover silage or barley–pea whole–crop silage), δ_l is the effect of parity of sows ($l= 2$ or >2), $(\alpha\beta)_{ij}$ is the interaction between dietary protein and day in gestation or lactation, ν_m is the random effect of sow ($m= 1, 2,\ldots, 20$), τ_{mn} is the random effect of piglet nested within sow ($n = 1, 2,\ldots, 343$), and ε_{ijklmn} is the residual random components. A compound symmetry structure was used to account for the correlation between repeated measures within animal across sampling days.

Plasma NEFA concentrations were transformed using the natural logarithm to obtain variance homogeneity, and the LS–means values and corresponding 95% confidence limits were reported.

Statistical analyses were performed with the software R (R Core Team, 2020), using the package lme4 to analyze mixed–effects models (Bates et al., 2015), and the package emmeans to analyze the effects of experimental factors (Lenth, 2021). Effects were considered significant if *P*–values were below 0.05 and tendencies were accepted at *P*–values below 0.10. If the dietary protein x day interaction was not significant, it was omitted from the model before analyzing the *P*–values for the main effects.

3. Results

The experimental period lasted from November 2020 to April 2021. On average, the air temperature was 1.9°C, wind speed 4.0 m/s, and it rained or snowed 1.6 mm/d, according to the local weather station (DMI, 2021).

The analyzed SID CP content of the control and low protein gestation diet was 88 g/kg DM, and 72 g/kg DM, respectively (Table 2). This is in accordance with the experimental design and a prerequisite for conducting the study.

3.1. *Intake of nutrients and sow characteristics*

Sows' nutrient intake from compound feed and changes in body composition are presented in Table 3. The sows' intake of compound feed followed the planned feeding curve throughout the experiment, except that some feed residues were collected during lactation. An interaction between dietary protein and reproductive stage was found and affect the sows' daily intake of SID CP ($P < 0.01$) and SID lysine ($P < 0.001$) from compound feed, showing that LP sows had a lower intake during gestation but higher intake during lactation (Fig. 2). In compliance, the average daily intake of compound feed and ME from compound feed throughout the study period tended to be higher in LP sows than control sows (6.1 kg/d vs. 6.4 kg/d, $P = 0.10$ and 79.8 MJ ME/d vs. 83.4 MJ ME/d, $P = 0.08$).

The intake of roughage during gestation was fairly low and lower than expected, and during lactation, the intake was negligible. During gestation, the average intake of roughage was 817 g DM/d; equal to 4.9 MJ ME/d and 1.3 g SID lysine/d. Fig. 3 displays the contribution of SID lysine from the compound feed and roughages in gestation relative to the requirement of the sow, based on Samuel et al. (2012). From d100 of gestation until d5 of lactation, the intake of roughage was almost absent. From d5 of lactation until weaning, sows consumed on average 175 g DM/d; equal to 1.7 MJ ME/d and 0.42 g SID lysine/d.

Dietary protein level did not affect the gain of LW, BF and estimated body protein and fat in organic sows. On average, sows gained 61.1 kg LW and 3.6 mm BF from d30 to d100 of gestation and lost 27.5 kg LW and 4.3 mm BF from d5 of lactation until weaning at d49. During the entire experimental period, second parity sows gained a total of 18.9 kg LW, while older sows lost 5.85 kg ($P < 0.05$).

The body composition and reproductive performance of sows are presented (Table 4). An interaction between dietary protein and reproductive stage tended to affect sow's estimated body protein pool, indicating a higher lactational body protein loss of LP sows as compared with control sows ($P = 0.06$). From d100 of gestation until d20 of lactation LP sows mobilized 6.4 kg body protein, while control sows only mobilized 4.0 kg body protein. Control sows weighed on average more than LP sows throughout the experimental period (269 kg vs. 246 kg, $P < 0.05$). Moreover, the estimated body fat pool was larger ($P < 0.05$) for control sows (83.8 kg) throughout the experimental period as compared with LP sows (70.7 kg).

On average, sows had 17.2 liveborn piglets with a mean birth weight of 1.90 kg and weaned 13.3 piglets with a mean weaning weight of 19.4 kg, and a total litter weight of 257 kg. Litters from LP sows gained on average 4.1 kg/d, while litters from control sows gained less (3.5 kg/d; $P < 0.05$). Thus, litters from LP sows gradually became heavier than litters from control sows as lactation progressed, and their litters at weaning weighed 276 kg, whereas litters from control sows weighed 238 kg ($P < 0.001$; Fig. 4). A similar effect was found on piglet weight, showing that LP sow's piglets became gradually heavier compared to control sow's piglets during the lactation period ($P < 0.001$). At weaning, piglets from LP sows weighed 20.2 kg, while piglets from control sows weighed 18.7 kg.

Table 3
Intake of compound feed, changes in body composition and litter gain of sows fed diets varying in dietary protein level.

	Protein level			Reproductive stage[1]					
	Control	Low	SEM	d30–60	d60–100	d100–5	d5–20	d20–49	SEM
Compound feed intake, kg/d[2]	6.1	6.4	0.12	3.8^{c}	3.9^{c}	4.1^{c}	8.4^{b}	11.1^{a}	0.15
ME intake, MJ/d	79.8	83.4	1.34	46.9^{c}	48.3^{c}	54.7^{c}	111.2^{b}	147.1^{a}	1.69
SID CP intake, g/d	616	624	10.9	264^{d}	272^{d}	448^{c}	911.0^{b}	1205^{a}	13.8
SID lysine intake, g/d	29.8	29.6	0.54	11.1^{d}	11.4^{d}	22.0^{c}	44.8^{d}	59.2^{a}	0.68
Liveweight gain, kg	4.2	8.9	1.60	27.5^{d}	33.6^{d}	–27.2^{c}	–15.2^{d}	–12.3^{b}	2.21
Backfat gain, mm	–0.8	1.0	0.29	2.2^{b}	1.4^{b}	0.9^{b}	–1.9^{a}	–2.4^{a}	0.41
Body protein gain, kg	–1.1	–0.6	0.42		4.8^{a}	–3.6^{c}	–2.0^{b}		0.49
Body fat gain, kg	–7.9	–12.9	1.54		12.4^{a}	–13.4^{b}	–9.3^{b}		1.83
Litter gain, kg/d[3]	3.49^{b}	4.10^{a}	0.15				3.93^{b}	5.60^{a}	0.13

	Parity			*P*–value			
	2	> 2	SEM	Protein	Stage	Parity	Protein x stage
Compound feed intake, kg/d	6.3	6.3	0.13	0.10	< 0.001	0.92	0.12
ME intake, MJ/d	82.1	81.1	1.44	0.07	< 0.001	0.61	0.14
SID CP intake, g/d	624	616	16.8	0.60	< 0.001	0.61	< 0.01
SID lysine intake, g/d	29.9	29.5	0.58	0.82	< 0.001	0.61	< 0.001
Liveweight gain, kg	18.9^{a}	–5.9^{b}	1.74	0.68	< 0.001	< 0.05	0.11
Backfat gain, mm	1.4	–1.3	0.29	0.37	< 0.001	0.18	0.29
Body protein gain, kg	–0.2	–1.5	0.46	0.77	< 0.001	0.46	0.46
Body fat gain, kg	–7.8	–13.0	1.71	0.75	< 0.001	0.65	0.06
Litter gain, kg/d	3.85	3.73	0.16	< 0.05	< 0.001	0.60	0.26

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

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

[2] When applying the same statistical model only from d5 to d49 of lactation the average compound feed intake was 9.4 kg and 10.2 kg for sows fed control and low protein diets during gestation, respectively ($P = 0.09$).

[3] Litter gain from birth until day 5 of lactation was 1.67 kg/d and 2.04 kg/d for sow fed control and low protein diets, respectively ($P = 0.19$).

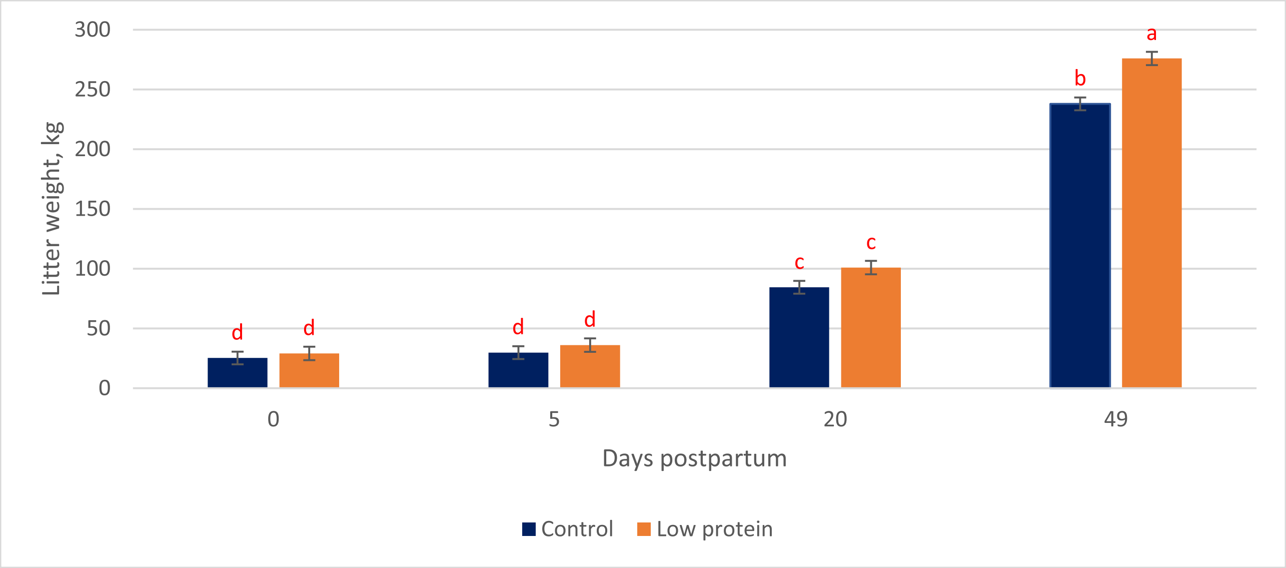

Fig. 2. Effect of the interaction between dietary protein and days postpartum on litter weight ($P < 0.001$). Error bars indicate SEM. Columns without common letters (a–d) differ ($P < 0.05$).

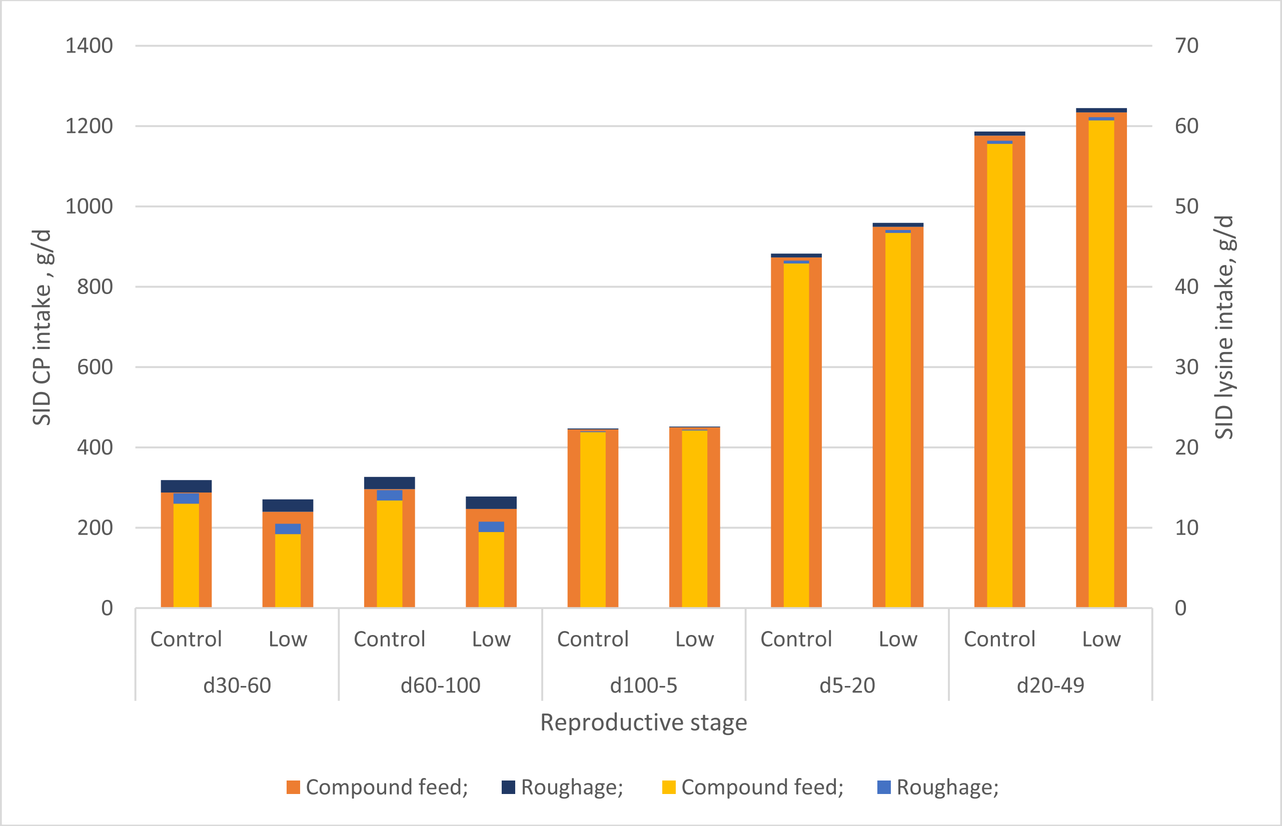

Fig. 3. Effect of the interaction between dietary protein level and reproductive stage on sow's daily intake of SID CP ($P < 0.01$) and SID lysine ($P < 0.001$) from compound feed. The average contribution of SID CP and SID lysine from roughage are included in the figure.

3.2. Metabolites in plasma and urine

Sow plasma and urine metabolite concentrations are seen in Table 5. No effect of dietary protein was found on plasma glucose, lactate, triglyceride, NEFA, and creatinine. An interaction between dietary protein and reproductive stage tended to affect plasma urea concentration ($P =$ 0.07): At d60 of gestation, control sows had a higher plasma urea concentration than LP sows (2.71 mM vs. 2.38 mM, respectively), but during lactation control sows had lower plasma concentration than LP sows (d5; 2.97 mM vs. 3.27 mM and d20; 3.60 mM vs 3.83 mM, respectively; Fig. 5A).

The reproductive stage affected all plasma metabolites. An interaction between dietary protein and reproductive stage was found for urinary urea to creatinine ratio, showing that LP sows have a lower ratio in

Table 4

Body composition and reproductive performance of sows fed diets varying in dietary protein level.

	Protein level			Reproductive stage[1]						
	Control	Low	SEM	30	60	100	5	20	49	SEM
Liveweight, kg[2]	269^{a}	246^{b}	6.15	234^{d}	261^{b}	294^{a}	266^{b}	251^{c}	238^{d}	4.63
Backfat, mm	14.4	12.6	0.91	11.4^{c}	13.7^{b}	15.0^{ab}	15.7^{a}	13.5^{b}	11.5^{c}	0.68
Body protein, kg	42.2	40.1	0.76		39.8^{c}	44.5^{a}	41.1^{b}	39.2^{c}		0.58
Body fat, kg	84.0^{a}	71.0^{b}	3.76		78.8^{b}	90.4^{a}	75.2^{b}	65.8^{c}		2.76
Piglet weight, kg[3]	7.40	8.02	0.52				2.49^{c}	7.04^{b}	19.4^{a}	0.35
Litter size, n[4]	14.6	14.7	1.23				14.7^{a}	13.6^{ab}	13.3^{b}	0.86
Litter weight, kg[5]	94.5^{b}	111^{a}	4.33				32.9^{c}	92.8^{b}	257.2^{a}	3.80
	Parity			*P*–value						
	2	> 2	SEM	Protein	Stage	Parity	Protein x stage			
Liveweight, kg	269^{a}	246^{b}	6.15	<0.05	< 0.001	<0.001	0.23			
Backfat, mm	14.4	12.6	0.91	0.18	< 0.001	0.06	0.82			
Body protein, kg	37.0^{b}	45.3^{a}	0.82	0.07	< 0.001	< 0.001	0.06			
Body fat, kg	66.0^{b}	89.0^{a}	4.03	< 0.05	< 0.001	< 0.001	0.12			
Piglet weight, kg	7.94	7.48	0.55	0.4	< 0.001	0.54	< 0.001			
Litter size, n	14.3	15.1	1.32	0.95	< 0.001	0.68	0.22			
Litter weight, kg	104	101	4.5	0.06	< 0.001	0.72	< 0.001			

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

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

[2] Birth weight of piglets was 1.83 kg for sows fed control diet and 1.96 kg for sow fed low dietary protein ($P = 1.00$).

[3] Liveborn piglets was in average 17.2.

[4] Litter weight at birth was 25.3 kg for sows fed control diet and 29.1 kg for sows fed low dietary protein ($P = 1.00$).

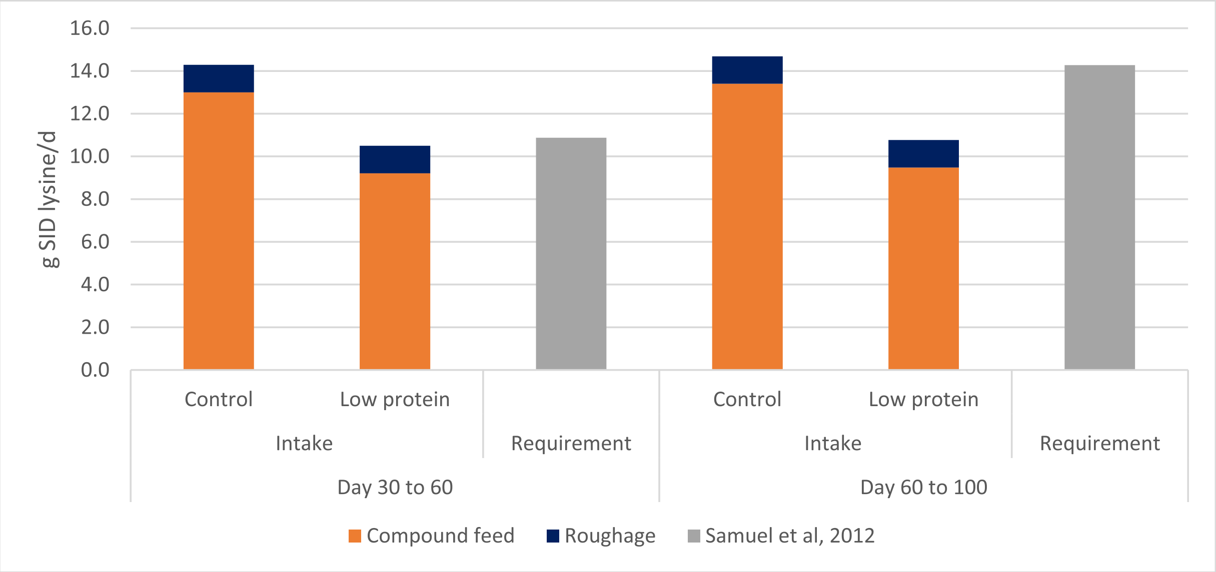

Fig. 4. The SID lysine intake of gestating organic sows fed control or low protein diets, including the contribution from roughage, and the daily SID lysine requirement according to Samuel et al. (2012).

gestation as compared with control sows, while no differences were observed during lactation ($P < 0.05$): At d60 of gestation the ratio was 12.0 and 9.23, and at d100 the ratio was 10.8 and 9.56 for control and LP sows, respectively; Fig. 5B).

3.3. *Milk yield and composition*

Milk yield, milk energy output, and milk chemical composition at d5 and d20 are presented in Table 6. An interaction between dietary protein and days in lactation was found on MY ($P < 0.05$), displaying that MY from LP sows increased more than MY from control sows as lactation progressed: At d5, MY of control and LP sows was 9.72 kg/d and 10.2 kg/d, and at d20 MY was 15.2 kg/d and 17.4 kg/d, respectively. The MY and milk energy output increased through lactation from 9.96 kg/d and 50.7 MJ/d on d5, reaching 16.3 kg/d and 81.7 MJ/d at d20.

4. Discussion

4.1. *Effect of dietary protein level during gestation*

In the current study, no indication was found that reduced dietary protein affected gestating sows' performance. In spite of the reduction in SID CP (from 88 to 72 g/kg DM) and SID lysine (from 4.0 to 2.8 g/kg DM) in compound feed, no differences in LW gain, BF gain, or body protein and fat gain between sows fed control and low protein diet were observed. This indicates that the current reduction in dietary SID CP (31% below recommended level) and lysine (32% recommended level) did not compromise retention of fat and protein during gestation when fed 3.8 kg and 3.9 kg compound feed from d30 to d100 of gestation. The results also indicate that LP sows were not undersupplied with dietary protein and lysine since this would have reduced the sows' ability to deposit protein (Kusina et al., 1999; Rehfeldt et al., 2011). In agreement with these observations, the daily intake of SID lysine of LP sows

Table 5
Plasma and urine metabolites from sows fed diets varying in dietary protein level.

	Protein level			Reproductive stage[1]				
	Control	Low	SEM	60	100	5	20	SEM
Plasma								
Glucose, mM	4.38	4.31	0.17	4.75^{a}	4.60^{a}	4.38^{ab}	3.64^{b}	0.16
Lactate, mM	3.31	2.87	0.33	3.92^{a}	2.57^{b}	2.76^{b}	3.10^{ab}	0.3
TG, mM [2]	0.49	0.502	0.032	0.447^{b}	0.567^{a}	0.403^{b}	0.567^{a}	0.035
NEFA, mM [2]	311	311		79.0^{c}	91.8^{c}	863^{b}	1495^{a}	
	(253–382)	(253–382)		(61.4–102)	(71.3–118)	(670–1111)	(1161–1925)	
Urea, mM	2.94	3.02	0.08	2.54c	2.59c	3.12b	3.67a	0.11
Creatinine, µM	134	143	5.57	140ab	137ab	148a	130b	4.64
Urine								
Urea: Creatinine[3]	11.5	10.6	0.48	10.6	10.2	9.59	13.9	0.51
	Parity			P–value				
	2	> 2	SEM	Protein	Stage	Parity	Protein x Stage	
Plasma								
Glucose, mM	4.36	4.30	0.18	0.77	<0.001	0.94	0.11	
Lactate, mM	3.56	2.63	0.34	0.37	<0.005	0.14	0.64	
TG, mM [2]	0.559^{a}	0.454^{b}	0.035	0.79	<0.001	0.2	0.15	
NEFA, mM [2]	330	293		0.95	<0.001	0.47	0.10	
	(264–414)	(234–367)						
Urea, mM	3.05	2.91	0.09	0.48	<0.001	0.2	0.07	
Creatinine, µM	137	141	6.11	0.28	<0.01	0.66	0.19	
Urine								
Urea: Creatinine[3]	11.7	10.5	0.53	0.21	< 0.001	0.10	< 0.05	

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

[1] Day 60 and 100 of gestation and day 5 and 20 of lactation.

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

[3] Ratio between concentration of urinary urea and creatinine.

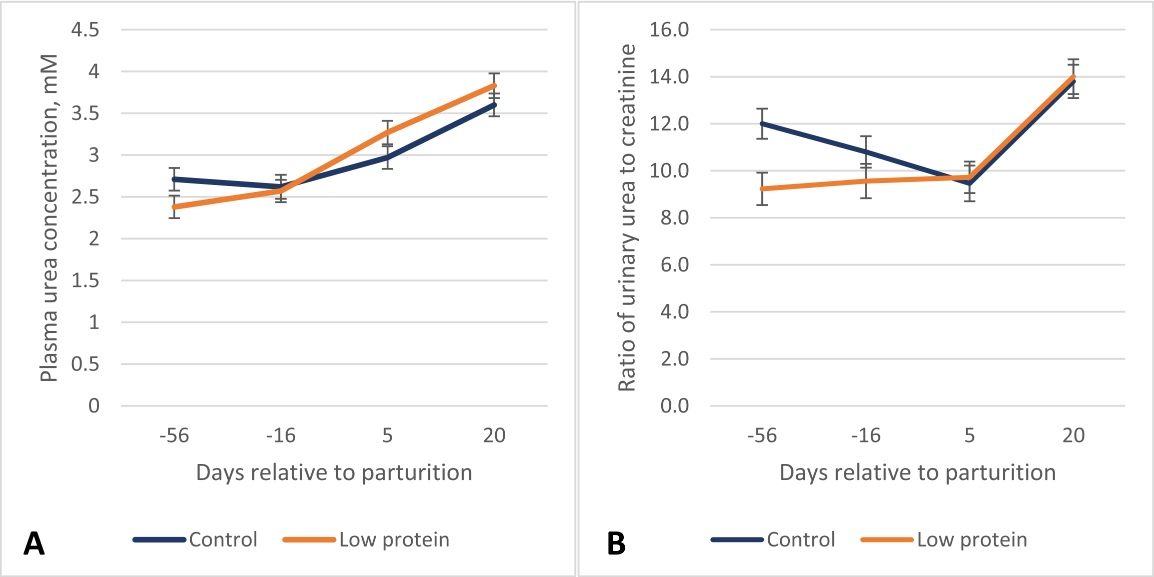

Fig. 5. Interaction between dietary protein level and reproductive stage on plasma urea concentration (Panel A; $P = 0.07$, and the ratio of urinary urea to creatinine concentrations (Panel B; $P < 0.05$). Error bars indicate SEM.

Table 6
Milk yield, energy output and composition at d5 and d20 in lactation of sows fed diets varying in dietary protein level.

	Protein level			Days in milk (DIM)			Parity			P–value			
	Control	Low	SEM	5	20	SEM	2	> 2	SEM	Protein	DIM	Parity	Protein x DIM
Milk yield, kg/d	12.4	13.8	0.80	9.96^{b}	16.3^{a}	0.56	13.3	12.9	0.86	0.25	< 0.001	0.78	< 0.05
Milk output, MJ/d	62.1	70.3	4.64	50.7^{b}	81.7^{a}	3.63	69.7	62.7	5	0.23	< 0.001	0.31	0.46
DM, %	18.5	18.4	0.37	18.4	18.5	0.36	18.9	18	0.4	0.85	0.7	0.11	0.09
Protein, %	5.28	5.22	0.16	5.45	5.06	0.15	5.33	5.18	0.17	0.80	0.06	0.51	0.87
Casein, %	4.24	4.18	0.15	4.32	4.10	0.13	4.27	4.16	0.16	0.76	0.19	0.63	0.98
Fat, %	7.31	7.8	0.36	7.42	7.68	0.35	8.11^{a}	6.99^{b}	0.4	0.35	0.59	< 0.05	0.22
Lactose, %	5.03	4.93	0.06	4.99	4.98	0.06	4.9	5.07	0.07	0.29	0.98	0.07	0.36

[a–b] Within a row, values without common subscriptions differ ($P < 0.05$).

complied well with the daily requirement throughout gestation, except for late gestation, where sows seemed to be undersupplied due to the increased requirement (Feyera and Theil, 2017). Thus, a high roughage intake and a greater intake of concentrate as compared with indoor sows were the key factors that allow the dietary protein concentration to be reduced. Control sows were oversupplied with lysine from d30–60 in spite of being feed below the recommended level, no matter whether roughage was considered or not.

Plasma and urinary urea are valuable indicators for AA oxidation and urea synthesis, and these metabolites increase if dietary protein is supplied in excess of their requirement (Hojgaard et al., 2019a; Pedersen et al., 2019a). Feeding the sow surplus protein is undesirable, both because dietary protein is a costly and limited resource and because urea synthesis is an energy–costly process, which reduces the feed efficiency and applies unnecessary metabolic stress to the sow (Pedersen et al., 2019b). In the current study, an interaction between dietary protein and reproductive stage tended to affect plasma urea concentration: At d60 of gestation, LP sows had lower plasma urea concentration and during lactation, they had higher plasma concentrations compared to control sows. This effect is correlated to the CP intake of sows and agrees with the literature, where similar effects have previously been reported for both gestating and lactating sows fed reduced dietary CP (Jang et al., 2014; Hojgaard et al., 2019a). Despite not being significant, this interaction is yet interesting to consider, as it supports the assumption that control sows were supplied with excessive dietary protein during gestation. In lactation, the carry–over effect causing an elevated plasma urea concentration in LP sows, are most likely due to the increased feed intake in lactation of these sows.

A similar and even more pronounced effect was observed on the urinary urea concentration. Due to the high correlation between urinary hydration and urinary urea concentration, the ratio of urea to creatinine was investigated in the current study. This reduces the impact of urinary hydration on urea concentration since creatinine is excreted into urine at a constant rate (Wyss and Kaddurah–Daouk, 2000). At d60 of gestation, an interaction showed that LP sows have a 23% lower urea to creatinine ratio than control sows, which however, decreased to 11% at d100 of gestation. The sow's protein requirement increases considerably with progress of gestation due to the growth of fetuses, placenta, mammary glands, and colostrum production, whereas the energy requirement only increases slightly (Feyera and Theil, 2017; Sola–Oriol and Gasa, 2017). Consequently, the feed supply should be increased to meet the sow's increasing nutritional demand because it is common to use a single gestation diet throughout gestation. Thus, in late gestation, the sows may be challenged earlier when fed low CP, which could explain why the percentual difference in urinary urea concentration between control and LP sows decreased from 23% to 11% in late gestation. Due to the changing protein requirement along the stage of gestation, literature suggests implementing phase–feeding of gestating sows (Samuel et al., 2012; Kraeling and Webel, 2015; Thomas et al., 2021). Replacing a single diet feeding strategy with phase–feeding or two–component feeding strategy would likely allow a further reduction of dietary protein in early and mid–gestation.

An unbalanced dietary AA composition relative to the requirement of the sow will increase the AA oxidation and thus increase urea production (Huber et al., 2015; Hojgaard et al., 2019b). In the current study, lysine was the first limiting AA in all diets; thus the ratio of the other essential AAs relative to lysine was higher compared to the nutritional recommendations (Tybirk et al., 2020). This is common in organic sows diets due to the limited range of feedstuff and feed additives available and the ban on using crystalline AAs (Regulation (EU), 2018). While the SID CP content was reduced by 18%, the SID lysine content was reduced by 31% in the LP diet, and consequently, the AA imbalance was most pronounced in this diet. This prevents fully benefitting from the reduced CP content, as the AAs in surplus relative to lysine are greater in the LP diet. Also, the relatively low SID lysine content in the LP diet is an obstacle to minimize dietary CP.

4.2. Carry–over effect of reduced dietary protein on lactating sows

Dietary protein level in gestation was found to affect the estimated milk production: As lactation proceeded, the MY of LP sows became increasingly higher compared to control sows. A high feed intake is a prerequisite for high milk production (Strathe et al., 2017b), and correspondingly, a tendency of increased daily compound feed and energy intake by LP sows compared to control sows during lactation was found. The increased milk production of LP sows is at least partially explained by low CP content of the diet during gestation, presumably because less protein is oxidized in LP sows, which improves the energy utilization and feed efficiency (Pedersen et al., 2019b). Thus, control sows might have had less energy available for mammary gland development during gestation than LP sows. Another possible explanation is that excess branch chain amino acids are oxidized in the mammary glands (and other peripheral tissues) and may affect the mammary gland function. Larger litters and greater piglet weight provide greater stimuli to the mammary gland, improving the development of the gland during lactation (Auldist et al., 1998; King, 2000). Despite only being a numerical difference, litters from LP sows were slightly heavier at birth than control sows' litters. A larger litter would have provided greater stimulus and improved mammary gland development, and thus contributed to the increased milk production of LP sows.

At birth, litter size and piglet weight did not differ significantly between dietary protein levels, indicating that feeding the LP diet did not compromise the reproductive performance of gestating sows. However, it should be emphasized that the experiment was not designed to evaluate differences on zootechnical traits as only 10 sows per treatment was studied. On average, sows had 17.2 liveborn piglets pr. litter, which is almost two piglets more than Danish organic sow herds (Rangstrup–Christensen et al., 2018; SEGES, 2019). Milk production is the primary factor affecting piglet growth (Auldist et al., 1998; Hansen et al., 2012). Concurrently with the increased MY, the gain of litters from LP sows was on average 17.5% higher than litters from control sows during the entire lactation period. From d5–20 the litter gain was 3.49 kg/d and 4.10 kg/d for control and LP sows, respectively. The litter gain of control sows corresponded well to the findings of Eskildsen et al. (2020a), who found an average litter gain of 3.45 kg/d from d5–20 for organic sows. However, the litter gain of LP sows was considerable higher. Due to the increased gain, litters from LP sows weighed 16.1% more than control sows' litters at weaning (276 kg vs. 238 kg). This is much higher than the average litter weight at weaning in Danish organic sow herds; 196 kg (SEGES, 2019). The high litter weight found in the current study is explained by the high individual piglet weight; 18.5 kg on average at weaning, which is considerably higher compared to a recent study of organic sows (14.2–14.9 kg) and Danish organic sow herds; 16.6 kg (SEGES, 2019). In addition, sows weaned a high number of piglets compared to other studies. However, litter size is also affected by managemental decisions, such as litter standardization or equalization.

Despite having a higher MY, body mobilization of LP sows was not increased compared to control sows, and sows lost on average 27.5 kg LW and mobilized 4.3 mm BF from d5 to weaning, and from d 5 to 20 they supported a substantial amount of the milk by mobilizing body fat (9.5 kg on average) and body protein (2.0 kg), and the high mobilization is supported by the high plasma NEFA. Moreover, plasma triglycerides were lowest at d 5 as they most likely were used for milk fat synthesis. Feed intake and MY are the two main factors affecting body mobilization (Strathe et al., 2017a). During lactation, LP sows had a slightly higher intake of compound feed compared to control sows, which likely explains why body mobilization was not affected in spite of the increased MY. However, a tendency of a higher body protein loss of LP sows during lactation as compared with control sows indicated that the higher feed intake did not entirely counterbalance the higher MY of LP sows. In comparison, Eskildsen et al. (2020a) found an average LW and body protein loss of 37 kg and 4.1 kg, respectively, from d5 to d40 of lactation, and Weissensteiner et al. (2018) registered an average LW loss of 25 kg from farrowing until d42, for organic sows. This corresponds well to the findings of the current study and supports that reducing the content of dietary protein during gestation did not compromise feed intake throughout the reproductive cycle and in fact increased the milk production in lactation after the dietary intervention ceased.

5. Conclusion

In conclusion, feeding organic gestating sows 31% as compared with 16% below the Danish recommendation for SID CP (72 *vs.* 88 g SID CP pr. kg DM, respectively) reduced the protein oxidation during gestation as evaluated from plasma urea while it had no impact on retained body protein, indicating that sows fed the low protein diet was not undersupplied with dietary protein and lysine. LP sows most likely excreted less urinary N as indicated by the 23% and 11% lower urinary urea concentration at d60 and d100 of gestation, respectively. As gestation progressed, the protein requirement increased, but no evidence of insufficient protein or lysine was observed with respect to sow productivity and in fact the subsequent milk production was greater, suggesting a beneficial carry–over effect of adequate protein supply during gestation on lactation performance of sows.

CRediT authorship contribution statement

J.C. Johannsen: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **M. Eskildsen:** Methodology, Investigation, Resources, Writing – review & editing, Supervision, Project administration. **A.G. Kongsted:** Writing – review & editing. **P.K. Theil:** Methodology, Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

All authors declare that they have no conflicts of interests.

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References

Auldist, D.E., Morrish, L., Eason, P., King, R.H., 1998. The influence of litter size on milk production of sows. Anim. Sci. 67, 333–337. https://doi.org/10.1017/s1357729800010109.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed–effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Bergeron, R., Bolduc, J., Ramonet, Y., Meunier–Salaün, M.C., Robert, S., 2000. Feeding motivation and stereotypies in pregnant sows fed increasing levels of fibre and/or food. Appl. Anim. Behav. Sci. 70, 27–40. https://doi.org/10.1016/S0168-1591(00)00142-8.

Bruun, T.S., Strathe, A.V., Krogsdahl, J., 2017. Effect of Feed Dose and Litter Size on Litter Growth and Weight Loss of Sows. SEGES Danish Pig Research Center.

L.J. Buckner, 1996. Physical and mathematical modelling of the energy requirements of the outdoor sow. PhD Thesis. The University of Aberdeen.

Close, W.H., Poornan, P.K., 1993. Outdoor Pigs-Their Nutrient Requirements, Appetite and Environmental Responses. Nottingham University Press, Loughborough.

Danielsen, V., Vestergaard, E.M., 2001. Dietary fibre for pregnant sows: effect on performance and behaviour. Anim. Feed Sci. Technol. 90, 71–80. https://doi.org/10.1016/S0377-8401(01)00197-3.

DMI, 2021. The Danish Meteorological Institute – Weather Archieve. DMI. https://www.dmi.dk/vejrarkiv/manedens–sasonens–og–arets–vejr/tabeller–maned/ (accessed 1 Maj 2021).

Edwards, S.A., 2003. Intake of nutrients from pasture by pigs. Proc. Nutr. Soc. 62, 257–265. https://doi.org/10.1079/pns2002199.

Eriksen, J., Petersen, S.O., Sommer, S.G., 2002. The fate of nitrogen in outdoor pig production. Agronomie 22, 863–867. https://doi.org/10.1051/agro:2002045.

Eskildsen, M., Krogh, U., Nørgaard, J.V., Hedemann, M.S., Sørensen, M.T., Kongsted, A.G., Theil, P.K., 2020a. Grass clover intake and effects of reduced dietary protein for organic sows during summer. Livest. Sci. 241, 104212 https://doi.org/10.1016/j.livsci.2020.104212.

Eskildsen, M., Krogh, U., Sørensen, M.T., Kongsted, A.G., Theil, P.K., 2020b. 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. 240, 104088 https://doi.org/10.1016/j.livsci.2020.104088.

Fernándéz, J.A., Danielsen, V., Søegaard, K., Poulsen, H.D., Jensen, S.K., 2006. Clover Grass – Grazed or Ensiled, Can Cover Half the Energy Requirement of Pregnant Sows. Aarhus University. The Faculty of Agricultural Sciences (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. https://doi.org/10.1016/j.livsci.2017.05.001.

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, 2285–2298. https://doi.org/10.2527/jas.2011-4788.

Hansen, B., 1989. Determination of nitrogen as elementary N, an alternative to Kjeldahl. Acta Agr. Scand. 39, 113–118. https://doi.org/10.1080/00015128909438504.

Hojgaard, C.K., Bruun, T.S., Theil, P.K., 2019a. Optimal crude protein in diets supplemented with crystalline amino acids fed to high–yielding lactating sows1. J. Anim. Sci. 97, 3399–3414. https://doi.org/10.1093/jas/skz200.

Hojgaard, C.K., Bruun, T.S., Theil, P.K., 2019b. Optimal lysine in diets for high–yielding lactating sows. J. Anim. Sci. 97, 4268–4281. https://doi.org/10.1093/jas/skz286.

Huber, L., de Lange, C.F.M., Krogh, U., Chamberlin, D., Trottier, N.L., 2015. Impact of feeding reduced crude protein diets to lactating sows on nitrogen utilization. J. Anim. Sci. 93, 5254–5264. https://doi.org/10.2527/jas.2015-9382.

Jang, Y.D., Jang, S.K., Kim, D.H., Oh, H.K., Kim, Y.Y., 2014. Effects of dietary protein levels for gestating gilts on reproductive performance, blood metabolites and milk composition. Asian Aust. J. Anim. Sci. 27, 83–92. https://doi.org/10.5713/ajas.2013.13369.

Jha, R., Fouhse, J.M., Tiwari, U.P., Li, L., Willing, B.P., 2019. Dietary fiber and intestinal health of monogastric animals. Front. Vet. Sci. 6 https://doi.org/10.3389/fvets.2019.00048.

King, R.H., 2000. Factors that influence milk production in well–fed sows. J. Anim. Sci. 78, 19–25. https://doi.org/10.2527/2000.78suppl_319x.

Kraeling, R.R., Webel, S.K., 2015. Current strategies for reproductive management of gilts and sows in North America. J. Anim. Sci. Biotechnol. 6, 3. https://doi.org/10.1186/2049-1891-6-3.

Kusina, J., Pettigrew, J., Sower, A.F., White, M., Crooker, B.A., Hathaway, M., 1999. Effect of protein intake during gestation and lactation on the lactational performance of primiparous sows. J. Anim. Sci. 77, 931–941. https://doi.org/10.2527/1999.774931x.

Lenth, R.V., 2021. emmeans: estimated marginal means, aka least–squares means. R Package Version 1.5.4.

Lindberg, J.E., 2014. Fiber effects in nutrition and gut health in pigs. J. Anim. Sci. Biotechnol. 5, 15. https://doi.org/10.1186/2049-1891-5-15.

Patience, J.F., 2012. Feed Efficiency in Swine. Wageningen Academic Press, Wageningen, The Netherlands.

Pedersen, T.F., Bruun, T.S., Trottier, N.L., Theil, P.K., 2019a. Nitrogen utilization of lactating sows fed increasing dietary protein1. J. Anim. Sci. 97, 3472–3486. https://doi.org/10.1093/jas/skz213.

Pedersen, T.F., Chang, C.Y., Trottier, N.L., Bruun, T.S., Theil, P.K., 2019b. Effect of dietary protein intake on energy utilization and feed efficiency of lactating sows. J. Anim. Sci. 97, 779–793. https://doi.org/10.1093/jas/sky462.

R Core Team, 2020. In: Computing, R.F.f.S. (Ed.), R: A language and environment for statistical computing. Vienna, Austria.

Rangstrup–Christensen, L., Krogh, M.A., Pedersen, L.J., Sørensen, J.T., 2018. Sow level risk factors for early piglet mortality and crushing in organic outdoor production. Animal 12, 810–818. https://doi.org/10.1017/S1751731117002178.

Regulation (EU), 2018. Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007.

Rehfeldt, C., Lang, I.S., Gors, S., Hennig, U., Kalbe, C., Stabenow, B., Brussow, K.P., Pfuhl, R., Bellmann, O., Nurnberg, G., Otten, W., Metges, C.C., 2011. Limited and excess dietary protein during gestation affects growth and compositional traits in gilts and impairs offspring fetal growth. J. Anim. Sci. 89, 329–341. https://doi.org/10.2527/jas.2010-2970.

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, 355–366. https://doi.org/10.2527/1994.722355x.

Samuel, R.S., Moehn, S., Pencharz, P.B., Ball, R.O., 2012. Dietary lysine requirement of sows increases in late gestation1. J. Anim. Sci. 90, 4896–4904. https://doi.org/10.2527/jas.2011-4583.

SEGES, 2019. Nøgetal for økologisk sohold. https://www.landbrugsinfo.dk/–/media/landbrugsinfo/basic/5/9/0/oe–noegletal–gns–oekologoisk_sohold–2018.pdf (accessed 14 December 2019).

Sola–Oriol, D., Gasa, J., 2017. Feeding strategies in pig production: sows and their piglets. Anim. Feed Sci. Technol. 233, 34–52. https://doi.org/10.1016/j.anifeedsci.2016.07.018.

Strathe, A.V., Bruun, T.S., Geertsen, N., Zerrahn, J.E., Hansen, C.F., 2017a. Increased dietary protein levels during lactation improved sow and litter performance. Anim. Feed Sci. Techno. 232, 169–181. https://doi.org/10.1016/j.anifeedsci.2017.08.015.

Strathe, A.V., Bruun, T.S., Hansen, C.F., 2017b. Sows with high milk production had both a high feed intake and high body mobilization. Animal 11, 1913–1921. https://doi.org/10.1017/S1751731117000155.

Sørensen, G., 2019. Feeding of gestating sows. Feed Seminar. SEGES Danish Pig Research Center, Kolding, Denmark.

Theil, P.K., Chwalibog, A., Jørgensen, H., 2020. Energy for pigs: metabolism, requirement, utilisation and prediction of dietary content. Textbook in Physiology. SEGES.

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 dtermination of deutrated water turnover on three piglets per litter. Acta Agric. Scand. A Anim. Sci. 52, 221–232. https://doi.org/10.1080/090647002762381104.

Thomas, L.L., Herd, L.K., Goodband, R.D., Tokach, M.D., Woodworth, J.C., DeRouchey, J.M., Dritz, S.S., Goncalves, M.A.D., Jones, D.B., 2021. Effects of increasing standardized ileal digestible lysine during gestation on reproductive performance of gilts and sows. Animal 15, 100221. https://doi.org/10.1016/j.animal.2021.100221.

Tybirk, P., Sloth, N.M., Kjeldsen, N., Shooter, L., 2020. Danish Nutrient Standards. SEGES Danish Pig Research Centre.

Weissensteiner, R., Baldinger, L., Hagmüller, 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. https://doi.org/10.1016/j.livsci.2018.06.006.

Wyss, M., Kaddurah–Daouk, R., 2000. Creatine and creatinine metabolism. Physiol. Rev. 80, 1107–1213. https://doi.org/10.1152/physrev.2000.80.3.1107.

Commission Regulation (EC), 2009. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union L 54/1; P 2-54.