Nitrogen utilization of lactating sows fed increasing dietary protein[1]

Trine F. Pedersen,* Thomas S. Bruun,† Nathalie L. Trottier,‡ and Peter K. Theil*[2]

*Department of animal Science, Faculty of Science and Technology, Aarhus University, DK-8830 Tjele, Denmark; †SEGES Danish Pig Research Centre, DK-1609 Copenhagen, Denmark; and ‡Department of Animal Science, Michigan State University, East Lansing 48824, MI

ABSTRACT: The objectives of the study were 1) to quantify dietary N utilized for milk N and N loss in urine and feces, in sows fed increasing dietary CP with a constant amount of Lys, Met, Thr, and Trp to meet their standardized ileal digestible (SID) requirement and 2) to determine the optimal dietary CP concentration based on dietary N utilization for milk production. Seventy-two sows were fed 1 of 6 dietary treatments, formulated to increase the SID CP as followed: 11.8, 12.8, 13.4, 14.0, 14.7, and 15.6% and formulated to be isocaloric (9.8 MJ NE/kg). Diets were fed from day 2 after parturition until weaning at day 28 (± 3 d). Litters were equalized to 14 piglets and weighed within 48 h following parturition. Sows were weighed and back fat scanned, at day 18 (± 3 d) and day 28 (weaning; ± 3 d). Litter weight was recorded at day 11, 18 (± 3 d), and 28 (± 3 d). Nitrogen balances were conducted on approximately day 4, 11, and 18 (± 3 d). Daily milk yield was estimated from recorded litter gain and litter size. To calculate sows mobilization of fat and protein, body pools of fat and protein were estimated by D_2O (deuterated water) enrichment on day 4 and 18 (± 3 d). No linear, quadratic, or cubic effects of increasing dietary CP was observed for sows total feed intake, sow BW, body pools of protein and fat, protein and fat mobilization, total milk yield, and piglet performance. The protein content in milk increased linearly with increasing dietary CP in week 1 ($P < 0.05$), week 2 ($P < 0.05$), and week 3 ($P < 0.001$). Urine production did not differ among treatments and N output in urine increased linearly with increasing dietary CP concentration in week 1 ($P = 0.05$), week 2 ($P < 0.001$), and week 3 ($P < 0.001$). Urine N excretion relative to N intake increased linearly with increasing dietary CP ($P < 0.001$). Milk N utilization relative to N intake decreased linearly from 77.8% to 63.1% from treatment 1 through 6 ($P < 0.001$). Corrected milk N utilization decreased from 68.6% to 64.2% from treatment 1 through 6 ($P < 0.05$). In conclusion, a low dietary CP concentration for lactating sows with supplemented crystalline AA improved the efficiency of dietary N utilization and reduced the N output in urine without affecting lactation performance.

Key words: body mobilization, feed efficiency, lactation, nitrogen metabolism, sow nutrition

J. Anim. Sci. 2019.97:3472–3486
doi: 10.1093/jas/skz213

INTRODUCTION

The productivity of lactating sows has improved during the past decades and modern hyper-prolific sows have a high milk yield with nursing up to 14 piglets per lactation cycle (Pedersen et al., 2016; Helverskov, 2017). The demand for nutrients has increased along with the improved productivity. A study by Strathe et al. (2017) with hyper-prolific sows found an improved ADG of the litter and lower BW loss of the sows when dietary standardized ileal digestible **(SID)** CP increased from 10.4% to 13.5% and from 10.4% to 14.3%, respectively. The study by Strathe et al. (2017) did, however, not

[1]This research project was funded by the Danish Pig Levy Foundation (project title RASP).

[2]Corresponding author: peter.theil@anis.au.dk

Received December 23, 2018.
Accepted June 24, 2019.

conclude whether dietary CP or Lys was the limiting factor for litter gain.

Excessive dietary protein reduced utilization of dietary energy (Pedersen et al., 2019) and it is important to feed close to requirement, both from an economical and environmental perspective (Just, 1982; Lenis and Jongbloed, 1999). Addition of crystalline L-Lys, DL-Met, L-Thr, and L-Trp is commonly applied in pig diets to improve the AA profile to allow the dietary CP content to be lowered (Kerr and Easter, 1995). By adding all 5 crystalline AA that are available on a feed-grade basis (Lys, Met, Thr, Trp, and Val) and Ile, it was possible to achieve a high level of CP utilization in low CP diets, including reduction in N excreted in urine and increased N secreted in milk (Huber et al., 2015). This knowledge cannot directly be applied in Danish farms, because crystalline Val and Ile are not used in practice and because Danish sows with large litters have a high milk yield.

The hypothesis was that sows milk yield will be maximized and excretion of N in urine will be reduced when fed optimally with dietary CP. The objectives of the current study were 1) to quantify N utilization for milk N and N loss in urine and feces, in sows nursing 14 piglets and fed decreasing CP with supplemental L-Lys, DL-Met, L-Thr, and L-Trp fed at the recommended level and 2) to determine the optimal dietary CP based on N utilization for milk production.

MATERIALS AND METHODS

All animals used in the current experiment were housed and reared in agreement with Danish laws and regulations for the humane care and use of animals in research (Law number 382, Act number 726 and 1081 of the Danish Ministry of Justice). Animal procedures were reviewed and approved by The Danish Animal Experimentation Inspectorate. Data from the same study regarding energy utilization were published in Pedersen et al. (2019).

Experimental Design

Seventy-two crossbred (Danish Landrace × Danish Yorkshire; DanBred, Herlev, Denmark) first to fifth parity sows and their litter were included in the experiment from parturition until weaning 28 d later. The experiment was conducted in the period from February 2016 to April 2016 in a commercial sow herd in Denmark. In 4 consecutive weeks (replicates), 6 sows in 3 blocks (18 sows per week) were chosen according to parity from a batch of 85 sows with the same expected farrowing date and randomly assigned to 1 of 6 dietary treatments. There were 2 blocks with first parity sows and 10 blocks with multiparous sows (mean parity of 3.6). Sows were moved to the farrowing unit 1 week before expected farrowing date. Litters were equalized to 14 piglets weighing above 900 g, during the first 48 h postpartum (Danish Duroc × Danish Landrace/Danish Yorkshire offspring). Excess piglets were moved to sows not included in the experiment.

Sows were housed in 4 different rooms, but within a replicate all 18 sows were housed in the same room. Sows and their litter were housed individually in farrowing pens (2.7 × 1.8 m) with a covered area for the piglets. The covered area for piglets was installed with a heating lamp (VengSystem A/S, Roslev, Denmark) and a rubber mattress. After parturition, the temperature in the covered piglet area was kept at 34 °C and decreased hereafter automatically to 22 °C 15 d after parturition. Before parturition, sawdust was provided as bedding material in the covered piglet area. Piglets had free access to water supplied by drinking nipple, and from 14 d after parturition until weaning piglets had free access to a low CP prestarter diet (9.3% CP, 0.24% SID Lys, 4.2% fat; Vestjyllands Andel, Ringkøbing, Denmark). The prestarter diet was intendedly low in CP and Lys as the diet should not contribute considerable to growth of piglets, instead, the purpose of the starter diet was to get piglets acquainted to solid feed to ease the transition from milk to solid feed after weaning. The room temperature in the farrowing unit was kept around 20 °C and the light was on from 0700 h to 1600 h.

Dietary Formulation and Feeding

Six experimental treatments were composed based on 4 feed components (Table 1): barley, fiber mix, supplementary feed mix 1 (FM1), and supplementary feed mix 2 (FM2). The FM1 and FM2 were purchased from DLG (Tjele, Denmark). The fiber mix was purchased from Vestjyllands Andel (Ringkøbing, Denmark). The FM1 was formulated to be low in CP and FM2 was formulated to be high in CP, obtained by replacing a part of the cereal fraction with soybean meal in FM2. To ensure a constant level of the 4 most limiting AA, crystalline L-Lys, DL-Met, L-Thr, and L-Trp was added to FM1, whereas the inclusion level of soybean meal in treatment 6 was chosen at the level where no addition of crystalline L-Lys, L-Thr, and L-Trp was needed. Thus, only crystalline DL-Met was added to FM2 to meet the requirement for

both Met and Cys in the low protein treatments (Ball et al., 2006). Crystalline L-Val was not added to the dietary treatments partly because crystalline Val is costly for the farmer and partly because no beneficial effect was observed on sow and piglet performance when increasing dietary Val to Lys ratio in the study by Strathe et al. (2016). The supplements were formulated to contain the same amount of supplemented fat, whereas the content of wheat and wheat bran was allowed to vary to obtain constant NE (MJ/kg).

Dietary treatments were mixed each day prior to each feeding, where barley and the fiber mix was included at a constant rate of 40% and 6%, respectively, in each treatment. The inclusion of FM1 decreased gradually (from 54% to 0%) at the expense of FM2 (0% to 54%) to make a CP gradient in treatment 1 through 6 (Table 2). Treatment 1 and 6 were formulated to supply dietary CP below and above the requirement, respectively (Tybirk et al., 2015), and the dose–response design was chosen to find the optimal CP level for lactating sows. Furthermore, the dietary treatments were formulated to be constant in energy (NE and Danish Feed units for sows) and SID levels of Lys, Thr, Met, Met + Cys, and Trp. As a consequence, treatment 1 was formulated to supply insufficient levels of AA's relative to Lys for the following AA according to the Danish recommendations: Val (58 vs. 76%), His (32 vs. 39%), Ile (49 vs. 56%), Leu (91 vs. 115%), and Phe+Tyr (98 vs. 113%). For treatment 6, all AA were fed in excess. The dietary treatments were formulated to meet the Danish recommendations for lactating sows nursing 14 piglets for 28 d while having an average feed intake of 6.5 kg/d for all other nutrients (Tybirk et al., 2015).

Sows were fed automatically by a SpotMix air-assisted feeding system (Schauer Agrotronic, Prambachkirchen, Germany). From day 1 to 9, sows were fed twice daily, the first meal between 0730 h and 0830 h and the second meal between 1400 h to 1500 h. After day 10 and until weaning, sows were fed 3 meals daily, the first meal between 0600 h and 0900 h, the second meal between 1230 h and 1500 h, and the third meal between 1900 h and 2000 h. First parity and multiparous sows followed 2 different feeding curves where first parity sows were fed 2.38 kg feed from day 1 in lactation and increased to 4.78 kg on day 7 in lactation. The feed supply increased from day 8 to 17 and reached 7.60 kg/d 17 d after parturition, where after it was kept constant until weaning. The multiparous sows were fed 2.38 kg feed from day 1 of lactation and increased to 5.26 kg on day 7 of lactation. The feed supply increased from day 8 to 17 and reached 8.56 kg/d 17 d after parturition,

Table 1. Ingredients and chemical composition of the supplements, fiber mix, and barley (as-fed basis)

Ingredient, %	Supplement 1	Supplement 2	Barley	Fiber mix
Barley	26.0	-	100	-
Wheat	33.8	38.5	-	-
Wheat bran	3.00	-	-	12.0
Soybean meal	23.0	50.0	-	-
Soy hulls	-	-	-	12.0
Sugar beet pulp	-	-	-	72.0
Sugar beet molasses	-	-	-	2.00
Soy oil	5.10	5.00	-	-
Leci E[1]	-	-	-	2.00
Premix[2]	4.50	4.50	-	-
Calcium carbonate	0.90	0.84	-	-
Sodium chloride	0.95	0.95	-	-
Monocalcium phosphate	0.33	0.10	-	-
L-Lys	1.73	-	-	-
DL-Met	0.25	0.07	-	-
L-Thr	0.33	-	-	-
L-Trp	0.12	-	-	-
Phytase[3]	0.04	0.04	-	-

[1]Phospolipids, FFA and triglycerides from rape seed oil.

[2]Provided vitamins and minerals per kg of diet: Retinol, 9418 IU; cholecalciferol, 1998 IU; α-tocopherol, 176 mg; thiamin, 2.35 mg; cyanocobalamin, 0.02 mg; riboflavin, 5.89 mg; pyridoxine, 3.53 mg; biotin, 0.43 mg; D-pantothenic acid, 17.65 mg; folic acid, 1.77 mg; niacin, 23.54 mg; 13.0 mg Cu as $CuSO_4$; 85.94 mg Fe as FeSO4; 0.23 mg I as $Ca(IO_3)_2$; 47.08 mg Mn as MnO; 0.37 mg Se as Na2SeO3.

[3]Provided 500 phytase activity (FTU) per kg of diet.

Table 2. Planned and analyzed chemical composition (as-fed) of experimental treatments

	Treatment					
Item[1]	1	2	3	4	5	6
Planned composition						
NE, MJ/kg[2]	9.8	9.8	9.8	9.8	9.8	9.8
FU_{sow}, kg[3]	1.07	1.07	1.07	1.07	1.07	1.07
CP	13.5	14.6	15.4	16.1	17.0	18.1
SID CP[4]	10.9	12.0	12.7	13.4	14.1	15.2
SID His	0.26	0.29	0.32	0.34	0.36	0.39
SID Ile	0.40	0.46	0.50	0.53	0.57	0.63
SID Leu	0.75	0.84	0.91	0.97	1.04	1.13
SID Lys	0.82	0.82	0.82	0.82	0.82	0.83
SID Met	0.30	0.29	0.29	0.28	0.28	0.27
SID Met + Cys	0.49	0.50	0.50	0.51	0.51	0.52
SID Phe	0.49	0.55	0.59	0.63	0.68	0.74
SID Thr	0.54	0.54	0.54	0.54	0.55	0.55
SID Trp	0.17	0.18	0.18	0.19	0.20	0.21
SID Tyr	0.32	0.37	0.40	0.43	0.46	0.51
SID Val	0.48	0.54	0.58	0.61	0.65	0.71
Analyzed composition						
DM, %	85.6	85.7	85.7	85.7	85.8	85.8
ME, MJ/kg[5]	13.1	13.1	13.0	12.8	12.8	12.8
FU_{sow}, kg[3]	1.04	1.04	1.04	1.04	1.04	1.04
CP	14.6	15.6	16.3	16.9	17.6	18.6
SID CP	11.8	12.8	13.4	14.0	14.7	15.6
Fat	4.7	4.7	4.7	4.7	4.7	4.6
Ash	5.4	5.3	5.3	5.2	5.2	5.1
Starch	40.5	39.2	38.2	37.4	36.5	35.1
Dietary fiber	17.4	17.4	17.3	17.3	17.3	17.3
Lignin	2.7	2.7	2.7	2.7	2.6	2.6
Supplemented AA[6]						
Lys	0.95 (0.83)	0.97 (0.83)	0.97 (0.84)	0.98 (0.84)	0.99 (0.84)	1.01 (0.85)
Met	0.30 (0.27)	0.29 (0.27)	0.29 (0.26)	0.29 (0.26)	0.28 (0.25)	0.28 (0.25)
Met + Cys	0.53 (0.49)	0.54 (0.50)	0.55 (0.50)	0.56 (0.51)	0.56 (0.51)	0.57 (0.52)
Thr	0.65 (0.54)	0.66 (0.55)	0.66 (0.55)	0.67 (0.55)	0.68 (0.56)	0.68 (0.56)
Trp	0.19 (0.16)	0.21 (0.18)	0.22 (0.18)	0.23 (0.19)	0.23 (0.20)	0.24 (0.21)
Variable AA						
His	0.32 (0.27)	0.36 (0.30)	0.38 (0.32)	0.39 (0.34)	0.42 (0.36)	0.45 (0.38)
Ile	0.52 (0.44)	0.58 (0.48)	0.61 (0.51)	0.64 (0.54)	0.68 (0.57)	0.73 (0.62)
Leu	0.98 (0.81)	1.07 (0.88)	1.13 (0.94)	1.18 (0.99)	1.24 (1.04)	1.33 (1.12)
Phe	0.67 (0.55)	0.72 (0.60)	0.76 (0.64)	0.79 (0.67)	0.83 (0.70)	0.88 (0.75)
Tyr	0.46 (0.37)	0.50 (0.41)	0.53 (0.43)	0.55 (0.46)	0.58 (0.48)	0.62 (0.52)
Val	0.66 (0.52)	0.71 (0.56)	0.74 (0.60)	0.77 (0.63)	0.80 (0.66)	0.85 (0.70)

[1]Items are presented as % on as fed basis unless otherwise is noted.

[2]Calculated according to EvaPig.

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

[4]SID: standardized ileal digestible (Pedersen and Boisen, 2002).

[5]Adapted from Pedersen et al. (2019).

[6]Values in brackets are calculated standardized ileal digestible (Pedersen and Boisen, 2002).

where after it remained constant until weaning. At feeding, water was added to the diet in the troughs. Sows had free access to water throughout the experiment and no straw was provided to the sows or piglets.

Recordings, Sampling, and Daily Management

Feed supply was recorded daily and adjustments to the planned feeding curve were made individually for each sow if feed leftovers were observed.

Sows with feed leftovers had their feed supply reduced by 50% the next day and then the planned feed supply the following day. Sows BW and back fat thickness (BF) were measured at litter equalization (within 2 d after farrowing), at day 18 ± 3 and day 28 ± 3. Back fat thickness was measured at P2 (on the right side 63 mm from the backbone of the last rib) using a SonoGrader II (RENCO, MN, USA). Samples of milk, urine, and feces were collected at 3 selected days, once during the first (day 4 ± 3), second (day 11 ± 3), and third week of lactation (day 18 ± 3), respectively. On collection day, feed leftovers were recorded to determine the total nutrient intake, whereas feed leftovers were not collected the remaining 6 d each week. Feed leftovers were drained from water and weighed. The conversion of drained feed leftovers to dry feed (as-fed) was made based on a conversion factor of 0.425, which was obtained from the regression curve made by soaking 100, 300, 600, and 1200 g of feed in water followed by draining in a sieve and weighing. The composition of the feed leftover was assumed to be equal to dry feed (as-fed). Sows were fitted with a urinary balloon catheter (Teleflex medical, Kamunting, Malaysia) for collection of urine on collection day. The urine collection started after the first or second feeding and continued for 6 h after insertion. Urine was collected quantitatively every second hour in a bucket by emptying the bladder to measure the total amount of urine produced daily. In between collections, the urinary catheter was blocked by a stopper. A subsample of urine was collected and stored at −20 °C until analysis. For calculation of protein and fat body pools, sows were administered i.m. in the neck (18G, 40 mm needle, 10 mL syringe) deuterium oxide (Sigma-Aldrich, MO, USA) on day 4 and 18. The deuterium solution was a mix of 40% deuterium oxide and 60% saline (9 mg NaCl/mL; B. Braun Melsungen AG, Melsungen, Germany) administered 0.0425 g per kg BW. A urine sample was drawn prior to enrichment to measure deuterium (D_2O) background level and another urine sample was collected 6 to 7 h post-D_2O administration to determine the D_2O after equilibration, and these were used to assess the total D_2O space as described by Theil et al. (2002). On each day 4, 11, and 18, a total of 50 to 60 mL milk was collected after separating the piglets from the sows and administering 2 mL oxytocin (Intervet Danmark A/S, Ballerup, Denmark) i.m. to induce milk let down. The milk was filtered for debris before subsampling and stored at −20 °C until analysis. For estimation of sow's milk yield, litter size and daily litter gain was recorded by weighing the litter at equalization within 48 h after parturition, at day 11, 18, and at weaning. On collection day, a fresh grab fecal sample was collected by rectal stimulation and stored at −20 °C until analysis.

According to herd procedures, piglets were given an iron injection (0.5 mL; Solofer Vet., Pharmacosmos A/S, Holbæk, Denmark) 3 to 4 d after birth. Throughout lactation piglets were supplied iron (1%; Opti-Jern, R2 Agro-Nutriscan, Hedensted, Denmark) in their drinking water. Piglets were tail docked, and males were castrated 3 to 4 d after birth. The health of sows and piglets was monitored daily by the farm staff and in case of illness; treatment was carried out according to standard operating procedures.

Chemical Analyses

Samples of FM1, FM2, barley, and fiber mix were collected upon delivery from supplier for chemical analysis. The components were analyzed for DM, ash, N, crude fat, total dietary fiber, starch, lignin, and AA. Prior to the chemical analysis, the dietary components were ground, using a 0.5-mm ultra-centrifugal mill (Model ZM200; RETSCH, Haan, Germany). Dry matter was determined after drying for 20 h at 103 °C in a forced air oven. After DM determination, the ash content was determined by combustion at 525 °C for 6 h. The N content was analyzed according to the Dumas method (Hansen, 1989) using a Vario Max CN Element analyzer (Elementar Analysensystem GmbH, Langenselbold, Germany), where aspartic acid was used as a calibration standard. Crude protein content was calculated as N × 6.25. The concentration of AA and crude fat in FM1, FM2, barley, and fiber mix was analyzed by Eurofins Steins Laboratorium A/S (Vejen, Denmark) according to the Official Journal of the European Union (EU; 152/2009). The content of starch was analyzed by enzymatic colorimetry as described by Bach Knudsen (1997). The total dietary fiber content was analyzed as total dietary fiber including lignin by enzymatic, chemical, and gravimetric determination of soluble and insoluble fibers according to Bach Knudsen (1997) with the modification that the polysaccharides were hydrolyzed with 2 M H_2SO_4 for 2 h. Fecal samples were freeze dried and subsequently ground to 0.5 mm before analyzed for DM, N, and lignin. Dry matter was determined after drying for 20 h at 103 °C in a forced air oven. Fecal N content was analyzed according to the Dumas method. Fecal lignin was analyzed gravimetric as Klason lignin according to Bach Knudsen (1997). Urine samples

were ultra-filtrated followed by analyzing for D_2O according to the method described for plasma by Theil et al. (2002). Urine samples were analyzed for N using a modified Kjeldahl method (KjelTec 2400, Hillerød, Denmark) according to the official method of AOAC (2012). Furthermore, urine samples were analyzed for urea and creatinine according to standard procedures (Siemens Diagnostics Clinical Methods for ADVIA 1650) using an auto analyzer (ADVIA 1650 Chemistry System, Siemens Medical Solution, Tarrytown, NY). Milk samples were analyzed for N according to the Dumas method (Hansen, 1989). Moreover, true milk protein and casein with infrared spectroscopy using a Milkoscan 4000 calibrated for cow milk (Foss MilkoScan, Hillerød, Denmark).

Calculations and Statistical Analysis

The SID content of CP and AA in the dietary treatments was calculated based on analyzed total content and estimated SID values (AgroSoft WinOpti.Net, AgroSoft A/S, Tjørring, Denmark; Pedersen and Boisen (2002). The allotment of FM1, FM2, barley, and fiber mix was recorded for each meal, which allowed the daily feed and nutrient supplies to be calculated. Protein and fat body pools at day 4 and 18 were estimated according to equations developed by Rozeboom et al. (1994) for Landrace × Yorkshire gilts, using with BW (kg), D_2O space (kg), and BF measurements (mm) as follows:

$$\begin{aligned}\text{Body protein [kg]} = {} & 1.3 + 0.103 \times \text{BW [kg]} \\ & + 0.092 \times D_2O \text{ space [kg]} \\ & 0.108 \times \text{BF [mm]}\end{aligned} \quad (1)$$

$$\begin{aligned}\text{Body fat [kg]} = {} & 7.7 + 0.649 \times \text{BW [kg]} \\ & - 0.610 \times D_2O \text{ space [kg]} \\ & + 0.299 \times \text{BF [mm]}\end{aligned} \quad (2)$$

Sow BW and BF at litter equalization and at day 18 were used as inputs. The D_2O space was estimated from urine samples collected at baseline (0 h) and 6 h after the D_2O enrichment at day 4 and day 18. The protein and fat mobilization from day 4 to day 18 of lactation was calculated as protein and fat loss as % of the initial protein and fat pools, respectively. Once per week (on day 4, 11, and 18 ± 3), N intake, N output, N balance (retention/mobilization), and N utilization was calculated. The N output in feces was calculated by determination of the N concentration in feces and fecal output. Fecal output was calculated as described by Pedersen et al. (2019) from DM intake and the DM digestibility adapted from equations of Stein et al. (2007) for apparent total tract digestibility (**ATTD**), using lignin as the internal marker. The daily N output in urine was calculated as 4 times the measured urine production per 6 h multiplied by the N concentration in urine. Sows milk production was estimated as total milk production for each week separately during lactation using inputs of litter weight gain (in kg/d) and litter size (Hansen et al. 2012) and the actual milk production at sampling days were used for estimating the milk N output and N balances. The input of litter weight gain as a determinant for estimating milk production may slightly overestimate the milk production, as piglets had access to creep feed. However, the creep feed had low CP and Lys contents and therefore likely did not contribute much to the estimated milk production. The N output in milk was calculated as the product of N concentration in milk at day 4, 11, and 18 (from Dumas analysis) and milk production at day 4, 11, and 18 (according to Hansen et al., 2012). Nitrogen balance (retention or mobilization) was calculated as the actual N intake minus N output in milk, urine, and feces. Milk N corrected for mobilization (retention) was calculated as milk N utilization divided by total N output relative to feed intake.

Data obtained in the current experiment were regarded as a dose–response design. Statistical analyses of total feed intake, BW, body protein and fat pools, protein and fat mobilization, total milk yield, litter weight, composition of milk, urine, and feces, and N metabolism (N output in milk, urine, and feces) were performed by applying the following model to the MIXED procedure in SAS (version 9.3; SAS Inst. Inc., Cary, NC):

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \sigma_k + \varepsilon_{ijk},$$

Y_{ijk} is the observed trait, μ is the overall mean of the observations, α_i is the main effect of the dietary treatment (i = 1, 2, 3, 4, 5, 6), β_j is the main effect of parity (j = first parity or multiparous), σ_k is the random effect of block (k = 1, 2, 3, 4), and ε_{ijk} is the residual random component. The model also contained a covariate (DIM), to account for the actual d of sampling within each week of lactation. Linear, quadratic, and cubic contrasts were constructed to determine the effect of increasing dietary CP content on the response parameter. Nitrogen utilization measurements were also analyzed as repeated measurements to test the main effects of treatment and week. The model for repeated measurements

included the effect of week (l = 1, 2, and 3), that is, γ_k (k =1, 2, and 3) was added to the model described above. To account for multiple mean comparison, the P-values were adjusted using a Tukey test. Piglet mortality from day 2 to 28 was analyzed as odds ratio using the GENMOD procedure in SAS (version 9.3; SAS Inst. Inc., Cary, NC). The estimates were considered significant when $P < 0.05$, and tendencies were accepted as $P \leq 0.10$. Mean values are presented as least squares means together with the highest value of the SEM. No interactions between treatment and parity or treatment and week were observed for any variables and are therefore omitted in the final model.

RESULTS

The calculated values of dietary SID CP, which were based on analyzed CP content and calculated SID CP increased from 11.8% for treatment 1 to 15.6% for treatment 6 (Table 2). The analyzed CP values were greater than the planned levels of 10.9% to 15.2% for treatment 1 through 6. The concentrations of SID Lys, Met, Met + Cys, and Thr were fairly constant, while a small increase in SID Trp was observed from treatment 1 through 6, except for Met which decreased slightly from 107% to 97% of the recommended level. The SID concentration of crystalline AA slightly exceeded the recommended level, 4% to 6% for Lys and 4% to 8% for Thr for diet 1 and 6, respectively, whereas Trp was supplied 2% to 30% above the Danish recommendations. This increase in SID Trp can be ascribed to the increased amount of soybean meal in FM2, whereby Trp slightly exceeded the recommended level. As intended, the following AA relative to Lys were below the Danish recommendations for treatment 1: Val (64 vs. 76%), His (34 vs. 39%), Ile (54 vs. 56%), and Leu (100 vs. 115%), whereas Phe+Tyr was at the recommended level (114 vs. 113%). For treatment 6, all AA were fed in excess.

Two sows were excluded from the experiment during lactation, one due to rectal prolapse (treatment 1) and the other one because of low feed intake (treatment 4). No linear, quadratic, or cubic effects of total feed intake, sow BW, BW loss, body protein and fat pools, protein and fat mobilization, litter weight at day 2 and at weaning, and piglet mortality were observed throughout lactation (Table 3). Estimated milk production decreased linearly ($P < 0.01$; Table 4) with increasing dietary CP in week 1, and did not differ among treatments in week 2 or 3. True protein concentration in milk increased linearly with increasing dietary CP content in week 1 ($P < 0.05$), week 2 ($P < 0.05$), and week 3 of lactation. The N concentration in milk did not differ in week 1 and 2, and increased linearly with increasing dietary CP content in week 3 ($P < 0.001$) of lactation. Milk casein content did not differ among dietary treatments in week 1. In week 2 and 3 of lactation, milk casein content increased linearly with increasing dietary CP content ($P < 0.001$). Milk casein to milk N, urine production, urine concentration of N, urea, and creatinine did not differ among treatments in any week.

Actual N intake increased linearly with increasing dietary CP content for week 1 ($P < 0.001$), week 2 ($P = 0.02$), and week 3 ($P < 0.001$) of lactation (Table 5). Nitrogen excreted in urine increased linearly with increasing dietary CP content in week 1 ($P < 0.05$), week 2 ($P < 0.001$), and week 3 ($P < 0.001$). Nitrogen secreted in milk did not differ among treatments in week 1 and 2, and in week 3, the amount of nitrogen secreted in milk increased linearly with increasing dietary CP content ($P < 0.001$). Nitrogen retention did not differ among treatments in any week. Increasing dietary CP had a cubic effect on fecal N output in week 1 and 2, N retention in week 2, and urine N output in week 3, which was caused by treatment 5. First parity sows had a lower N intake compared with multiparous sows in week 1($P = 0.01$), week 2 ($P < 0.01$), and week 3 ($P < 0.001$). Nitrogen excreted in urine did not differ between parities in week 1 and 2, and multiparous sows excreted more N in urine in week 3 of lactation ($P = 0.03$). Nitrogen secreted in milk was higher for first parity sows in week 1 compared to multiparous sows ($P = 0.01$), and no difference was observed between parities in week 2 and week 3. Nitrogen retention was lower in all weeks for first parity sows compared with multiparous sows ($P = 0.01$ and $P = 0.03$) for week 1 and 3, respectively, although it did not differ statistically in week 2.

Realized N intake and SID N intake increased linearly from treatment 1 through 6 ($P < 0.001$; Table 6). Milk N utilization relative to N intake decreased linearly from 77.8% to 63.1% with increasing dietary CP content ($P < 0.001$) and was higher in week 1 and 2 compared with week 3 ($P < 0.001$). Also, the utilization of N for milk N relative to N intake when corrected for protein mobilization decreased linearly from treatment 1 (68.6%) through treatment 6 (64.2%; $P < 0.05$) and was higher in week 1 and 2 compared with week 3 ($P < 0.001$). Urine N excretion relative to N intake increased linearly with increasing dietary CP content ($P < 0.001$) and decreased from week 1 to week

Table 3. Lactation performance over a 28-day lactation period of sows fed increasing dietary SID CP levels

	Treatment, % SID CP							Parity			*P*-value[1]	
	11.8	12.8	13.4	14	14.7	15.6						
Item	1	2	3	4	5	6	SEM	First	Multi	SEM	Trt[2]	Parity
No. sows	11	12	12	11	12	12	-	12	60	-	-	-
Mean parity	3.2	3.2	3.2	3.3	3.2	3.2	-	1	3.6	-	-	-
Total FI day 2–27, kg	169[a]	160[ab]	172[a]	165[ab]	147[b]	170[a]	4.85	139	169	4.64	<0.01	<0.001
Sow BW day 2, kg	266	255	269	269	262	263	8.37	227	272	8.54	0.52	<0.001
Sow BW loss day 2–28, %	6.4	8.6	8.9	8.0	7.0	7.3	1.63	9.1	7.4	1.56	0.89	0.32
Body protein day 4, kg[3]	42.2	39.7	42.3	42.3	41.3	42.1	1.61	35.8	42.9	1.64	0.27	<0.001
Protein mobilization day 4–18, %	1.6	-0.2	0.7	3.0	0.0	0.6	1.56	0.8	1.0	1.58	0.53	0.92
Body fat day 4, kg[3]	68.3	67.9	72.4	71.4	66.6	66.7	2.35	60.7	70.5	2.25	0.36	<0.001
Fat mobilization day 4–18, %	16.3	14.0	15.5	14.9	10.6	17.0	3.95	23.4	12.9	3.99	0.82	0.01
Total milk yield day 2–27, kg[4]	344[ab]	333[ab]	357[a]	337[ab]	307[b]	340[ab]	11.4	319	339	11.3	0.03	0.09
Piglets												
Piglets per litter day 2	14	14	14	14	14	14	-	14	14	-	-	-
Mortality day 2–28, %	8.4	7.0	5.1	8.1	13.2	7.9	-	8.3	7.6	-	0.19	0.81
95% CI	[4.7–14.6]	[3.8–12.5]	[2.4–10.3]	[4.5–14.0]	[8.3–20.2]	[4.5–13.7]		[4.5–14.9]	[5.8–10.0]			
Litter weight day 2, kg	23.5	23.9	22.7	25.2	23.1	21.9	1.04	20.5	23.9	1.04	0.24	<0.01
Litter weight day 28, kg	105[ab]	101[ab]	113[a]	104[ab]	89.0[b]	103[ab]	5.39	84.1	106	5.42	0.04	<0.001
Litter weight gain day 2–27, kg/d	3.26[ab]	3.15[ab]	3.53[a]	3.32[ab]	2.84[b]	3.39[ab]	0.16	2.63	3.37	0.16	0.04	<0.001

[a,b]Treatment means with different superscript letters differ significantly ($P < 0.05$).

[1]No linear, quadratic and cubic effects of dietary treatments were observed $P > 0.05$.

[2]Dietary treatment.

[3]Body fat and protein pool is calculated from Rozeboom et al. (1994).

[4]Milk yield estimated by equations from Hansen et al. (2012).

Table 4. Composition of milk, urine, and feces for week 1, 2, and 3 in lactation in sows fed increasing dietary SID CP levels

	Treatment, % SID CP							Parity			*P*-value[1]			
	11.8	12.8	13.4	14.0	14.7	15.6								
Item	1	2	3	4	5	6	SEM	First	Multi	SEM	Trt[2]	Parity	Linear	Cubic
No. sows	11	12	12	11	12	12	-	12	60	-	-	-	-	-
Mean parity	3.2	3.2	3.2	3.3	3.2	3.2	-	1	3.6	-	-	-	-	-
Week 1 [day 4 ± 3]														
Milk production, kg/d[3]	8.14^{ab}	8.24^{ab}	8.31^{a}	7.97^{ab}	8.02^{ab}	7.83^{b}	0.12	8.27	8.04	0.12	0.02	0.09	**	NS
Milk protein, %	5.39^{ab}	5.56^{ab}	5.21^{b}	5.51^{ab}	5.70^{a}	5.66^{ab}	0.12	5.75	5.46	0.10	0.04	0.02	*	NS
Milk N, %	0.92	0.93	0.89	0.92	0.96	0.95	0.02	0.97	0.92	0.02	0.07	<0.01	NS	NS
Milk casein, %	4.73	4.79	4.57	4.59	4.87	4.75	0.16	4.73	4.72	0.15	0.22	0.91	NS	NS
Milk casein to N	0.87	0.85	0.87	0.84	0.84	0.84	0.03	0.82	0.86	0.03	0.30	0.01	NS	NS
Urine production, kg/d	7.16	6.37	8.10	12.7	8.11	9.41	2.17	6.88	8.98	2.16	0.10	0.28	NS	NS
Urine N, %	0.39	0.39	0.49	0.35	0.51	0.41	0.09	0.61	0.39	0.09	0.54	0.01	NS	NS
Urine urea, mM	114	115	156	119	171	112	25.4	198	117	25.6	0.19	<0.01	NS	NS
Urine creatinine, mM	8.94	10.8	12.2	7.64	11.1	7.56	1.97	11.8	9.26	1.98	0.22	0.19	NS	NS
Feces N, g/kg DM	23.9^{b}	24.0^{b}	26.1^{ab}	23.8^{b}	29.4^{a}	24.5^{b}	1.42	27.6	24.8	1.22	<0.01	0.03	NS	*
Week 2 [day 11 ± 3]														
Milk production, kg/d[3]	13.9	13.5	14.2	13.4	12.6	13.5	0.49	13.3	13.6	0.53	0.27	0.63	NS	NS
Milk protein, %	4.96^{ab}	4.81^{b}	4.77^{b}	5.12^{ab}	5.37^{a}	5.09^{ab}	0.25	5.21	4.98	0.24	0.02	0.15	*	*
Milk N, %	0.58	0.51	0.52	0.57	0.54	0.51	0.06	0.56	0.53	0.06	0.32	0.29	NS	NS
Milk casein, %	3.63^{bc}	3.66^{bc}	3.54^{c}	3.76^{abc}	4.10^{a}	3.96^{ab}	0.14	3.78	3.78	0.13	<0.001	0.99	***	NS
Milk casein to N	0.75	0.76	0.74	0.75	0.77	0.78	0.02	0.74	0.76	0.02	0.34	0.10	NS	NS
Urine production, kg/d	9.97	9.84	12.1	12.8	9.11	14.2	1.81	9.57	11.7	1.88	0.21	0.30	NS	NS
Urine N, %	0.31	0.38	0.33	0.32	0.54	0.30	0.06	0.41	0.36	0.06	0.05	0.39	NS	NS
Urine urea, mM	101	118	114	121	148	106	17.2	120	118	18.2	0.44	0.93	NS	NS
Urine creatinine, mM	7.75	7.48	7.57	7.14	9.19	5.05	1.21	8.43	7.13	1.13	0.23	0.31	NS	NS
Feces N, g/kg DM	28.1^{bc}	28.7^{bc}	29.8^{ab}	29.1^{abc}	26.4^{c}	31.6^{a}	0.8	28.8	29.0	0.82	<0.001	0.81	*	***
Week 3 [day 18 ± 3]														
Milk production, kg/d[3]	15.3	14.7	15.6	15.1	13.7	15.4	0.69	14.3	15.1	0.73	0.24	0.26	NS	NS
Milk protein, %	4.46^{d}	4.81^{bc}	4.71^{cd}	5.04^{ab}	5.09^{ab}	5.18^{a}	0.08	4.98	4.87	0.08	<0.001	0.23	***	NS
Milk N, %	0.73^{c}	0.77^{bc}	0.78^{bc}	0.83^{ab}	0.84^{ab}	0.85^{a}	0.02	0.83	0.80	0.02	<0.001	0.09	***	NS
Milk casein, %	3.39^{c}	3.64^{bc}	3.69^{bc}	3.86^{ab}	3.99^{ab}	4.04^{a}	0.08	3.80	3.77	0.08	<0.001	0.75	***	NS
Milk casein to N	0.76	0.78	0.77	0.77	0.79	0.78	0.01	0.76	0.78	0.01	0.26	0.10	NS	NS
Urine production, kg/d	11.7	8.81	10.6	11.0	11.9	11.1	1.69	8.03	11.5	1.60	0.76	0.06	NS	NS
Urine N, %	0.28	0.44	0.35	0.34	0.48	0.41	0.06	0.41	0.38	0.06	0.22	0.72	NS	NS
Urine urea, mM	81.0	127	117	113	151	134	21.6	124	121	21.9	0.23	0.87	*	NS
Urine creatinine, mM	5.83	8.15	6.56	5.36	6.92	5.09	1.25	6.36	6.33	1.28	0.32	0.98	NS	NS
Feces N, g/kg DM	30.9^{b}	30.3^{b}	32.4^{ab}	32.3^{ab}	31.3^{b}	34.4^{a}	0.79	34.4	31.4	0.84	<0.01	<0.01	**	NS

[a,b,c]Treatment means with different superscript letters differ significantly ($P < 0.05$).

[1]No quadratic effects of dietary treatments were observed $P > 0.05$. *P*-values for linear and cubic treatment effects: NS = not significant, * < 0.05, ** < 0.01, *** < 0.001.

[2]Dietray treatment.

[3]Milk production estimated by equations from Hansen et al. (2012).

Table 5. Daily N intake, output, and retention (g/d) in sows fed increasing dietary SID CP levels

	Treatment, % SID CP							Parity			*P*-value[1]			
	11.8	12.8	13.4	14.0	14.7	15.6								
Item, (g/d)	1	2	3	4	5	6	SEM	First	Multi	SEM	Trt[2]	Parity	Linear	Cubic
No. sows	11	12	12	11	12	12		12	60		-	-	-	-
Week 1 (day 4 ± 3)														
Realized N intake	93.2^{c}	102.2^{bc}	95.2^{c}	113.2^{ab}	112.0^{ab}	116.7^{a}	2.93	97.8	107.3	3.24	<0.001	0.01	***	NS
Urine N output	20.5	22.7	30.2	34.1	28.0	31.8	4.04	28.9	27.7	4.15	0.05	0.77	*	NS
Fecal N output	9.8^{ab}	9.7^{ab}	9.2^{ab}	10.7^{ab}	11.3^{a}	9.1^{b}	0.60	9.9	10.0	0.52	0.04	0.97	NS	**
Milk N output	74.3	75.4	73.8	75.7	75.8	74.0	1.97	78.3	74.1	1.82	0.94	0.04	NS	NS
Retention[3]	−12.9	−9.33	−19.1	−14.8	−13.4	−1.84	5.35	−23.8	−9.19	5.13	0.24	0.01	NS	NS
Week 2 (day 11 ± 3)														
Realized N intake	133.5^{b}	157.7^{ab}	171.8^{a}	164.4^{ab}	157.6^{ab}	174.5^{a}	12.2	128	167	12.0	0.02	<0.001	*	NS
Urine N output	25.0^{ab}	24.1^{b}	32.7^{ab}	38.6^{ab}	38.5^{ab}	39.4^{a}	4.15	27.6	34.3	4.29	<0.01	0.12	***	NS
Fecal N output	18.0^{ab}	20.2^{a}	22.2^{a}	20.0^{ab}	14.8^{b}	19.6^{ab}	1.78	14.8	20.0	1.73	<0.01	<0.01	NS	**
Milk N output	115.1	112.3	118.7	117.7	109.4	117.6	6.23	110.2	116.1	6.29	0.77	0.35	NS	NS
Retention[3]	−27.5	−1.64	−11.8	−29.6	−17.9	−0.43	17.7	−30.1	−11.2	17.7	0.32	0.16	NS	*
Week 3 (day 18 ± 3)														
Realized N intake	186.3^{c}	198.4^{bc}	211.4^{b}	215.7^{b}	213.1^{b}	241.8^{a}	5.68	183	217	5.36	<0.001	<0.001	***	NS
Urine N output	27.1^{b}	30.2^{b}	33.8^{b}	34.0^{b}	50.0^{a}	40.7^{ab}	4.32	27.9	37.9	4.47	<0.001	0.03	***	*
Fecal N output	28.9^{ab}	27.2^{ab}	30.0^{ab}	29.4^{ab}	25.0^{b}	31.3^{a}	1.53	27.7	28.8	1.64	0.02	0.56	NS	NS
Milk N output	106.1^{b}	113.4^{ab}	121.7^{ab}	125.3^{a}	114.6^{ab}	130.5^{a}	5.17	112.1	120.0	5.37	<0.01	0.15	***	NS
Retention[3]	15.3	12.1	26.1	26.9	18.0	39.3	7.97	6.70	26.4	8.35	0.10	0.03	***	NS

[a,b,c]Treatment means with different superscript letters differ significantly ($P < 0.05$).

[1]No quadratic effects were observed $P > 0.05$. *P*-values for linear and cubic treatment effects: NS = not significant, * < 0.05, ** < 0.01, *** < 0.001.

[2]Dietray treatment.

[3]Realized N intake – (N in milk + N in urine + N in feces).

Table 6. Nitrogen intake and utilization of dietary N (in % of intake) in sows fed increasing dietary SID CP levels

	Treatment, % SID CP							Week				*P*-value[1]			
	11.8	12.8	13.4	14	14.7	15.6									
Item[2]	1	2	3	4	5	6	SEM	1	2	3	SEM	Trt[3]	Week	Linear	Cubic
No. sows	11	12	12	11	12	12		72	72	72		-	-	-	-
Realized N intake, g/d	141.2^{c}	151.6^{bc}	157.3^{ab}	165.7^{ab}	156.9^{ab}	173.9^{a}	4.99	105.2^{C}	156.7^{B}	212.0^{A}	3.15	<0.001	<0.001	***	NS
Realized SID N intake, g/d	124.0^{c}	131.7^{bc}	138.4^{ab}	142.6^{ab}	136.6^{ab}	147.8^{a}	4.00	90.6^{C}	137.7^{B}	182.5^{A}	2.63	<0.001	<0.001	***	NS
Milk N utilization	77.8^{a}	69.5^{ab}	68.2^{ab}	66.3^{b}	71.0^{ab}	63.1^{b}	3.54	72.0^{A}	78.3^{A}	57.4^{B}	2.96	<0.01	<0.001	***	*
Urine N excretion	18.5^{b}	17.4^{b}	23.4^{ab}	23.4^{ab}	27.4^{a}	24.7^{ab}	2.38	27.0^{A}	23.1^{B}	17.1^{C}	1.85	<0.01	<0.001	***	*
Fecal N excretion	13.0^{a}	12.1^{ab}	12.2^{ab}	11.6^{bc}	10.6^{c}	10.8^{c}	0.35	9.53^{C}	12.0^{B}	13.6^{A}	0.24	<0.001	<0.001	***	NS
Total N output	110.7	101.9	108.1	103.8	110.2	98.9	5.37	111.8^{A}	116.4^{A}	88.4^{B}	4.24	0.31	<0.001	NS	NS
Corrected milk N utilization[4]	68.6	67.3	64.7	63.5	61.6	64.2	2.51	61.6^{B}	64.3^{B}	68.8^{A}	1.90	0.22	<0.01	*	NS

^{a,b,c}Treatment means with different superscript letters differ significantly ($P < 0.05$).

$^{A\text{-}C}$Wk means with different superscript letters differ significantly ($P < 0.05$).

[1]No quadratic effects were observed $P > 0.05$. *P*-values for linear and cubic treatment effects: NS = not significant, * < 0.05, ** < 0.01, *** < 0.001.

[2]Items are presented as % of N intake unless otherwise is noted.

[3]Dietray treatment.

[4]Milk N utilization corrected for N retention was calculated for individual sows each week as milk N utilization divided by total N output relative to N intake.

3 ($P < 0.001$). Overall, the output of N relative to N intake did not differ among treatments but was higher in week 1 and 2 than in week 3 ($P < 0.001$). Increasing dietary CP had a cubic effect on milk N utilization and N excreted in urine, which was caused by treatment 5.

DISCUSSION

Nitrogen Output

The output of N in milk, urine, and feces exceeded N intake in week 1 and 2 (112% and 116%, respectively), whereas it was lower (88%) in week 3 of lactation, indicating that sows mobilized protein in early lactation. The N output in urine (relative to intake) decreased with progress of lactation (27, 23, and 17%, respectively), indicating that the feeding curve and dietary nutrient composition was more suitable at peak lactation than in early lactation. This is in line with the N output in urine found by Huber et al. (2015), who reported that sows excreted 21% to 26% of their N intake as urine in early lactation (day 3 to 7) and 12% to 16% at peak lactation (day 14 to 18), when crystalline L-Lys, L-Ile, DL-Met, L-Thr, L-Trp, and L-Val was added to the diets. Nitrogen excretion was reduced when comparing this study to that of Theil et al. (2004) who reported an urinary excretion of 28% and 31% of N intake in week 2 and 3, respectively, when fed a diet without addition of crystalline AA. The higher excretion of N in urine in week 1 and 2 could also be due to body protein catabolism (Pedersen et al., 2016) and AA oxidization to partly cover energy requirement. The higher excretion of N in urine in week 1 and 2 may also indicate that the dietary AA profile was less balanced compared with peak lactation. The optimal AA profile may change during lactation, as Krogh et al. (2017) found that the AA profile taken up by the mammary gland in early lactation (day 3) differ from that at peak lactation (day 17). The change in AA profile during lactation might be due to mammary tissue accretion during lactation (Kim et al., 1999). The higher excretion of N in urine in early lactation could also be due to regression of the uterus after parturition (Palmer et al., 1965) and the release of AA to the systemic circulation. In particular, the concentration of Leu, Val, and Phe is high in the uterus relative to Lys compared to milk (Jang et al., 2017).

No difference in urine production was found between treatments, and sows produced from 6.4 to 12.7 kg, 9.1 to 14.2 kg, and 8.8 to 11.9 kg urine daily in week 1, 2, and 3, respectively. This is in agreement with Huber et al. (2015) who found that sows produce 7.2 to 12.0 kg and 7.8 to 11.9 kg urine daily in early (day 3 to 7) and peak lactation (day 14 to 18), respectively. In the study of Huber et al. (2015), urine production was presented as the average for a 4-d period, where sows were fitted with a urinary catheter and tubes leading into a bucket. However, urinary catheters increase the risk of infections in the urinary tract, which may decrease feed intake and in turn the nutrient balance. Reducing the time sows were fitted with urinary catheters is desirable. Collecting urine every second hour for 6 h in the current study instead of using connection tube to a bucket prevents the balloon catheter from being pushed in and out of the vulva. In the current study, the urine production was measured for a 6-h period and extrapolated to 24 h, and it is possible that some diurnal variation affected the 24-h estimates. However, the mean urine production in the current study and in Huber et al. (2015) is almost identical, suggesting that the use of this method to estimate urine production is acceptable. In addition, Feyera et al. (2018) showed no differences in urine production measured by total collection for 6 h or by using a more invasive method, where *para*-aminohippuric acid (pAH) was infused intravenously to measure kidney clearance of pAH and hereby urine production. The urine production was 330, 443, and 407 mL/h in the current study for week 1, 2, and 3, respectively, which is comparable to Huber et al. (2015) who found 363 and 399 mL/h for early lactation (day 3 to 7) and peak lactation (day 14 to 18), respectively. Furthermore, similar urine production levels were reported by Theil (2002), who found an average of 333 mL/h for week 2 to 4 and Feyera et al. (2018), who found 361 mL/h during the first 24 h after birth of the first piglet.

The ATTD of N is rather high in the current study (87.0% to 89.4%). This is markedly higher than what was observed in the study of Theil et al. (2004), where the ATTD of N was 82.2% to 83.2% from week 2 to 4 in lactation. The study of Theil et al. (2004) was carried out without the addition of crystalline AA, which likely caused the lower ATTD of N. The average N digestibility for sows fed diets with high inclusion of crystalline AA was 88.5% in week 1 of lactation and 88.0% in week 3 of lactation (Huber et al., 2015) and is comparable to that found in the present study. The ATTD of N decreased from 90.5% in week 1 to 86.4% in week 3 of lactation in the current study, and this is likely due to the increased feed allowance from day 2 to 18 of lactation. In line with that the study of Zhou et al. (2018), reported a decreased digestibility of N from 83.4 at

day 3 to 81.6% at day 17 of lactation when sows were fed a coarsely ground diet.

Utilization of N for Milk Production

The crude efficiency of N for milk production relative to N intake decreased from 77.8% to 63.1% as dietary CP increased. These efficiencies are substantially greater than observed previously (Everts and Dekker, 1994; Dourmad et al., 1998; van den Brand et al., 2000; Huber et al., 2015) and is likely due to the very high milk production of the sows combined with a substantial protein mobilization in the present study. Potentially, the high efficiency could be due to an overestimation of the milk yield because piglets were offered dry feed, but dry feed intake is normally very low until fourth week of lactation and does not affect piglet growth rate in the first 3 wk (Pluske et al., 2007). The model developed by Hansen et al. (2012) was used to quantify milk production and milk N output was calculated from milk yield and analyzed milk N concentration in the current study and gave on average 103 g N/d. This was 16% higher than that estimated milk N output using the equation reported by NRC. This discrepancy may well be due to the underestimation of milk yield when using the weigh suckle weigh technique, which was used to derive the equations reported in NRC (2012). The presented crude efficiencies relative to intake are not very useful because mobilization of muscle mass cause biased estimates. Therefore, milk N utilization was corrected for N mobilization from the body, which is much more reliable and this efficiency decreased from 71.6% to 63.2% as the dietary CP increased.

The use of crystalline L-Lys, DL-Met, L-Thr, and L-Trp in the current study is likely the possible reason for the improved utilization of N, which appear from the lower excretion of N in urine and greater utilization for milk N in comparison with earlier studies. Along with the increasing N intake, the dietary AA profile changed and indeed was close to the recommended level in treatment 2 (Leu 106%, His 36%, Ile 58%, Val 67%, and Phe 72% relative to lysine) while they were supplied at increasing levels relative to lysine in diets 3 through 6. Especially valine was clearly lower than recommended by NRC (85% relative to lysine). There are indications that Val and Leu interact and that a high dietary Leu content when feeding diets based on corn and soybean increases the Val requirement. However, in Europe, wheat and barley are the main ingredients then Val recommendation is low. The Danish recommendation for Val was 76% when this experiment was carried out (Tybirk et al., 2015) and this was later reduced to 69% relative to Lys (Tybirk et al., 2018).

In the current study, sows secreted as much as 72, 78, and 57% of the dietary N intake in milk in week 1, 2, and 3, respectively. This is clearly better than that reported by Strathe et al. (2017) who found that sows secreted 47% of their N intake in milk at day 17 when sows were fed optimally in that experiment (13.5% SID CP, which corresponded to 16.2% dietary CP). The improved utilization of N for milk production in the current study therefore indicates an improved AA profile in the diet due to the addition of crystalline L-Lys, DL-Met, L-Thr, and L-Trp. Ideally, by formulating diets using all essential AA as crystalline supplementation, the dietary CP could be minimized along with the excretion of N in urine. This was done in a study by Huber et al. (2018) who added crystalline L-Ile, L-His, L-Leu, L-Lys, L-Met, L-Phe, L-Thr, L-Trp, and Val to the diet. In that study, dietary CP was reduced from 16.2% to 12.7% CP, without having any impact on performance throughout lactation and a concomitant reduction in urinary N excretion from 25% to 13% of the N intake at day 13 to 17 in lactation. Furthermore, the study showed an improved efficiency of AA utilization and improved utilization of N for milk production at peak lactation (from 48% to 65% of the N intake). In the present study, only 4 crystalline AA were used, and no effect on BW loss or milk yield was observed with CP reduced from 18.6% to 14.6%. The dietary CP could possibly be reduced further by adding the remaining 5 essential AA as crystalline AA which is currently not economically applicable.

Milk N Secretion and Protein Content

Output of N in milk increased from 106 to 131 g/d in week 3 of lactation when the N intake increased by 30% from 186 to 242 g N/d due to the increasing CP level (14.6% to 18.6% dietary CP). The increased milk N output was due to the increased casein synthesis by the mammary gland which increased the true protein content of the milk. In line with this, Strathe et al. (2017) also found an increased milk N output (from 89 to 104 g/d at day 17), when dietary protein increased from 12.8% to 16.6% CP. However, in the current study litter weight gain throughout lactation did not increase even though milk protein and milk casein concentration increased along with the increased dietary CP inclusion, suggesting that piglets do not benefit from an elevated protein to

energy ratio in sow milk. These findings suggest that the sow likely produce excessive casein and milk protein relative to milk energy, when supplied with excess dietary protein. The N retention was positive for all treatments in week 3, which further support that sows were fed above their requirement and therefore disposed excess AA and N for milk synthesis. The fact that sows secreted more N in milk when fed high CP diets without increasing the litter gain is in line with Chisoro et al. (unpublished data), who found a slightly negative correlation between protein concentration in sow milk and piglet weight gain.

Sow Mobilization

Sows mobilized 11% to 17% of their body fat pool from day 4 to 18 of lactation, while sow protein mobilization ranged from 0% to 3% of their body protein pool, indicating energy intake was limiting, while protein intake was adequate. A previous study carried out in the same herd (i.e., same genetics, housing conditions, management, and feeding curve) found that sows on average mobilized 21% of their body fat and mobilized 6% to 1% of their body protein when dietary CP increased from 10.4% to 15.0% SID CP (Strathe et al., unpublished data). The lower mobilization in the present study as compared with that reported by Strathe et al. (2017), indicates that an improved AA profile improved utilization of dietary energy (Pedersen et al., 2019) and N (this study). Moreover, the litter weight gain peaked at 3.07 kg/d when sows were fed at or above the breakpoint 135 g SID CP/kg by Strathe et al. (2017). In the current study, sows had a mean litter gain of 3.25 kg/d, emphasizing that the lactation performance is really high in modern hyper prolific sows. In line with that, Thingnes et al. (2012) and Craig et al. (2016) reported a daily litter gain of 3.12 and 3.24 kg/d, respectively. For comparison, daily litter gain is normally considerably lower in sows with less suckling piglets (10 to 12 littermates), and the productivity of these sows are typically 2.3 kg/d to 2.7 kg/d (e.g., Huber et al., 2015 and Greiner et al., 2018).

Based on fat mobilization (D_2O technique) and protein mobilization (D_2O technique or balance method), sow weight change during lactation can be estimated as 5 times protein balance plus fat mobilization. When comparing with the recorded weight change with that obtained based on the D_2O and balance techniques, the mean weight changes were 10.3 kg weight loss (based on weighings), and 12.5 kg weight loss based on fat and protein mobilization (D_2O technique) and 10.3 kg weight loss based on fat mobilization (D_2O technique) and protein mobilization (balance method). Possibly, the weight loss estimated from the D_2O technique is slightly more accurate than the observed weight change based on weighing because the latter causes an underestimation due to higher gut fill at day 18 than at day 4.

CONCLUSION

A low dietary CP concentration for lactating sows with supplemented crystalline AA improved the efficiency of N utilization and reduced the N output in urine as compared with high CP inclusion levels. No optimal dietary CP level for N utilization for milk production was found across dietary treatments. Future studies should focus on optimizing dietary CP and AA of sows in early lactation.

LITERATURE CITED

AOAC. 2012. Official methods of analysis of AOAC International. 19th ed. AOAC International, Gaithersburg, MD.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319−338. doi:10.1016/S0377-8401(97)00009-6

Ball, R. O., G. Courtney-Martin, and P. B. Pencharz. 2006. The in vivo sparing of methionine by cysteine in sulfur amino acid requirements in animal models and adult humans. J. Nutr. 136(6 Suppl):1682S–1693S. doi:10.1093/jn/136.6.1682S

van den Brand, H., M. J. Heetkamp, N. M. Soede, J. W. Schrama, and B. Kemp. 2000. Energy balance of lactating primiparous sows as affected by feeding level and dietary energy source. J. Anim. Sci. 78:1520–1528. doi:10.2527/2000.7861520x

Craig, A., W. Henry, and E. Magowan. 2016. Effect of phase feeding and valine-to-lysine ratio during lactation on sow and piglet performance. J. Anim. Sci. 94:3835–3843. doi:10.2527/jas.2016-0648

Dourmad, J. Y., J. Noblet, and M. Etienne. 1998. Effect of protein and lysine supply on performance, nitrogen balance, and body composition changes of sows during lactation. J. Anim. Sci. 76:542–550. doi:10.2527/1998.762542x

Everts, H., and R. A. Dekker. 1994. Effect of nitrogen supply on nitrogen and energy metabolism in lactating sows. Anim. Prod. 59:445–454. doi:10.1017/s0003356100007972

Feyera, T., P. Zhou, T. F. Pedersen, U. Krogh, and P. K. Theil. 2018. Comparison of sow urine production measured by total collection and para-aminohippuric acid infusion. In: Book of Abstracts. 14th International Symposium. Digestive Physiology of Pigs, Brisbane, Australia. p. 107.

Greiner, L., P. Srichana, J. L. Usry, C. Neill, G. L. Allee, J. Connor, K. J. Touchette, and C. D. Knight. 2018. The use of feed-grade amino acids in lactating sow diets. J. Anim. Sci. Biotechnol. 9:3. doi:10.1186/s40104-017-0223-z

Hansen, B. 1989. Determination of nitrogen as elementary-n, an alternative to kjeldahl. Acta. Agr. Scand. 39:113−118.

Hansen, A. V., A. B. Strathe, E. Kebreab, J. France, and P. K. Theil. 2012. Predicting milk yield and composition in lactating sows: A Bayesian approach. J. Anim. Sci. 90:2285–2298. doi:10.2527/jas.2011-4788

Helverskov, O. 2017. National average productivity in Danish swine production 2016 (in Danish: landsgennemsnit for produktivitet i svineproduktionen 2016). Report no. 1716. SEGES Pig Research Centre, Axelborg, Denmark.

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

Huber, L. A., M. Rudar, N. L. Trottier, J. P. Cant, and C. F. M. de Lange. 2018. Whole-body nitrogen utilization and tissue protein and casein synthesis in lactating primiparous sows fed low- and high-protein diets. J. Anim. Sci. 96:2380–2391. doi:10.1093/jas/sky047

Jang, Y. D., Y. L. Ma, R. L. Payne, and M. D. Lindemann. 2017. Amino acid composition of fetus, placenta, and uterus in gilts throughout gestation. J. Anim. Sci. 95:4448–4461. doi:10.2527/jas2017.1836

Just, A. 1982. The net energy value of crude (catabolized) protein for growth in pigs. Livest. Prod. Sci. 9:349–360. doi:10.1016/0301-6226(82)90041-0

Kerr, B. J., and R. A. Easter. 1995. Effect of feeding reduced protein, amino acid-supplemented diets on nitrogen and energy balance in grower pigs. J. Anim. Sci. 73:3000–3008. doi:10.2527/1995.73103000x

Kim, S. W., W. L. Hurley, I. K. Han, and R. A. Easter. 1999. Changes in tissue composition associated with mammary gland growth during lactation in sows. J. Anim. Sci. 77:2510–2516. doi:10.2527/1999.7792510x

Krogh, U., N. Oksbjerg, A. C. Storm, T. Feyera, and P. K. Theil. 2017. Mammary nutrient uptake in multiparous sows fed supplementary arginine during gestation and lactation. J. Anim. Sci. 95:2517–2532. doi:10.2527/jas.2016.1291

Lenis, N. P., and A. W. Jongbloed. 1999. New technologies in low pollution swine diets: diet manipulation and use of synthetic amino acids, phytase and phase feeding for reduction of nitrogen and phosphorus excretion and ammonia emission - Review. Asian-Australas. J. Anim. Sci. 12:305–327. doi:10.5713/ajas.1999.305

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Palmer, W. M., H. S. Teague, and W. G. Venzke. 1965. Macroscopic observations on the reproductive tract of the sow during lactation and early postweaning. J. Anim. Sci. 24:541–545. doi:10.2527/jas1965.242541x

Pedersen, C., and S. Boisen. 2002. Establishment of tabulated values for standardized ileal digestibility of crude protein and essential amino acids in common feedstuffs for pigs. Acta. Agr. Scand. A-An. 52:121–140. doi:10.1080/090647002320229374

Pedersen, T. F., T. S. Bruun, T. Feyera, U. K. Larsen, and P. K. Theil. 2016. A two-diet feeding regime for lactating sows reduced nutrient deficiency in early lactation and improved milk yield. Livest. Sci. 191:165–173. doi:10.1016/j.livsci.2016.08.004

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

Pluske, J. R., J. C. Kim, C. F. Hansen, B. P. Mullan, H. G. Payne, D. J. Hampson, J. Callesen, and R. H. Wilson. 2007. Piglet growth before and after weaning in relation to a qualitative estimate of solid (creep) feed intake during lactation: a pilot study. Arch. Anim. Nutr. 61:469–480. doi:10.1080/17450390701664249

Rozeboom, D. W., J. E. Pettigrew, R. L. Moser, S. G. Cornelius, and S. M. el Kandelgy. 1994. In vivo estimation of body composition of mature gilts using live weight, backfat thickness, and deuterium oxide. J. Anim. Sci. 72:355–366. doi:10.2527/1994.722355x

Stein, H. H., B. Seve, M. F. Fuller, P. J. Moughan, and F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J. Anim. Sci. 76:1433–1436. doi:10.2527/jas.2005–742

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

Strathe, A. V., T. S. Bruun, J. E. Zerrahn, A. H. Tauson, and C. F. Hansen. 2016. The effect of increasing the dietary valine-to-lysine ratio on sow metabolism, milk production, and litter growth. J. Anim. Sci. 94:155–164. doi:10.2527/jas.2015-9267

Theil, P. K. 2002. Energy and protein metabolism in pregnant and lactating sows. PhD Diss. The Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Theil, P. K., H. Jørgensen, and K. Jakobsen. 2004. Energy and protein metabolism in lactating sows fed two levels of dietary fat. Livest. Prod. Sci. 89:265–276. doi:10.1016/j.livprodsci.2004.01.001

Theil, P. K., T. T. Nielsen, N. B. Kristensen, R. Labouriau, V. Danielsen, C. Lauridsen, and K. Jakobsen. 2002. Estimation of milk production in lactating sows by determination of deuterated water turnover in three piglets per litter. Acta. Agr. Scand. A-An. 52:221–232. doi:10.1080/090647002762381104

Thingnes, S. L., A. S. Ekker, A. H. Gaustad, and T. Framstad. 2012. Ad libitum versus step-up feeding during late lactation: The effect on feed consumption, body composition and production performance in dry fed loose housed sows. Livest. Sci. 149:250–259. doi:10.1016/j.livsci.2012.07.015

Tybirk, P., N. M. Sloth, N. Kjeldsen, and L. Shooter. 2018. Danish nutrient requirement standards (In Danish: Normer for næringsstoffer). 28th rev. ed. SEGES Pig Research Centre, Axelborg, Denmark.

Tybirk, P., N. M. Sloth, T. B. Sønderby, and N. Kjeldsen. 2015. Danish nutrient requirement standards (In Danish: Normer for næringsstoffer). 22th rev. ed. SEGES Pig Research Centre, Axelborg, Denmark.

Tybirk, P., A. B. Strathe, E. Vils, N. M. Sloth, and S. Boisen. 2006. The Danish feed evaluation system for pig feeds (In Danish: Det Danske fodervurderingssytem for svinefoder). SEGES Pig Research Centre, Axelborg, Denmark.

Zhou, P., M. Nuntapaitoon, T. F. Pedersen, T. S. Bruun, B. Fisker, and P. K. Theil. 2018. Effects of mono-component xylanase supplementation on nutrient digestibility and performance of lactating sows fed a coarsely ground diet. J. Anim. Sci. 96:181–193. doi:10.1093/jas/skx042