animal

Increased dietary protein for lactating sows affects body composition, blood metabolites and milk production

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*Hyper-prolific sows nurse more piglets than less productive sows, putting a high demand on the nutrient supply for milk production. In addition, the high production level can increase mobilization from body tissues. The effect of increased dietary protein (104, 113, 121, 129, 139 and 150 g standardized ileal digestible (**SID**) CP/kg) on sow body composition, milk production and plasma metabolite concentrations was investigated from litter standardization (day 2) until weaning (day 24). Sow body composition was determined using the deuterium oxide dilution technique on days 3 and 24 postpartum. Blood samples were collected weekly, and milk samples were obtained on days 3, 10 and 17 of lactation. Litter average daily gain (**ADG**) peaked at 135 g SID CP/kg (P < 0.001). Sow BW and back fat loss reached a breakpoint at 143 and 127 g SID CP/kg (P < 0.001). Milk fat increased linearly with increasing dietary SID CP (P < 0.05), and milk lactose decreased until a breakpoint at 124 g SID CP/kg and 5.3% (P < 0.001) on day 17. The concentration of milk protein on day 17 increased until a breakpoint at 136 g SID CP/kg (5.0%; P < 0.001). The loss of body protein from day 3 until weaning decreased with increased dietary SID CP until it reached a breakpoint at 128 g SID CP/kg (P < 0.001). The body ash loss declined linearly with increasing dietary SID CP (P < 0.01), and the change in body fat was unaffected by dietary treatment (P=0.41). In early lactation (day 3 + day 10), plasma urea N (**PUN**) increased linearly after the breakpoint at 139 g SID CP/kg at a concentration of 3.8 mmol/l, and in late lactation (day 17 + day 24), PUN increased linearly after a breakpoint at 133 g SID CP/kg (P < 0.001) at a concentration of 4.5 mmol/l. In conclusion, the SID CP requirement for sows was estimated to 135 g/kg based on litter ADG, and this was supported by the breakpoints of other response variables within the interval 124 to 143 g/kg.*

Implications

Feeding excessive dietary protein to sows increases nitrogen excretion into the environment. Hence, it is important that the protein provision matches the actual amino acid requirement of the sow. This study showed that increased protein intake of hyper-prolific lactating sows prevents excessive BW loss and decreases body protein mobilization, as the extra dietary protein was utilized for milk production. Minimizing the body mobilization can ensure better performance of sows in the following reproductive cycle. The higher milk yield and nutrient concentrations resulted in higher litter gains, which is of great economic importance to the pig producer.

Introduction

Sows have become larger and leaner during the last decades, and their milk yield has increased (Hansen *et al.*, 2012) to cover the requirement of the large litters. These tremendous changes probably have increased the dietary requirement of protein and amino acids of the sow. For many years focus has been on determining the requirement of single amino acids, especially lysine, and not the requirement of balanced CP. The supply of protein above the requirement will cause deamination of the surplus amino acids and increased excretion of N into the environment (Hansen *et al.*, 2014; Huber *et al.*, 2015). In another part (Strathe *et al.*, 2017a) of the current study, the dietary standardized ileal digestible (**SID**) CP concentration for maximizing litter gain was determined to be 135 g/kg. Litter gain is regarded as an important determinant of nutrient requirements of lactating sows (Trottier *et al.*, 2015). However, it is also important to

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understand how the dietary provision of CP influences the mobilization of body tissues and milk production when determining the requirement of lactating sows. Sows often turn catabolic in order to maintain their milk production (Strathe *et al.*, 2017b). Under-supplying dietary protein can cause a high mobilization of body protein (King *et al.*, 1993; Mejia-Guadarrama *et al.*, 2002), since milk production has a very high priority. Depleted body protein pools must be restored during the following gestation, which is energetically unfavourable (Dourmad *et al.*, 1996; Bender, 2012) and should therefore be avoided. The extent of the catabolic state will be reflected in changes in body composition, plasma metabolite concentrations and milk production (Dourmad *et al.*, 1998). Measurements of plasma urea N (**PUN**) concentrations, milk composition and body composition can be used as alternative markers for the optimum SID CP (Coma *et al.*, 1996; Yang *et al.*, 2000), because it is not given that the metabolism of the sow is optimized at the same dietary SID CP concentration as the litter gain.

Hence, the objective of this experiment was to estimate the dietary SID CP requirement of lactating sows using litter gain as the main determinant supported by measurements on several traits reflecting sow metabolism. It was hypothesized that increased dietary protein provision would decrease body protein mobilization and improve milk production through increased yield and nutrient concentrations as well as affect sow plasma concentrations of metabolites related to protein and fat metabolism.

Material and methods

This experiment was carried out on a subsample of 92 sows from a larger trial with 544 sows (Strathe *et al.*, 2017a).

Treatments, animals and housing

The study was conducted in a commercial piggery using a total of 92 parity 2 and 3 sows (Danish Landrace × Danish Yorkshire, DanAvl) inseminated with Duroc semen (Hatting KS, Horsens, Denmark). The animals were studied from 4 days *prepartum* (−4.1 ± 1.6) to 24 days *postpartum* (24.4 ± 1.2) when piglets were weaned. Sows were randomly allocated to one out of six dietary treatments with increasing dietary provision of SID protein (Table 1) and stratified for parity in a complete block design from day 2 *postpartum*. Diets were formulated to be isoenergetic on net energy basis (EvaPig®, 2008). Sows were fed using a SpotMix feeding system (Schauer Agrotronic, Prambachkirchen, Austria), and meals for each sow were weighed and registered by the feeding system. From day 4 *prepartum* to day 2 *postpartum* all sows were fed the same commercial formulated lactation diet based on wheat, soybean meal and sugar beet pulp complying with Danish recommendations (Tybirk *et al.*, 2014). From day 2 to day 10 of lactation, sows were fed twice daily, and from day 10 onwards, sows were fed three times per day. The sows were fed 2.3 kg from day 2 *postpartum*, and feed allowance was gradually increased to 8.4 kg at day 17. Sows had

drinking nipples in the trough and free access to water. Feed residuals were not recorded, but feed allowance was adjusted for individual sows daily, and the feed allowances mentioned above were used as maximum for the sows, so some sows would consume less.

Each week a batch of six sows was selected for the experiment, moved to the farrowing unit day 4 *prepartum* and placed in individual farrowing crates. Twenty-four hours *postpartum* (day 2) the litters of the experimental sows were standardized to 14 piglets (average piglet weight 1.76 ± 0.24 kg). Apart from the observations made in the experiment all animals were managed according to the general routines of the herd. Health was monitored by the stock personnel, and normal herd practices for management, treatments and vaccinations were followed. When dead or very weak piglets were removed, the date and weight of the excluded piglets were recorded.

Data collection, measurements and chemical analyses

Information on the collection of feed samples and analyses is given in Strathe *et al.* (2017a). On 544 sows, BW and back fat (**BF**) thickness were measured at day 2 *postpartum* and at weaning, and litter weight was recorded at litter standardization and at weaning (Strathe *et al.*, 2017a).

On 70 sows (10 to 12 per dietary treatment), blood, urine and milk samples were collected. Blood samples were collected in 10 ml EDTA tubes (BD Vacutainer, Plymouth, UK) from the jugular vein at days −4, 3, 10, 17, and 24 *postpartum* 4 h after the morning feeding. Samples were placed on ice and centrifuged for 10 min (1560×**g**) at room temperature, and plasma was then harvested and stored at −20°C in 1.5-ml microcentrifuge tubes (Kruuse, Langeskov, Denmark) until analysis. Plasma was analysed for concentrations of PUN, creatinine, glucose, lactate and non-esterified fatty acids (**NEFA**), triglycerides, cholesterol, total protein, albumin, alanine amino transferase (**ALT**), aspartate amino transferase (**AST**), gamma-glutamyl transferase (**GGT**) and alkaline phosphatase (Advia 1800 Chemistry System; Siemens, Denmark).

Milk samples were collected on days 3, 10 and 17 *postpartum*. Piglets were removed from the sow for at least 30 min, and 2 ml oxytocin (Oxytocin 'Intervet' Vet, 10 IE/ml; Intervet International B. V., Boxmeer, Netherlands) was injected intramuscularly to induce milk letdown. Milk was sampled from four to five teats and stored in 50-ml tubes (Sarstedt, Nümbrecht, Germany) at −20°C until analysis for DM, lactose, fat and true protein (MilcoScan FT2, Foss Electric, Denmark).

On another subsample of 60 sows (10 per treatment), body water content was measured by the deuterium oxide (**D₂O**) dilution technique (Theil *et al.*, 2002) on days 3 and 24 *postpartum*. An initial blood sample was drawn from the jugular vein into a 4-ml lithium-heparinized tube (BD Vacutainer, Plymouth, UK) just before D₂O injection, and then 5 h after injection with D₂O, a second sample was taken. A dose of 0.2 ml (10% solution of D₂O; Sigma Aldrich, Brøndby, Denmark) per kg BW was injected i.m. in the neck

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Table 1 *Dietary composition (as-fed) of diets fed to sows from day 2 postpartum to weaning*

	Diet					
	1	2	3	4	5	6
Ingredient (%)						
Barley	46.0	44.8	43.8	42.8	41.4	40
Wheat	36.2	35.0	34.0	33.0	31.6	30.2
Soy bean meal	8.4	11.1	13.4	15.7	18.9	22.1
Wheat bran	1.80	1.44	1.14	0.84	0.42	0.00
Sugar beet pellets	2.00	2.00	2.00	2.00	2.00	2.00
Vegetable oil[1]	1.73	1.76	1.78	1.80	1.84	1.87
Monocalcium phosphate	1.15	1.13	1.11	1.10	1.07	1.05
Limestone	1.63	1.62	1.61	1.61	1.59	1.58
Salt	0.52	0.52	0.52	0.52	0.52	0.52
L-Lys (65%)	0.34	0.34	0.34	0.34	0.34	0.33
L-Thr	0.06	0.07	0.07	0.07	0.08	0.08
DL-Met	0.03	0.04	0.05	0.06	0.07	0.08
L-Val	0.00	0.01	0.01	0.01	0.02	0.02
Vitamin and mineral premix[2]	0.18	0.19	0.19	0.19	0.20	0.20
Phyzyme XP[3]	0.01	0.01	0.01	0.01	0.01	0.01
Microgrits	0.05	0.05	0.05	0.05	0.05	0.05
Composition (calculated)						
DM (%)	86.3	86.4	86.5	86.6	86.7	86.8
CP (g/kg)	120	130	139	147	159	171
Starch (g/kg)	452	440	429	419	405	391
Soluble fibre (g/kg)	42	43	43	44	45	46
Insoluble fibre (g/kg)	126	126	126	126	126	126
SID[4] Lys (g/kg)	5.8	6.5	7.0	7.5	8.3	9.0
Energy (MJ NE/kg)	7.9	7.9	7.9	8.0	8.0	8.0
Composition (analysed)[5]						
CP (g/kg)	128.4	138.0	146.4	154.6	166.3	177.9
Lys (g/kg)	7.04	7.71	8.26	8.82	9.60	10.38
Met (g/kg)	2.21	2.41	2.58	2.75	2.98	3.22
Thr (g/kg)	4.80	5.26	5.64	6.02	6.55	7.08
Trp (g/kg)	1.61	1.75	1.86	1.97	2.13	2.29
Leu (g/kg)	8.48	9.28	9.95	10.6	11.6	12.5
Ile (g/kg)	4.51	5.00	5.41	5.82	6.39	6.96
His (g/kg)	2.92	3.19	3.41	3.63	3.95	4.26
Phe (g/kg)	5.81	6.33	6.77	7.20	7.81	8.42
Val (g/kg)	5.74	6.21	6.60	6.99	7.53	8.08
SID CP[6] (g/kg)	104.3	113.3	120.9	128.5	139.2	150.0
SID Lys[6] (g/kg)	5.85	6.46	6.98	7.52	8.25	8.98
SID Met[6] (g/kg)	1.91	2.12	2.28	2.45	2.68	2.91
SID Thr[6] (g/kg)	3.86	4.29	4.64	4.98	5.47	5.99
SID Trp[6] (g/kg)	1.30	1.44	1.54	1.64	1.79	1.94
SID Leu[6] (g/kg)	6.92	7.65	8.25	8.86	9.70	10.6
SID Ile[6] (g/kg)	3.68	4.13	4.50	4.88	5.40	5.92
SID His[6] (g/kg)	2.39	2.82	2.85	3.05	3.34	3.63
SID Phe[6] (g/kg)	4.84	5.32	5.73	6.13	6.70	7.26
SID Val[6] (g/kg)	4.52	4.97	5.35	5.71	9.12	6.75

Lys=lysine; Met=methionine; Thr=threonine; Trp=tryptophan; Leu=leucine; Ile=isoleucine; His=histidine; Phe=phenylalanine; Val=valine; SID=standardized ileal digestible.
[1] Leci E Basic, Evilec ApS, Sandbjergvej 26, 6000 Kolding, Denmark.
[2] Provided per kilogram of the diet: 8900 IU vitamin A; 890 IU vitamin D3; 167 mg DL-alfa-tocopherol, 4.24 mg vitamin K3; 2.23 mg vitamin B1; 5.57 mg vitamin B2; 3.34 mg vitamin B6; 0.02 mg vitamin B12; 16.70 mg D-pantothenic acid; 22.26 mg niacin; 1.67 mg folic acid; 89.04 mg iron (FeSO$_4$); 13.00 mg copper (CuSO$_4$); 44.52 mg manganese (MnO); 0.22 mg iodine (Ca(IO$_3$)$_2$); 0.37 mg selenium (Na$_2$SeO$_3$).
[3] Phyzyme XP provided 500 phytase activity (FTU) per kilogram of diet (Danisco Animal Nutrition DuPont, Marlborough, UK).
[4] SID values used in diet formulation were from Pedersen and Boisen (2002).
[5] Feed samples were sampled regularly during the experimental period, and 12 feed samples were analysed per dietary treatment.
[6] Calculated from the analysed composition and digestibilities used when formulating the diets.

of the sow. The exact amount of D_2O injected was determined by weighing the syringe before and after injection. The blood samples were centrifuged for 10 min (1560×**g**) at room temperature, and plasma was then recovered and stored in 1.5-ml microcentrifuge tubes (Kruuse, Langeskov, Denmark) at −20°C until analysis. The total D_2O space was determined as described by Theil *et al.* (2002).

Calculations and statistical analysis
Calculations and statistical analyses were carried out using the statistical software R (R Core Team, Vienna, Austria) with the individual sow regarded as the experimental unit. Average daily gain (**ADG**) of the litter was calculated from litter size and weight at standardization and at weaning. Weights of dead piglets or piglets removed from the litter during the experiment were included in the litter gain. Milk yield was estimated from ADG of the litter and litter size (data not shown) using equations by Hansen *et al.* (2012). The content of body water was estimated based on the measured concentrations of D_2O in plasma. From body water, BF and BW, the body protein, fat and ash contents were estimated using equations by Rozeboom *et al.* (1994). In the analysis of the dose–response data, the dietary SID CP concentrations were used as an explanatory variable. Based on the recordings of individual daily feed intakes of the sows, chemical analysis of dietary composition, and digestibility coefficients of CP of the six diets, an average dietary SID CP concentration was calculated for the individual sow.

Data were fitted using a mixed-effects model in ANOVA form using the nlme package in R to describe the effect of the six dietary treatments. Feed intake, sow BW and BF, sow body composition and changes in body composition, and litter weight were analysed using model (1). The interaction between diet and parity was tested, but the interaction and the effect of parity were not significant and therefore eliminated in the final model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk} \qquad (1)$$

where Y_{ijk} is the response variable measured on the kth sow ($k = 1, \ldots, n_{ij}$) in the jth block ($j = 1, 2, \ldots, 16$) receiving the ith diet ($i = 1, \ldots, 6$). Here μ is the overall mean, α_i is the fixed effect of diet, β_j is the random effect of block that was assumed to be $N(0, \sigma_B^2)$, and ε_{ijk} is the random error component, which was assumed to be $N(0, \sigma_e^2)$.

When analysing milk yield and composition, daily secretions and concentrations of plasma metabolites, a repeated-measures model was fitted to data. The three-way interaction between diet, parity and day were included in model (2), but none of the three-way or two-way interactions and the effect of parity were significant and therefore excluded in the final model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \nu_l + \varepsilon_{ijkl} \qquad (2)$$

where Y_{ijkl} is the response variable measured on the lth sow ($l = 1, \ldots, n_{ijk}$) in the kth block ($k = 1, 2, \ldots, 16$) receiving the ith diet ($i = 1, \ldots, 6$) on the jth day *postpartum* ($j = 1, \ldots, 5$). Here μ is the overall mean, α_i is the fixed effect of diet, β_j is the fixed effect of day *postpartum*, γ_k is the random effect of block that was assumed to be $N(0, \sigma_B^2)$, ν_l is the random effect of sow that was assumed to be $N(0, \sigma_S^2)$, and ε_{ijkl} is the random error component that was assumed to be $N(0, \sigma_e^2)$. Plasma concentrations of lactate, NEFA, alkaline phosphatase and GGT were log-transformed, because of lacking variance homogeneity. Results are given as least squares means and SE. Log-transformed data were back-transformed and given as least squares means and 95% confidence interval (**CI**). Statistical significance was declared at $P < 0.05$ and tendency at $P < 0.10$.

All response variables were analysed using either a linear dose–response function or nonlinear dose–response function, being the linear broken-line and quadratic broken-line. Here dietary CP concentration was treated as a continuous variable. The nonlinear dose–response models were fitted using nonlinear mixed-effects models, where the random effect of block was incorporated into the plateau parameter of the models as described by Robbins *et al.* (2006). Taking the linear plateau model as an example, then the nonlinear mixed-effect model was formulated as:

$$y_{ij} = \begin{cases} k * (R - CP_{ij}); CP_{ij} < R \\ y_{\max} + b_i; CP_{ij} > R \end{cases} + e_{ij} \qquad (3)$$

where y_{ij} denotes response variable for the jth sow in the ith block. Population parameters are the slope k in response to dietary CP_{ij} below the requirement R and $y_{\max}$ is plateau level, where the dietary CP_{ij} is not limiting sow performance. The random effect b_i captures the block-to-block variation in sow performance and is assumed to be $N(0, \sigma_B^2)$, and ε_{ij} is the random error component, which was assumed to be $N(0, \sigma_e^2)$

The model output was evaluated using three criteria: (1) comparison of Akaike Information Criteria and −2 log likelihood fit statistics for nested models, (2) models with breakpoint outside the range of the tested SID CP concentrations was regarded inappropriate, and (3) evaluation of visual fit to data. The nonlinear models were compared to the linear model using the abovementioned criteria. However, only results of variables with a significant effect of dietary CP concentration are shown in the Results section. All data were used to fit the models, but in Figures 1 and 2 the least square means for the six groups are also presented together with the best model as described above.

When the ANOVA revealed that there was no difference between sampling days ($P > 0.10$), data for these days were pooled. For PUN, data from days 3 and 10, and days 17 and 24, respectively, were combined. Data for plasma albumin at days 17 and 24 were united. For GGT, data from days 3, 10, 17 and 24 were pooled.

Results

Results for the model with the best fit to data according to the criteria given above are presented. The nonlinear models
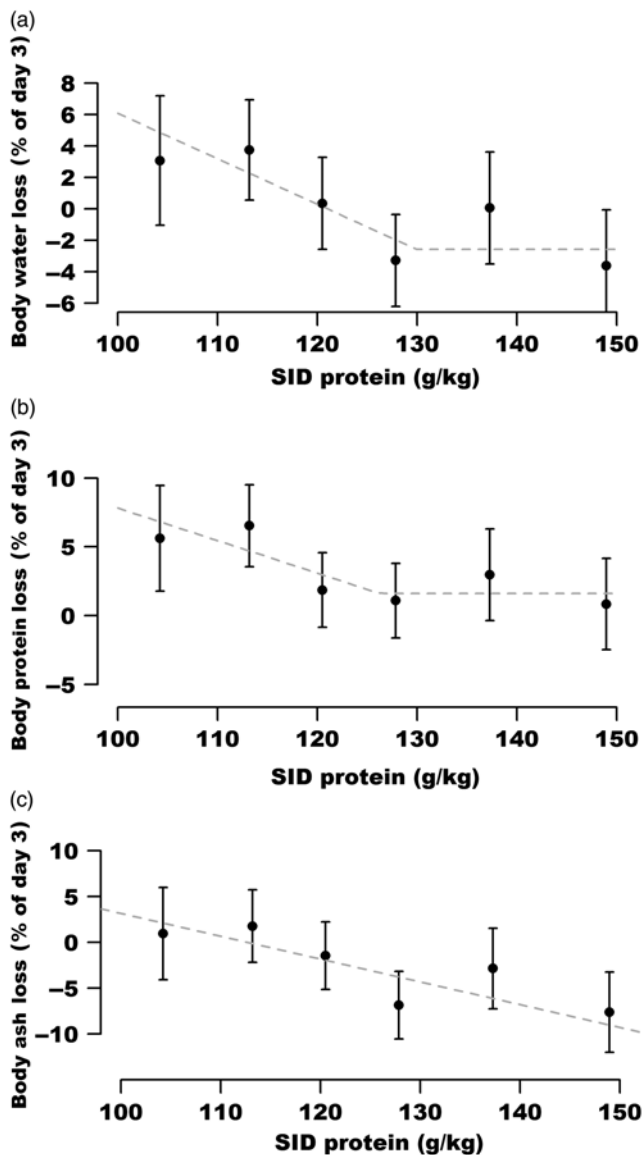
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Figure 1 Changes in the contents of body water, protein and ash from day 3 to day 24 *postpartum* with increased standard ilea digestible (SID) dietary protein. The content of body water was determined using the deuterium dilution technique (Theil *et al.*, 2002). Body protein and ash were estimated from body water, BW and back fat thickness. The loss is given as the percentage of tissue mass at day 3. Data were fitted using linear regression, linear broken-line and quadratic broken-line models using nonlinear mixed-effects models. The model with the best fit is presented in the figure. Data points in the figures are least square means and SE of the six dietary groups, and the linear broken-line model or the linear regression model using data of individual sows are also presented. (a) Body water loss decreased linearly ($-0.3 \times$ (SID CP $- 130$) $\times$ (SID CP ≤ 130) $- 2.5$) until 130 g SID CP/kg at a loss of 2.5% ($P<0.001$), (b) body protein decreased linearly ($-0.2 \times$ (SID CP $- 128$) $\times$ (SID CP ≤ 128) $+ 1.5$) until 128 g SID CP/kg at a loss of 1.5% ($P<0.001$) and (c) body ash loss decreased linearly ($Y = 25.5 - 0.23 \times$ SID CP (g/kg); $P<0.001$). Negative values indicate a gain.

were compared to the linear regression model, and for the change in body water and protein, concentrations of milk lactose and CP and PUN, the linear broken-line model was chosen over other models. For plasma concentrations of albumin, GGT and ALT, change in body ash and concentration of milk fat, the linear regression model was the best fit to data.

Diets and sow performance

The concentration of protein was slightly higher in the six diets than planned, because wheat and barley had a higher content of crude protein than expected in the feed formulation. The average daily feed intake of the sows was similar in all groups (Table 2; $P=0.78$), and as planned, the daily intake of protein increased with increasing SID CP concentrations of the six diets ($P < 0.001$).

Litter size (12.8 $\pm$ 1.2 piglets) at weaning was unaffected by dietary SID CP concentration, whereas ADG of the litters ($0.01 \times$ (SID CP $- 135$) $\times$ (SID CP ≤ 135) $+ 2.53_{\text{first parity}}$ or $3.07_{\text{multiparity}}$) and BF loss ($-0.03 \times$ (SID CP $- 127$) $\times$ (SID CP ≤ 127) $- 2.97$) of sows increased and BW loss ($0.007 \times$ (SID CP $- 143$) $\times$ (SID CP ≤ 143) $- 0.58$) decreased with increasing dietary SID CP ($P < 0.001$; data not shown). For a detailed description of these results, see Strathe *et al.* (2017a).

Milk composition and nutrient output

Milk composition was only affected by dietary SID CP at day 17 of lactation. Milk fat and DM contents decreased throughout lactation ($P < 0.001$), and at day 17, milk fat concentration increased linearly with increasing dietary SID CP ($P < 0.05$), as an increase by 1 g SID CP/kg increased the fat content with 0.02 percentage units. Milk lactose concentration on day 17 of lactation decreased with increasing dietary SID CP until a breakpoint at 124 g SID CP/kg (5.3%; $P < 0.001$), but the lactose concentration of the milk did not change throughout lactation ($P=0.15$). Milk protein concentration decreased from day 3 to day 10 of lactation ($P < 0.001$). The concentration of milk protein on day 17 increased until a breakpoint at 136 g SID CP/kg (5.0%; $P < 0.001$). Results of milk yield and composition are given in Table 3.

Body composition of sows

Results of body composition measurements are given in Table 4 and Figure 1. The loss of body water from day 3 of lactation to weaning decreased with increasing dietary SID CP until a dietary SID CP of 130 g/kg ($P < 0.001$), and a similar pattern was seen for the loss of protein, which reached a breakpoint at 128 g SID CP/kg ($P < 0.001$). The body ash loss declined linearly with increasing dietary SID CP ($P < 0.01$), whereas the change in body fat was unaffected by dietary treatment ($P=0.41$).

Plasma metabolites

As expected, the concentrations of metabolites in plasma were similar for all groups at day -4 ($P > 0.05$). The concentrations of PUN increased with increasing dietary SID CP intake (Figure 2; $P < 0.001$). The concentrations of PUN were similar at days 3 and 10, and at days 17 and 24, respectively, and there was an interaction between diet and stage of lactation ($P < 0.05$). In early lactation (day 3 + day 10), PUN increased linearly after the breakpoint at 139 g SID CP/kg at a concentration at 3.8 mmol/l, but in late lactation (day 17 + day 24), PUN increased linearly after a breakpoint
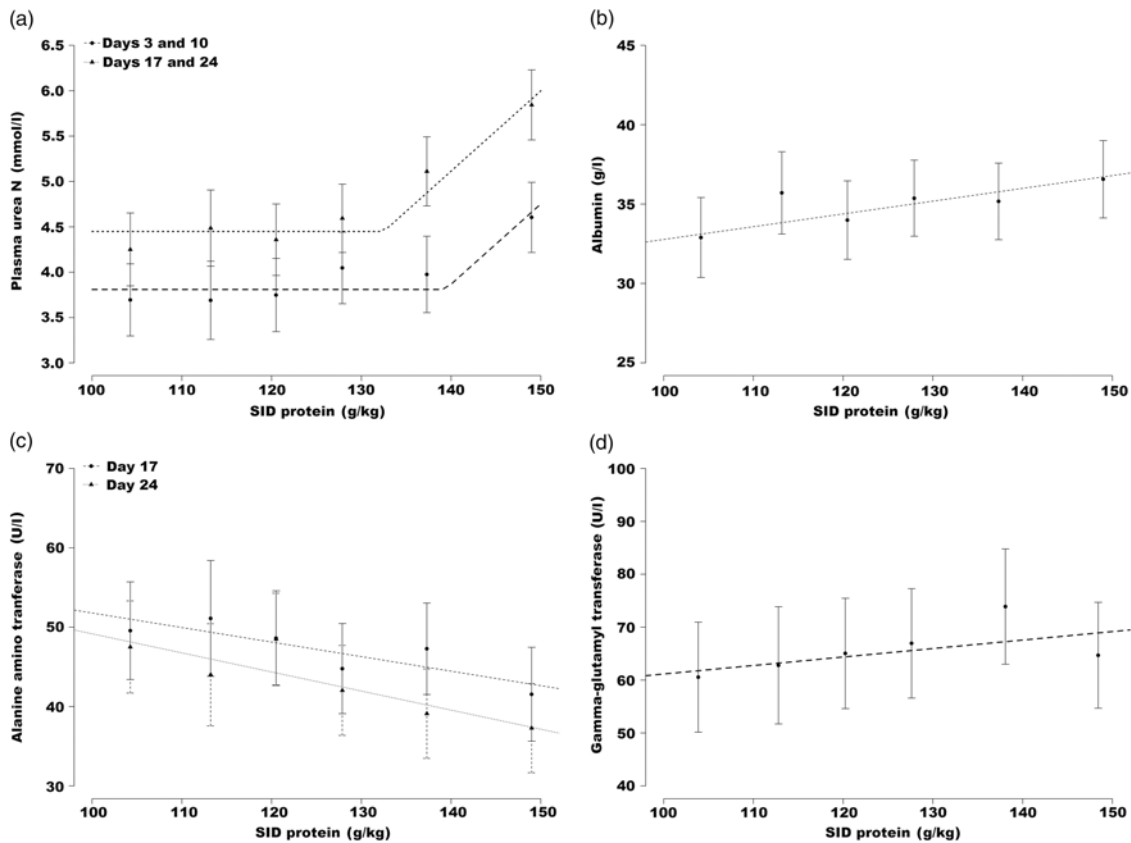
Figure 2 Effect of dietary standardized ileal digestible (**SID**) CP concentrations on the plasma concentrations of metabolites. Data were fitted using linear regression, linear broken-line and quadratic broken-line models using nonlinear mixed-effects models. The model with the best fit is presented in the figure. Data points in the figures are least square means and SE of the six dietary groups, and the linear broken-line model or the linear regression model using data of individual sows are also presented. (a) Urea N was at a stable level until a linear increase from 139 g SID CP/kg ($0.09 \times (SID\ CP - 139) \times (SID\ CP \geq 139) + 3.8$) in early lactation (day 3 + day 10) and at a stable level until a linear increase from 133 g SID CP/kg ($0.09 \times (SID\ CP - 133) \times (SID\ CP \geq 133) + 4.5$) in late (day 17 + day 24) lactation, respectively ($P < 0.001$); (b) albumin increased linearly ($Y = 27.7 + 0.06 \times SID\ CP\ (kg/day)$) at late (day 17 + day 24) lactation ($P < 0.05$); (c) alanine amino transferase increased linearly at day 17 ($Y = 72.8 - 0.23 \times SID\ CP\ (g/kg)$; $P < 0.01$) and day 24 ($Y = 80.1 - 0.26 \times SID\ CP\ (g/kg)$; $P < 0.05$) of lactation and (d) gamma-glutamyl transferase (days 3 + 10 + 17 + 24) increased linearly ($Y = 31.2 + 0.05 \times SID\ CP\ (g/kg)$) with increasing dietary SID CP ($P < 0.05$), respectively.

at 133 g SID CP/kg ($P < 0.001$, Figure 2) at a concentration of 4.5 mmol/l. Albumin concentration increased linearly in late lactation with increasing dietary SID CP (day 17 + day 24; $P < 0.05$), and GGT also increased linearly ($P < 0.05$) at all stages of lactation, whereas the concentration of ALT decreased linearly at day 17 ($P < 0.01$) and day 24 ($P < 0.05$) with increasing dietary SID CP concentration (Figure 2).

The dietary SID CP did not affect plasma concentrations of glucose, lactate, NEFA, triglycerides, cholesterol, creatinine, total protein, AST and alkaline phosphatase ($P > 0.05$; data not shown), whereas all of the abovementioned metabolites were affected by day ($P < 0.001$; data not shown) except for lactate ($P > 0.05$).

Discussion

The current study estimated the SID CP requirement of hyperprolific lactating sows using litter gain as the main determinant supported by measurements on several traits of sow metabolism. Based on the average feed intake and the SID CP concentration of 135 g/kg (Strathe *et al.*, 2017a), the daily requirement for optimal litter ADG was estimated to be 850 g SID CP/day. The 850 g SID CP/day found in the current study was considerably higher than the 700 g SID CP/day estimated in the NRC model (National Research Council, 2012) and slightly lower than the 880 g SID CP/day (±70 g/day) estimated in the requirement model by Strathe *et al.* (2015). The largest difference between the two models is the equation used to determine milk production: the one used by NRC (2012) is based on weigh-suckle-weigh data, which underestimates milk yield and thereby estimates a lower requirement (Hansen *et al.*, 2012).

Milk production

The main purpose of the lactating sow is to produce milk with a nutrient composition and yield that matches the requirements of the piglets. Dietary SID CP concentration did only affect milk nutrient concentration and nutrient yield on day 17 of lactation. The improved ADG of the litter with increasing dietary SID CP most likely was a combined result of increased milk yield throughout lactation and an increased concentration of protein at peak lactation. Many studies have

Table 2 *Effect of increased dietary standardized ileal digestible (SID) CP on sow and piglet performance (n = 92)[1]*

| | SID CP (g/kg) | | | | | | | *P*-value |
	104.3	113.3	120.9	128.5	139.2	150.0	SE	Diet
Parity	2.7	2.7	2.7	2.7	2.7	2.7	0.12	0.95
Feed intake (kg/day)	6.31	6.24	6.52	6.62	6.42	6.34	0.22	0.78
Protein intake (g/day)	808	861	951	1019	1053	1122	33.9	<0.001
SID protein intake (g/day)	656	707	785	847	882	945	28.1	<0.001
Lysine intake (g/day)	42.1	46.4	51.9	57.1	60.3	65.0	2.10	<0.001
SID lysine intake (g/day)	35.7	39.8	44.7	49.4	52.5	56.8	1.81	<0.001
Litter weight day 2 (kg)	24.9	26.4	24.1	24.9	23.7	24.8	1.08	0.47
BW sow day 2 (kg)	239	251	252	248	253	248	6.30	0.31
Back fat sow day 2 (mm)	13.4	14.5	14.2	14.4	14.4	13.7	0.72	0.44
Litter weight day 25 (kg)	85.1	92.6	93.8	93.7	93.0	96.3	4.88	0.31
BW sow day 25 (kg)	221	225	227	235	237	240	6.80	0.05
Back fat sow day 25 (mm)	11.7	11.9	11.8	12.3	11.1	10.6	0.71	0.29

[1] The interaction of Diet × Parity was not significant (*P* > 0.05).

Table 3 *Effect of increased dietary standardized ileal digestible (SID) CP on milk yield, composition and nutrient output of lactating sows[1]*

| | *n* | SID protein (g/kg) | | | | | | SE | *P*-values | |
		104.3	113.3	120.9	128.5	139.2	150.0		Diet	Day
Milk yield[2] (kg/day)	92	10.5	11.2	11.5	11.3	11.4	11.4	0.49	0.26	–
Milk composition										
DM (%)									<0.05	<0.001
Day 3	37	17.9	18.9	18.5	19.2	19.0	18.4	0.62		
Day 10	56	17.2	18.6	18.1	17.9	18.9	17.8	0.50		
Day 17	64	16.5	17.2	17.3	17.1	17.8	17.6	0.47		
Lactose (%)									<0.10	0.15
Day 3	38	5.33	5.18	5.10	5.45	4.99	5.05	0.27		
Day 10	57	5.29	5.11	5.66	5.76	5.14	5.09	0.19		
Day 17	59	5.58	5.47	5.22	5.47	5.44	5.15	0.19		
Fat (%)									0.14	<0.001
Day 3	38	7.36	8.13	8.69	8.70	8.05	7.58	0.67		
Day 10	57	6.79	7.73	7.43	7.46	7.54	7.20	0.43		
Day 17	60	6.06	6.49	6.35	6.63	7.02	6.91	0.43		
Protein (%)									<0.05	<0.001
Day 3	36	5.13	5.54	5.62	5.80	5.59	5.46	0.23		
Day 10	56	4.66	5.02	4.99	4.97	5.08	4.95	0.16		
Day 17	61	4.61	4.72	4.71	4.89	5.10	4.94	0.15		

[1] The interactions of Diet × Day and Diet × Parity and the effect of Parity were not significant (*P* > 0.05).
[2] Estimated from equation by Hansen *et al.* (2012).

proven it difficult to increase the concentration of protein in sow milk above 5.0% to 5.5% (Hurley, 2015). The true milk protein content at the breakpoint on day 17 reached only 5.0%, which was not unusually high compared to other studies (Guan *et al.*, 2004; Laspiur *et al.*, 2009) although it was higher than shown in another recent trial with hyper-prolific sows where the true milk protein content was approximately 4.5% throughout lactation (Pedersen *et al.*, 2016). The higher protein intake resulted in concomitant higher plasma concentrations of GGT, which supported the view that increased milk protein concentration was caused by increasing the uptake of amino acids, because this enzyme has been shown

to be involved in the mammary gland uptake of amino acids in rats (Viña *et al.*, 1981a and 1981b) The piglet has a high rate of muscle growth and therefore a high protein requirement (Theil and Jørgensen, 2016), and the litter gain reached maximum at 135 g SID CP/kg, which is identical to the dietary SID CP concentration which resulted in maximum milk protein content (136 g SID CP/kg).

Changes in body composition
Lactating sows often turn catabolic, and therefore milk nutrients are derived from both diet and body reserves. The decrease in BW loss in the current experiment was mainly a result of

Table 4 *Body composition and changes in body composition in the sows measured by deuterium dilution technique[1]*

	SID CP (g/kg)							P-value
	104.3	113.3	120.9	128.5	139.2	150.0	SE	Diet
Body composition day 3								
Water[2] (kg)	120	125	123	120	126	127	13.1	0.46
Ash (kg)	7.7	8.1	8.0	7.7	8.1	8.2	0.20	0.51
Fat (kg)	56.9	62.9	59.5	61.3	64.2	61.2	3.51	0.59
Protein (kg)	38.9	41.0	40.0	39.4	41.3	41.3	0.97	0.50
Fat:protein (kg/kg)	1.47	1.53	1.50	1.55	1.56	1.49	0.07	0.85
Water (% of BW)	52.3	49.9	51.4	50.0	50.8	50.9	1.39	0.37
Ash (% of BW)	3.3	3.2	3.3	3.2	3.2	3.2	0.11	0.80
Fat (% of BW)	22.1	25.1	23.1	24.2	24.1	24.0	1.23	0.40
Protein (% of BW)	16.7	16.2	16.6	16.3	16.5	16.6	0.39	0.21
Body composition day 24								
Water[2] (kg)	116	120	123	124	126	131	3.99	0.16
Ash (kg)	7.7	7.9	8.1	8.2	8.3	8.8	0.29	0.08
Fat (kg)	46.5	50.6	52.2	47.7	52.8	47.9	3.70	0.55
Protein (kg)	37.1	38.4	39.3	39.1	40.4	41.1	1.28	0.24
Fat:protein (kg/kg)	1.25	1.31	1.33	1.21	1.30	1.15	0.08	0.20
Water (% of BW)	53.1	52.04	52.21	53.5	52.3	54.6	0.96	0.10
Ash (% of BW)	3.5	3.4	3.4	3.6	3.5	3.7	0.09	0.10
Fat (% of BW)	20.9	21.3	22.2	20.0	21.5	19.5	1.17	0.26
Protein (% of BW	16.8	16.5	16.7	16.8	16.7	17.1	0.19	0.13
Change in body composition (% of mass at day 3)2								
Water loss3	2.9	4.2	0.3	−3.2	0.1	−3.5	2.00	<0.01
Fat loss	19.4	20.3	16.1	23.8	20.9	22.9	4.50	0.41
Protein loss	5.8	6.3	1.8	1.0	2.9	0.7	1.92	0.06
Ash loss[3]	0.8	2.2	−1.5	−6.8	−2.8	−7.3	2.43	<0.01

SID=standardized ileal digestible.
[1] The interaction of Diet × Parity was not significant (*P*>0.05). For body composition on days 3 and 24, there was an effect of Parity (*P*<0.05), because of different BW of parity 2 and 3 sows. There was no effect of Parity on changes in body composition (*P*>0.05).
[2] Body water was determined by the technique described by Theil *et al.* (2002).
[3] Negative values indicate a gain.

decreased body protein mobilization, because BF mobilization continued to increase with increased protein intake. A similar response to increased dietary protein was reported by Dourmad *et al.* (1998). This suggests that sows fed high levels of dietary SID CP use the dietary protein as substrate for milk protein and thereby save body protein. The lower protein mobilization in sows fed high SID CP levels were followed by decreased plasma ALT concentrations as a result of the down-regulation of urea cycle enzymes (Das and Waterlow, 1974). Feeding protein-deficient diets results in a reduction of the body protein turnover to ensure a higher utilization of dietary protein. A lower plasma albumin concentration observed in sows fed low SID CP concentrations has also been shown in pigs suffering protein deficiency (Wykes *et al.*, 1996), and this indicated that these sows had a reduced albumin synthesis. In this study, minimum body protein loss (128 SID CP g/kg) occurred at a dietary SID CP concentration close to that where milk protein was maximized (136 g SID CP/kg). In addition, the N balance was optimized at SID CP levels close to these concentrations as indicated by an increase in PUN in both early (139 g SID CP/kg) and late (133 g SID CP/kg) lactation above these concentrations. The increase in PUN concentrations after the breakpoint indicated that protein intake above this dietary protein concentration was in excess of the requirement (Coma *et al.*, 1995 and

1996). The breakpoint for PUN was reached at higher SID CP concentrations in early compared to late lactation, because of the lower daily CP intake in early lactation. Minimizing body protein mobilization during lactation is important, because it can be difficult for the sow to regain the lost body protein during the following gestation. This is in accordance with Dourmad *et al.* (1996), who reported that sows losing 35 *v.* 12 kg during lactation were unable to restore the body reserves, especially body protein, during subsequent gestation.

Body ash is a measure of bone mass, and therefore the lower mobilization of ash with a higher dietary protein intake suggests that bone mobilization was reduced. Low protein intakes reduce intestinal absorption of calcium and increase the concentration of parathyroid hormone, resulting in calcium being released from the bones (Heaney and Layman, 2008). This can negatively affect the longevity of the sows, because leg disorders are a cause of culling in sow herds (Engblom *et al.*, 2007).

In the current study, milk fat content and BF mobilization increased with increasing dietary protein intake, which was in accordance with the results by King *et al.* (1993). This indicated that the mobilized body fat was redirected towards the mammary glands for milk fat production. Back fat loss increased with increased dietary protein, but changes in body fat estimated by D_2O dilution technique was unaffected

by the tested dietary protein levels, which is in accordance with Mejia-Guadarrama et al. (2002). The lacking response of body fat might be ascribed to the used equations (Rozeboom et al., 1994), which were developed on data from gilts. The increased BF mobilization suggests that the sows fed high levels of dietary SID CP most likely were in an energy deficit. Increased energy demand will often result in increased body fat mobilization, when energy intake is not increased at the same rate as the requirement. The six diets were isoenergetic, and this resulted in diets with different ratios between energy and protein. It has been observed in other studies that sows fed high CP levels increase BF mobilization (Tritton et al., 1996; McNamara and Pettigrew, 2002; Heo et al., 2008). However, it was also shown that both increasing dietary CP and energy level could minimize BF loss (Tritton et al., 1996; McNamara and Pettigrew, 2002), which emphasizes the importance of also determining the energy requirement of sows when increasing protein intake.

Conclusions

The SID CP requirement of hyper-prolific lactating sows was estimated at 135 g/kg based on the ADG of the litter. At this level of SID CP, BW loss, body protein loss and body ash loss were considerably reduced, and N balance of the sows measured by PUN was optimized. The higher litter gain was a result of increased milk yield and higher contents of protein and fat in milk at peak lactation.

Acknowledgements

Declaration of interest

None.

Ethics statement

The Danish Animal Experimentation Inspectorate (Authorization No. 2013-15-2934-00961) approved the trial.

Software and data repository resources

None of the data were deposited in an official repository.

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