



Dietary CP and digestion kinetics influence BW loss, litter weight gain, and reproduction by affecting postprandial amino acid metabolism in lactating sows



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ABSTRACT

To avoid a high body protein mobilization in modern lean sows during lactation, an adequate dietary amino acid (AA) supply and an efficient AA utilization are crucial. This study evaluated the effects of dietary CP and *in vitro* protein digestion kinetics on changes in sow body condition, litter weight gain, milk composition, blood metabolites, protein utilization efficiency and subsequent reproductive performance. We hypothesized that a slower digestion of dietary protein would improve AA availability and utilization. In total, 110 multiparous sows were fed one of four lactation diets in a 2 × 2 factorial design, with two CP concentrations: 140 g/kg vs 180 g/kg, and two protein digestion kinetics, expressed as a percentage of slow protein (*in vitro* degradation between 30 and 240 min): 8 vs 16% of total protein. Feeding sows the high CP diets reduced sow weight loss ($\Delta = 7.6$ kg, $P < 0.01$), estimated body fat loss ($\Delta = 2.6$ kg, $P = 0.02$), and estimated body protein loss ($\Delta = 1.0$ kg, $P = 0.08$), but only at a high percentage of slow protein. A higher percentage of slow protein increased litter weight gain throughout lactation ($\Delta = 2.6$ kg, $P = 0.04$) regardless of CP concentrations, whereas a higher CP only increased litter weight gain during week 3 of lactation ($\Delta = 1.2$ kg, $P = 0.01$). On Day 15 postfarrowing, serial blood samples were taken from a subsample of sows fed with the high CP diets. In these sows, a high percentage of slow protein resulted in higher plasma AA concentrations at 150 and 180 min after feeding ($\Delta = 0.89$, $P = 0.02$, $\Delta = 0.78$, $P = 0.03$, mmol/L, respectively) and lower increases in urea at 90 and 120 min after feeding ($\Delta = 0.67$, $P = 0.04$, $\Delta = 0.70$, $P = 0.03$, mmol/L, respectively). The higher dietary CP concentration increased total nitrogen loss to the environment ($\Delta = 604$ g, $P < 0.01$) with a reduction of protein efficiency ($\Delta = 14.8\%$, $P < 0.01$). In the next farrowing, a higher percentage of slow protein increased subsequent liveborn litter size ($\Delta = 0.7$, $P < 0.05$). In conclusion, feeding sows with a high dietary CP concentration alleviated maternal weight loss during lactation when the dietary protein digestion rate was slower, but lowered protein efficiency. A slower protein digestion improved litter weight gain, possibly by reducing AA oxidation and improving plasma AA availability, thus, improving protein efficiency.

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Implications

In modern lean sows, body protein reserves are mobilized to produce milk, which can adversely affect both sow and litter performance. The current study shows that feeding protein sources with a slower digestion rate to lactating sows improved litter weight gain by optimizing postprandial amino acid utilization. A high dietary CP reduced sow body loss and benefited litter weight gain, but this depended on protein digestion kinetics and came at a

higher environmental cost. Thus, applying lactation diets with slower protein digestion may be preferable from a production as well as sustainability point of view.

Introduction

Over the last decades, genetic selection has increased litter size in modern hybrid sows (Kemp et al., 2018). To support milk production for increasingly larger litters, sows need to mobilize their body reserves as their voluntary feed intake does not cover their nutritional demands during lactation (Tokach et al., 2019). Moreover, as body fat reserves have decreased in modern lean geno-

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types, sow BW loss during lactation increasingly consists of protein loss (Ball et al., 2008). High maternal protein loss has been associated with decreased piglet growth and lower milk protein concentration in the ongoing lactation (Clowes et al., 2003). Furthermore, a high BW loss during lactation adversely affects sow reproductive performance parameters, including a prolonged weaning-to-estrus interval (WOI), a reduced farrowing rate at the first postweaning estrus and litter size (Quesnel et al., 2005; Thaker and Bilkei, 2005; Schenkel et al., 2010), and an increased within-litter variation in birth weight in the subsequent cycle (Wientjes et al., 2013).

Dietary protein that supplies sufficient amino acids (AAs) with appropriate composition is therefore crucial for both sow and litter performance. Elevating dietary CP can reduce sow BW losses, thereby improving reproductive performance parameters (Huang et al., 2013), and improving litter weight gain during lactation (Quesnel et al., 2005; Strathe et al., 2017). However, a higher dietary CP content may also have adverse consequences for the animals and the environment. For instance, a high dietary CP can increase protein fermentation in the hindgut, and amplify the production of detrimental metabolites, such as cadaverine and sulfides (Mu et al., 2016). In addition, a high dietary CP can increase nitrogen losses in animal excreta (Pedersen et al., 2019), with a negative impact on the environment (Oenema, 2004). Thus, improvement of sow dietary protein utilization is preferred over a higher dietary CP concentration. The proteolysis pattern of protein in the small intestine, known as protein digestion kinetics, was indicated to be a critical factor affecting dietary protein utilization (Dangin et al., 2001; Heyer et al., 2022). Previous research with humans has shown that proteins that are slowly digested (e.g. casein) saved body protein breakdown during the postprandial period compared to proteins with a faster digestion (e.g. whey protein) (Dangin et al., 2001; Fouillet et al., 2009). Similar beneficial effects of a slow digestion of dietary protein emerged in our previous study using multiparous sows (Ye et al., 2022); feeding a higher percentage of slow digestion protein in total protein reduced sow protein tissue loss (i.e. reduced loin muscle depth losses) and AA oxidation after feeding, as suggested by lower postfeeding plasma urea concentrations during lactation (Ye et al., 2022). Questions that remained were if these beneficial effects of slowly degraded proteins depend on total dietary CP concentrations and if the positive effects on protein loss translate into reproductive benefits.

Therefore, in this study, we investigated the effects of protein digestion kinetics and CP concentrations in lactation diets on sow and litter characteristics, in a 2×2 factorial design. Diets contained low or high dietary CP (140 g/kg vs 180 g/kg CP) and a low or high percentage of slow protein (8 vs 16% in total protein). We expected that both a high dietary CP and a high percentage of slow protein in total protein will benefit sow milk production and will reduce sow BW loss, thereby benefiting subsequent farrowing rate and litter size.

Material and methods

Animals, housing and management

The experiment was conducted at the Swine Research Facility of Trouw Nutrition R&D (Boxmeer, the Netherlands). A total of 110 multiparous sows of a Yorkshire \times Landrace genetic line (Hypor Libra, Hendrix Genetics, Boxmeer, the Netherlands) were used in four consecutive cohorts. Sows were moved to the farrowing rooms 6.9 \pm 0.1 days prior to expected farrowing and were housed in crates in individual farrowing pens. The temperature of the farrowing rooms was set at 22 °C around farrowing and gradually decreased to 20 °C at weaning, and lights were on from 0600–2200 h.

After parturition (Day 0), piglets were treated according to the routine management practices on the farm, which included tail docking within 24 h after birth. For sows that completed parturition after 1630 h, the next day was considered as Day 0. Piglets with birth weight lower than 750 g were euthanized. Within 48 h after farrowing, litters were standardized to 13–15 piglets, depending on the number of functional teats, and taking care that litter size was similar across treatments. To maintain litter size during lactation, any mortalities in litters were compensated by transferring a piglet with birth weight > 1 000 g from reserve sows. In all sows, weaning took place at Day 22.1 \pm 0.1 postfarrowing.

From Day 1 postweaning, sows were moved to the gestating room and monitored for signs of standing estrus twice per day using a boar. Once the sows showed standing estrus, the date was recorded and artificial inseminations were performed once/day until the end of the estrus. The room temperature during gestation was controlled at 20 °C. In the next farrowing, the liveborn litter size, total born litter size and the number of stillborn piglets were recorded.

Feeding and diets

Sows were allocated to treatments ensuring equal distribution of parity (4.0 \pm 0.2), BW (271.5 \pm 2.5 kg), estimated body protein (43.8 \pm 0.4 kg) and estimated body fat reserves (50.6 \pm 0.7 kg) on the day of entering the farrowing crates. There were two concentrations of dietary CP (140 or 180 g/kg) and 2 concentrations of slow protein as a percentage of total protein (8 or 16%). The change in dietary CP was achieved mostly by changes in soybean meal inclusion and synthetic amino acid inclusion while the change in percentage of slow protein to total protein contrast to fast protein was achieved mostly by replacing rapeseed meal with sunflower seed meal and wheat with maize (See Table 1). The percentages of slow protein in total protein were used to differentiate protein sources with varying protein digestion rates. The percentage of fast, slow and resistant protein were defined as the nitrogen solubility reached at cut-off time points in an *in vitro* digestion model (fast: 0–30 min; slow: 30 – 240 min; resistant: undigested protein at 240 min), which was previously established for a range of ingredients based on Chen et al. (2019). All diets were formulated to contain the same net energy level and apparent total tract digestible phosphorus concentration. Ileal digestible essential AAs were formulated at or above the estimated requirements. Low CP diets had a lower level of non-essential AAs but a similar level of essential AAs compared to high CP diets.

After entering the farrowing crates, sows received 3.5 kg/d of the allocated experimental lactation diet. On Day 1 postfarrowing, sows received 3.0 kg/d of their allocated experimental lactation diets, and the daily feed allowance was then increased stepwise to 7.7 kg/d on Day 21 postfarrowing. The feed curves were strictly followed to ensure equal intake across treatments. Sows had free access to water throughout the experimental period. The daily feed intake was calculated as feed allowance minus feed refusal, if any. During the following gestating period, all sows were fed the same diet.

BW, backfat and loin muscle

Sow BW and backfat and loin muscle thickness were measured on Day 1, Day 7, Day 14, and Day 21 postfarrowing before the morning feeding. The thickness of backfat and loin muscle were measured with a Brightness mode ultrasound device (MyLab™-touchVet, Esaote S.p.A, Genoa, Italy) equipped with a 15 cm, 3.5 MHz probe. The probe was placed 50 mm from the midline at the last rib and the backfat and loin muscle thicknesses were

Table 1
Ingredients and nutrient composition of experimental diets (as fed basis) for lactating sows.

Item	L _{cp} L _{kin}	L _{cp} H _{kin}	H _{cp} L _{kin}	H _{cp} H _{kin}
Ingredients (%)				
Wheat	34.47	–	39.43	35.93
Maize	14.39	40.06	5.05	23.23
Rye	23.90	34.00	20.30	3.80
Sunflower meal 38% CP	9.66	–	21.20	–
Soybean meal 46% CP	2.02	7.07	–	21.24
Soya hulls	–	7.61	–	7.90
Rapeseed meal, CP < 38%	–	1.52	5.76	–
Calcium Carbonate	1.53	1.45	1.37	1.40
Soybean oil	1.27	0.60	2.92	0.96
Wheat bran	5.10	–	–	–
Monocalcium phosphate	1.09	1.24	1.01	1.03
Potato protein, Ash < 1.0%	–	4.00	–	–
Diamol DI 100 K	1.00	1.00	1.00	1.00
Oats grain	3.60	–	–	–
Palm kernel flakes CF < 180	–	–	–	2.58
Na Bicarbonate	0.79	0.69	0.51	–
L-Lysine HCl 98%	0.52	0.23	0.37	0.10
Premix ^a	0.25	0.25	0.25	0.25
Salt (NaCl)	–	0.08	0.18	0.55
Wheat gluten meal	–	–	0.62	–
L-Threonine 98%	0.16	0.12	0.03	–
L-Iso + L-Leu	0.11	–	–	–
DL-Methionine 99%	0.03	0.05	–	0.02
Enzyme premixture ^b	0.02	0.02	0.02	0.02
L-Valine 96.5%	0.06	–	–	–
L-Tryptophan 98%	0.02	0.03	–	–
Total	100	100	100	100
Composition (Formulated)				
DM (g/kg)	886	887	900	886
CP (g/kg)	140	140	180	180
Net energy (MJ/kg)	9.7	9.7	9.7	9.6
Digestible energy (MJ/kg)	13.4	13.7	13.8	14.1
Metabolizable energy (MJ/kg)	13.1	13.3	13.4	13.5
Total dietary fiber (%)	17.5	17.4	18.8	18.7
Crude fiber (%)	4.3	4.7	6.0	5.4
Crude fat (%)	3.8	3.5	5.1	3.8
Total AA (g/kg)	122	132	157	169
SID Lysine (g/kg)	8.1	8.0	8.1	8.3
SID Methionine + Cysteine (g/kg)	4.7	4.7	6.0	5.1
SID Methionine (g/kg)	2.5	2.7	3.1	2.5
SID Cysteine (g/kg)	2.2	2.0	2.8	2.6
SID Threonine (g/kg)	5.2	5.9	5.2	5.3
SID Tryptophan (g/kg)	1.6	1.6	1.9	1.9
SID Valine (g/kg)	5.7	6.0	6.9	7.1
SID Isoleucine (g/kg)	4.4	5.0	5.6	6.3
SID Leucine (g/kg)	8.6	10.5	9.7	11.8
SID Tyrosine (g/kg)	3.2	4.6	3.9	5.4
SID Phenylalanine (g/kg)	5.1	6.0	6.6	7.4
SID Arginine (g/kg)	7.3	6.8	10.5	10.2
Slow Protein (% of total protein)	8.0	16.0	8.0	16.0

L_{cp}L_{kin} = low CP (140 g/kg) and low percentage of slow protein (8%) diet, L_{cp}H_{kin} = low CP (140 g/kg) and high percentage of slow protein (16%) diet, H_{cp}L_{kin} = high CP (180 g/kg) and low percentage of slow protein diet (8%), H_{cp}H_{kin} = high CP (180 g/kg) and high percentage of slow protein diet (16%), AA = amino acids, SID = standardized ileal digestibility.

Nutrients in diets were based on CVB (2018).

^a FARMIX ZEUGEN (Trouw Nutrition, Putten, Netherlands).

^b Enzyme Axta PHY 10000 T Premixture (Trouw Nutrition, Putten, Netherlands).

recorded at both sides of the midline. On the same days, the individual BW of the piglets were recorded.

Collection of blood, milk and feces samples

On Day 12 postfarrowing, 30 ml of milk was collected from each sow. First, 4 ml oxytocin (20 IU) was injected i.m. to induce milk let-down, and then, milk was collected in equal proportions from all functional teats and pooled in 50 ml tubes for each sow. Samples were stored at – 20 °C until analysis for urea, lactose, fat and protein.

On Day 14 postfarrowing, a 10 ml blood sample was collected by jugular vein puncture, before and 4 h after the morning feeding,

respectively, using heparin-coated tubes. The samples were placed in an ice box for transportation to the laboratory and centrifuged at 3 000 × g for 15 min at 4 °C. After centrifugation, the plasma samples were stored at –20 °C prior to the assays of non-esterified fatty acid (NEFA), urea, creatinine and IGF-1.

In cohorts 2 and 3, a total of 8 sows fed with a low percentage of slow protein at high dietary CP and 8 fed with a high percentage of slow protein at high dietary CP sows of similar parity (3.9 ± 1.9) were fitted with semi-permanent ear vein catheters on Day 14 for serial blood sample collection on Day 15. In brief, sows were treated with 5 ml analgesic (Novem, Boehringer Ingelheim, Ingelheim am Rhein, Germany), and then, a 1 × 1.5-mm PVC catheter (Microtube Extrusions, New South Wales, Australia) was inserted

50 cm into a lateral or intermediate auricular vein. After an overnight rest, serial blood samples were collected before the first-morning feeding (T0) and at 30, 60, 90, 120, 150, 180, 240 and 300 min relative to feeding (T30, T60, T90, T120, T150, T180, T240, T300, respectively), with 10 ml per sample. No extra feed was given to the sows until the serial blood sampling was finished. Filled tubes were placed in an ice box for transportation to the laboratory and centrifuged at $3\,000 \times g$ for 15 min at 4 °C. After centrifugation, plasma samples were stored at -20 °C prior to the assay of total AA and urea concentrations. In addition, samples that were collected at T0 and T240 were assayed for non-esterified fatty acid, creatinine and IGF-1, as for the blood samples collected from other sows on Day 14 postfarrowing.

On Day 11.1 ± 0.2 postfarrowing, feces samples were taken from the rectum for analysis of nitrogen and acid-insoluble ash (AIA). In our previous study (Ye et al., 2022), in which feces samples were collected on Days 6, 13 and 20 postfarrowing, the days of lactation did not affect the CP digestibility (88.6, 87.6 and 88.1, %, respectively, original data not shown). Additionally, the digestibility assessment is considered reliable based on a sufficient number of samples. Thus, the feces samples were collected only once in the middle of lactation (Day 11.1 ± 0.2) in the current study.

Chemical analyses

The Dumas method (ISO 16634-1, 2008) was used to determine the nitrogen (N) concentration of feed and fecal samples, and protein was calculated as $N \times 6.25$. AIA was selected as the internal digestibility marker in this study and was spiked by adding diatomaceous earth. The recovery rate of AIA across different studies in pigs ranged from 0.91 to 1.00 as reviewed by Sales and Janssens (2003). The AIA concentration of feed and fecal samples was measured as described by Atkinson et al. (1984).

Plasma samples were thawed before analyses. The urea and creatinine concentrations in sow blood plasma were measured by using corresponding commercial colorimetric kits (HUMAN, Wiesbaden, Germany) with a UV-Visible Spectrophotometer (Evolution™ 201, Thermo Scientific, Waltham, USA). The intra-assay CVs for urea and creatinine analyses were 4.5 and 3.3%, respectively, and their detection levels were 0.8 mmol/L, and 31 $\mu\text{mol/L}$, respectively. Sow plasma NEFA was measured by a commercial colorimetric kit (Wako Chemicals, Neuss, Germany), with inter-assay CV and detection level at 2.0% and 10 $\mu\text{mol/L}$, respectively. The concentration of IGF-1 in sow plasma was detected by an ELISA kit (Cloud-Clone Corp., US; detection level = 3.12 ng/ml; no replicates), and the concentration of total AA was measured according to Lee and Takahashi (1966) (Inter-assays CV = 7.5%). The IGF-1, NEFA and total AA were measured by a UV/VIS spectrophotometer (Multiskan® GO, Thermo Scientific, Waltham, USA).

Sow milk samples were analyzed for protein, fat, urea, and lactose concentrations using Fourier Transform mid InfraRed (FTIR) spectrophotometry, by a qualified external laboratory (Qlip, Zutphen, Netherlands).

Calculations

Protein digestibility was calculated as follows:

$$\text{Digestibility (\%)} = 100 - \left[\frac{(\text{CIn}_{\text{input}} \times \text{CC}_{\text{output}}) / (\text{CC}_{\text{input}} \times \text{Cl}_{\text{output}})}{\text{Cl}_{\text{input}}} \right] \times 100,$$

where $\text{CIn}_{\text{input}}$ and $\text{Cl}_{\text{output}}$ were the concentration of AIA in feed and feces, respectively; CC_{input} and $\text{CC}_{\text{output}}$ were the concentration of components in feed and feces, respectively.

To estimate sow body protein and fat mass, the equations of Dourmad et al. (1997) were used, considering their estimations

were based on a high number of sows and are still valid for current sows (van der Peet-Schwering and Bikker, 2019; Muller et al., 2021). Body protein and fat mass were estimated as follows: Protein (kg) = $2.28 + (0.178 \times 0.96 \times \text{Sow BW, kg}) - (0.333 \times \text{backfat, mm})$ and Fat (kg) = $-26.4 + (0.221 \times 0.96 \times \text{Sow BW, kg}) + (1.331 \times \text{backfat, mm})$ (Dourmad et al., 1997).

Litter weight gain as a proxy for milk production was calculated as the sum of the individual piglet gains from birth to weaning. Piglets did not receive any creep feed during lactation. The piglet gains before and after the cross-fostering were added separately to the corresponding litters. Weight gain for piglets with decreased BW in a certain lactating period was corrected to 0 in the litter gain calculation. The BW of piglets taken out of the experiment because of death or weakness was recorded to calculate the gain until removal, and the gain of replacement piglets was calculated from the time of replacement.

The total nitrogen loss of sows to the environment (g) was calculated as dietary nitrogen intake, g + nitrogen mobilized from body reserves, g – nitrogen output in milk, g, where nitrogen mobilized from body reserves = estimated protein loss / 6.25, and the total nitrogen output in milk was calculated according to Noblet and Etienne (1989): milk nitrogen output = $((0.0257 \times \text{AGD, g/d}) + (0.42 + \text{LS})) \times 19, \text{ d}$, where the AGD = average daily weight gain, LS = litter size at weaning. The nitrogen output in feces (g) was calculated as total dietary nitrogen intake, g * (1 – dietary protein digestibility), and the nitrogen in urine as total nitrogen loss, g – nitrogen in feces, g.

Additionally, the protein utilization was defined as follows: protein output in milk, g/d / (digestible protein intake, g/d + maternal protein loss, g/d – protein for sow maintenance, g/d, where protein for sow maintenance = $1.32 \times ((\text{Sow BW on Day 1 postfarrowing} + \text{Sow BW on Day 21 postfarrowing})/2)^{0.75}$) (Dourmad et al., 2008).

Statistics

Relationships between sow and litter characteristics were analyzed with SAS OnDemand for Academics (SAS Institute Inc., Cary, North Carolina, USA), using MIXED models. Data normality was checked as the model assumption using the UNIVARIATE procedure.

To analyze the effects of dietary CP, protein digestion kinetics and their interactions on feed intake, digestible protein intake and protein efficiency between Day 1 to Day 21 postfarrowing and sow subsequent reproductive performance, the following MIXED model was used:

$$Y = \mu + \text{dietary CP} + \text{protein digestion kinetics} + \text{dietary CP} \times \text{protein digestion kinetics} + \text{parity classes} + e \text{ (Model 1)}$$

where Y = average daily feed intake (ADFI), net energy intake, digestible protein intake, protein efficiency Day 1 to Day 21 postfarrowing, WOI, total born litter size, or total born litter weight in the next farrowing, μ = overall mean, dietary CP = high (180 g/kg CP in the diet, n = 56) or low (140 g/kg CP in the diet, n = 54), protein digestion kinetics = high (16%, N = 54) or low (8%, n = 56) percentage of slow protein in total protein, parity classes = parity 2 (n = 30), parity 3 (n = 25), parity 4–6 (n = 41), or parity 7–9 (n = 14), e = residual error. Cohorts (1–4) were included as a random factor.

Subsequently, a series of models were established based on Model 1. ADFI was added as a covariate to Model 1 to analyze the effects on sow body losses including BW, backfat thickness, loin muscle thickness, estimated body protein and estimated body fat losses, on sow blood plasma concentrations of IGF-1, NEFA, creatinine and urea on Day 14, on milk composition including milk fat,

protein and lactose concentrations on Day 12, on nitrogen loss to environments and nitrogen output in feces and urine.

Litter weight postcross fostering (Day 2 postfarrowing) and ADFI were added as covariates to Model 1 to analyze the effects on litter weight gain during lactation and different weeks of lactation.

Concerning the measurements at the next farrowing, liveborn litter size and liveborn litter weight were corrected for total born litter size adding it as a covariate to Model 1.

For all models, interactions between CP, protein digestion kinetics and parity class were excluded from the model if they were not significant. Results are presented as least square means ± SEM in all MIXED models.

Plasma urea and AA concentrations and postprandial increases in AA and urea sows fed with low and high percentages of slow protein at high dietary CP from T0 to T300 after the morning feeding were compared in MIXED models as follows:

$$Y = \mu + \text{treatment} + \text{time} + \text{treatment} * \text{time} + e$$

where Y = plasma urea, AA concentrations, postprandial increases in urea, postprandial increases in AA relative to T0, treatment = low (n = 8) or high (n = 8) percentage of slow protein at high dietary CP, e = residual. Sows were included in the model as a random factor. The repeated measure option was not used.

The codes of all statistical models were displayed in [Supplementary Material S1](#).

Results

Feed intake

Sows fed with a high percentage of slow protein tended to have a lower ADFI than sows fed with a low percentage of slow protein ($\Delta = 0.11$ kg/d, $P = 0.07$, [Table 2](#)). Sows fed a high CP diet had significantly higher digestible protein intake than sows fed a low CP diet ($\Delta = 213$ g/d, $P < 0.01$, [Table 2](#)). Sows–fed diets with a high percentage of slow protein had a lower formulated net energy intake than sows–fed diets with a low percentage of slow protein ($\Delta = 1.4$ MJ/d, $P = 0.02$, [Table 2](#)).

Sow BW, backfat and loin muscle losses

On Day 1 postfarrowing, sow BW, loin muscle and backfat thicknesses, estimated body protein and fat reserves were similar across treatments.

During the 21-day lactation, interactions between dietary CP and protein digestion kinetics were found in sow BW and estimated body protein and fat losses: at a high percentage of slow protein, the higher dietary CP reduced BW loss (in kilogram $\Delta = 7.6$ kg, $P < 0.01$, in percentage $\Delta = 3.3\%$, $P < 0.01$, respectively)

Table 2

ADFI, digestible protein and net energy intake between Day 1 and Day 21 postfarrowing in sows fed with different lactation diets (Least square means ± SEM).

Item	Lactation diets				SEM	P-value ^a		
	L _{cp} L _{kin}	L _{cp} H _{kin}	H _{cp} L _{kin}	H _{cp} H _{kin}		CP	Kin	Par
Sows (n)	29	25	27	29				
ADFI (kg/d)	5.68	5.56	5.67	5.57	0.09	0.95	0.07	0.33
Measured dietary CP (g/kg)	136	138	172	177				
Digestible protein intake (g/d) ^b	667	650	865	876	12	< 0.01	0.71	0.17
Net energy intake (MJ/d)	54.9	53.6	55.1	53.6	0.9	0.88	0.02	0.34

L_{cp}L_{kin} = low CP and low percentage of slow protein diet, L_{cp}H_{kin} = low CP and high percentage of slow protein diet, H_{cp}L_{kin} = high CP and low percentage of slow protein diet, H_{cp}H_{kin} = high CP and high percentage of slow protein diet, CP = low(140 g/kg)/high(180 g/kg), Kin = Dietary protein digestion kinetics [8% slow protein in total CP/16% slow protein in total CP], Par = parity classes[parity 2/3/4–6/7–9]. ADFI = average daily feed intake.

^a P-values for main effects with interaction being excluded.

^b Calculations based on apparent protein digestibility L_{cp}L_{kin}/L_{cp}H_{kin}/H_{cp}L_{kin}/H_{cp}H_{kin} = 86.7/85.1/88.5/88.8.

and estimated body fat ($\Delta = 2.6$ kg, $P = 0.02$) loss, and tended to reduce estimated body protein loss ($\Delta = 1.0$ kg, $P = 0.08$) ([Table 3](#)). The loss in loin muscle and backfat thickness did not show significant differences between diets ([Table 3](#)).

Litter weight gain

At the start of lactation (Day 2), litter size and litter weights were not different between the different diets ([Table 4](#)). At weaning, litter sizes were also similar across treatments. No interactions between dietary CP and protein digestion kinetics were found on litter weight gain throughout the lactating period. A high dietary CP increased litter weight gain only in week 3 of lactation ($\Delta = 1.2$ kg, $P = 0.01$; [Table 4](#)). A high percentage of slow protein improved litter weight gain by 2.6 kg over the whole lactation ($P = 0.04$, [Table 4](#)), similarly in all 3 weeks (week 1: $\Delta = 1.1$ kg, $P = 0.09$, week 2: $\Delta = 0.9$ kg, $P = 0.09$, week 3: $\Delta = 1.1$ kg, $P = 0.03$, [Table 4](#)).

Milk composition

On Day 12 postfarrowing, neither dietary CP nor protein digestion kinetics showed significant effects on milk fat and lactose concentrations ([Table 5](#)). An interaction between dietary CP and protein digestion kinetics on milk protein concentration was found: a higher dietary CP increased milk protein concentration only at a low percentage of slow protein ($\Delta = 0.28\%$, $P = 0.01$, [Table 5](#)). Besides, high dietary CP increased sow milk urea concentration on Day 12 postfarrowing compared to low dietary CP ($\Delta = 15.3$ mg/L, $P < 0.01$, [Table 5](#)), whereas protein digestion kinetics did not affect milk urea concentration.

Plasma metabolites and IGF-1

On Day 14 postfarrowing, no interactions were found between dietary CP and protein digestion kinetics as to their effect on sow blood plasma metabolites. A high dietary CP induced both higher prefeeding and postfeeding plasma urea concentrations ($\Delta = 2.0$ mmol/L, $P < 0.01$, $\Delta = 2.2$ mmol/L, $P < 0.01$, respectively; [Table 6](#)). A high percentage of slow protein reduced the postprandial increase in plasma urea relative to the basal concentration compared to a low percentage of slow protein ($\Delta = 0.15$ mmol/L, $P = 0.04$, [Table 6](#)). For plasma creatinine and NEFA, neither dietary CP nor protein digestion kinetics showed significant effects on prefeeding or postfeeding concentrations, or on postprandial changes, except that high dietary CP tended to increase prefeeding NEFA compared to low dietary CP ($\Delta = 119.9$ μ mol/L, $P = 0.07$; [Table 6](#)). A high dietary CP led to higher postprandial increase of IGF-1 ($\Delta = 35.7$ ng/mL, $P < 0.01$; [Table 6](#)) and postfeeding plasma IGF-1 than a low dietary CP ($\Delta = 20.8$ ng/mL, $P = 0.03$; [Table 6](#)). A high percentage of slow protein resulted in both lower prefeed-

Table 3
Sow body condition parameters on Day 1 postfarrowing and sow body losses during 21-day lactation of sows fed with different lactation diets (Least square means ± SEM).

Item	Lactation diets				SEM	P-value ^c			
	L _{cp} L _{kin}	L _{cp} H _{kin}	H _{cp} L _{kin}	H _{cp} H _{kin}		CP	Kin	CP*kin	Par
Sows (n)	29	25	27	29					
Parity	4.0	3.6	4.0	4.1	0.4	0.47	0.73		–
Day 1 postfarrowing									
BW (kg)	254.0	249.0	254.7	252.2	4.4	0.59	0.30		< 0.01
Loin muscle (mm)	55.3	55.3	57.3	54.9	1.3	0.40	0.22		0.63
Backfat (mm)	14.9	15.0	14.5	14.7	0.6	0.58	0.87		0.02
Estimated body protein (kg)	40.7	39.8	41.0	40.5	0.8	0.45	0.26		< 0.01
Estimated body fat (kg)	47.3	46.4	46.9	46.6	1.3	0.95	0.61		< 0.01
Day 1 to Day 21 postfarrowing									
BW loss (kg)	24.0 ^{ab}	26.9 ^a	24.0 ^{ab}	19.4 ^b	2.0	0.01	0.59	0.01	0.19
BW loss (%)	9.5 ^{ab}	11.0 ^a	9.5 ^{ab}	7.7 ^b	0.8	< 0.01	0.76	0.01	< 0.01
Loin muscle loss (mm)	6.2	6.1	6.2	4.7	1.0	0.47	0.41		0.27
Backfat loss (mm)	3.2	3.7	3.9	3.7	0.5	0.51	0.45		0.16
Estimated body protein loss (kg)	3.1 ^{xy}	3.3 ^x	3.0 ^{xy}	2.3 ^y	0.3	0.03	0.37	0.08	0.39
Estimated body fat loss (kg)	9.5 ^{ab}	10.8 ^a	9.7 ^{ab}	8.3 ^b	0.8	0.07	0.94	0.01	0.08

L_{cp}L_{kin} = low CP and low percentage of slow protein diet, L_{cp}H_{kin} = low CP and high percentage of slow protein diet, H_{cp}L_{kin} = high CP and low percentage of slow protein diet, H_{cp}H_{kin} = high CP and high percentage of slow protein diet, CP = low(140 g/kg)/high(180 g/kg), Kin = Dietary protein digestion kinetics [8% slow protein in total CP/16% slow protein in total CP], Par = parity classes[parity 2/3/4–6/7to9].

^{ab} Least square means with different superscripts differ significantly at P < 0.05.

^{xy} Least square means with different superscripts differ significantly at P < 0.10.

^c P-values for main effects with interaction being excluded if CP*kin is not shown.

Table 4
Litter size and weight on Day 2 postfarrowing, litter weight gain during 21-day lactation of sows fed with different lactation diets (Least square means ± SEM).

Item	Lactation diets				SEM	P-value ^a		
	L _{cp} L _{kin}	L _{cp} H _{kin}	H _{cp} L _{kin}	H _{cp} H _{kin}		CP	Kin	Par
Sows (n)	29	25	27	29				
Litter weight on Day 2 postfarrowing (kg)	25.9	26.5	26.1	24.7	0.8	0.32	0.62	0.35
Litter size on Day 2 postfarrowing	13.9	14.2	14.1	14.0	0.2	0.88	0.61	0.83
Litter size on Day 21 postfarrowing	14.2	14.3	14.2	14.2	0.1	0.57	0.68	0.47
Litter weight gain Day 1 to Day 21 (kg)	68.6	71.3	70.6	73.0	1.3	0.13	0.04	< 0.01
Litter weight gain Week 1 (kg)	20.5	21.4	20.0	21.3	0.7	0.64	0.09	< 0.01
Litter weight gain Week 2 (kg)	23.3	24.0	23.7	24.9	0.6	0.24	0.09	0.02
Litter weight gain Week 3 (kg)	24.9	25.8	26.0	27.1	0.5	0.01	0.03	0.29

L_{cp}L_{kin} = low CP and low percentage of slow protein diet, L_{cp}H_{kin} = low CP and high percentage of slow protein diet, H_{cp}L_{kin} = high CP and low percentage of slow protein diet, H_{cp}H_{kin} = high CP and high percentage of slow protein diet, CP = low(140 g/kg)/high(180 g/kg), Kin = Dietary protein digestion kinetics [8% slow protein in total CP/16% slow protein in total CP], Par = parity classes[parity 2/3/4–6/7to9].

^a P-values for main effects with interaction being excluded.

Table 5
Milk fat, protein, lactose and urea concentrations of sows fed with different lactation diets on Day 12 postfarrowing (Least square means ± SEM).

Item	Lactation diets				SEM	P-value ^c			
	L _{cp} L _{kin}	L _{cp} H _{kin}	H _{cp} L _{kin}	H _{cp} H _{kin}		CP	Kin	CP*kin	Par
N	29	25	27	29					
Milk fat (%)	9.19	8.91	8.52	9.32	0.06	0.60	0.29		0.70
Milk protein (%)	4.56 ^a	4.76 ^{ab}	4.84 ^b	4.74 ^{ab}	0.06	0.03	0.39	0.01	0.94
Milk lactose (%)	5.56	5.60	5.57	5.5	0.05	0.24	0.70		0.57
Milk urea (mg/L)	114.0	107.4	125.5	126.7	3.3	< 0.01	0.30		0.18

L_{cp}L_{kin} = low CP and low percentage of slow protein diet, L_{cp}H_{kin} = low CP and high percentage of slow protein diet, H_{cp}L_{kin} = high CP and low percentage of slow protein diet, H_{cp}H_{kin} = high CP and high percentage of slow protein diet, CP = low(140 g/kg)/high(180 g/kg), Kin = Dietary protein digestion kinetics [8% slow protein in total CP/16% slow protein in total CP], Par = parity classes[parity 2/3/4–6/7to9].

^{ab} Least square means with different superscripts differ significantly at P < 0.05.

^c P-values for main effects with interaction being excluded if CP*kin is not shown.

ing and postfeeding IGF-1 concentrations compared to a low percentage of slow protein ($\Delta = 16.4$ ng/mL, $P = 0.03$, $\Delta = 19.9$ ng/mL, $P = 0.03$, respectively; Table 6).

Postprandial plasma amino acids and urea concentrations

Serial blood samples were collected on Day 15 postfarrowing in sows fed with a high dietary CP. A high percentage of slow protein

induced numerically higher plasma AA concentrations at all times, with differences becoming significant at T150 and T180 ($\Delta = 0.89$ mmol/L, $P = 0.02$, $\Delta = 0.78$ mmol/L, $P = 0.04$, respectively; Fig. 1A). Compared to the basal concentration at T0, a higher percentage of slow protein elicited significantly higher plasma AA concentrations from T60 until T300 (Fig. 1A), while a low percentage of slow protein only led to higher plasma AA concentrations from T60 to T90 and T180 to T300 (Fig. 1A). Postprandial increases

Table 6

Plasma urea, creatinine, NEFA and IGF-1 concentrations before and 4 h after feeding in sows fed with different lactation diets on Day 14 postfarrowing (Least square means ± SEM).

Item	Lactation diets				SEM	P-value ^a		
	L _{cp} L _{kin}	L _{cp} H _{kin}	H _{cp} L _{kin}	H _{cp} H _{kin}		CP	Kin	Par
N	54	56	56	54				
Urea (mmol/L)								
Prefeeding,	4.1	4.0	5.9	6.2	0.2	< 0.01	0.69	0.18
Postfeeding,	4.8	4.4	6.9	6.8	0.3	< 0.01	0.21	0.50
Postprandial changes,	0.7	0.5	1.0	0.6	0.2	0.11	0.04	0.66
Creatinine (μmol/L)								
Prefeeding,	162.5	160.6	159.4	151.6	3.8	0.11	0.19	0.42
Postfeeding,	162.0	159.9	162.1	157.6	4.9	0.77	0.35	0.65
Postprandial changes,	-0.4	-1.1	2.7	6.2	4.0	0.13	0.66	0.11
NEFA (μmol/L)								
Prefeeding,	375.8	377.7	444.7	550.3	72.9	0.07	0.41	0.76
Postfeeding,	171.2	209.3	206.5	209.3	26.1	0.48	0.44	0.49
Postprandial changes,	-205.4	-173.8	-237.2	-340.2	76.5	0.15	0.57	0.66
IGF-1 (ng/ml)								
Prefeeding,	165.4	128.9	135.9	125.9	22.7	0.12	0.03	0.30
Postfeeding,	129.0	98.9	140.3	129.8	14.8	0.03	0.03	0.34
Postprandial changes,	-36.2	-26.9	4.1	3.9	14.9	< 0.01	0.64	0.45

L_{cp}L_{kin} = low CP and low percentage of slow protein diet, L_{cp}H_{kin} = low CP and high percentage of slow protein diet, H_{cp}L_{kin} = high CP and low percentage of slow protein diet, H_{cp}H_{kin} = high CP and high percentage of slow protein diet, CP = low (140 g/kg)/high (180 g/kg), Kin = Dietary protein digestion kinetics [8% slow protein in total CP/16% slow protein in total CP], Par = parity classes[parity 2/3/4-6/7to9], NEFA = non-esterified fatty acid.

^a P-values for main effects with interaction being excluded.

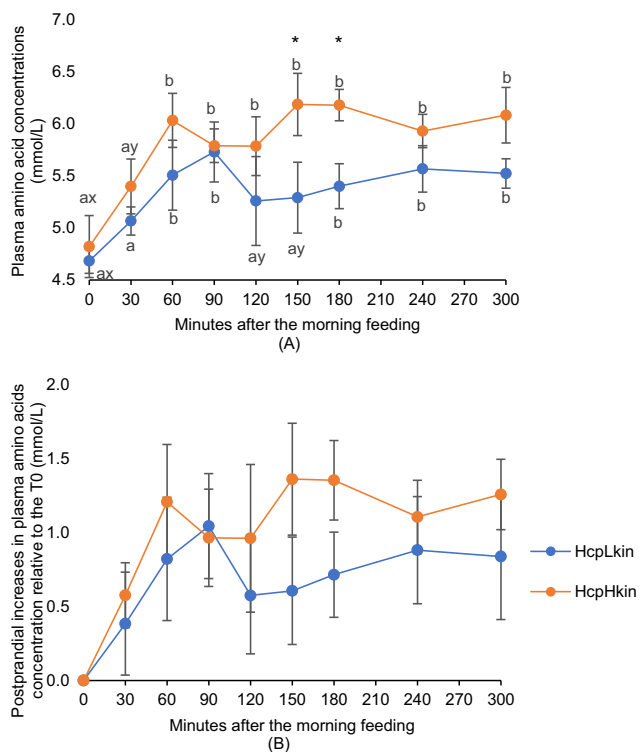


Fig. 1. A, B. Plasma amino acid (AA) concentrations (A) and postprandial increases in plasma AA concentration relative to T0 (B) over 300 min after the morning feeding of sows fed with diets containing high CP, low percentage of slow protein (H_{cp}L_{kin}) and high CP, high percentage of slow protein (H_{cp}H_{kin}) (LSmeans). ^{a,b} Least square means within a line with different letters differ significantly at P < 0.05. ^{x,y} Least square means within a line with different letters differ significantly at P < 0.1. * Least square means at the same time point differ significantly at P < 0.05.

in AA concentration relative to T0 were not significantly different between the sows fed with high and low percentages of slow protein (Fig. 1B).

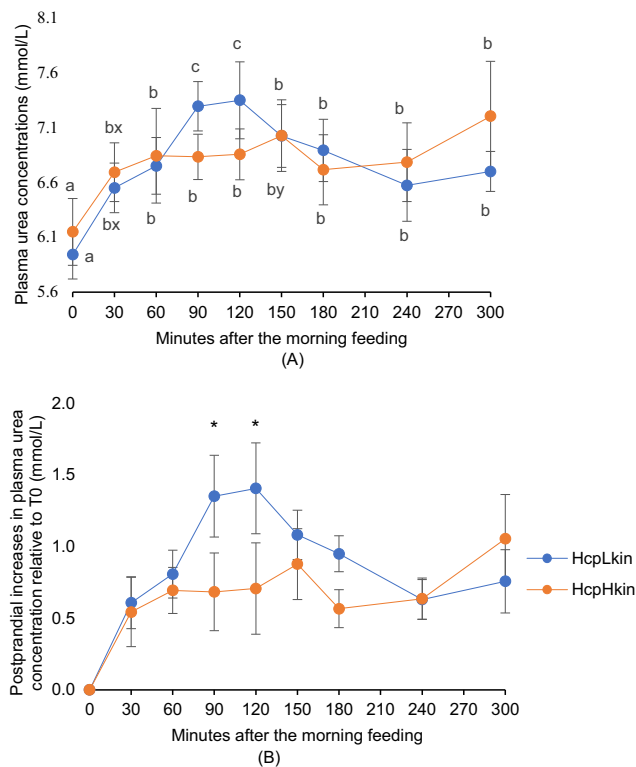


Fig. 2. A, B. Plasma urea concentrations (A) and postprandial increases in plasma urea concentration relative to T0 (B) over 300 min after the morning feeding of sows fed with diets containing high CP, low percentage of slow protein (HcpLkin) and high CP, high percentage of slow protein (HcpHkin) (LSmeans). At T150, HH = 7.03, HL = 7.02. ^{a,b} Least square means within a line with different letters differ significantly at P < 0.05. ^{x,y} Least square means within a line with different letters differ significantly at P < 0.1. * Least square means at the same time point differ significantly at P < 0.05.

Plasma urea concentrations were not different between the sows fed with high and low percentages of slow protein over the 300-minutes postprandial period when comparing absolute val-

ues (Fig. 2A). A high percentage of slow protein induced a higher plasma urea concentration at T30 than T0 ($\Delta = 0.54$ mmol/L, $P = 0.03$), but then remained at a similar concentration to T30 till the end of the sampling period (Fig. 2A). Meanwhile, a low percentage of slow protein increased urea plasma concentration from 5.94 mmol/L at T0 to 6.55 mmol/L at T30 ($P = 0.01$), and reached a peak of 7.35 mmol/L at T120 (Fig. 2A). Subsequently, sows fed with a low percentage of slow protein maintained higher plasma AA concentrations from T150 to T300 than T0 (Fig. 2A). When taking urea concentration at T0 as basal concentration, sows fed with a low percentage of slow protein exhibited significantly higher increases in postprandial plasma urea concentration at T90 and T120 than sows fed with a high percentage of slow protein ($\Delta = 0.67$ mmol/L, $P = 0.04$, $\Delta = 0.70$ mmol/L, $P = 0.03$, respectively; Fig. 2B).

Nitrogen loss to the environments

During a 21-day lactation, sows fed with a high dietary CP had significantly higher total nitrogen intake ($\Delta = 720$ g, $P < 0.01$), higher total nitrogen loss to the environment ($\Delta = 604$ g, $P < 0.01$), and higher urinary nitrogen output ($\Delta = 595$ g, $P < 0.01$; Table 7). Dietary protein digestion kinetics did not elicit any significant differences in total nitrogen intake, total nitrogen loss to the environment and urinary nitrogen output during lactation. An interaction was found between dietary CP and protein digestion kinetics, in that a higher percentage of slow protein increased fecal nitrogen output at a low dietary CP ($\Delta = 48$ g, $P < 0.01$; Table 7), but led to a similar fecal nitrogen output to the lower percentage of slow protein when dietary CP was high (Table 7). A high dietary CP led to a lower protein efficiency than sows fed with a low CP diet ($\Delta = 14.8\%$, $P < 0.01$; Table 7), while protein digestion kinetics did not affect protein efficiency significantly.

Subsequent reproductive performance

Sow WOI was not affected by either dietary CP or protein digestion kinetics in the previous lactation. The number of culled sows after inseminations due to non-reproductive problems was displayed (Table 8), which were excluded from the calculation of pregnancy rate. In the next farrowing, no significant differences were found in the total born litter size and total born litter weight between diets. Sows fed with a higher percentage of slow protein during lactation had a greater liveborn litter size in the subsequent farrowing ($\Delta = 0.7$, $P = 0.04$; Table 8), but a similar liveborn litter weight compared to the sows fed with a low percentage of slow protein (Table 8).

Table 7

Total nitrogen intake, loss to the environment, fecal nitrogen output, urinary nitrogen output and protein efficiency between Day 1 and Day 21 postfarrowing of sows fed with different lactation diets (Least square means \pm SEM).

Item	Lactation diets				SEM	P-value ^c			
	L _{cp} L _{kin}	L _{cp} H _{kin}	H _{cp} L _{kin}	H _{cp} H _{kin}		CP	Kin	CP*kin	Par
N	54	56	56	54					
Total N intake (g)	2 649	3 369	3 006	3 012	29	< 0.01	0.84		0.22
Total N loss to the environment (g)	1 237	1 225	1 866	1 804	48	< 0.01	0.42		< 0.01
Fecal N output (g)	351 ^b	399 ^c	384 ^a	384 ^a	0.2	< 0.01	< 0.01	< 0.01	0.66
Urinary N output (g)	886	826	1 482	1 420	48	< 0.01	0.12		< 0.01
Protein efficiency (%)	77.5	80.0	63.2	64.8	1.5	< 0.01	0.16		< 0.01

L_{cp}L_{kin} = low CP and low percentage of slow protein diet, L_{cp}H_{kin} = low CP and high percentage of slow protein diet, H_{cp}L_{kin} = high CP and low percentage of slow protein diet, H_{cp}H_{kin} = high CP and high percentage of slow protein diet, CP = low (140 g/kg)/high (180 g/kg), Kin = Dietary protein digestion kinetics [8% slow protein in total CP/16% slow protein in total CP], Par = parity classes[parity 2/3/4–6/7to9], N = nitrogen.

^{a,b} Least square means with different superscripts differ significantly at $P < 0.05$.

^c P-values for main effects with interaction being excluded if CP*Kin is not shown.

Discussion

The aim of the current study was to investigate how dietary protein digestion kinetics, dietary CP and their interactions affect the availability and utilization of dietary protein during lactation. We hypothesized that a slower rate of protein digestion kinetics would increase protein efficiency and utilization, thereby increasing sow lactation performance. Therefore, we assessed postprandial blood metabolites and dietary consequences for maternal protein and fat loss, milk composition, litter weight gain and subsequent reproductive performance. We found that a higher dietary CP reduced BW loss and estimated body protein and fat losses, however, only at the higher percentage of slow protein. In corroboration with our hypothesis, a slower digestion of dietary protein benefitted milk production (litter weight gain) throughout lactation. A slower digestion of protein resulted in greater plasma AA concentrations and lower increases in plasma urea concentrations during the postprandial period, which suggested reduced AA oxidation and more efficient use of dietary protein.

In this study, a higher percentage of slow protein in total dietary protein sustained a higher plasma AA concentration over the postprandial period in lactating sows. This was in line with previous studies in humans, in which ingestion of proteins with a slower digestion rate induced a prolonged plasma hyperaminoacidemia compared to the ingestion of fast protein (Dangin et al., 2001). For instance, feeding casein to young men led to a greater leucine balance over a 7-hour postprandial period than feeding free AAs that have a faster digestion rate but with identical AA composition to casein (Dangin et al., 2001). Intake of fast protein induced a strong but transient AA delivery, while only a part of dietary AAs could be taken up for protein synthesis (Dangin et al., 2001; Fouillet et al., 2009), possibly because intracellular AA concentration outreached the maximum for processing protein synthesis (Earl and Hindley, 1971). The AAs that are not deposited will be catabolized as an energy source through oxidative deamination, leading to an increase in plasma urea after fast protein intake in human studies (Dangin et al., 2001; Brady, 2005; Fouillet et al., 2009). This was confirmed by the present study, in which a greater AA catabolism might be induced by feeding a low percentage of slow protein, as suggested by the greater increases in concentrations of plasma urea over the postprandial period. Collectively, feeding lactating sows a higher percentage of slow protein leading to prolonged higher plasma AA availability and might reduce postprandial oxidation of AAs. Since the blood AAs are the precursors for milk protein synthesis (Mephram, 1982), a higher percentage of slow protein could sustain a greater abundance of substrates for protein synthesis, and benefit milk production. As expected, we observed a greater litter weight gain by feeding sows a high percentage of slow protein, which indicated a higher total milk production. While the total milk production was elevated, the milk

Table 8

Reproductive performance at the first estrus of sows fed with different lactation diets, including WOI, farrowing rate, total born litter size and weight, liveborn litter size and weight in the next farrowing (Least square means \pm SEM).

Item	Lactation diets				SEM	P-value ^a		
	L _{cp} L _{kin}	L _{cp} H _{kin}	H _{cp} L _{kin}	H _{cp} H _{kin}		CP	Kin	Par
Post weaning								
N	28	24	25	27				
WOI (d)	3.9	3.9	3.9	3.9	0.1	0.66	0.73	0.81
Pregnancy rate (%) ^b	100	100	98.0	100				
Number of culled sows ^c	2	1	2	2				
Next farrowing								
N	26	22	22	24				
Total born litter size	16.6	17.2	17.3	17.7	0.6	0.43	0.47	0.23
Total born litter weight (kg)	23.2	24.5	24.0	24.0	0.9	0.78	0.67	0.56
Liveborn litter size ^{d1}	14.6	15.8	15.4	15.5	0.4	0.48	0.04	0.35
Liveborn litter weight (kg) ^{d2}	21.4	23.2	22.6	22.1	0.8	0.93	0.31	0.73

L_{cp}L_{kin} = low CP and low percentage of slow protein diet, L_{cp}H_{kin} = low CP and high percentage of slow protein diet, H_{cp}L_{kin} = high CP and low percentage of slow protein diet, H_{cp}H_{kin} = high CP and high percentage of slow protein diet, CP = low (140 g/kg)/high (180 g/kg), Kin = Dietary protein digestion kinetics [8% slow protein in total CP/16% slow protein in total CP], Par = parity classes[parity 2/3/4–6/7to9], WOI = weaning-to-estrus interval.

^a P-values for main effects with interaction being excluded.

^b Raw means of sow pregnancy rates.

^c Number of culled sows after the first insemination

^d Total born litter size was added as covariates into the models: ¹ $\beta = 0.8$ piglets/piglet, $P < 0.01$; ² $\beta = 1.0$ kg /piglet, $P < 0.01$.

protein concentration on Day 12 postfarrowing was unaffected by a high percentage of slow protein, probably due to the dilution effect of a high total milk yield.

Elevating dietary CP increased litter weight gain, however, only during the third week of lactation. A number of studies have demonstrated that increasing both essential (EAA) and non-essential amino acids (NEAAs), will increase litter gain, at least up to a breakpoint above which the EAAs are no longer limiting and litter gain reaches a plateau (Guan et al., 2004; Quesnel et al., 2005). From work by Hojgaard et al. (2019b), the breakpoint seems to be somewhere around 8.1 g/kg SID lysine. Interestingly, work from the same authors (Hojgaard et al., 2019a) seems to suggest that NEAA may also limit litter gain in some conditions, since increasing dietary CP from 96 to 152 g/kg SID CP (corresponding to 115–183 g/kg CP) whilst maintaining EAA (SID lysine at 8.1 g/kg), increased litter gain up to a breakpoint at 125 g/kg SID CP (or 149 g/kg CP). The current study seems to confirm this, because EAAs were maintained at 8 g/kg SID lysine, and increasing CP from 140 to 180 g/kg CP increased litter gain. This effect was not observed in the first 2 weeks of lactation, possibly because at that stage, with a lower milk production, the intake of protein was more than required. In other words, the breakpoint may be lower in the first part of lactation.

Dietary protein and AAs affect not only sow milk production, but also the extent of sow BW loss during lactation. In studies where dietary inclusion of both NEAA and EAA were increased, it was demonstrated that providing more AAs for milk production had a sparing effect on maternal body tissue (Mejia-Guadarrama et al., 2002; Quesnel et al., 2005; Strathe et al., 2017). Again, it seems that in certain conditions, NEAA can be limiting in this effect, since reducing dietary CP results in more loss of BW, even if at lower dietary CP, the supply of EAA is maintained at requirements by supplementing crystalline amino acids (Huber et al., 2015; Huber et al., 2018). In the current study, the effects of dietary CP appeared to depend on the protein digestion kinetics of diets. A high dietary CP reduced sow body mobilization during lactation compared to a low dietary CP, however, only at a high percentage of slow protein. This suggests that the beneficial effects on body condition associated with a higher dietary CP are limited when protein digestion is rapid. This is somewhat mirrored in the work by Huber et al. (2015, 2018), where the diets with higher dietary CP included less (fast degradable) crystalline amino acids, demonstrating that increasing CP and at the same time the proportion of slow protein, reduces weight loss.

It is relevant to note that in the current study, diets were deliberately formulated to maintain EAA, in order not to confound the effects of changing dietary CP and digestion kinetics with changes in EAA. In this way, the increase in CP was not associated with an increase in EAA and allowed to establish the role of NEAA at the same time. The results of this study emphasize the earlier conclusion that, besides essential AAs, non-essential AAs in a certain range can also limit or contribute to milk production and BW losses in lactating sows. Additionally, the requirement for both NEAA and EAA could increase with higher milk production in late lactation (Feyera and Theil, 2017). A review by Wu et al. (2013) pointed out that the importance of non-essential AAs in formulating a sow diet has been long-overlooked. For instance, supplementation of arginine, as a non-essential AA in the sow diet, effectively increased sow milk production by enhancing blood flow and nutrient transfer to the mammary gland (Mateo et al., 2008; Kim and Wu, 2009).

Changes in sow body condition during lactation have been associated with a number of plasma metabolites and biomarkers, including urea, NEFA, creatinine, and IGF-1 (Yang et al., 2000; Xue et al., 2012; Costermans et al., 2020). In the current study, a higher percentage of slow protein reduced the increase in plasma urea concentration at 4 h after feeding, which suggested a reduced AA catabolism, coinciding with the results of serial blood samples previously discussed. Plasma IGF-1 concentration, as a key metabolic indicator, is associated with both energy and protein availability (Ketelslegers et al., 1995). It was surprising to find that both prefeeding and postfeeding plasma IGF-1 concentrations were lower in sows fed with a high percentage of slow protein, since greater postprandial plasma AA concentrations were found in those sows. The unexpected results might be the consequence of the increased milk production at a high percentage of slow protein, and therefore a stronger IGF-1 delivery from blood plasma to milk. As indicated in a previous study, milk IGF-1, which is likely derived from IGF in maternal circulation (Donovan et al., 1994), can be increased by supplementing arginine to multiparous sows (Krogh et al., 2016). However, how the fluxes of IGF-1 between milk and systemic circulation influence its plasma concentrations needs to be further verified.

A high dietary CP increased both prefeeding and postfeeding plasma urea concentrations, indicating that the associated higher AA oxidation could last overnight to the next morning feeding. As an indicator of body protein mobilization, the plasma creatinine concentrations were unaffected by dietary CP, although sow BW

loss and estimated protein loss were reduced by a higher dietary CP at a high percentage of slow protein. A possible explanation could be that the plasma samples that were collected only on 1 day of lactation (Day 14) may not reflect the body mobilization throughout the 21-day period. Besides, it may be argued that creatinine is not a trustful indicator of protein mobilization in lactating sows. A previous study found that sows with a higher BW loss during lactation showed higher serum concentrations of 3 methyl-histidine and urea but similar creatinine concentrations to sows with significantly lower BW loss, suggesting that 3 methyl-histidine could be a more reliable biomarker for body protein tissue loss than creatinine (Patel et al., 2013). As a marker of fat mobilization, prefeeding plasma NEFA concentration tended to be higher at a high dietary CP. The implied higher body fat catabolism during the fasting period might be elicited by the increased energy demand of the higher milk production driven by high CP diets (King et al., 1993; Strathe et al., 2017). However, plasma NEFA was reduced to a similar concentration at 4 h after feeding for all diets, which might suggest that the diets sustained a similar energy balance and therefore led to a similar body fat catabolism after feeding. The high CP diets resulted in a higher postfeeding plasma IGF-1 concentration on Day 14, suggesting an improved metabolic status of these sows, in line with their lower BW loss. In previous studies, feeding sows increasing dietary CP and SID-lysine also significantly increased plasma IGF-1 and reduced BW loss (Yang et al., 2000; Mejia-Guadarrama et al., 2002; Xue et al., 2012). However, when sows were fed diets ranging from 11.9 to 19.8% CP, a higher dietary CP did not affect blood IGF-1 on Days 7, 13 and 21 postfarrowing, but reduced BW loss during lactation (Clowes et al., 2003). The lack of an effect on blood IGF-1 in that study might be explained by the limited number of animals (8–10 sows) in each treatment group (Clowes et al., 2003).

The metabolism of dietary protein is closely associated with nitrogen output to the environment, which plays a key role in achieving a sustainable pig industry. While increases in postprandial plasma urea concentration were reduced by a higher percentage of slow protein, suggesting less AA oxidation, protein digestion kinetics did not significantly affect neither total nitrogen loss to the environment ($P = 0.42$) nor nitrogen output in urine ($P = 0.12$). It should be realized that the current urinary nitrogen output was calculated based on the estimations of nitrogen mobilization from sow body reserve and nitrogen output in milk. To have better accuracy, measured nitrogen output by total urine collection is preferred in future studies. The fecal nitrogen output is largely dependent on protein digestibility, and therefore the lowest apparent protein digestibility of the diet with a low CP and a high percentage of slow protein resulted in the highest fecal nitrogen output. Conversely, high CP diets with high and low percentages of slow protein had a similar digestibility, and therefore a similar fecal nitrogen output. These differences in protein digestibility explained why an interaction was found between the protein digestion kinetics and dietary CP for nitrogen output. A high CP in diets reduced protein efficiency, since it did not enhance the milk production proportionately with the higher protein intake. When comparing the 4 diets, feeding a low dietary CP with a high percentage of slow protein led to the highest protein efficiency in our sows (Table 7), and reducing dietary CP at a high percentage of slow protein did not compromise litter weight gain (Table 4). Thus, increasing slow protein content in lactating sows' diets has more benefits from a sustainability point of view compared to increasing total dietary CP.

Sow nutritional status and BW loss during lactation have been closely associated with their subsequent reproductive perfor-

mance, such as WOI, farrowing rate and subsequent litter size (Zak et al., 1997; Thaker and Bilkei, 2005; Schenkel et al., 2010). In the current study, neither dietary protein digestion kinetics nor CP affected WOI. On the one hand, sow weight loss across the treatment groups was modest (9.3–10.2%) compared to the previously identified threshold value of 10% weight loss and protein loss that prolongs WOI and reduces subsequent litter size (Thaker and Bilkei, 2005; Schenkel et al., 2010). On the other hand, it was indicated that weight loss during lactation does not prolong the WOI anymore in modern sows due to genetic selection towards shortened WOI, although it can still affect subsequent embryo survival during gestation (Kemp et al., 2018). This might also explain the greater subsequent liveborn litter size of sows fed with a higher percentage of slow protein. The nutritional history and AA supply during lactation might still affect subsequent reproduction irrespective of total weight loss (Zak et al., 1997; Clowes et al., 2003). Previous studies showed that with a similar total weight loss, a better dietary AA supply during lactation enhanced oocyte maturation and follicular estradiol level at weaning (Clowes et al., 2003), which can potentially improve subsequent embryo development and survival (Driancourt and Thuel, 1998). In the current study, a high dietary CP did not show beneficial effects on liveborn litter size in the next farrowing, possibly related to lower CP concentrations (140 and 180 g/kg) than in the study of Clowes et al. (2003) (150.6 vs 197.9 g/kg). Collectively, the current results suggested that the increased AA availability induced by a higher percentage of slow protein affects subsequent litter size more than a higher dietary CP in the current range.

In the current study, a range of ingredients was used in diets to create the contrasts in dietary CP and protein digestion kinetics, and therefore, potential confounding of treatment effects with ingredients cannot be excluded. To minimize the risk of confounding, a range of protein sources was used with overlap between the treatment diets. Equally, there were slight differences in some nutrients, despite our efforts to keep all nutrients other than those of interest equal across diets. For example, dietary fiber and fat differed slightly between diets. However, these differences were very small and negligible compared to differences in nutrients of interest and moreover, the effects of these differences on dietary energy were limited because diets were formulated to have equal NE. Due to a trend for lower average daily feed intake, sows fed a higher percentage of slow protein had a lower net energy intake. Nevertheless, milk production was improved by the high–slow diets. The current results at slower protein digestion, including the higher plasma AA concentration and lower plasma urea concentration, were in line with the previous studies in humans, in which protein digestion kinetics were studied using isonitrogenous single protein sources with identical AA composition (Boirie et al., 1997; Dangin et al., 2001). The potential of confounding effects with ingredients was therefore not considered as the main explanation for the dietary effects.

Conclusions

Feeding protein sources with slower digestion improved litter weight gain by 4% (+2.6 kg), probably due to reduced AA oxidation and therefore a higher substrate availability for milk production. A high percentage of slow protein also benefitted liveborn litter size in the next cycle. Increasing dietary CP from 140 to 180 g/kg increased litter weight gain by 4.8%, but only in week 3, and reduced (by 28%) sow BW loss at a high percentage of slow protein. Reducing dietary CP in the current range could considerably elevate protein efficiency during lactation and would not compromise

sow lactation performance when the percentage of slow protein is increased to 16% of total protein.

Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.animal.2024.101184>.

Ethics approval

The animal use protocols were reviewed and approved by the central committee for animal experiments in The Netherlands (approval number AVD20400202013805).

Data and model availability statement

None of the data were deposited in an official repository. The data/models that support the study findings are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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