Whole-body nitrogen utilization and tissue protein and casein synthesis in lactating primiparous sows fed low- and high-protein diets¹

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ABSTRACT: Twenty-eight lactating Yorkshire and Yorkshire × Landrace primiparous sows were used to test the hypothesis that feeding a diet with reduced CP concentration and supplemented with crystalline AA (CAA) does not decrease milk protein yield and litter growth but improves apparent N utilization for milk protein production. Sows were assigned to 1 of 2 dietary treatments: 1) control (CON; 16.2% CP; analyzed content) or 2) low CP with CAA to meet estimated requirements of limiting AA (LCP; 12.7% CP) over a 17-d lactation period. A N balance was conducted for each sow between days 13 and 17 of lactation. On day 17, a 12-h primed continuous infusion of L-[ring-²H₅]-Phe was conducted on 12 sows ($n = 6$) with serial blood and milk sampling to determine plasma AA concentrations and Phe enrichment, and milk casein synthesis, respectively. Thereafter, sows were sacrificed and tissues were collected to determine tissue protein fractional synthesis rates (FSR). Litter growth rate and milk composition

did not differ. Sows fed the LCP diet had reduced N intake (122.7 vs. 153.2 g/d; $P < 0.001$) and maternal N retention (13.5 vs. 24.6 g/d; $P < 0.05$) and greater apparent efficiency of using dietary N intake for milk production (85.1% vs. 67.5%; $P < 0.001$). On day 17 of lactation, all plasma essential AA concentrations exhibited a quartic relationship over time relative to consumption of a meal, where peaks occurred at approximately 1- and 4-h postprandial ($P < 0.05$). Protein FSR in liver, LM, gastrocnemius muscle, mammary gland, and in milk caseins did not differ between treatments. Feeding primiparous sows with a diet containing 12.7% CP and supplemented with CAA to meet the limiting AA requirements did not reduce milk protein yield or piglet growth rate and increased the apparent utilization of dietary N, Arg, Leu, Phe+Tyr, and Trp for milk protein production. The improved apparent utilization of N and AA appears to be related exclusively to a reduction in N and AA intake.

Key words: AA intake, lactating primiparous sows, nitrogen retention, tissue fractional protein synthesis

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INTRODUCTION

Feeding diets reduced in CP concentration and supplemented with crystalline AA (CAA) to meet estimated requirements of limiting AA of multiparous lactating sows improves dietary AA balance, increases apparent N utilization efficiency, and decreases N losses to the environment (Huber et al., 2015, 2016). In several studies, feeding reduced-CP diets with improved AA balance increased milk protein yield and piglet growth rates (Laspiur et al., 2009; Manjarín et al., 2012; Huber et al., 2015). The benefits of reduced-CP diets may come at the expense of maternal N retention. We reported that multiparous sows fed a low CP diet supplemented with CAA retained less N in the maternal body pool (Huber et al., 2015). Even though litter size and growth rate are important determinants of AA requirements, the partitioning of dietary AA between maternal and milk protein pools should be considered in the empirical estimations of AA requirements (Noblet et al., 1990; Wilson et al., 1996). These findings point to potential impact of dietary AA balance on lactation AA requirement, in particular for primiparous sows that have not reached mature body protein mass (Clowes et al., 1998; Jones and Stahly, 1999). The effect of feeding a reduced-CP diet with balanced AA to primiparous sows on their maternal N utilization and lactation performance has not been documented to date.

Based on the previous findings in multiparous sows, we hypothesized that feeding a diet reduced in dietary CP and supplemented with CAA decreases maternal N retention, increases milk production, and improves apparent utilization efficiency of dietary N and AA for milk protein production. The objectives were to 1) determine maternal protein retention and muscle protein synthesis and 2) estimate N and AA utilization efficiency in primiparous sows fed either a reduced-CP diet supplemented with CAA or a diet formulated to provide Lys using only corn and soybean meal.

MATERIALS AND METHODS

The experimental protocol was approved by the University of Guelph Animal Care Committee and followed Canadian Council of Animal Care guidelines (CCAC, 2009).

Animals and Feeding

Twenty-eight primiparous sows (Yorkshire or Yorkshire × Landrace) were selected at day 110 of gestation and randomly assigned to 1 of 2 dietary treatments over 4 replicates of 4, 12, 6, and 6 sows, respectively. Sows were housed in conventional

farrowing crates, and litter sizes were standardized to 12 piglets within the first 24 h of birth (day 0 of lactation). After farrowing, sows were fed progressively to reach a feed intake of 4.5 kg/d by day 6 of lactation and an average of 5.0 kg/d over a 21-d lactation period (NRC, 2012). Feed was provided in 3 equal meals per day between days 1 and 9 of lactation (i.e., at 0800, 1200, and 1600 h) and in 4 equal meals per day thereafter and until day 17 of lactation (i.e., at 1000, 1600, 2000, and 0400 h). Feed intake and feed refusals were monitored daily. Water was provided ad libitum to both sows and piglets. Injection of iron and surgical castration of male piglets were conducted on days 5 and 10, respectively. Piglets were not supplied with creep feed. Individual piglets were weighed on days 1 (i.e., 24 h postpartum and after standardization of litter size), 4, 7, 10, 13, and 17, and sows were weighed on days 1, 7, 13, and 17 of lactation. Litter weigh-suckle-weigh data were collected from a total of 9 sows fed the control diet and 8 sows fed the low CP diet, which included all sows that underwent isotopic infusion (described later). Isotopic infusions were conducted on 6 sows from each dietary treatment across all replicates. Four sows (1 fed the control diet and 3 fed the low CP diet) were removed from the study due to farrowing complications or poor venous access and were not included in analysis.

Dietary Treatments

Ingredient and nutrient contents of diets are presented in Table 1. Diets were formulated based on nutrient requirements that were predicted using the NRC (2012) model and based on performance variables determined in a previous study using similar genetics (Miller et al., 2016). The performance variables included mean sow BW at farrowing of 180 kg, litter size of 12 piglets, mean piglet gain during a 21-d lactation period of 200 g/d, mean feed intake of 5.0 kg/d, minimum maternal protein loss of 5 g/d, and protein to lipid ratio in BW change of 0.01 (NRC, 2012). Primiparous sows were randomly assigned to 1 of 2 dietary treatments: 1) control (CON; 16.2% CP) or 2) low CP (LCP; 12.7% CP), which were formulated to provide identical levels of standardized ileal digestible (SID) Lys (0.77%) at 20% below predicted requirements to optimize the estimation of SID Lys utilization efficiency (Huber et al., 2015). The CON diet was formulated using soybean meal and corn as the only sources of Lys, while the LCP diet was formulated to meet the minimum estimated N requirement of 107 g/d by feeding an average of 5.0 kg/d over a 21-d lactation

Table 1. Ingredient composition and nutrient content of experimental diets (as-fed)

Ingredient	CON ¹	LCP
Corn	68.07	73.72
Soybean meal, dehulled, 48% CP	24.40	10.52
Fat, A/V blend	3.55	3.64
Soy hulls	0	6.22
L-Lys·HCl	0	0.42
L-Val	0.17	0.40
L-Phe	0	0.31
L-Thr	0.08	0.27
L-Ile	0	0.17
DL-Met	0.04	0.17
L-Leu	0	0.14
L-His	0	0.12
L-Trp	0.01	0.09
Sodium chloride	0.50	0.50
Limestone, ground	1.23	1.16
Monocalcium phosphate	1.25	1.45
Vitamin and mineral premix ²	0.60	0.60
Titanium dioxide	0.10	0.10
Total	100.00	100.00
Calculated nutrient content ³		
NE, kcal/kg	2,600	2,600
CP, %	17.48	13.37
SID Lys, % ⁴	0.77	0.77
SID Ile, %	0.62	0.55
SID Met, %	0.29	0.35
SID Met + Cys, %	0.53	0.53
SID Thr, %	0.61	0.61
SID Trp, %	0.19	0.19
SID Val, %	0.85	0.85
SID Arg, %	1.01	0.61
SID His, %	0.42	0.40
SID Leu, %	1.35	1.12
SID Phe, %	0.74	0.79
SID Phe + Tyr, %	1.22	1.11
STTD P, % ⁵	0.42	0.42
Total Ca, %	0.83	0.83
Fermentable fiber, %	10.00	10.00
Analyzed nutrient content, %		
DM	89.76	89.69
CP	16.24	12.68
Lys	0.89 (0.89) ⁶	0.82 (0.87)
Ile	0.76 (0.71)	0.61 (0.62)
Met	0.30 (0.32)	0.33 (0.38)
Met + Cys	0.60 (0.62)	0.54 (0.61)
Thr	0.74 (0.72)	0.68 (0.69)
Trp	— (0.21)	— (0.21)
Val	0.98 (0.97)	0.92 (0.94)
Arg	1.20 (1.09)	0.71 (0.67)
His	0.48 (0.48)	0.42 (0.45)
Leu	1.63 (1.54)	1.26 (1.28)

Table 1. Continued

	CON ¹	LCP
Phe	0.93 (0.85)	0.86 (0.88)
Phe + Tyr	— (1.42)	— (1.27)

¹CON = control, 16.2% CP (as-fed; analyzed contents); LCP = low crude protein, 12.7% CP.

²Provided the following amounts of vitamins per kilogram of diet: vitamin A, 3,000 IU; vitamin D₃, 300 IU; vitamin E, 20 IU; menadione (vitamin K), 1 mg; vitamin B₁₂, 20 µg; riboflavin, 4 mg; D-pantothenic acid, 10 mg; niacin, 15 mg and provided the following amounts of trace minerals per kilogram of diet: Fe, 640 mg as FeCO₃; Zn, 260 mg as ZnO; Mn, 36 mg as MnO₂; Cu, 20 mg as CuCl₂; I, 0.58 mg as ethylenediamine dihydroiodide.

³Based on nutrient concentrations in feed ingredients according to the [NRC \(2012\)](#).

⁴SID = standardized ileal digestible ([NRC, 2012](#)).

⁵STTD = standardized total tract digestible.

⁶Calculated amino acid concentrations are shown in parentheses.

period ([NRC, 2012](#)). Crystalline AA were added to meet estimated requirements for each essential AA (EAA) that became limiting. A new batch of feed was mixed for each replicate, and the average nutrient composition is presented in [Table 1](#).

Nitrogen Balance, Continuous Infusion, Blood, and Milk Sampling

Nitrogen balance was conducted during peak lactation between days 13 and 17 on each primiparous sow. Total urine collection and fecal grab sampling were performed as previously described ([Huber et al., 2015](#)). On day 16 of lactation, piglets were removed and catheters (1.78 mm o.d., 1.02 mm i.d.; TYGON, Saint-Gobain Performance Plastics Corp., Cleveland, OH) were inserted through an external ear vein to the jugular vein according to the methods of [de Ridder et al. \(2014\)](#) in a total of 6 LCP- and 6 CON-fed sows. Before piglets were returned to the sow, a 50-mL background milk sample was collected from all mammary glands on one side after administering oxytocin intramuscularly (20 IU oxytocin, sodium chloride 0.9% wt/vol, and chlorobutanol 0.5% wt/vol, VetTek™, Blue Springs, MO).

On day 17 of lactation, a primed continuous infusion of filter-sterilized (0.22-µm syringe filter units) L-[ring-²H₃]-Phe (Cambridge Isotopes Laboratories Inc., Tewksbury, MA; 146.9 mmol/L L-[ring-²H₃]-Phe dissolved in sterile saline; sodium chloride 0.9% wt/vol, pH 7) at a rate of 3.67 mmol/h was conducted for 12 h between 0400 and 1600 h. A previous study in lactating goats demonstrated a plateau in Phe enrichment can be achieved in blood plasma approximately 6 h, and in casein

approximately 14 h, after the initiation of a non-primed continuous infusion (Bequette et al., 1994). A priming dose equivalent to 1 h of infusion (i.e., 3.67 mmol L-[ring- $^2\text{H}_5$]-Phe) was given directly before initiating the continuous infusion.

Samples of blood, obtained via the indwelling infusion catheter, and milk were collected each hour. During infusion and sampling, piglets were kept in an adjacent pen equipped with a heat source. For each sampling time, the infusion pump was stopped for at least 30 s before the infusion line was disconnected from the catheter. An initial 20 mL of blood were first removed before sampling 18 mL of blood into a clean syringe, at which point the initial 20 mL of blood were returned. Oxytocin (5 IU oxytocin, sodium chloride 0.9% wt/vol, and chlorobutanol 0.5% wt/vol, VetTekTM, Blue Springs, MO) was injected into the catheter (to minimize multiple IM injections and to ensure rapid and consistent action of oxytocin), and the catheter was flushed with 10 mL of sterile saline before the infusion line was reconnected and infusion resumed. Blood samples were transferred into ethylenediamine tetraacetic acid tubes (BD Vacutainers, Mississauga, Ontario, Canada) and centrifuged for 20 min at $1,500 \times g$ at 4 °C within 2 h of collection. The resulting plasma was aliquoted into microcentrifuge tubes, and subsequently stored at -20 °C until further analysis. Fifteen milliliters of milk was collected from all glands on one side of the udder after each blood sampling and stored at 4 °C until further analysis. The blood and milk sampling procedure took less than 5 min. Piglets were weighed (GSE 450, GSE Canada Inc., Airdrie, Alberta, Canada) returned to the sow and allowed to suckle. Immediately following cessation of the milk secretory phase, piglets were removed from the sow and the litter rapidly weighed. The difference in litter weight between pre- and post-suckling represented the amount of milk extracted from the mammary glands by the piglets. The weigh-suckle-weigh approach was repeated each hour.

Immediately after the final blood and milk sampling and suckling bout at 1600 h, sows were euthanized using an injection of sodium pentobarbital into the catheter (50 mg/kg BW; Schering-Plough Canada Inc., Kirkland, Québec, Canada). Samples of LM and gastrocnemius muscles (representative of fast-twitch and mixed muscle fiber muscles, respectively), mammary gland, and liver were immediately excised, rinsed with ice cold saline, and plunged into liquid N_2 . The time of tracer incorporation in tissue protein was recorded from the start of the infusion to the time that tissue samples were frozen in liquid N_2 . Whole livers were then removed and weighed.

Nutrient Analysis

Feed was subsampled from each 25-kg bag at manufacturing, pooled within batch and diet, and homogenized before analysis. Approximately 100 g of subsampled feed was shipped to Degussa AG (Hanua, Germany) to analyze AA content by ion-exchange chromatography coupled with post-column derivatization with ninhydrin (AOAC, 2006; Method 982.30). Fecal samples were pooled per sow and N balance period, blended, and a 200-g sample was freeze-dried and homogenized using a commercially available coffee grinder. The DM content of diets, feed refusals, and freeze-dried feces was measured by oven drying for 2 h at 135 °C according to AOAC (1997; Method 930.15).

Nitrogen concentration in feed, feces, and urine were measured at Agrifood Laboratories (Guelph, Ontario, Canada) using combustion analysis (LECO-FP 428; LECO Instruments Ltd., Mississauga, Ontario, Canada) according to AOAC (1997; Method 990.03). Titanium concentration in feces (in duplicate) and diets (in quadruplicate; $90.7 \pm 4.7\%$ recovery) was measured as described by Myers et al. (2004), with minor adaptations (digestion for 24 h at 120 °C in 10 mL tubes and addition of H_2O_2 after precipitate settled in 100-mL volumetric flasks). Absorbance of standards and samples were measured at 408 nm by spectrophotometry (Power Wave XS KC4; BioTek Instruments, Inc., Winooski, VT).

Whole milk samples were analyzed for true protein, lactose, and total solids with infrared spectroscopy by the Agriculture and Food Laboratory (Guelph, Ontario, Canada). Milk casein concentration was determined according to the methods of Guan et al. (2002). Plasma free AA concentrations were analyzed according to the methods of Boogers et al. (2008) and using Ultra Performance Liquid Chromatography and Empower Chromatography Data Software (Waters Corporation, Milford, CT).

For Phe enrichment analysis, plasma and tissue samples were prepared according to Litvak et al. (2013). The isotopic enrichment of L-[ring- $^2\text{H}_5$]-Phe in the tissue free and protein-bound pools was determined as the tert-butyldimethylsilyl derivative (Burd et al., 2012) by gas chromatography-mass spectrometry (Bruker Ltd., Milton, Ontario, Canada). Ions were monitored at 336 and 341 m/z , and ion ratios were converted to molar percent excess based on calibration curves from mixtures of labeled and unlabeled Phe. Duplicate injections were conducted for plasma free Phe and singles for tissue free and protein-bound pools and casein protein-bound pools.

Intra- and interassay variation in enrichment analysis were, respectively, 2.7% and 3.3% for plasma, 1.2% and 2.2% for tissue free, 3.9% and 3.9% for tissue bound, and 1.4% and 1.8% for casein bound.

Calculations and Statistical Analysis

Daily N intake, retention, and excretion were calculated as in Huber et al. (2015). Maternal N retention was calculated by subtracting true milk protein N output from N retention, where milk protein N output was based on weigh-suckle-weigh milk production and true milk protein concentration. The efficiencies of using dietary N, absorbed N, or retained N for true milk protein production were calculated. The apparent efficiencies of using SID AA and N (based on analyzed total dietary AA content and estimated SID) for AA output in milk protein over the entire 17-d lactation period were calculated according to NRC (2012) and Huber et al. (2016), which accounts for maternal maintenance requirements and the estimated contribution of maternal body protein mobilization to milk production, based on sow BW change.

Predicted Phe enrichment (E_p , mol %) at any time (t) in both plasma and milk casein was estimated using Eq. 1 (Waterlow and Stephen, 1967), with a rate constant (k) and enrichment at plateau ($E_{\max}$ and $E_{\max c}$ for plasma and casein, respectively) as parameters. Background plasma samples had non-detectable levels of L-[ring- 2 H $_5$]-Phe (data not shown); therefore, no corrections were required for natural abundance of L-[ring- 2 H $_5$]-Phe. Parameter values for Eq. 1 were estimated by minimizing the residual sum of squares between observed and predicted enrichment (mol %) using Solver in Microsoft Excel (Microsoft Corp., Redmond, WA). Sows were fed every 6 h, and it was assumed that all tracee fluxes were constant across the hourly time intervals.

$$E_p = E_{\max}(1 - e^{-kt}) \quad (1)$$

Plateau Phe enrichment in plasma was much higher and more variable than free Phe enrichment in the various tissues that were sampled at the end of the infusion (Supplementary Appendix 1), which was probably due to contamination of blood samples with infusate during sampling. Therefore, E_p values were adjusted by the ratio between mammary free Phe enrichment and plasma free Phe enrichment at 12 h, and the adjusted values were used to estimate parameters for Eq. 1 and all subsequent calculations.

Tissue protein fractional synthesis rate (FSR; percentage per day) was calculated according to Eq. 2 on day 17 of lactation:

$$\text{FSR} = \frac{E_b}{\frac{\sum_{t=1}^{12} \frac{E_{p,t}}{12}}{\sum_{t=9}^{12} \frac{E_{p,t}}{4}}} \times 24 \times E_f \times t \quad (2)$$

where E_b is enrichment of Phe in the tissue protein-bound pool (mol %), E_f is the enrichment of Phe in the tissue free pool (mol %), and t is the incorporation period in hours (Garlick et al., 1980). Because the tracer was administered by continuous infusion, the ratio of mean E_p across the 12-h sampling period to mean E_p during the final 4 h, when E_p had reached plateau (data not shown), was used to correct for the time that E_f was not at plateau.

Daily casein FSR (FSR_c) was calculated according to Eq. 3 on day 17 of lactation:

$$\text{FSR}_c = \frac{\text{AUC}_c}{\text{AUC}_p \times t} \times 24 \quad (3)$$

where AUC_c and AUC_p are the areas under the casein and plasma enrichment curves for each individual sow, calculated as the integral of Eq. 1. Daily casein fractional degradation rate (FDR_c) was considered as the difference between FSR_c and 100.

Statistical analyses for sow and litter performance, apparent AA utilization efficiency for milk production, milk composition, N utilization, whole-body Phe flux, and tissue and casein protein FSR were conducted using the mixed model procedure of SAS (SAS Institute, Inc., Cary, NC) with dietary treatment as the fixed effect, and replicate and sow within replicate as random effects. Individual sows (and when appropriate, the litter) were considered the experimental unit. Sow and piglet initial BW were tested as covariates and when appropriate ($P > 0.10$), a reduced model was used. Statistical analyses for plasma AA concentrations were also conducted using the mixed model procedure of SAS with repeated measures; the fixed effects of dietary treatment, feeding cycle (i.e., meal 1 or meal 2) or sampling time (i.e., hour), and the interactive effect of dietary treatment and feeding cycle or sampling time were tested for each AA with replicate as a random effect. First, using all 12 sampling time points, the main effects of cycle (i.e., the overall concentration of AA between 0500 and 1000 h vs. 1100 and 1600 h) and time (i.e., 0500 to 1600 h) were tested. Given the absence of interaction between cycle or sampling time and dietary treatment (data not shown), the data were combined such that each sow had 6 plasma AA measurements relative to one feeding time (i.e., 0-, 1-, 2-, 3-, 4-, and 5-h postprandial; each

measurement represents the average of 2 plasma AA concentrations per sow relative to feeding time). The mixed procedure of SAS was used with the fixed effects of dietary treatment, time after feeding, and the interactive effect of dietary treatment and time after feeding. Replicate and individual sow within block were included as random effects. Linear, quadratic, cubic, quartic, and quintic contrasts were written to describe the main effect of time after feeding and the highest polynomial degree (**PD**) is reported for the significant time contrast. Probability levels less than 0.05 were considered significant, whereas $0.05 < P \leq 0.10$ was considered a trend and $P > 0.10$ was considered not significant.

RESULTS

Analyzed dietary CP and AA concentrations were comparable to calculated values (Table 1). In the present study, urinary catheterization was conducted on 13 and 11 primiparous sows for CON and LCP, respectively, after 1 CON- and 3 LCP-fed sows were removed due to farrowing complications or poor venous access (N balance: $n = 13$ and $n = 11$ for CON- and LCP-fed sows, respectively). Data from one CON-fed sow were excluded from enrichment calculations, due to unsuccessful infusion, and for 2 additional CON-fed sows, due to biologically questionable $E_{\max}$ estimates. Those estimates were approximately 3 times greater than those of other sows and 4 times greater than enrichments in the tissue free pools. Time to reach 90% of plateau was also approximately 6 times greater than for other sows (data not shown; plasma fractional rate constants: $n = 3$ and $n = 6$ for CON- and LCP-fed sows, respectively). Enrichments in the tissue free and protein-bound pools, including caseins, were reasonable in the latter 2 sows and were therefore included in statistical analysis for FSR (tissue protein FSR: $n = 5$ and $n = 6$ for CON- and LCP-fed sows, respectively).

Sow and litter performance across the 17-d lactation period were not different between sows fed CON and LCP diets (Table 2), nor were milk yield or milk composition (Table 3). Primiparous sows fed LCP diet had lower N intake ($P < 0.001$), N digestibility ($P < 0.005$), total N excretion ($P < 0.001$), urinary N excretion ($P < 0.001$), absorbed N ($P < 0.001$), total N retention ($P = 0.067$), and maternal N retention ($P < 0.05$) than sows fed CON diet (Table 4). Sows fed LCP diet had greater efficiency of retaining consumed N ($P < 0.005$), retaining absorbed N ($P < 0.001$), and using retained N for true milk protein output ($P < 0.05$) than sows fed CON diet. Apparent utilization efficiency of Lys, Met + Cys, Phe, Thr, and Val for milk production across the

Table 2. Overall 17-d lactation performance for primiparous sows fed control or low CP diets

Item	Diet		SEM ¹	P value
	CON ²	LCP		
Number of sows ³	13	11	—	—
Sow initial BW, kg ⁴	183	177	4	0.182
Sow ADFI, kg/d, as-fed	4.68	4.66	0.07	0.727
Litter size at weaning	11.6	11.7	0.2	0.582
Litter growth rate, kg/d	2.56	2.40	0.08	0.156
Piglet ADG, g/d	218	204	7	0.137
Sow BW change, kg	−5.5	−6.0	1.1	0.741

¹Maximum value of the SEM.

²CON = control, 16.2% CP (as-fed; analyzed contents); LCP = low crude protein, 12.7% CP.

³One CON and 3 LCP sows were removed before analysis due to farrowing complications or poor venous access.

⁴Collected on day 1 of lactation, after litter size standardization.

Table 3. Milk composition on day 16 of lactation for primiparous sows fed control or low CP diets

Item	Diet		SEM ¹	P value
	CON ²	LCP		
Number of sows	9	8	—	—
Milk yield, kg/d ³	8.83	9.28	0.40	0.423
True protein content, %	5.36	5.52	0.11	0.322
True protein output, g/d	471	511	19	0.142
Casein content, % in defatted milk ⁴	3.79	3.74	0.23	0.833
Casein output, g/d	329	329	20	0.979
Fat content, %	8.48	8.38	0.42	0.831
Fat output, g/d	743	777	40	0.543
Lactose content, %	5.83	5.92	0.054	0.237
Lactose output, g/d	516	550	25	0.349

¹Maximum value of the SEM.

²CON = control, 16.2% CP (as-fed; analyzed contents); LCP = low CP, 12.7% CP.

³Estimated milk yield based on weigh-suckle-weigh approach, which was conducted successfully on 9 and 8 sows fed CON and LCP diets, respectively.

⁴Casein concentration in defatted milk for 12 sows $n = 6$ for CON and LCP.

17-d lactation period did not differ between LCP- and CON-fed sows. Apparent utilization efficiency of Arg, His, Ile, Leu, and N were greater for sows fed the LCP diet than for sows fed the CON diet ($P < 0.001$, $P = 0.100$, $P < 0.005$, $P < 0.001$, and $P < 0.001$, respectively; Table 4).

Overall plasma AA concentrations were greater in the second feeding cycle (i.e., between 1100 and 1600 h) than in the first feeding cycle (i.e., 500 to 1000 h; $P < 0.05$; data not shown); however, there was no interactive effect of feeding cycle and dietary treatment (data not shown). Given the absence of interaction between cycle or sampling time and dietary treatment, the data were combined such that

Table 4. Nitrogen utilization between days 13 and 17 of lactation (peak lactation) for primiparous sows fed control or low-protein diets

Item	Diet		NRC (2012) ¹	SEM ²	P value
	CON ³	LCP			
Number of sows ⁴	13	11		—	—
Sow body weight change, kg/d	−0.62	−0.87		0.29	0.498
N intake, g/d	153.2	122.7		1.7	<0.0001
Apparent fecal N digestibility, %	88.9	85.8		0.7	0.0002
Total N excretion, g/d	55.9	33.1		3.0	<0.0001
Fecal N, g/d	17.0	17.3		0.8	0.720
Urinary N, g/d	38.6	15.9		2.5	<0.0001
N absorbed, g/d ⁵	136.2	105.5		2.2	<0.0001
N retention, g/d ⁶	97.2	89.7		3.9	0.067
True milk protein N, g/d ⁷	73.8	80.2		3.0	0.142
Maternal N retention, g/d ⁸	24.6	13.5		3.7	0.045
N retained, % of intake	63.5	72.9		2.4	0.001
N retained, % of absorbed	71.6	84.7		2.2	0.0001
True milk protein N output, % of retained N ⁹	75.4	86.0		3.5	0.040
AA utilization efficiency, % ¹⁰					
Arg	37.2	61.4	81.6	2.4	<0.0001
His	64.7	69.8	72.2	2.7	0.100
Ile	54.6	64.1	69.8	2.5	0.002
Leu	53.8	65.9	72.3	2.5	0.0002
Lys	76.6	77.1	67.0	3.0	0.877
Met	63.8	53.0	67.5	2.2	0.0003
Met + Cys	64.9	66.9	66.2	2.6	0.494
Phe	45.6	45.0	73.3	1.7	0.750
Phe + Tyr ¹¹	57.0	65.7	70.5	2.5	0.004
Thr	65.2	65.2	76.4	2.6	0.996
Trp ¹¹	64.0	58.7	67.4	2.4	0.050
Val	53.8	53.1	58.3	2.1	0.747
N	67.5	85.1	75.9	3.0	<0.0001

¹Biological maximum AA utilization efficiency for milk protein production for groups of sows according to the NRC (2012).

²Maximum value of the SEM.

³CON = control, 16.2% CP (as-fed; analyzed contents); LCP = low crude protein, 12.7% CP.

⁴Variables dependent on milk yield (i.e., true milk protein N, maternal N retention, and efficiency of retaining N in true milk protein) are based on 9 and 8 sows for CON and LCP diets, respectively.

⁵N intake − N excreted in feces.

⁶N intake − N excreted in feces − N excreted in urine.

⁷Calculated based on true protein content of milk (day 16) and milk yield estimated via weigh-suckle-weigh on day 17 of lactation.

⁸N retention − true milk protein N output.

⁹Retained N includes N retained in maternal and milk protein pools.

¹⁰AA utilization efficiency for milk production across the 17-d lactation period calculated according to the NRC (2012) using analyzed dietary AA concentration and estimated digestibility (NRC, 2012).

¹¹Calculated values for dietary Tyr and Trp, respectively, were used for the AA utilization efficiency calculation.

each sow had 6 plasma AA measurements relative to one feeding time (i.e., 0-, 1-, 2-, 3-, 4-, and 5-h postprandial; see Materials and Methods); there was no interaction between time after feeding and dietary treatment (data not shown), therefore only the main effects of diet are presented in Table 5. Sows fed the LCP diet had lower overall plasma concentrations of Arg ($P < 0.05$), Leu ($P < 0.005$) and higher overall plasma concentrations of Met ($P < 0.001$), Met + Cys ($P < 0.001$), Phe ($P < 0.05$), Thr ($P < 0.005$), Val ($P < 0.05$), and total EAA ($P = 0.08$) than sows fed

the CON diet. For nonessential AA (NEAA), sows fed the LCP diet had lower overall plasma concentrations of Asn ($P < 0.05$), Asp ($P < 0.005$), Pro ($P = 0.092$), and Ser ($P < 0.05$) than sows fed the CON diet. The main effect of time was significant for each AA (data not shown); the P values of the contrast with the highest significant PD are presented in Table 5 for each AA. All of the EAA exhibited a quartic relationship over time relative to feeding where peaks occurred at approximately 1- and 4-h postprandial ($P < 0.05$ for Arg, His, Ile, Leu, Met,

Table 5. Serum concentrations of postprandial essential and nonessential AA on day 17 of lactation for primiparous sows fed control or low-protein diets

Item	Diet		SEM ³	<i>P</i> value ¹	Time relative to feeding						SEM	<i>P</i> value	
	CON ²	LCP			0 h	1 h	2 h	3 h	4 h	5 h		PD ⁴	Time ⁵
Number of sows ⁶	6	6											
Essential AA, µmol/L													
Arg	166	120	17	0.030	125	170	140	145	148	131	17	4	0.015
His	144	147	11	0.792	134	152	140	153	153	141	10	4	0.005
Ile	142	126	7	0.124	115	154	138	136	139	124	11	4	0.010
Leu	257	203	9	<0.001	212	258	229	225	236	219	12	4	0.010
Lys	130	156	16	0.279	117	168	144	150	151	128	25	4	0.052
Met	44	86	5	<0.001	57	68	65	68	70	60	5	4	0.014
Met + Cys	49	93	6	<0.001	63	75	71	74	76	66	5	4	0.011
Phe	158	224	17	0.023	176	186	188	204	212	179	21	—	NS ⁷
Phe + Tyr	337	392	26	0.167	344	365	356	379	396	347	24	4	0.068
Thr	257	366	16	0.001	271	335	315	326	324	298	15	4	0.004
Trp	48	58	15	0.499	46	56	54	57	59	45	17	4	0.092
Val	471	579	29	0.024	457	564	539	545	545	498	26	4	0.010
Total essential AA	1,835	2,075	89	0.080	1,720	2,121	1,961	2,018	2,047	1,866	97	4	0.027
Nonessential AA, µmol/L													
Ala	688	706	70	0.863	625	782	697	695	697	685	55	4	0.001
Asn	94	65	11	0.047	67	84	95	86	88	79	10	4	0.004
Asp	27	19	2	0.003	21	25	23	24	22	23	2	5	0.077
Cys	5.4	7.1	0.9	0.136	5.9	7.1	6.3	6.0	6.1	5.9	0.8	4	0.010
Gln	600	561	79	0.683	603	556	524	578	607	614	65	3	0.089
Glu	385	416	38	0.574	387	434	391	384	389	416	30	3	0.017
Gly	1,058	1,055	138	0.981	1,073	1,031	968	1,081	1,112	1,075	120	3	0.078
Pro	466	386	30	0.092	404	457	405	420	442	426	27	4	0.002
Ser	144	113	13	0.042	129	138	120	126	130	139	12	4	0.024
Tyr	180	168	14	0.554	168	179	168	175	184	168	12	4	0.100
Total nonessential AA	3,699	3,329	320	0.294	3,430	3,641	3,324	3,524	3,625	3,542	338	4	0.006

¹Main effect of dietary treatment; the interactive effect between day of lactation and dietary treatment was $P > 0.05$, therefore data are not shown.

²CON = control, 16.2% CP (as-fed; analyzed contents); LCP = low crude protein, 12.7% CP.

³Maximum value of the SEM.

⁴Highest polynomial degree (PD) of significant time effect.

⁵*P* value of specific contrast reported as highest PD.

⁶Six sows sampled over 12 time points, with 2 time points averaged relative to feeding time.

⁷NS = nonsignificant; no significant polynomial contrast.

Met + Cys, Thr, Val, and total EAA, and $P = 0.052$, 0.068, 0.092 for Lys, Phe + Tyr, and Trp, respectively), except for Phe, which was not influenced by the main effect of time. The NEAA exhibited cubic, quartic, and quintic relationships as outlined in Table 5.

Temporal changes in isotopic enrichment of adjusted plasma free Phe and casein protein-bound Phe are presented in Fig. 1. Model parameters for plasma free and casein-bound Phe are shown in Supplementary Appendix 1, none of which differed between treatments. Liver, mammary gland, and LM and gastrocnemius muscle FSR, and casein FSR and FDR rates did not differ between treatments (Table 6). Liver wet weights were lower for LCP-fed sows compared those fed CON ($P < 0.05$) and did not differ when expressed as a percent of BW (Table 6).

DISCUSSION

In a previous study, we reported that reducing dietary CP concentration and supplementing with CAA to meet the requirements of limiting AA, increased milk casein yield and apparent efficiency of N and AA utilization for milk protein production in sows (Huber et al., 2015, 2016). The increase in apparent AA utilization efficiency and casein yields were not associated with any increase in AA transporter mRNA abundance in mammary tissue (Huber et al., 2016). We suggested that improving dietary AA balance may decrease competitive inhibition between AA at the blood-facing aspect of mammary epithelial cells and possibly increase partitioning of dietary AA toward milk protein production by

Figure 1. Temporal changes in adjusted plasma free (A) and casein-bound (B) isotopic enrichment of Phe on day 17 of lactation for primiparous sows fed control (open circle; $n = 3$ for plasma and $n = 5$ for casein; 16.0% CP) and low CP diets (solid circle; $n = 6$; 12.7% CP).

decreasing AA utilization in muscle protein in multiparous sows. The aim of the present study was to determine N partitioning between maternal and milk protein pools in lactating primiparous sows fed either a control diet with limited CAA supplementation or a low CP diet supplemented with CAA to meet the estimated requirements of limiting AA.

In the present study, lactating primiparous sows fed reduced dietary CP supplemented with CAA had lower maternal N retention and greater apparent N utilization efficiency for milk protein production relative to those fed the control diet, which is consistent with multiparous sows (Huber et al., 2015). In the present study, however, primiparous sows fed LCP diet did not increase milk production and casein yield. The reduced maternal N retention in the LCP-fed primiparous sows indicates that one or several AA or N was limiting maternal protein deposition. In fact, apparent N utilization efficiency for milk production was greater than the maximum biological efficiency of utilization reported by the NRC (2012) for groups of sows, and remarkably similar to the biological maximum for individual sows (85%). The NEAA supply in the LCP diet may have been too low to support maternal protein synthesis, and if so, EAA may have provided N for synthesis of NEAA. This suggests that the NRC (2012) estimated requirement for N for primiparous sows may be too low. Conversely, the reduction in N retention for LCP-fed sows was not associated with a reduction in maternal tissue protein FSR including muscle. It is possible that protein FSR was decreased in muscle groups other than the LM or gastrocnemius or that muscle protein FDR may have increased (Baracos et al., 1991). In addition, the reduction in maternal N retention in LCP-fed primiparous sows must be a result of changes in absolute protein synthesis or degradation rates which, unlike factorial rates, are dependent on protein pool

sizes and merit further consideration. We acknowledge that the number of sows used to determine Phe kinetics however was low and may have precluded us from showing differences in FSR between CON- and LCP-fed sows. Nonetheless, the data are novel, and this is the first study to date to report on N utilization in primiparous sows fed a low CP diet with CAA supplementation.

The positive maternal N balance and negative BW change in the present study suggest that the composition of BW change may differ depending on the specific demands of lactation and the supply of nutrients from the diet. Indeed, this has recently been demonstrated in lactating multiparous sows as well (Pedersen et al., 2016). Therefore, the assumption that protein comprises 10% sow BW change during lactation (NRC, 2012) may not be appropriate, which has implications for the calculation of AA utilization efficiency for milk protein production. Furthermore, the assumption that the partial efficiencies of using dietary AA and AA liberated from body protein degradation are static throughout the lactation period and across parities may not be accurate. This issue has been noticed in lactating dairy cows, which exhibit substantial changes in energy status and protein balance throughout lactation (Lee et al., 2015). The increased concentration of plasma EAA (Met + Cys, Phe, Thr, Val) in the LCP-fed primiparous sows may reflect an improved bioavailability of CAA versus protein-bound AA. The quartic relationship of plasma EAA concentrations relative to meal consumption, with peaks occurring at approximately 1- and 4-h postprandial, may simply be a reflection of dietary AA absorption and AA mobilized from maternal protein stores. Conversely, the apparent utilization efficiency of N and all EAA in the present study were greater than those of sows in peak lactation fed nearly identical diets (Huber et al., 2016). Thus, it is likely that

Table 6. Mean isotopic enrichment of Phe in protein-bound pools and fractional synthesis rate of protein in select tissues on day 17 of lactation for primiparous sows fed control or low CP diets

Item	Diet		SEM ¹	P value
	CON ²	LCP		
Number of sows ³	5	6	—	—
Protein-bound enrichment, mol %				
Liver	1.63	1.70	0.10	0.588
Mammary gland ⁴	4.40	4.13	0.30	0.529
LM	0.15	0.18	0.01	0.068
Gastrocnemius	0.18	0.22	0.03	0.259
Protein fractional synthesis rate, %/d				
Liver	29.9	28.2	0.18	0.586
Mammary gland ⁴	66.9	55.8	0.98	0.267
LM	2.21	2.20	0.20	0.993
Gastrocnemius	2.44	2.56	0.32	0.792
Casein fractional synthesis rate, %/d ⁵	128	127	11	0.923
Casein fractional degradation rate, %/d ⁵	28	27	11	0.923
Liver weight, kg ⁶	3.96	3.45	0.14	0.039
Liver weight, % of BW	2.21	2.11	0.12	0.552

¹Maximum value of the SEM.²CON = control, 16.2% CP (as-fed; analyzed contents); LCP = low crude protein, 12.7% CP.³One sow was removed due to catheter malfunction.⁴The litter was allowed to suckle after administration of 5 IU oxytocin to facilitate milk removal before euthanasia and tissue collection.⁵*n* = 3 for CON-fed sows.⁶Wet weight after euthanasia; *n* = 6 for each CON and LCP.

differences exist in the utilization of dietary AA for milk protein production between sows of different parities or genetics. These concepts require further development, especially with respect to the factorial estimations of AA and N requirements for lactating primiparous and multiparous sows.

A concern in the present study is the unrealistically high and variable plasma Phe enrichment, which was attributed to the use of a single catheter for tracer infusion and blood sampling. This approach may have resulted in contamination of blood samples with infusate. Due to the high plateau Phe enrichment in plasma, subsequent calculation of whole-body Phe flux and release of Phe from body protein (i.e., breakdown) were well below practical estimates (and even negative in the case of breakdown; data not shown). Therefore, within the constraints of this study, it was deemed reasonable to correct the observed plasma free Phe enrichment values with the ratio between mammary tissue free and plasma enrichment values determined at 12 h, which carries the assumption that the free pool in mammary tissue had also reached an “uncontaminated” plateau.

Of all the tissues sampled, enrichment in the free pool of the mammary gland was least variable at time of tissue collection, and therefore, it was selected as a reasonable candidate for the correction. The correction only affected estimates of whole-body Phe flux, but does not influence estimates obtained for FSR of the various tissues; this requires careful consideration in future studies. In fact, the protein FSR values for liver and muscle (both LM and gastrocnemius) were within the range of those determined by others in other lactating species using either flooding dose or continuous infusion techniques (e.g., [Lobley et al., 1980](#); [Pine et al., 1994](#); [Tauveron et al., 1994](#)). Though Phe kinetics were determined in a relatively small sample size of lactating primiparous sows in the present study, the data are novel and essential to further our understanding of AA nutrition for lactating sows. In future lactating sow studies, replication should be increased and the procedure adjusted to minimize potential contamination of the blood samples with the infusate (e.g., contralateral jugular catheters for infusion and blood sampling or a jugular catheter for infusion and an arterial catheter for blood sampling).

In the present study, the coefficients of variation for Phe kinetic parameters were generally larger than 20%, however relative to E_p , the measurement of E_c had low variation. Additionally, the times to reach 90% of plateau enrichment in plasma (3.6 h) and caseins (13.5 h) were comparable to values from lactating goats administered a nonprimed continuous infusion (5.8 and 13.8 h for plasma and caseins, respectively; [Bequette et al., 1994](#)). As well, the rate constants for Phe labeling of caseins were similar between these studies. Therefore, the present study yielded reasonable values for casein FSR and FDR under an hourly milking regime. In both the present study and an in vitro study conducted by [Hanigan et al. \(2009\)](#), considerable casein degradation occurred before excretion into milk protein. Indeed, it has been suggested previously that a portion of intracellular AA used for milk casein synthesis in the mammary gland are supplied by breakdown of rapidly turning over proteins (e.g., casein signal peptides, nonexported milk proteins, enzymes, structural proteins; [Bequette et al., 1998](#)), which results in a rather significant inefficiency of energy use for milk protein production ([Hanigan et al., 2009](#)). These data are new for lactating sows and provide important insight into the inefficiency of AA and energy utilization for milk (protein) production and will aid in the refinement of factorial estimations of AA requirements.

Feeding lactating primiparous sows low CP diets supplemented with CAA to provide limiting AA does not negatively affect lactation

performance and improves apparent N and Arg, Ile, Leu, Phe + Tyr, and Trp utilization efficiency for milk production and, therefore, can be used as a strategy to mitigate N losses to the environment. The effect of feeding a low CP diet over multiple consecutive lactation periods on sow longevity and lifetime performance should also be examined. Differences in maternal N retention, however, were not mirrored by differences in maternal tissue protein and casein FSR.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

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