Dietary protein concentration affects plasma arteriovenous difference of amino acids across the porcine mammary gland[1]

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ABSTRACT: The objective of this study was to determine whether the porcine mammary gland responds to increasing dietary CP concentration through changes in AA arteriovenous difference (a-v). Sixteen Landrace × Yorkshire lactating sows were provided ad libitum access to one of four isocaloric diets varying in CP concentration (7.8, 13.0, 18.2, and 23.5 %; as-fed basis). Litters were adjusted to 11 pigs within 48 h of birth. Sows were fitted with catheters in the carotid artery and main mammary vein on d 4. On d 10, 14, 18, and 22 of lactation, arterial and venous blood samples were obtained every 30 min over 6 h. Milk yield was estimated on d 11 and 21 using the D_2O dilution technique. Final litter sizes on d 21 were 10.3, 11, 9.5, and 11 piglets for sows fed the 7.8, 13.0, 18.2, and 23.5% CP diets, respectively. Piglet ADG tended ($P = 0.088$) to increase with increasing dietary CP concentration and were 186, 221, 220, and 202 g for sows fed the 7.8, 13.0, 18.2, and 23.5% CP diet, respectively. Daily total milk yield on d 21 (kg milk/d) tended ($P = 0.099$) to increase, and average milk yield per nursed piglet (kg of milk·pig^{-1}·d^{-1}) increased ($P < 0.05$) with increasing CP concentration and were, on a per-piglet basis, 0.95, 1.19, 1.14 and 1.13 kg of milk/d for the 7.8, 13.0, 18.2, and 23.5% CP diets, respectively. As dietary CP increased from 7.8 to 23.5%, isoleucine and leucine a-v increased linearly only (linear, $P < 0.01$); all other AA a-v increased, reached a maximum in sows fed 18.2% CP, and decreased thereafter in sows fed 23.5% CP (quadratic, from $P = 0.10$ to $P < 0.05$). Amino acid uptake by the entire udder and by each gland increased (linear, $P < 0.05$) with increasing dietary CP. Arteriovenous differences response to increasing day of lactation varied among AA, from no change for histidine, isoleucine, lysine, methionine, tryptophan, and valine, to a linear trend increase for arginine ($P = 0.055$), leucine ($P = 0.064$), phenylalanine ($P = 0.101$), and threonine ($P = 0.057$). In summary, for the majority of AA, a-v increased with increasing dietary CP concentration from 7.8 to 18.2%, but decreased when CP concentration exceeded 18.2%. In contrast, mammary AA uptake, piglet ADG and milk yield per pig increased linearly with increasing dietary CP, suggesting a coordinated regulation between AA delivery and transport to meet the demand for milk yield.

Key Words: Amino Acid Transport, Dietary Crude Protein, Lactation, Mammary Gland, Porcine

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Introduction

Empirical studies have provided excellent information to allow development of models to estimate AA requirements for lactation. According to recent NRC (1998) recommendations, estimates of AA requirements for lactation can be predicted based on individual pig ADG and litter size, and hence milk production level. Refinement of existing models will require more mechanistic descriptions necessary to predict how the mammary gland responds to nutrients. There is a paucity of in vivo information investigating how the porcine mammary gland responds to limited or excess dietary CP and/or AA. This lack of information contributes partly to the limited understanding of mechanisms regulating dietary AA use by mammary tissue.

Arteriovenous difference (**a-v**) and mammary AA uptake responses to production factors known to modulate milk yield, such as stage of lactation and litter size,

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have been assessed. Litter size has little effect on AA a-v across the porcine udder, as a-v across the porcine udder reflects a-v across each single mammary gland (Nielsen et al., 2002). Feeding graded levels of dietary lysine and/or protein to lactating sows results in a curvilinear response, whereby piglet ADG increases to plateau when lysine and/or protein requirement is met (Stahly et al., 1992; King et al., 1993; Yang et al., 2000). It is unknown whether the porcine mammary gland responds to increasing CP concentration through changes in AA transport as measured by a-v. It has been suggested that the mammary gland limits the entry of additional AA as suggested in the dairy cow (Guinard and Rulquin, 1994). We hypothesized that AA a-v and uptake across the porcine mammary gland is a function of dietary CP intake. Objectives of this study were to test whether indispensable AA a-v and uptake across the porcine mammary gland respond to varying concentrations of dietary CP, and to determine whether the magnitude of the response is similar among AA.

Materials and Methods

This study was approved by the Michigan State University All-University Committee on Animal Use and Care.

Experimental Design and Diets

Sixteen Landrace × Yorkshire lactating sows (Parity 2 or 3) were allocated to dietary treatments according to a randomized block design. Each block consisted of a time period with four sows. Each sow in one block was provided ad libitum access to one of four diets. Diets contained different CP concentrations in an attempt to create sufficient variation to assess if AA a-v and uptake are affected by AA availability. Treatments consisted of Deficient, Low, Normal, and Excess CP diets containing 7.8, 13.0, 18.2, and 23.5% CP (as-fed basis), respectively. The Normal diet was formulated to meet the CP and lysine requirements of a sow nursing an average litter of 10 pigs with an ADG of 200 g/pig (NRC, 1998). To optimize similarity in AA concentrations relative to lysine across diets, corn and soybean meal were included in a fixed ratio of 1.05:1. Thus, the Excess CP diet was diluted with cornstarch and sucrose in a fixed ratio of 3:1 to obtain the Normal, Low and Deficient CP diets. Sucrose and tallow were used to improve palatability and decrease dustiness of diets resulting from the addition of cornstarch and solka flock. Diets were balanced to be isocaloric (14.3 MJ of ME/kg). Methionine, threonine, and valine were included so that all diets contained a minimum ratio of methionine, threonine and valine to lysine of 0.28, 0.68, and 0.88 (Trottier et al., 1997). Ingredient and nutrient composition of diets are given in Table 1.

Animals

Litters were cross-fostered to 11 pigs per sow within 48 h of birth. Sows were housed individually in farrowing crates in a thermally controlled room (21°C), and provided ad libitum access to feed and water. Feed intake was recorded daily. On d 1 and 21 of lactation, backfat depth and loin eye area were measured using B-mode ultrasound equipped with a 3.5-MHz 18-cm probe (Pie Medical Scanner 200, Pie Medical Equipment, Maastricht, The Netherlands), 5 cm lateral to the spine and centered over the 10th rib. Sows and piglets were weighed individually every week. The anterior main mammary vein and a carotid artery were cannulated on d 4 ± 1 of lactation as described by Trottier et al. (1995). At 24, 48, and 72 h after surgery, sows were administered an antibiotic (Naxcel, Pharmacia and Upjohn Co., Kalamazoo, MI) and an antiinflammatory (Banamine, Schering-Plough Animal Health Corp., Kenilworth, NJ). Catheters were flushed once daily with heparinized saline (20 IU of heparin/mL).

Milk Yield Estimation

Milk intake by piglets was estimated on d 11 and 21 by the D_2O dilution method of Pettigrew et al. (1987) as modified by Pluske et al. (1997) using piglet serum. In brief, piglets were fasted for 1 h, weighed, and injected i.m. (1 mg/kg BW) with D_2O (Cambridge Isotope Laboratories, Andover, MA). One hour after D_2O administration, a blood sample was taken via jugular puncture from each piglet and kept on ice until centrifuged. At 24 h, piglets were fasted for 1 h, weighed, and a blood sample taken as before. During the 24-h period following D_2O administration, piglets were not given access to water. Blood samples were centrifuged at 1,500 × *g* for 15 min at 4°C, and serum was separated and stored at –20°C. Serum D_2O concentration was determined by infrared spectrophotometry (The Foxboro Co., East Bridgewater, MA) set at a fixed-filter length of 4 μm. Milk intake was estimated on the basis of the D_2O dilution principle (Pettigrew et al., 1987).

Blood and Milk Sampling Protocol

Carotid arterial and mammary venous blood samples were collected simultaneously every 30 min over 6 h from each sow on d 10, 14, 18, and 22. Samples were kept on ice for a maximum of approximately 30 min until centrifugation. Sows were fed 1 h before blood sampling and provided ad libitum access to feed and water during the sampling period. In total, 13 blood samples were collected from each sow per sampling day. Blood was centrifuged at 1,500 × *g* for 15 min at 4°C, plasma removed and stored at –20°C. Approximately 2 h following the 6-h blood sampling period, a total of approximately 40 mL of milk was collected from each sow. Milk was obtained in equal proportion from all functional teats following i.v. administration of oxytocin (10 IU) and pooled for each sow. One aliquot of milk was defatted by centrifugation at 1,500 × *g* for 15 min at 4°C, and stored at –20°C for subsequent analysis of AA concentration. One aliquot of milk was kept fresh for analysis of fat, lactose, and true protein concentration.

Table 1. Ingredient and nutrient composition of experimental diets, as-fed basis

Item	Dietary CP, %: 7.8 Deficient	13.0 Low	18.2 Normal	23.5 Excess
Ingredients				
Corn	15.43	25.72	36.00	46.37
Soybean meal (44% CP)	14.75	24.58	34.41	44.32
Corn starch	42.53	28.39	14.25	0
Sugar	14.18	9.46	4.75	0
Tallow	5.00	5.00	5.00	5.00
Solka floc	3.02	2.02	1.02	0
Dicalcium phosphate	3.14	2.64	2.14	1.63
Calcium carbonate	0.51	0.70	0.89	1.08
Salt	0.25	0.25	0.25	0.25
Trace mineral and vitamin premix[a]	1.13	1.13	1.13	1.13
DL-Methionine	0.027	0.044	0.061	0.079
L-Threonine	0.011	0.019	0.025	0.033
L-Valine	0.038	0.064	0.090	0.115
Nutrients, analyzed				
ME, MJ/kg[b]	14.3	14.3	14.3	14.3
CP, %	8.2	13.2	18.2	23.0
Amino acids, %				
Arginine	0.60 (1.46)[c]	0.93 (1.38)	1.28 (1.42)	1.60 (1.34)
Histidine	0.30 (0.73)	0.45 (0.67)	0.58 (0.64)	0.72 (0.61)
Isoleucine	0.37 (0.90)	0.57 (0.85)	0.78 (0.87)	1.00 (0.84)
Leucine	0.64 (1.56)	1.01 (1.51)	1.36 (1.51)	1.73 (1.45)
Lysine	0.41	0.67	0.90	1.19
Methionine	0.18 (0.43)	0.27 (0.40)	0.34 (0.38)	0.42 (0.35)
Phenylalanine	0.42 (1.02)	0.66 (0.99)	0.89 (0.99)	1.13 (0.95)
Threonine	0.32 (0.78)	0.53 (0.79)	0.66 (0.73)	0.93 (0.78)
Tryptophan[b]	0.11 (0.27)	0.18 (0.27)	0.25 (0.28)	0.33 (0.28)
Valine	0.46 (1.12)	0.71 (1.06)	0.96 (1.07)	1.21 (1.02)

[a]Provided the following amounts of trace minerals and vitamins in milligrams per kilogram of diet: copper, 5; iodine, 0.075; iron, 50; manganese, 5; selenium, 0.15; zinc, 50; retinyl acetate, 2.84; cholecalciferol, 0.0138; DL-alpha-tocopherol, 44.1; menadione, 4.5; vitamin B_{12}, 0.033; riboflavin, 4.5; d-pantothenic acid, 17.6; niacin, 26.4; thiamin, 1.1; pyridoxine, 1.0; choline, 385.0; folic acid, 1.65; and d-biotin, 0.22.
[b]Calculated values (NRC, 1998).
[c]Values in parenthesis represent the analyzed AA:lysine concentration ratio.

Sample Analyses

Milk. Milk fat, lactose, and protein concentrations were assayed using midinfrared spectroscopy (Multispec M, Berwind Instrument Ltd., York, U.K.). Milk energy content was estimated by multiplying the percentage of milk protein, fat, and lactose by 5.70, 9.40, and 4.15 kcal/g, respectively (Jenness, 1974). For AA analysis in defatted milk, milk samples were hydrolyzed in 6 *N* HCl at 110°C for 24 h before analysis by reverse-phase HPLC (Alliance 2690, Waters Corp., Milford, MA), as described by Guan et al. (2002). Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis.

Plasma. For each sampling day, the 13 plasma samples obtained per sow were pooled, glucosaminic acid was added as an internal standard, and samples were deproteinized by addition of 35% (wt/vol) sulfosalicylic acid followed by centrifugation at 4°C. Plasma AA concentrations were determined by a commercial laboratory (Missouri Agric. Exp. Stn. Chem. Laboratory, Univ. of Missouri, Columbia) on a Beckman 6300 AA analyzer (Fullerton, CA), as described by Guan et al. (2002).

Diets. Feed samples were finely ground using a Cyclotec 1093 Sample Mill (Foss Tecator, Hoeganaes, Sweden). Feed N was analyzed by the microKjeldahl method (AOAC, 1990). Amino acid concentrations in feed samples were analyzed by reverse-phase high-performance liquid chromatography (Alliance 2690, Waters Corp.) as described by Guan et al. (2002) following hydrolysis in 6 *N* HCL at 110°C for 24 h. Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis.

Calculations

Net output of AA in milk (g/d) was calculated as described by Guan et al. (2002):

$$\text{Milk yield (L/d)} \times (1 - \text{lipid concentration in whole milk}) \times \text{AA concentration } (\mu\text{mol/L}) \text{ in defatted milk} \times \text{AA molecular weight (g/mol)} \times 10^{-6}.$$

Mammary uptake of AA (g/d) was calculated as described by Trottier et al. (1997) and Nielsen et al. (2002):

Table 2. Effect of dietary CP concentration on sow and litter performance[a]

	Dietary CP, %						
Item	7.8 Deficient	13.0 Low	18.2 Normal	23.5 Excess	SEM	L *P* values[b]	Q *P*-values[c]
No. of sows	4	4	4	4	—		
Intake, kg/d							
Feed	4.62	5.75	4.67	4.62	0.20	0.099	0.014
Protein	0.366	0.750	0.845	1.070	0.037	0.001	0.060
Sow BW, kg							
d 1	209.5	216.1	220.0	230.3	8.4	—	—
Change, d 1 to 21	–25.8	–25.4	–16.8	–26.6	4.6	0.462	0.363
Backfat depth, mm							
d 1	22.5	21.1	24.4	20.8	2.6	—	—
Change, d 1 to 21	–4.6	–0.2	–6.8	–5.8	1.4	0.874	0.483
Loin muscle area, cm^2							
d 1	46.1	47.2	44.8	51.2	2.4	—	—
Change, d 1 to 21	–8.46	–3.72	–1.26	–3.70	1.44	0.141	0.018
Litter size							
d 1	11	11	11	11	—	—	—
d 21	10.3	11.0	9.5	11.0	0.4	0.146	0.290
Litter wt, kg							
d 1	19.1	19.3	19.3	18.5	0.8	—	—
Gain, d 1 to 21	37.5	48.7	41.4	44.5	2.9	0.106	0.203
Pig ADG, g							
d 1 to 21	186	221	220	202	14	0.088	0.310
Milk yield, kg/d							
d 11	10.03	13.07	10.57	12.99	1.25	0.294	0.809
d 21	9.18	13.21	10.82	12.43	1.25	0.099	0.351
Average milk yield, kg/pig	0.95	1.19	1.14	1.13	0.08	0.017	0.461

[a]Data are least squares means ± standard error.
[b]*P*-values for linear (L) contrast.
[c]*P*-values for quadratic (Q) contrast.

Mammary arterio-venous difference (μmol/L) of an AA × mammary plasma flow rate (L/d)

Daily mammary plasma flow rate was estimated based on the conservation of lysine across the mammary glands as described by Trottier et al. (1997) and Nielsen et al. (2002). Mammary plasma flow rate was estimated for d 10 and 14 using d 10 and 14 plasma lysine a-v and milk concentration values, respectively, and d 11 milk yield estimate. Similarly, for d 18 and 22, mammary plasma flow rate was estimated using d 18 and 22 plasma lysine a-v and milk concentration values, respectively, and d-22 milk yield estimate. For estimation of mammary plasma flow on d 11 and 21, d 10 and 22 plasma lysine a-v and milk concentration values were used, respectively, along with milk yield estimates for d 11 and d 21.

Statistical Analyses

Data were analyzed using the MIXED Procedure of SAS/STAT (Version 8, SAS Inst., Inc., Cary, NC) and the first-autogressive covariance structure as best fit. Individual sow was considered as the experimental unit and day of lactation as the repeated effect. The model for milk and plasma measurements included the random effect of sow within diet and fixed effects of block, diet, and day of lactation, and all two-way interactions with day of lactation in a repeated statement. The interaction between diet and day of lactation was not significant ($P > 0.10$); therefore, only main effects (diet or day of lactation) are presented. The model for performance measurements included sow, block, and dietary treatment as classification effects; sow body weight, backfat depth, and loin eye area on d 1 of lactation were used as covariates. Relationships between dietary CP intake or day of lactation and outcome variables were determined using linear and quadratic contrasts. Significant effects were considered at $P < 0.05$ and significant trends at $P \leq 0.10$.

Results

Lactation Performance

Feed and CP intake over the 21-d lactation period increased (linear, $P = 0.099$ and $P < 0.01$, respectively; quadratic, $P < 0.05$ and $P = 0.06$, respectively) with increasing dietary CP concentration (Table 2). Sow BW and backfat change was not affected by dietary CP intake, and loss in loin eye area decreased (quadratic, $P < 0.05$) with increasing dietary CP intake, reaching a minimum in sows fed the Normal CP diet. Sows fed the Deficient and Normal CP diet lost, on average, 0.7 and 1.5 pigs, respectively, but there was no relationship between dietary CP intake and litter size on d 21 of

lactation. Piglet ADG tended (linear, $P = 0.088$) to increase with increasing dietary CP concentration and was 186, 221, 220, and 202 g for sows fed the Deficient, Low, Normal, and Excess CP diets, respectively. Over the 21-d lactation period, daily (or 21-d) litter weight gain tended (linear, $P = 0.106$) to increase with increasing dietary CP concentration. Daily total milk yield (kg of milk/d) on d 21 tended (linear, $P = 0.099$) to increase and milk yield per pig (kg milk·pig^{-1}·d^{-1}) increased (linear, $P < 0.05$) with increasing CP concentration and was, on a per-pig basis, 0.95, 1.19, 1.14 and 1.13 kg of milk/d for the Deficient, Low, Normal, and Excess CP diets, respectively.

Milk Composition

Milk nutrient composition is presented in Tables 3 and 4. True protein concentration in whole milk increased and lactose concentration decreased with increasing dietary CP concentration (linear, $P < 0.01$; Table 3). True milk protein concentration was approximately 10% lower for sows fed the Deficient and Low CP diets compared with that in sows fed Normal and Excess CP diets. Whole milk DM, fat, and energy concentrations were not different between diets. True milk protein concentration decreased with day of lactation, reaching a minimum of d 18 and increasing thereafter to d 22 (quadratic, $P < 0.05$).

Generally, AA concentrations in defatted milk (Table 4) reflected changes described above for true milk protein concentration. Except for histidine and methionine, where no change occurred, AA concentrations in defatted milk increased linearly (from $P < 0.05$ to 0.01) with increasing dietary CP concentration. Concentration in defatted milk decreased for most AA, with increasing day of lactation from 10 to 18 and increased thereafter to d 22 (quadratic, $P < 0.05$).

Data on daily milk AA output are presented in Table 5. Increasing dietary CP linearly increased ($P < 0.05$) AA output in milk. For sows fed the Deficient diet, milk output of individual AA was lower by 25 to 30% compared with that in other diets. There were no differences in individual AA and protein output in milk (0.55 ± 0.03 vs. 0.55 ± 0.03 kg/d, $P > 0.10$) between d 11 and 21 of lactation.

Arterial Concentration and Arteriovenous Difference

Arterial AA concentrations and a-v data are presented in Tables 6 and 7, respectively. Except for histidine, where no change in arterial concentration occurred, arterial concentrations for most AA, and notably so for the branched-chain AA (**BCAA**), increased linearly (from $P < 0.05$ to 0.01) with increasing CP concentration (Table 6). For some AA, such as arginine, methionine, threonine, and tryptophan, arterial concentrations increased, reaching a maximum on the Low CP diet and remaining relatively constant thereafter as CP increased (quadratic, from $P < 0.05$ to 0.01). Ex-

Table 3. Effect of increasing dietary CP concentration and day of lactation on sow milk composition (as-is basis)[a]

	Dietary CP, %				Day of lactation					Diet		Day	
	7.8 Deficient	13.0 Low	18.2 Normal	23.5 Excess	10	14	18	22		L[c]	Q[d]	L	Q
No.[b]	16	13	14	13	15	13	13	15		*P*-values		*P*-values	
DM, %	18.42 ± 0.32	18.27 ± 0.39	18.56 ± 0.39	18.91 ± 0.39	18.65 ± 0.32	18.66 ± 0.39	18.26 ± 0.39	18.59 ± 0.32		0.361	0.516	0.726	0.674
True protein, %	4.53 ± 0.07	4.51 ± 0.11	4.94 ± 0.08	5.02 ± 0.11	4.77 ± 0.08	4.71 ± 0.09	4.61 ± 0.09	4.90 ± 0.08		0.001	0.590	0.434	0.041
Fat, %	6.29 ± 0.22	6.31 ± 0.34	6.37 ± 0.27	6.76 ± 0.34	6.82 ± 0.24	6.53 ± 0.27	6.15 ± 0.27	6.22 ± 0.24		0.263	0.538	0.057	0.483
Lactose, %	5.83 ± 0.09	5.84 ± 0.14	5.61 ± 0.11	5.47 ± 0.14	5.62 ± 0.10	5.72 ± 0.11	5.81 ± 0.11	5.61 ± 0.10		0.018	0.502	0.881	0.144
Energy, Mcal/kg	1.09 ± 0.02	1.09 ± 0.03	1.11 ± 0.03	1.15 ± 0.03	1.15 ± 0.02	1.12 ± 0.03	1.08 ± 0.03	1.10 ± 0.02		0.138	0.560	0.089	0.409

[a]Data are least squares means ± standard error.
[b]Number of milk samples analyzed.
[c]*P*-values for linear (L) contrast.
[d]*P*-values for quadratic (Q) contrast.

Table 4. Effect of increasing dietary CP concentration and day of lactation on amino acid concentrations (mmol/L) in defatted porcine milk[a]

	Dietary CP, %				Day of lactation				Diet		Day	
									L[c]	Q[d]	L	Q
Item	7.8 Deficient	13.0 Low	18.2 Normal	23.5 Excess	10	14	18	22	*P*-values		*P*-values	
No.[b]	16	13	14	13	15	13	13	15				
Arg	16.7 ± 0.5	16.4 ± 0.7	17.7 ± 0.6	18.6 ± 0.7	17.6 ± 0.5	17.5 ± 0.6	16.2 ± 0.6	18.2 ± 0.5	0.014	0.345	0.900	0.054
His	11.0 ± 0.3	11.2 ± 0.7	11.3 ± 0.4	11.8 ± 0.5	11.5 ± 0.3	11.6 ± 0.4	10.1 ± 0.4	12.2 ± 0.4	0.165	0.778	0.795	0.018
Ile	17.9 ± 0.4	18.3 ± 0.6	19.7 ± 0.4	20.9 ± 0.6	19.3 ± 0.4	19.3 ± 0.5	18.4 ± 0.5	19.9 ± 0.4	0.001	0.394	0.597	0.090
Leu	31.9 ± 0.6	32.0 ± 0.8	34.7 ± 0.7	36.3 ± 0.8	33.8 ± 0.6	33.8 ± 0.7	32.4 ± 0.7	34.8 ± 0.6	0.001	0.286	0.564	0.070
Lys	25.2 ± 0.7	24.5 ± 1.0	27.9 ± 0.8	28.5 ± 1.0	26.7 ± 0.7	26.3 ± 0.8	25.0 ± 0.8	28.1 ± 0.7	0.003	0.469	0.379	0.032
Met	9.1 ± 0.5	9.0 ± 0.6	8.7 ± 0.5	9.5 ± 0.5	9.1 ± 0.4	9.1 ± 0.5	8.6 ± 0.5	9.6 ± 0.4	0.589	0.448	0.589	0.263
Phe	13.3 ± 0.3	13.2 ± 0.4	14.5 ± 0.3	15.0 ± 0.4	14.0 ± 0.3	14.0 ± 0.3	13.4 ± 0.3	14.6 ± 0.3	0.001	0.388	0.383	0.054
Thr	21.0 ± 0.5	19.8 ± 0.8	21.8 ± 0.6	22.9 ± 0.8	21.8 ± 0.6	21.2 ± 0.7	20.0 ± 0.7	22.5 ± 0.6	0.023	0.110	0.708	0.018
Val	26.4 ± 0.4	25.9 ± 0.7	28.1 ± 0.5	29.5 ± 0.7	27.7 ± 0.5	27.5 ± 0.5	26.2 ± 0.5	28.5 ± 0.5	0.001	0.117	0.661	0.017

[a]Data are least squares means ± standard error.
[b]Number of milk samples analyzed.
[c]*P*-values for linear (L) contrast.
[d]*P* values for quadratic (Q) contrast.

cept for arginine, threonine, and tryptophan, where no change occurred, arterial concentrations of other AA increased curvilinearly with day of lactation, reaching a maximum between d 14 and 18 (quadratic, from $P = 0.082$ to 0.05).

As dietary CP increased, a-v increased curvilinearly (quadratic) for arginine ($P < 0.01$), histidine ($P = 0.078$), lysine ($P < 0.05$), methionine ($P < 0.05$), phenylalanine ($P = 0.071$), threonine ($P < 0.05$), tryptophan ($P = 0.109$), valine ($P = 0.061$), and total indispensable AA ($P < 0.05$), reaching a maximum on the Normal diet, and decreasing thereafter for sows fed the Excess CP diet (Table 7). For isoleucine and leucine, a-v increased linearly only ($P < 0.01$) with increasing dietary CP. For a majority of AA, day of lactation varying from 10 to 22 had little effect on a-v. Arteriovenous differences response to increasing day of lactation varied among AA, from no change for histidine, isoleucine, lysine, methionine, tryptophan, and valine, to a linear trend increase for arginine ($P = 0.055$), leucine ($P = 0.064$), phenylalanine ($P = 0.101$), and threonine ($P = 0.057$). There was no quadratic relationship between AA a-v and day of lactation.

Plasma Flow and Net Mammary Uptake of Amino Acids

Data on the relationship between dietary CP intake, mammary plasma flow, and net uptake of AA are presented in Table 8. There was no relationship (linear or quadratic) between dietary CP and plasma flow. There was no difference in plasma flow between d 11 and 21 of lactation. For most AA, in particular isoleucine and leucine, mammary (total udder) uptake increased linearly (from $P < 0.05$ to 0.01) with increasing dietary CP.

Table 5. Effect of increasing dietary CP concentration and day of lactation on amino acid output (g/d) in sow milk[a]

	Dietary CP, %				Day of lactation		Diet	
							L[c]	Q[d]
Item	7.8 Deficient	13.0 Low	18.2 Normal	23.5 Excess	11	21	*P*-values	*P*-values
No.[b]	8	7	8	7	15	15		
Arg	26.0 ± 2.4	36.3 ± 2.6	30.7 ± 2.4	38.6 ± 2.6	33.3 ± 1.6	32.5 ± 1.6	0.019	0.644
His	15.3 ± 1.7	21.6 ± 2.0	17.9 ± 1.7	22.0 ± 1.8	19.5 ± 1.1	18.9 ± 1.2	0.066	0.558
Ile	21.2 ± 2.1	29.8 ± 2.28	25.8 ± 2.1	32.6 ± 2.3	27.6 ± 1.4	27.2 ± 1.4	0.014	0.691
Leu	37.3 ± 3.7	52.2 ± 3.9	45.5 ± 3.7	56.6 ± 3.9	48.3 ± 2.5	47.5 ± 2.5	0.015	0.639
Lys	33.0 ± 3.6	44.7 ± 3.9	41.1 ± 3.6	50.5 ± 3.9	42.4 ± 2.4	42.3 ± 2.4	0.017	0.772
Met	11.1 ± 1.4	14.9 ± 1.6	13.4 ± 1.2	17.4 ± 1.2	14.2 ± 0.9	14.2 ± 0.9	0.023	0.946
Phe	19.7 ± 2.0	27.0 ± 2.1	23.8 ± 2.0	29.5 ± 2.1	25.1 ± 1.3	24.9 ± 1.3	0.018	0.703
Thr	22.6 ± 2.4	30.0 ± 2.5	26.0 ± 2.4	32.6 ± 2.5	28.0 ± 1.5	27.7 ± 1.5	0.040	0.868
Val	27.7 ± 2.7	38.1 ± 2.9	32.7 ± 2.7	41.2 ± 2.9	35.2 ± 1.8	34.6 ± 1.8	0.021	0.740

[a]Data are least squares means ± standard error.
[b]Number of observations per treatment.
[c]*P*-values for linear (L) contrast.
[d]*P*-values for quadratic (Q) contrast.

Table 6. Effect of increasing dietary CP concentration and day of lactation on arterial plasma amino acid concentrations (μmol/L)[a]

	Dietary CP, %				Day of lactation				Diet		Day	
									L[c]	Q[d]	L	Q
Item	7.8 Deficient	13.0 Low	18.2 Normal	23.5 Excess	10	14	18	22	*P*-values		*P*-values	
No.[b]	12	9	12	12	12	11	11	11				
Arg	109.0 ± 12.7	185.5 ± 22.0	219.2 ± 12.7	198.7 ± 12.7	148.9 ± 12.7	190.5 ± 14.7	194.1 ± 14.7	178.9 ± 14.7	0.001	0.007	0.148	0.542
His	93.0 ± 2.9	95.0 ± 5.1	94.6 ± 2.9	88.7 ± 2.9	87.6 ± 2.9	96.7 ± 3.4	96.0 ± 3.4	91.1 ± 3.4	0.348	0.285	0.507	0.044
Ile	117.0 ± 6.7	107.0 ± 11.5	122.0 ± 6.7	139.3 ± 6.7	99.7 ± 6.7	121.7 ± 7.7	136.9 ± 7.7	127.0 ± 7.7	0.019	0.116	0.009	0.042
Leu	83.6 ± 9.2	130.3 ± 15.9	161.2 ± 9.2	202.9 ± 9.2	120.4 ± 9.2	151.9 ± 10.6	156.1 ± 10.6	149.5 ± 10.6	0.001	0.828	0.056	0.074
Lys	126.8 ± 8.6	126.4 ± 14.9	184.0 ± 8.6	162.0 ± 8.6	127.0 ± 8.6	171.4 ± 9.9	156.0 ± 9.9	144.8 ± 9.9	0.001	0.319	0.372	0.009
Met	36.6 ± 2.6	48.4 ± 4.5	48.6 ± 2.6	46.3 ± 2.6	41.2 ± 2.6	48.7 ± 3.0	46.4 ± 3.0	43.5 ± 3.0	0.029	0.043	0.716	0.082
Phe	52.9 ± 4.1	74.3 ± 7.1	80.3 ± 4.1	96.2 ± 4.1	61.2 ± 4.1	78.5 ± 4.7	83.0 ± 4.7	81.0 ± 4.7	0.001	0.580	0.005	0.044
Thr	85.3 ± 8.6	134.8 ± 15.0	160.1 ± 8.6	160.0 ± 8.6	126.3 ± 8.6	142.6 ± 10.0	136.4 ± 10.0	134.8 ± 10.0	0.001	0.033	0.649	0.352
Trp	36.4 ± 2.5	53.9 ± 4.3	56.5 ± 2.50	56.5 ± 2.5	48.5 ± 2.5	51.7 ± 2.9	53.8 ± 2.9	49.4 ± 2.9	0.001	0.012	0.704	0.179
Val	244.5 ± 14.5	288.3 ± 25.2	313.1 ± 14.5	354.6 ± 14.5	245.7 ± 14.5	314.5 ± 16.8	328.4 ± 16.8	311.8 ± 16.8	0.001	0.949	0.009	0.016

[a]Data are least squares means ± standard error.
[b]Number of plasma samples analyzed.
[c]*P*-values for linear (L) contrast.
[d]*P*-values for quadratic (Q) contrast.

Table 7. Effect of increasing dietary CP concentration and day of lactation on arterio-venous (μmol/L) differences of plasma amino acids across the mammary glands in lactating sows[a]

	Dietary CP, %				Day of lactation				Diet		Day	
									L[c]	Q[d]	L	Q
Item	7.8 Deficient	13.0 Low	18.2 Normal	23.5 Excess	10	14	18	22	*P*-values		*P*-values	
No.[b]	12	9	9	12	12	10	10	10				
Arg	26.56 ± 3.44	31.15 ± 6.01	62.81 ± 6.01	38.77 ± 3.44	34.22 ± 3.43	35.43 ± 4.31	46.89 ± 4.31	42.77 ± 4.31	0.002	0.010	0.055	0.507
His	9.22 ± 1.58	7.58 ± 2.77	21.99 ± 2.77	12.03 ± 1.58	12.66 ± 1.58	9.88 ± 1.99	15.56 ± 1.99	12.72 ± 1.99	0.014	0.078	0.481	0.989
Ile	24.27 ± 2.76	33.32 ± 4.83	44.99 ± 4.83	43.81 ± 2.76	32.69 ± 2.76	33.27 ± 3.46	43.69 ± 3.46	36.74 ± 3.46	0.001	0.199	0.133	0.252
Leu	41.63 ± 3.30	53.73 ± 5.78	67.06 ± 5.78	72.01 ± 3.30	53.00 ± 3.30	53.67 ± 4.14	68.20 ± 4.14	59.56 ± 4.14	0.001	0.443	0.064	0.238
Lys	33.23 ± 2.59	36.99 ± 4.53	57.00 ± 4.53	41.98 ± 2.59	39.93 ± 2.59	36.13 ± 3.25	48.61 ± 3.25	44.53 ± 3.25	0.004	0.017	0.390	0.343
Met	10.04 ± .91	11.85 ± 1.59	17.74 ± 1.59	13.91 ± .91	12.47 ± .91	12.22 ± 1.14	15.42 ± 1.14	13.42 ± 1.14	0.002	0.042	0.213	0.411
Phe	17.76 ± 1.41	20.78 ± 2.47	28.07 ± 2.47	23.47 ± 1.41	20.80 ± 1.41	19.87 ± 1.77	26.59 ± 1.77	22.81 ± 1.77	0.005	0.071	0.101	0.390
Thr	24.68 ± 2.27	25.35 ± 3.97	46.19 ± 3.97	31.90 ± 2.27	29.14 ± 2.27	27.42 ± 2.84	37.80 ± 2.84	33.77 ± 2.84	0.003	0.032	0.057	0.660
Trp	3.54 ± 1.07	2.65 ± 1.86	10.14 ± 1.86	4.23 ± 1.07	5.01 ± 1.07	4.48 ± 1.34	5.98 ± 1.34	5.09 ± 1.34	0.097	0.109	0.753	0.882
Val	35.13 ± 5.64	47.99 ± 9.88	77.02 ± 9.88	58.02 ± 5.64	47.16 ± 5.64	47.51 ± 7.07	68.41 ± 7.07	55.08 ± 7.07	0.005	0.061	0.145	0.306
Total	226.05 ± 23.55	271.38 ± 41.18	433.00 ± 41.18	340.13 ± 23.55	287.08 ± 23.55	279.86 ± 29.53	377.14 ± 29.53	326.48 ± 29.53	0.001	0.052	0.096	0.436

[a]Data are least squares means ± standard error.
[b]Number of plasma samples analyzed.
[c]*P*-values for linear (L) contrast.
[d]*P*-values for quadratic (Q) contrast.

Table 8. Effect of increasing dietary CP concentration and day of lactation on plasma flow and net uptake of essential amino acids (g/d) across the udder of lactating sows[a]

	Dietary CP, %				Day of lactation		Diet	
Item	7.8 Deficient	13.0 Low	18.2 Normal	23.5 Excess	11	21	L[c] *P*-values	Q[d] *P*-values
No.[b]	8	7	5	6	14	12		
Blood flow, L × 10^3/d	7.14 ± 0.81	7.84 ± 0.81	5.24 ± 0.81	8.41 ± 0.97	7.74 ± 0.56	6.58 ± 0.56	0.764	0.185
Amino acid								
Arg	30.5 ± 7.0	49.2 ± 7.3	50.9 ± 9.0	58.0 ± 8.6	46.4 ± 4.6	47.9 ± 4.8	0.082	0.746
His	9.8 ± 2.4	19.8 ± 3.0	14.5 ± 3.3	16.5 ± 3.1	17.0 ± 1.9	13.3 ± 1.9	0.358	0.908
Ile	21.1 ± 2.5	36.2 ± 2.6	31.3 ± 3.2	46.2 ± 3.0	34.2 ± 1.8	33.1 ± 1.9	0.003	0.889
Leu	36.5 ± 3.3	57.6 ± 3.6	49.2 ± 4.0	76.2 ± 4.1	56.2 ± 2.5	53.5 ± 2.8	0.001	0.449
Lys	33.0 ± 3.9	43.8 ± 4.2	41.0 ± 4.6	50.2 ± 4.7	44.4 ± 2.8	39.6 ± 3.2	0.036	0.866
Met	9.8 ± 1.0	15.5 ± 1.0	12.1 ± 1.3	16.8 ± 1.2	13.7 ± 0.8	13.4 ± 0.8	0.009	0.664
Phe	19.6 ± 2.6	28.9 ± 2.7	25.0 ± 3.4	31.4 ± 3.2	26.7 ± 1.8	25.8 ± 1.9	0.036	0.629
Thr	19.2 ± 3.4	29.0 ± 3.5	26.1 ± 4.4	32.0 ± 4.1	26.2 ± 2.3	27.0 ± 2.3	0.050	0.619
Trp	4.4 ± 1.4	10.7 ± 1.7	7.9 ± 2.0	9.7 ± 1.9	10.0 ± 1.2	6.4 ± 1.2	0.051	0.920
Val	24.8 ± 5.8	46.7 ± 6.0	44.2 ± 7.5	54.5 ± 7.1	42.4 ± 3.9	42.7 ± 4.1	0.023	0.399

[a]Data are least squares means ± standard error.
[b]Number of observations per treatment.
[c]*P* values for linear (L) contrast.
[d]*P* values for quadratic (Q) contrast.

Discussion

Concentration of true protein in milk ranged between 4.5 and 5.0% in the current study, which agrees with previously reported values (Revell et al., 1998; Sauber et al., 1998; Kusina et al., 1999). Although true protein concentration in milk increased linearly with increasing dietary CP intake, the largest increase (approximately 9%) occurred in sows fed the Normal diet (18.2% CP) relative to those fed the Deficient (7.8%) and Low (13.0%) CP diets. Milk protein concentration increased by only 1.6% in sows fed the Excess (23.5%) CP diet relative to that in sows fed the Normal diet.

Change in AA a-v across the mammary glands with increasing dietary CP concentration paralleled, to some extent, change in true milk protein concentration, whereby as arterial AA availability increased, mammary AA a-v increased. However, at the highest dietary CP intake (Excess CP diet), a-v for the majority of AA decreased relative to that of Normal CP diet, despite the fact that arterial AA availability was not limiting. Similarly, in dairy cows, lysine a-v response across the mammary gland to graded amounts of duodenal infusions of lysine and/or to increasing lysine arterial flux was curvilinear, suggesting the mammary gland was unable to utilize additional lysine (Guinard and Rulquin, 1994). The mechanisms involved in modulating the transport of AA by mammary tissue are not known. Each mammary gland extracts AA from blood by specific AA transport systems situated in the blood-facing aspect of mammary epithelial cells, as reflected in mammary a-v of blood AA (Shennan et al., 1997). Substrate-induced uptake in mammalian cells has been demonstrated where increasing AA availability increases AA transport activity via posttranslational transstimulation mechanisms (Munir et al., 2000; Pan et al., 2002). Conversely, there are several mechanisms by which AA influx into cells and/or tissues can be inhibited: 1) high concentrations of intracellular AA can inhibit the additional uptake of extracellular AA, a process referred as transinhibition (Hyde et al., 2003; Palii et al., 2004); 2) high concentrations of extracellular AA can inhibit further uptake of those AA for which transport systems exhibit a low Michaelis constant ($\boldsymbol{K_m}$) relative to the AA concentration (high affinity, low capacity), a process known as saturation; and finally 3) high extracellular concentrations of certain AA can inhibit the uptake of other AA (Meier et al., 2002).

In lactating mammary tissue, a paucity of information pertaining to the transport of AA as affected by AA availability exists and is limited to in vitro studies. Nonetheless, these studies provide critical information allowing one to decipher some of the regulatory mechanisms behind AA transport in mammary tissue and also to substantiate in vivo data. For instance, in this study, saturation for lysine and valine was unlikely, as arterial concentrations for the cationic and unbranched neutral AA were relatively similar in sows fed the Excess CP diet compared with that of sows fed the Normal and Low CP diet. Indeed, Hurley et al. (2000) demonstrated that concentrative lysine uptake in porcine mammary tissue is facilitated via a sodium-independent transport mechanism with a K_m of 1.4 m*M*, and Jackson et al. (2000) identified a transport system for valine in porcine mammary tissue with a K_m of 640 μmol/L. These K_m are approximately ten- and twofold higher than the arterial concentration measured in this study for lysine and valine, respectively, indicating that porcine mammary tissue possesses high capacity and nonsaturable systems for transporting lysine and valine.

Uptake inhibition among AA has been shown to occur in a number of tissues, including mammary tissue, and thus may explain the curvilinear relationship between CP intake and AA a-v observed in this study, as well as that observed by Guinard and Rulquin (1994). In this study, in contrast to the cationic and small neutral AA, arterial concentrations of the large neutral AA, in particular the BCAA leucine and isoleucine, largely increased in sows fed the Excess CP diet, which may have contributed to the quadratic response, albeit a trend, between CP intake and valine a-v. For instance, uptake of valine by the lactating sow mammary tissue has been shown to be strongly inhibited by physiological concentration of leucine (Jackson et al., 2000). A similar explanation for the quadratic response in lysine a-v may be proposed, based on the findings that high concentrations of neutral AA both inhibit lysine influx into and stimulate lysine efflux from rodent mammary tissue (Shennan et al., 1994; Calvert and Shennan, 1996). So far, in the aforementioned studies, the nature of these inhibitions (i.e., competitive vs. noncompetitive) has not been determined. In fact, some transport systems in mammary and other tissues largely responsible for uptake of large neutral AA function as obligatory exchangers between cationic and neutral AA (Sharma and Kansal, 2000; Meier et al., 2002; Shennan et al., 2002), and thus promote the efflux of cationic AA. If true, the decrease in lysine a-v in this study would be apparent, rather than true, and would suggest that lysine entry per se in mammary tissue is not limited. In vivo, increasing dietary concentration of the large neutral BCAA valine in excess of dietary requirement decreased net lysine uptake by porcine mammary glands (Guan et al., 2002). Conversely, Hurley et al. (2000) also demonstrated in vitro that valine uptake is strongly inhibited (67% inhibition) by lysine, and Richert et al. (1996) showed in vivo that dietary lysine supplementation may create a deficiency in valine. Thus, in the current study, an interaction between lysine and valine transport may have occurred. Finally, the interaction between BCAA and the transport of other indispensable AA in mammary tissue has not been reported, but one should not rule out the possibility that the BCAA, in particular isoleucine and leucine, may also affect the transport of AA other than lysine. For instance, in this study, the a-v response to increasing dietary CP to excess level was quadratic for all AA except isoleucine and leucine.

The magnitude of a-v response to AA availability varied among AA. For instance, the relationship between CP intake and a-v for isoleucine and leucine in particular was strictly linear in contrast to other AA. Arterial availability may have contributed partly to the large a-v, at least for leucine, as arterial concentration of leucine increased by more than twofold from the Deficient to the Excess CP diet. Furthermore, high oxidative capacity in mammary tissue for leucine and isoleucine (Bequette et al., 1996; DeSantiago et al., 1998; Richert et al. 1998) may promote relatively lower intracellular concentration due to rapid oxidative degradation and removal, thus allowing accelerated inward transfer in the presence of increasing extracellular or arterial concentrations.

Despite the limited number of sows to assess lactation performance, the data indicate that total milk yield, milk yield per piglet, and piglet ADG were not negatively affected in sows, despite the quadratic response in a-v for most AA, including arginine, histidine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. In fact, true milk protein concentration and milk AA output increased with increasing CP. Correspondingly, daily AA uptake per udder also increased with increasing dietary CP. Presumably, the linear increase in AA uptake was accompanied by a change in blood flow across the udder. Admittedly, our blood flow data are limited and consequently preclude identifying a relationship with dietary CP intake. Nevertheless, some inferences may still be made from the blood flow data. For instance, it is apparent that sows fed the Excess CP diet had a numerically higher mammary plasma flow (either total udder plasma flow, as shown, or per suckled mammary gland plasma flow [not shown]), doubtlessly resulting in higher daily AA uptake per udder (shown) with increasing CP intake. On a per-suckled-gland basis (data not shown), lysine and threonine uptakes for sows fed the Excess and Normal CP diet were 4.56 vs. 4.32 g/d for lysine and 2.91 vs. 2.75 g/d for threonine, respectively. Likewise, in sows fed the Normal and Low CP diet, daily lysine and threonine uptakes were 4.32 and 3.98 g/d for lysine and 2.75 vs. 2.64 g/d for threonine, respectively. For sows fed the Deficient CP diet, plasma flow did not seemingly "compensate" for the low AA a-v, hence these sows had lower AA uptake across the total udder (33 g/d for lysine and 19.2 g for threonine, as shown) or each gland (3.2 g/d for lysine and 1.86 g/d for threonine) relative to all other treatments.

Nonetheless, across dietary treatments, AA uptake increased, but the most striking increase was observed for isoleucine and leucine, with little relative improvement in total milk yield and ADG. We recognize that results presented herein on AA uptake are limited due to the Fick-calculated plasma flow, as it is unknown whether our dietary treatments altered mammary metabolism of lysine. Furthermore, due to variation, albeit small, in litter size, data for plasma flow across dietary treatments were erratic because they are in part inherently related to total milk yield. With this notion in mind, we attempted to correct for this discrepancy by expressing data, when appropriate in the discussion, on a per-suckled-gland basis. Nevertheless, as a whole, the data are novel for the porcine mammary gland AA metabolism and indicate that two processes are involved in modulating AA uptake when challenged with different levels of CP.

The physiological importance, if any, of the large uptake of the BCAA and their oxidation in mammary tissue as demonstrated by others (Bequette et al., 1996;

DeSantiago et al., 1998; Richert et al. 1998) is unknown. For instance, in lactating dairy cows fed supplemental dietary CP, leucine oxidation by the mammary gland increased with no increase in milk protein yield (Bequette et al., 1996). In the current study, a small increase (1.6%) in milk protein concentration in sows fed the Excess CP diet relative to that of sows fed the Normal diet was found, compared with as much as a 22 and 28% increase in daily leucine and isoleucine uptake per suckled gland (5.8 vs. 6.3 g of leucine in Normal vs. Excess CP diet, respectively, and 3.29 vs. 4.2 g of isoleucine in Normal and Excess CP diet, respectively). Bequette et al. (1996) suggested that reducing activities, such as oxidation, not seemingly crucial for milk synthesis, might improve the efficiency of AA conversion into milk protein. In this study, AA output in milk relative to AA uptake by the mammary glands decreased with increasing dietary CP concentration (data not shown). For example, leucine output to uptake ranged from 100% in sows fed the Deficient CP diet to 71% in sows fed the Excess CP diet, with 82% in sows fed Low and Normal CP diets. Similar changes in output to uptake ratios were observed for isoleucine, valine, and arginine. Amino acids such as threonine, phenylalanine and methionine had consistently high output to uptake ratios (close to 100%) across dietary treatments, suggesting minimum use, if any, of these AA into oxidative pathways. Output to uptake ratios for histidine were consistently higher than 100%, indicating in situ secretion of histidine by mammary tissue.

The relationship between day of lactation and a-v for the majority of AA in this study was unclear, which is in contrast with Nielsen et al. (2002). Nielsen et al. (2002) demonstrated a clear curvilinear a-v response for the majority of AA to increasing day of lactation from 9 to 24. Additional sampling before d 10 and after d 22, as performed by Nielsen et al. (2002), may be essential in order to better define the relationship between day of lactation and AA a-v.

Transport of AA per plasma unit in the porcine mammary gland can be modulated by extracellular (arterial) AA concentration: a-v increased with increasing extracellular (arterial) AA availability, but decreased when arterial AA concentration, in particular that of the BCAA, exceeded requirement. The largest AA a-v was achieved in sows fed dietary CP corresponding to their CP requirements; however, this did not translate into superior piglet ADG relative to the other dietary CP concentrations. Net AA compensatory uptake was achieved across dietary CP intake, presumably via modulation in plasma flow to meet demand for milk and piglet growth; consequently, total udder AA uptake increased with increasing CP. The fact that a-v for the first-limiting AA, such as lysine, methionine, threonine, and tryptophan (but also arginine, histidine, phenylalanine, and valine), was highest for sows fed the 18% CP diet and decreased for sows fed the 23.5% CP diet suggests that the proportion of these AA relative to other AA may have optimized their transport into mammary tissue and thus minimized their rate of delivery to mammary tissue. The latter suggests the presence of a coordinated regulation between rate of AA delivery to mammary tissue and their respective processes for transport. The data do not support the concept that mammary gland does not have the ability to utilize additional AA. Rather, the mammary gland response to AA availability is a coordinated uptake to meet AA requirement for milk demand. It is still unclear, however, as to why leucine and isoleucine uptake seems to be unregulated.

Implications

Regulatory mechanisms behind modulation of mammary amino acid uptake by dietary proteins are unknown, but the arterial proportion of each amino acid relative to others, in particular to large neutral amino acids such as the branched-chain acids, may play an important role. Although a coordinated regulation between rate of delivery of amino acids to mammary tissue and their respective transport per se may ensure uptake of amino acids in sufficient quantities to meet requirements for those that are limiting, this may decrease efficiency of overall amino acid use for milk protein synthesis. Thus, interactions between large neutral amino acids, in particular isoleucine and leucine, and transport of cationic and small neutral amino acids in mammary tissue may affect the efficiency of dietary protein and amino acid use. Such knowledge may have important implications regarding the biological significance behind balancing dietary amino acids relative to the first limiting one.

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