The Amino Acid Need for Milk Synthesis Is Defined by the Maximal Uptake of Plasma Amino Acids by Porcine Mammary Glands[1–3]

Xinfu Guan,[4,5] Brian J. Bequette,* Pao K. Ku, Robert J. Tempelman, and Nathalie L. Trottier

*Department of Animal Sciences, Michigan State University, East Lansing, MI 48824 and *Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742*

ABSTRACT To define dietary indispensable amino acid (IAA) needs for milk synthesis by the mammary glands (MG), 16 lactating sows were fed 1 of 4 isocaloric diets varying in protein concentrations (from 78 to 235 g/kg) with an ideal amino acid (AA) pattern. On d 9, 13, 17, and 21 of lactation, blood samples were obtained simultaneously from a carotid artery and the main mammary vein every 30 min over 6 h. A quadratic regression model of the log mammary arteriovenous difference (AVD) of plasma IAA ($\hat{y}$) against daily intake of dietary IAA (X) was established. First, the reverse log intercept, defined as the mammary AVD at zero dietary AA supply, was used to quantify the contribution of endogenous IAA. The quantification was validated by body N balance coupled with AA composition analysis. Then, the estimated vertex ($\hat{y}_{max}$, X_i) was used in 2 aspects: *1*) The maximal mammary uptake of plasma IAA, quantified by multiplying the maximal mammary AVD and plasma flow rate, was considered the physiological IAA need for milk synthesis. *2*) Corresponding to the $\hat{y}_{max}$, dietary IAA intake (X_i) would represent the total dietary IAA requirement, i.e., the sum of maintenance need and milk synthesis need after adjustment for body weight loss. Thus, dietary IAA needs for milk synthesis were derived. Moreover, the estimate of lysine need for milk synthesis in this study was identical to an estimate obtained from multiple regression analysis of feeding trial data. We conclude that dietary IAA needs for milk synthesis can be quantified by the maximal uptakes of plasma IAA by porcine MG. J. Nutr. 134: 2182–2190, 2004.

KEY WORDS: • *amino acid requirement* • *mammary arteriovenous difference* • *amino acid uptake* • *pigs* • *lactation*

Fn5 Fn6 Lactating sows must be provided with adequate amounts of indispensable amino acids (IAA)[6] in optimal proportions for maternal health, reproductive performance, and dietary protein utilization. Dietary IAA requirements (i.e., adequate intakes) of sows were estimated using both an empirical method and a factorial approach. The empirical method involves exploring physiological and/or reproductive response curves in animals fed graded levels of the test amino acid (AA). This method only estimates total dietary requirement for 1 AA under the specified conditions. Moreover, it is difficult to extrapolate total dietary requirement to other conditions such as different rates of lactation performance and body weight change. In contrast, the factorial approach derives total dietary requirements of the test AA by summing up individual physiological needs of various functional components (e.g., body maintenance, milk synthesis, and body protein accretion). The factorial approach was used to predict dietary IAA requirements of sows under various conditions (1); however, none of the individual physiological needs was defined experimentally.

Of the dietary IAA requirements, lysine has been the most intensively investigated because it is usually the first limiting AA for lactating sows. Empirical estimates of dietary lysine requirements of sows vary widely from 40 to 55 g/d (2–5). The wide variation in estimates may be attributed to changes in maternal body weight, differences in lactation performance, and/or diversity in response criteria used (6,7). When dietary intake of protein and/or lysine is inadequate during lactation, sows mobilize body proteins to support milk synthesis (8,9). In this situation, the contribution of endogenous lysine mobilized from body protein reserves will compensate for part of the total dietary lysine requirement. It is this lactation homeorrhesis that renders an inherent uncertainty of any empirical estimates of minimal IAA requirements of lactating sows. The

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[3] The fitting analysis between the 2 models is included in a supplemental table with the online posting of this paper at www.nutrition.org.

[4] To whom correspondence should be addressed. E-mail: xguan@bcm.tmc.edu.

[5] Present address: U.S. Department of Agriculture, Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030.

[6] Abbreviations used: AA, amino acid; AVD, arteriovenous difference; BCCA, body composition change approach; IAA, indispensable AA; IAA_e, contribution of endogenous IAA; MAVD, regression model for mammary AA AVD; MG, mammary glands; TDIAA, true digestible IAA.

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total dietary lysine requirement was also estimated by the factorial approach (1). However, neither endogenous lysine contribution nor dietary lysine need for milk synthesis by the mammary glands (MG) per se was defined experimentally.

Dietary requirements of IAA other than lysine have not been fully established in the lactating sow. The NRC (1) derived dietary requirements for IAA other than lysine by multiplying dietary lysine needs and ideal ratios of other IAA to lysine for maintenance, milk synthesis, and body protein accretion (or body tissue), respectively. The AA pattern in milk was assumed to be an ideal profile of dietary AA for milk synthesis (1). However, the AA pattern in milk is not identical to the mammary AA uptake pattern (10). Specifically, the mammary uptake of branched-chain AA and arginine exceeds the output in milk by ~20 to 50% (10). In addition to their incorporation into milk, branched-chain AA may provide α-amino nitrogen, carbon skeleton, and energy for synthesis of dispensable AAs and other compounds (e.g., lactose and fatty acids) in the MG (11). Arginine is also the precursor for de novo synthesis of nitric oxide, which regulates mammary blood flow (12). Furthermore, a recent study indicated that the ideal ratios of dietary IAA are dynamic and highly related to exogenous intake of dietary IAA and endogenous contribution of body protein (13). Therefore, estimates of dietary IAA needs for milk synthesis based only on the AA pattern in milk are appropriate in the absence of empirical data, but they are the minimum values.

Our recent study indicates that the mammary net uptake of some IAA (e.g., lysine) is a rate-limiting step for the synthesis of milk protein in vivo (14). Maximal mammary uptake of plasma IAA will theoretically reflect the intramammary IAA potentially required for milk synthesis. Thus we hypothesized that the maximal mammary uptake of plasma IAA can be defined as the mammary physiological need for IAA and further presumed as the dietary true digestible IAA (TDIAA) need for milk synthesis. Dietary TDIAA needs for milk synthesis defined by the maximal mammary uptake not only include their output in milk, but also their metabolic need by the MG. Moreover, a set of dietary true digestible IAA needs for milk synthesis could be concurrently estimated by this method. In the present study we quantified the maximal mammary uptakes of plasma IAA and estimated a set of dietary true digestible IAA needs for milk synthesis. In addition, our estimate of dietary requirement for true digestible lysine for milk synthesis was supported by a multiple regression analysis of the total dietary lysine requirements against litter weight gain and maternal body weight loss. The estimates of this study can be used to construct an ideal pattern of dietary IAA for milk synthesis and predict total dietary IAA requirements of lactating sows.

MATERIALS AND METHODS

The All-University Committee on Animal Use and Care of Michigan State University approved all procedures used in the study.

Dietary treatments. On d 1 of lactation, 16 sows (Landrace × Yorkshire, parity 2 or 3) were allocated to dietary treatments according to a completely randomized block design. Each block consisted of 4 sows. Each sow in 1 block was allowed to eat ad libitum from 1 of 4 isocaloric diets (14.3 MJ ME/kg) containing different protein concentrations (78, 130, 182, and 235 g/kg) with an ideal pattern of dietary IAA (1). The composition of the diets is given in **Table 1**.

Animals and cannulation. Litters with 11 piglets per sow were cross-fostered within 48 h after birth. Sows were individually housed in farrowing crates in a mechanically ventilated, thermally controlled room (21°C). The anterior main mammary vein and a carotid artery were cannulated on d 4 ± 1 of lactation following the surgical procedure described by Trottier et al. (15). Either the arterial or the venous catheter did not function in 3 of 16 sows. The sows were fed twice daily to appetite and given free access to water. Sow food intake was recorded daily during a 21-d lactation. Sow body weight was recorded after farrowing (d 1) and at weaning (d 21). Piglets were individually weighed weekly.

TABLE 1

Composition of diets for lactating sows

Item	Dietary protein 235	182	130	78
	g/kg (as fed)			
Ingredient				
Corn	463.7	360.0	257.2	154.3
Soybean meal (44% CP)	443.2	344.1	245.8	147.5
Corn starch	0	142.5	283.9	425.3
Sugar	0	47.5	94.6	141.8
Tallow	50.0	50.0	50.0	50.0
Solka floc	0	10.2	20.2	30.2
Dicalcium phosphate	16.3	21.4	26.4	31.4
Calcium carbonate	10.8	8.9	7.0	5.1
Sodium chloride	2.5	2.5	2.5	2.5
Trace mineral premix[1]	2.3	2.3	2.3	2.3
Vitamin premix[2]	9.0	9.0	9.0	9.0
DL-Met	0.79	0.61	0.44	0.27
L-Thr	0.33	0.25	0.19	0.11
L-Val	1.15	0.90	0.64	0.38
Calculated nutrient content				
ME, *MJ/kg*	14.3	14.3	14.3	14.3
Calcium	9.0	9.0	9.0	9.0
Phosphorus	7.2	7.2	7.2	7.2
Analyzed nutrient content				
Protein	230	182	132	82
Arg	16.0	12.8	9.3	6.0
His	7.2	5.8	4.5	3.0
Ile	10.0	7.8	5.7	3.7
Leu	17.3	13.6	10.1	6.4
Lys	11.9	9.0	6.7	4.1
Met	4.2	3.4	2.7	1.8
Cys[3]	4.0	3.1	2.2	1.3
Phe	11.3	8.9	6.6	4.2
Thr	9.3	6.6	5.3	3.2
Trp[3]	3.3	2.5	1.8	1.1
Val	12.1	9.6	7.1	4.6

[1] Provided the following amounts of trace minerals in milligrams per kilogram of diet: copper, 5; iodine, 0.075; iron, 50; manganese, 5; selenium, 0.15; and zinc, 50.

[2] Provided the following amounts of vitamins in milligrams per kilogram of diet: retinyl acetate, 8.3; cholecalciferol, 0.0138; D-α-tocoperol, 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.

[3] Calculated value (1).

Milk yield estimation. Milk yield was estimated on d 11 and 21 by the D_2O dilution method of Pettigrew et al. (16). In brief, piglets were deprived for 1 h, weighed, and injected i.m. at a dose (1 mg/kg BW) of D_2O (Cambridge Isotope Laboratories). At 1 h after the dose, piglets were bled from a jugular vein. At 24 h after the dose, the piglets were deprived of food for 1 h, weighed, and bled as before. During this 24-h period, piglets were not given access to water. Blood samples were centrifuged at 1500 × *g* for 15 min at 4°C, and then serum samples were separated and stored at −20°C. Serum D_2O concentration was determined by an infrared spectrophotometer (Foxboro) using a fixed filter length of 4 μm. Milk yield was calculated on the basis of the D_2O dilution principle (16).

Sampling protocol. Blood samples were collected on d 9 ± 1, 13 ± 1, 17 ± 1, and 21 ± 1 (referred to as d 9, 13, 17, and 21,

respectively). Sows were fed 1 h prior to blood sampling and still given ad libitum access to food and water. Carotid arterial and main mammary venous blood samples (10 mL each) were simultaneously collected every 30 min over a 6-h period. Thus a total of 13 blood samples were collected from each sow per sampling day. Blood samples were centrifuged at 1500 × *g* for 15 min at 4°C. Plasma samples were removed and stored at −20°C. On sampling days, milk was collected following an i.v. administration of oxytocin (10 IU) and pooled from all functional teats on each sow. An aliquot of pooled milk was stored at 4°C until assayed for milk composition; the remainder was defatted by centrifugation at 1500 × *g* for 15 min at 4°C and stored at −20°C until assayed for AAs.

Amino acid analysis. Food samples were finely ground using a sample mill. Total nitrogen content in food and milk was determined using a nitrogen analyzer (FP-2000, LECO) using EDTA (Sigma) as a calibration standard. Milk lipid and protein contents were assayed by a mid-infrared spectrophotometry (Multispec M, Berwind Instrument). Concentrations of AAs in the defatted milk and food were analyzed using reversed-phase HPLC by precolumn derivatization with phenylisothiocyanate (14). Concentrations of AAs in the pooled plasma samples were determined using a Beckman 6300 amino acid analyzer (14).

Calculations

Mammary plasma flow rate. Mammary plasma flow rate was estimated by Fick's principle as described previously (14). Given that the amount of lysine output in milk contributed from plasma peptides is negligible (17), mammary uptake of plasma lysine can be calculated as

$$\text{Mammary uptake of plasma lysine} = \text{Plasma lysine AVD} \times \text{mammary plasma flow rate} = \text{Output of lysine in milk} + \text{amount of lysine metabolized in the MG} \quad (1)$$

In addition to incorporation into milk, lysine has 2 major metabolic pathways: oxidation and accretion in the MG. Because dietary ME intake was adequate, lysine as the first limiting AA might be minimally oxidized in the MG. In addition, accretion of lysine in the MG was calculated from the study of Kim et al. (18) to be around 1 g/d, which is negligible compared to the measured lysine output in milk (41 g/d) in the present study. Therefore, Eq. (1) was simplified as follows:

$$\text{Mammary uptake of plasma lysine} = \text{Plasma lysine AVD} \times \text{mammary plasma flow rate} = \text{Output of lysine in milk, i.e.,} \quad (2)$$

$$\text{Mammary plasma flow rate (L/d)} = \text{Lysine concentration in milk (mmol/L)} \times \text{milk yield (L/d)/mammary lysine AVD (mmol/L)} \quad (3)$$

The vertex of the quadratic regression. The relationship of log mammary AVD of plasma IAA ($\hat{y}$) and daily intake of each dietary IAA (X) was fitted by a quadratic regression curve: $\hat{y} = \beta_0 + \beta_1 X + \beta_2 X^2$, with $\hat{y}$ being the predicted variable, X being the predictor variable, and β_0, β_1, and β_2 being the parameter estimates. The vertex ($\hat{y}_{max}$, X_i) of this regression was predicted at $d\hat{y}/dX = 0$, i.e., $X_i = -\beta_1/2\beta_2$, thus $\hat{y}_{max} = \beta_0 - \beta_1{}^2/4\beta_2$.

Dietary TDIAA needs for milk synthesis derived by the factorial approach. Based on the factorial approach, the total dietary requirement of true digestible IAA (i.e., daily intake of true digestible IAA at $\hat{y}_{max}$) = maintenance need + milk synthesis need − the contribution of endogenous IAA. Thus dietary true digestible IAA need for milk synthesis = daily intake of true digestible IAA at $\hat{y}_{max}$ + the contribution of endogenous IAA − maintenance need. Daily intake of true digestible IAA at $\hat{y}_{max}$ was converted from daily intake (X_i) of IAA at $\hat{y}_{max}$ (1).

Modeling and statistical analyses

The regression models were computed with the REG and NLIN procedures (SAS/STAT Version 8, SAS Institute). Mammary AVD of plasma IAA were log-transformed based on their residual error distribution. Based on the sum of squared residuals (see the supplemental table), the quadratic regression model was better fitted than a nonlinear exponential model [$\hat{y} = b_0(1 - \text{EXP}(-b_1 X)$]. Confidence intervals were estimated with a total of 750 bootstrapping samples using the bootstrapping reflection method (19). Milk yield and plasma flow rate were analyzed by the MIXED procedure (SAS/STAT), in which dietary treatment and lactation day as the fixed effects and sow as the random effect were included in the model; lactation day as repeated measures was included in the REPEATED statement; and least-square means and SEM were presented.

RESULTS

Mammary plasma flow. Plasma flow rate across the MG was estimated to be 6440 ± 765 L/d based on Fick's principle using lysine as an internal indicator and was not different from that using phenylalanine plus tyrosine as an internal indicator. In the present study, the AVD of plasma lysine over the 21-d lactation period was 43.4 ± 3.6 μmol/L whereas the concentration of lysine in skin milk was 26.0 ± 0.9 mmol/L. The milk yield was 11.48 ± 0.98 L/d, which did not differ ($P > 0.10$) between d 11 and 21 of lactation (11.7 ± 0.6 and 11.4 ± 0.6 L/d, respectively). The mammary plasma flow rate was not affected ($P > 0.10$) by dietary treatments. The mean ratio of mammary plasma flow to milk yield was 560:1 (v:v) over the 21-d lactation period.

Contributions of endogenous IAA (IAA_c) predicted by the mammary uptake of plasma IAA. A quadratic regression

TABLE 2

Regressions of log mammary AVD of plasma IAA against daily intake of dietary IAA in lactating sows over a 21-d lactation period[1]

	Parameter estimate				*P*-value			
Amino acid	β_0	β_1	β_2	R^2	Model	β_0	β_1	β_2
Arg	1.2767	0.0992	−0.0009	0.64	0.006	0.055	0.004	0.007
His	−1.4929	0.4054	−0.0090	0.53	0.022	0.257	0.007	0.007
Lys	2.4104	0.0790	−0.0011	0.49	0.036	0.000	0.015	0.020
Met	0.7216	0.2710	−0.0091	0.60	0.015	0.254	0.022	0.039
Phe	1.6599	0.0859	−0.0012	0.54	0.020	0.003	0.012	0.018
Thr	1.6842	0.1263	−0.0020	0.59	0.028	0.012	0.012	0.017
Trp	−0.8173	0.6476	−0.0373	0.63	0.007	0.217	0.002	0.002
Val	1.7441	0.1120	−0.0013	0.63	0.007	0.017	0.012	0.026

[1] A polynomial regression model was best fitted by: $\hat{y} = \beta_0 + \beta_1 X + \beta_2 X2$ where β_0, β_1, and β_2 are parameter estimates and $\hat{y}$ and X are the log mammary AVD of plasma IAA (the predicted variable) and daily intake of dietary total IAA (the predictor variable), respectively.

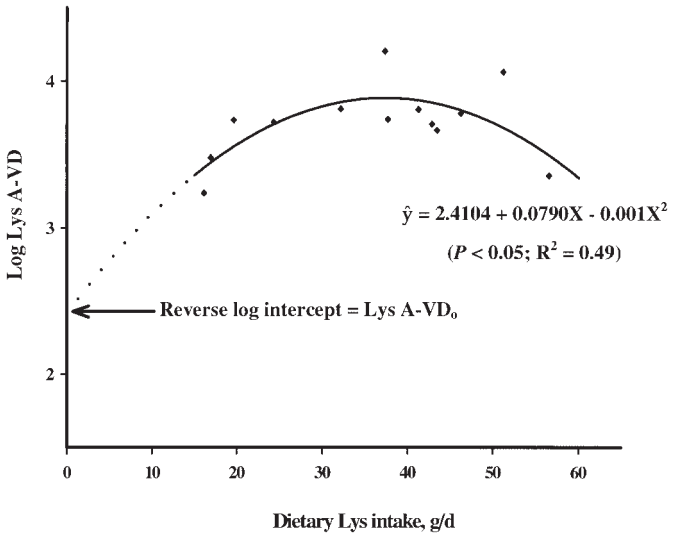

FIGURE 1 Relationship between the log mammary AVD of plasma lysine and daily intake of dietary lysine in lactating sows over a 21-d lactation period. The solid curve is best fitted by a polynomial regression model: $\hat{y} = 2.4104 + 0.07902X - 0.001059X^2$ ($P = 0.036$; $R^2 = 0.49$). Extrapolation of the curve (dotted line) is predicted on the basis of this model. Each diamond represents an individual sow. The reverse log intercept is defined as the plasma lysine AVD_0 when dietary lysine intake is extrapolated to zero.

model for mammary AVD (MAVD) of plasma IAA was developed to predict the IAA_c. The relationship was best fitted by a quadratic regression model between log mammary AVD of plasma IAA and daily intake of IAA over the 21-d period of lactation (**Table 2**). Uptake of indispensable AAs by the MG at zero intake of protein can be attributed to 2 sources: *1*) release from (muscle) protein breakdown and *2*) endogenous de novo synthesis (e.g., arginine). Thus estimation of the mammary uptake by the model in the present study includes both sources of endogenous indispensable AAs. Because arginine can be synthesized in the body (1), the contribution of endogenous arginine released from body proteins could not be defined. In addition, either estimates of intercepts for histidine and tryptophan or estimates of the quadratic term for isoleucine and leucine were not different from zero ($P > 0.05$). Endogenous contributions of lysine, methionine, phenylalanine, threonine, and valine were determined in the study. The relationship between log mammary AVD of plasma lysine and daily intake of lysine is shown as an example in **Figure 1**. The reverse log intercept (β_0) is defined as the mammary AVD of plasma lysine when lysine intake is extrapolated to zero. The mammary uptake of plasma IAA at zero intake of dietary IAA was attributed to the IAA_c (mobilized from extramammary tissue proteins). A small proportion of endogenous IAA would be first used for obligatory metabolism, and a predominant proportion of endogenous IAA would be then taken up by the MG for milk synthesis. Thus, the IAA_c is defined as the sum of these 2 parts. The amount of IAA_c used for obligatory metabolism was considered maintenance need (1). The amount of IAA_c used for milk synthesis was predicted using the regression equations (**Table 3**).

Contributions of endogenous IAA derived from body protein loss. To validate the MAVD, IAA_c was quantified as the algebraic product of body protein loss during lactation and the respective AA concentration in body protein (**Table 4**). We refer to this method as the body composition change approach (BCCA). First, nitrogen balance in lactating sows was predicted from the following regression equation: Nitrogen balance (g/d) = −15.8 + 1.22 dietary lysine intake (g/d) − 0.63 nitrogen output in milk (g/d) (8). Negative nitrogen balance of the whole body represented the amount of nitrogen loss from body protein reserves. Second, AA concentrations in body protein were derived from ARC (20) and NRC (1). Lysine concentration was estimated to be 7 g/100 g body protein (20). Methionine, phenylalanine, threonine, and valine concentrations were calculated on the basis of the amino acid profile of body protein (1).

Physiological amino acid needs for milk synthesis predicted by the maximal mammary uptake of plasma IAA. Mammary IAA needs should not only include their incorporation into milk, but also cover their mammary metabolic needs (e.g., oxidation, accretion, and conversion to other substrates such as dispensable AAs, lactose, and fatty acids). Thus, mammary physiological IAA needs could be defined by the maximal mammary uptakes of plasma IAA. Quadratic coefficients in the regressions (Table 2) were negative ($P < 0.05$), indicating that $\hat{y}$ can be maximized in the vertex. A representative vertex of the quadratic regression for valine is shown in **Figure 2**. Maximal mammary uptakes of plasma IAA were predicted by multiplying maximal AVD of plasma IAA (the reverse log $\hat{y}_{max}$) and mammary plasma flow rate. Sows

TABLE 3

Contributions of endogenous IAAIAA in lactating sows predicted by the mammary AVD of plasma[1]

Amino acid	Intercept	AVD_0[2]	Molecular weight	$Uptake_0$[3]	OM[4]	IAA_c
	Log μmol/L	*μmol/L*	*g/mol*	*g/d*		
Lys	2.4104	11.14	146.19	10.49	1.96	12.45
Met	0.7216	2.06	149.21	1.98	0.55	2.53
Phe	1.6599	5.26	165.19	5.60	0.98	6.58
Thr	1.6842	5.38	119.12	4.13	2.96	7.09
Val	1.7441	5.72	117.15	4.32	1.31	5.63

[1] The contributions of endogenous IAA are defined as the sum of the amount of the IAA captured for obligatory metabolism and the amount of the IAA taken up ($uptake_0$) by the MG in lactating sows when daily intake of dietary IAA is extrapolated to zero.

[2] Mammary AVD_0 of plasma IAA = the reverse log intercept.

[3] Mammary $uptake_0$ of plasma IAA (g/d) = Mammary AVD_0 of plasma IAA (μmol/L) × mammary plasma flow rate (6440.7 L/d) × molecular wt of IAA (g/mol) × 10^{-6}.

[4] Obligatory metabolism (OM) is considered the maintenance requirement (1). Daily maintenance need of the lactating sows (g/d) = Metabolic body weight (54.36, kg $BW^{0.75}$) × maintenance need (10^{-3} × mg · kg $BW^{-0.75}$ · d^{-1}).

TABLE 4

Contributions of endogenous IAA in lactating sows estimated by the body composition change approach

Item	Mean	Equation
Dietary Lys intake, *g/d*	38.48	= Food intake (kg/d) × dietary Lys concentration (g/kg diet)[1]
Milk N output, *g/d*	94.36	= Milk N concentration (8.227 g/L) × milk yield (11.47 L/d)[1]
Nitrogen balance, *g/d*	−28.30	= −15.8 + 1.22 dietary Lys intake (g/d) − 0.63 N output in milk (g/d)[2]
Body protein loss, *g/d*	176.90	= Nitrogen balance (g/d) × 6.25
Amino acid from body protein loss, *g/d*		
Lys	12.38	= Body protein loss (g/d) × Lys concentration in body tissue (g/100 g protein) = Body protein loss × 7.0 %[3]
Met	3.36	= Body protein loss × 1.9 %[4]
Phe	7.43	= Body protein loss × 4.2 %[4]
Thr	7.25	= Body protein loss × 4.1 %[4]
Val	8.49	= Body protein loss × 4.8 %[4]

[1] Obtained from sows in the present study (n = 16).
[2] This regression equation was established by Dourmad et al. (8).
[3] Lysine concentration in body tissue was estimated at 7.0 g/100 g protein (20).
[4] Methionine, phenylalanine, threonine, and valine concentrations in body tissue were calculated to be 1.9, 4.2, 4.1, and 4.8 g/100 g protein, respectively, from the amino acid profile in body tissue (1).

had a mean litter weight gain of 2.15 kg/d and a mean milk yield of 11.48 kg/d over the 21-d lactation period in this study. Thus, the maximal mammary uptake was normalized for milk synthesis need by dividing the maximal mammary uptake by the litter weight gain (**Table 5**). The maximal mammary uptakes were defined as mammary physiological IAA needs and further considered dietary true digestible (absorbed) IAA needs for milk synthesis. We assumed that the loss of absorbed IAA on the first pass is attributable to the body maintenance need. Thus, most of the absorbed IAA is contributed to its postsplanchnic availability (to the MG). Ratios relative to lysine for the maximal mammary uptake were estimated at 1.43, 0.50, 0.34, 0.60, 0.70, 0.22, and 1.11:1, respectively, for arginine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine.

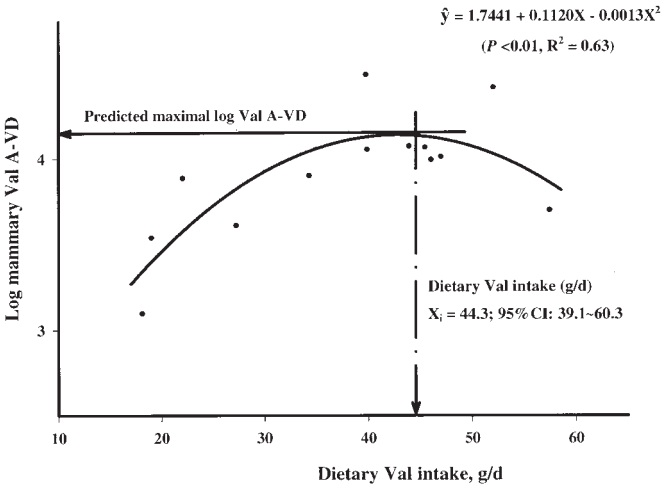

FIGURE 2 The relation between log mammary AVD of plasma Val ($\hat{y}$) and daily intake of dietary total Val (X) in lactating sows over a 21-d lactation period. The curve was best fitted by a quadratic regression model: $\hat{y}$ = 1.7441 + 0.1120X - 0.001308X^2 (P = 0.0066, R^2 = 0.63). Each dot represents an individual sow. The vertex ($\hat{y}_{max}$, X_i) derived from this regression had 2 implications: *1*) The maximal mammary uptake of plasma Val could be quantified by multiplying the maximal mammary AVD of plasma Val (i.e., the reverse log $\hat{y}_{max}$) and mammary plasma flow rate. *2*) The maximal mammary uptake of plasma Val was defined as mammary physiological need for Val and further considered the dietary true digestible Val need for milk synthesis by the MG. Daily intake of dietary Val (X_i) would represent the total requirement of dietary Val by lactating sows, i.e., the sum of maintenance need and dietary need for milk synthesis after adjusted by the maternal body weight loss in this study. Thus dietary true digestible Val need for milk synthesis could be derived by the reverse factorial approach. A bootstrapping reflection method was employed to compute the 95% CI for daily intake of dietary Val (19).

Dietary TDIAA needs for milk synthesis derived from the factorial approach. Corresponding to the maximal AVD of plasma IAA (i.e., the reverse log $\hat{y}_{max}$), daily intake of dietary IAA (X_i) was estimated from regression equations in Table 2. The dietary IAA intake means, SD, and 95% CI were computed by the bootstrapping reflection method (**Table 6**). The dietary IAA intake, after being subtracted from the mainte-

TABLE 5

Physiological IAA needs for milk synthesis by lactating sows predicted at the maximal mammary uptake of plasma IAA

Amino acid	$\hat{y}_{max}$[1]	AVD_{max}[2]	Mammary $uptake_{max}$[3]		Milk synthesis need[4]
	log μmol/L	*μmol/L*	*mmol/d*	*g/d*	*g/kg LWG*[5]
Arg	4.01	55.16	355.22	61.88	28.78
His	3.07	21.59	139.06	21.58	10.04
Lys	3.829	46.0	296.3	43.31	20.15
Met	2.739	15.5	99.7	14.87	6.92
Phe	3.197	24.5	157.5	26.02	12.10
Thr	3.678	39.6	254.9	30.30	14.12
Trp	1.994	7.3	47.3	9.66	4.49
Val	4.156	63.8	411.1	48.17	22.40

[1] When $X_i = -\beta_1/2\beta_2$, the $\hat{y}_{max} = \beta_0 - \beta_1^2/4\beta_2$, where β_0, β_1, and β_2 are the intercept, the linear coefficient, and the quadratic coefficient in the regression, respectively (see Table 2).
[2] Maximal mammary AVD of plasma IAA (μmol/L) = the reverse log $\hat{y}_{max}$.
[3] Maximal mammary uptake of plasma IAA (mmol/d) = mammary AVD_{max} (μmol/L) × plasma flow rate (6440.7 L/d) × 10^{-3}. Maximal mammary uptake in mass (g/d) = mammary $uptake_{max}$ (mmol/d) × molecular weight (g/mol) × 10^{-3}.
[4] Milk synthesis need is defined by mammary $uptake_{max}$ (g/d) after being normalized by LWG of 2.15 kg/d over a 21-d lactation period.
[5] LWG, litter weight gain.

TABLE 6

Daily dietary IAA intakes by lactating sows predicted at the maximal log mammary A-VD of plasma IAA over a 21-d lactation period[1]

Amino acid	Mean ± SD[2]	95% CI[2]
	g/d	
Arg	53.35 ± 3.50	48.91 ~ 63.05
His	22.58 ± 0.93	20.85 ~ 24.51
Lys	38.08 ± 4.65	33.48 ~ 47.53
Met	15.49 ± 2.42	13.68 ~ 21.13
Phe	37.59 ± 3.52	33.86 ~ 48.58
Thr	29.88 ± 2.17	26.74 ~ 34.65
Trp	8.65 ± 0.34	7.93 ~ 9.26
Val	44.29 ± 6.30	39.05 ~ 60.27

[1] Daily dietary IAA intakes (X, g/d) were predicted at $\hat{y}_{max}$ based on the regressions in Table 2. When $d\hat{y}/dX = 0$, i.e., $X_i = -\beta_1/2\beta_2$, $\hat{y}$ reaches its maximal value because of negative β_2.

[2] Mean, SD, and 95% CI for daily intake of dietary IAA were computed using the bootstrapping reflection method with a bootstrapping sample size of 750.

nance need, was considered the dietary IAA need for milk synthesis at a given contribution of endogenous IAA. We designated this the reverse factorial approach. Based on this approach, dietary true digestible IAA intake at $\hat{y}_{max}$ = dietary true digestible IAA need for maintenance + dietary true digestible IAA need for milk synthesis − the contribution of endogenous IAA. Thus the dietary true digestible IAA need for milk synthesis was derived (**Table 7**). Ratios of other true digestible IAA to lysine required for milk synthesis (determined by the reverse factorial approach) were estimated to be 1.44, 0.58, 0.37, 0.90, 0.68, 0.19, and 0.99 for arginine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine, respectively.

DISCUSSION

The porcine mammary gland has a large demand for IAA to meet its high rate of milk production (1). Our recent study showed that mammary net uptake of some IAA (e.g., lysine) is a rate-limiting step for the synthesis of milk protein in vivo (14). It has been well documented that mammary uptake of plasma branched-chain AA and arginine exceeds their output in milk by ~20 to 50%. Those IAA needs for milk synthesis might be underestimated if they were only based on the AA profile in milk (7). It is the mammary intracellular AAs that act as the immediate precursors for protein synthesis and provide the metabolic support for other cellular functions in the MG. In the present study, we hypothesized that the maximal mammary uptake of plasma IAA can be defined as the dietary true digestible IAA need for milk synthesis. To our knowledge, this study is the first to quantify the maximal mammary uptake of plasma IAA and thus to establish the physiological mammary IAA needs for milk synthesis in lactating sows. In addition, our estimate of dietary lysine need for milk synthesis was supported by a multiple regression analysis of data available in the literature.

Mammary IAA uptake and their dietary intake. Mammary net uptake (transport) of plasma AAs is a tightly regulated and highly coordinated process that provides AAs to the organ for milk protein synthesis and specific metabolic functions. The process is not only determined by arterial AA availability and mammary blood flow, but also controlled by AA transporter capacity in the mammary epithelium, AA transporter competition, intracellular metabolic driving (e.g., metabolic activity and milk synthesis potential), and hormone action (21,22). However, the quantitative relationship between mammary AA uptake and dietary AA intake has not been described in vivo. To quantify mammary uptake of plasma IAA, we measured the product of mammary AVD for IAA and plasma flow estimated by Fick's method (10,14). In the present study, lysine was used as the internal indicator to estimate mammary blood flow, based on the assumption that lysine metabolic loss and accretion in the MG are negligible relative to its output in milk. Mammary plasma flow rate was estimated to be 6440 L/d, equivalent to a volume ratio of plasma flow to milk yield of 560:1, which is in agreement with an estimate of 540:1 in lactating sows (10). It is important to note that mammary plasma flow was not affected by dietary IAA intake. Therefore, mammary IAA uptake was largely driven by increases in arterial IAA concentrations, which

TABLE 7

Dietary true digestible IAA needs for milk synthesis by lactating sows derived by the reverse factorial approach

Amino acid	Maintenance need[1]	Contribution of endogenous IAA[2]	Intake of TDIAA[4]	Milk synthesis need[5]	
		g/d		*g/d*	*g/kg LWG*
Arg	0	13.07[3]	49.48	62.55	29.09
His	0.63	5.60[3]	20.21	25.18	11.71
Lys	1.96	12.45	33.07	43.56	20.26
Met	0.55	2.53	14.04	16.02	7.45
Phe	0.98	6.58	33.60	39.20	18.23
Thr	2.96	7.09	25.45	29.58	13.76
Trp	0.51	1.25[3]	7.65	8.39	3.90
Val	1.31	5.63	38.84	43.16	20.07

[1] Based on mean body weight of 206 kg over a 21-d lactation period (1).

[2] Estimated by the MAVD (Table 3).

[3] Calculated by multiplying endogenous lysine contribution and the ratio of a respective AA to lysine in body tissue, respectively (1).

[4] Dietary true digestible IAA intake was converted from dietary IAA intake (Table 6) based on the regressions of dietary true digestible IAA against dietary IAA (1).

[5] Dietary true digestible IAA need for milk synthesis was derived by a reverse factorial approach: daily true digestible IAA intake = maintenance need + milk synthesis need − the contribution of endogenous IAA. The lactating sows had a mean body weight loss of 1.18 kg/d and litter weight gain of 2.15 kg/d over a 21-d lactation period in this study. Milk synthesis need was normalized by litter weight gain.

were positively correlated with their dietary intake. For most IAA the relationship between mammary AVD (i.e., uptake) and dietary intake was quadratic, reaching a vertex at ~182 g protein/kg diet; however, above this dietary intake, the mammary IAA AVD declined. In contrast, the mammary AVD of leucine and isoleucine increased linearly with increasing dietary intake. We postulate that the decline in the mammary AVD of most IAA at their highest intake was not due to substrate saturation of the AA transport system, but to competitive inhibition of AA transport by large neutral amino acids (e.g., leucine and isoleucine). In fact, arterial concentrations of those AAs (e.g., lysine and methionine) were much lower than their K_m at the highest intake. Increasing evidence suggests that there is a substantial interaction in mammary AA transport between cationic and neutral amino acids. It has been previously demonstrated in our laboratory that high physiological concentrations of valine significantly inhibit in vivo net uptake of lysine by the porcine MG (14). In addition, high physiological concentrations of leucine strongly inhibit in vitro net uptake of both valine by the porcine mammary tissue (23) and lysine by rodent mammary tissue (24).

Estimation of endogenous IAA contribution. Sows mobilize body protein reserves to support milk synthesis when the intake of protein or IAA is not adequate. This mobilization is mediated mainly through the regulation of interorgan protein metabolism [e.g., decreasing protein synthesis and increasing protein degradation in muscle tissues (25,26)]. Through this mobilization, IAA from extramammary tissue proteins are partitioned to the MG for milk synthesis. Moreover, nutrients are used first for maintenance and second for milk synthesis in sows (27). Therefore we hypothesized that IAA_c mobilized from extramammary tissue proteins can be predicted as the sum of the amount of the IAA captured for obligatory metabolism (or maintenance need) and the amount of the IAA taken up by the MG when dietary intake of the IAA is zero.

To validate the MAVD estimation, we quantified the contributions of endogenous IAA by multiplying body protein loss and the concentrations of IAA in body protein. In the present study, body protein loss was estimated at 177 g/d or 150 g/kg BW loss, assuming that negative N balance of the whole body represented body protein loss. This estimate is in agreement with previous studies (9). In the present study, lysine concentration in body protein was assumed to be 70 g/kg body protein, which was based on estimates of 70 (20) and 70.5 (28) g/kg body protein. Moreover, AA composition of the whole body protein was assumed to represent that of mobilized body protein (i.e., mainly muscle protein) during lactation. However, AAs are not released from muscle in an exact proportion to the occurrence in AA sequence of muscle protein. Within muscle branched-chain AAs are metabolized substantially through transamination and decarboxylation and methionine to some extent through transulfuration; however, no other AA (including lysine, phenylalanine, and threonine) are metabolized (29,30). The first 2 enzymes (i.e., branched-chain aminotransferase and branched-chain α-keto acid dehydrogenase) in the catabolic pathway are highly expressed in skeletal muscle (29,31) and upregulated significantly in the MG during lactation (32). As a result, these highly metabolized AAs (especially valine) from muscle proteolysis will become less available to the MG. Therefore, the estimates of lysine, phenylalanine, and threonine predicted by the MAVD could be close to those defined by the BCCA, whereas the estimates of methionine and valine predicted by the MAVD are slightly lower than those defined by the BCCA. It should be pointed out that the MAVD predicted the available amount of IAA from body protein loss contributed to the MG at zero dietary AA supply, whereas the BCCA estimated the maximal amount of IAA released from body protein loss at a negative body N balance. Furthermore, measurements of the nonmetabolized AA AVD and blood flow across the hindquarter would be a direct estimation of the AAs released from muscle proteolysis, which can be used to validate the MAVD estimates. Finally, the contribution of endogenous lysine (a mean of 10.5 g/kg BW loss) is in agreement with results from the study of Jones and Stahly (9), but is much higher than an estimate of 6.2 g/kg BW loss assumed by the NRC (1). However, contributions of endogenous AAs are not only determined by gross loss of body weight, but also by the lean proportion of mobilized body reserves and the aforementioned AA metabolism in muscle. Thus, it is not appropriate to estimate the contributions if they are only based on body weight loss and AA composition in body protein as modeled by the NRC (1).

Estimation of IAA need for milk synthesis. Output of IAA in milk might not be a sensitive endpoint because of the inherent lactation homeorrhesis. A certain level of milk synthesis can be maintained at the cost of body protein loss when intake of dietary protein (IAA) is not adequate (8,9). In contrast, mammary uptake of plasma IAA is specifically responsive to intake of dietary IAA (14), which can be considered a new response criterion for estimating dietary IAA needs for milk synthesis (7). In the present study, we defined the maximal mammary uptake of plasma IAA as its mammary physiological need for milk synthesis and further considered the dietary true digestible IAA need for milk synthesis. Estimates of dietary true digestible IAA (except for phenylalanine) needs for milk synthesis defined by the maximal mammary uptake are comparable to those derived independently using the reverse factorial approach (see Tables 5 and 7). Quantitatively, the maximal mammary uptakes of plasma IAA not only encompass their output in milk, but also includes their metabolic need by the MG. The metabolic need may include oxidation, tissue protein accretion, de novo synthesis of dispensable AAs, and/or conversions to other substrates (33).

The total requirement for dietary lysine of lactating sows was estimated empirically based on reproductive performance and nutritional status of body nitrogen (7). The total requirements of dietary lysine are strongly related to lactation performance (6), which can be met by both exogenous intake of dietary lysine and endogenous contribution of lysine mobilized from body protein reserves. It is this endogenous contribution that complicates the empirical estimation of total requirements of dietary lysine for lactating sows (6). To compare empirical estimates of total requirements of dietary lysine, we developed a response surface model to fit data reported in the literature (2–5,8,34–39). The response surface of the total requirements of dietary true digestible lysine was regressed against litter weight gain and maternal body weight loss during lactation. The total requirements (Y) of dietary true digestible lysine by lactating sows can be predicted by the response surface model: Y (g/d) = 0.83 + 20.20 litter weight gain (kg/d) − 7.28 body weight loss (kg/d) ($P < 0.0001$, $R^2 = 0.64$). The partial coefficient of litter weight gain (20.20 g/kg, $P < 0.0001$) can be considered the dietary true digestible lysine need for milk synthesis at zero body weight loss and agrees closely with an estimate of 20.15 g/kg predicted by the maximal mammary uptake in this study. The total requirement of

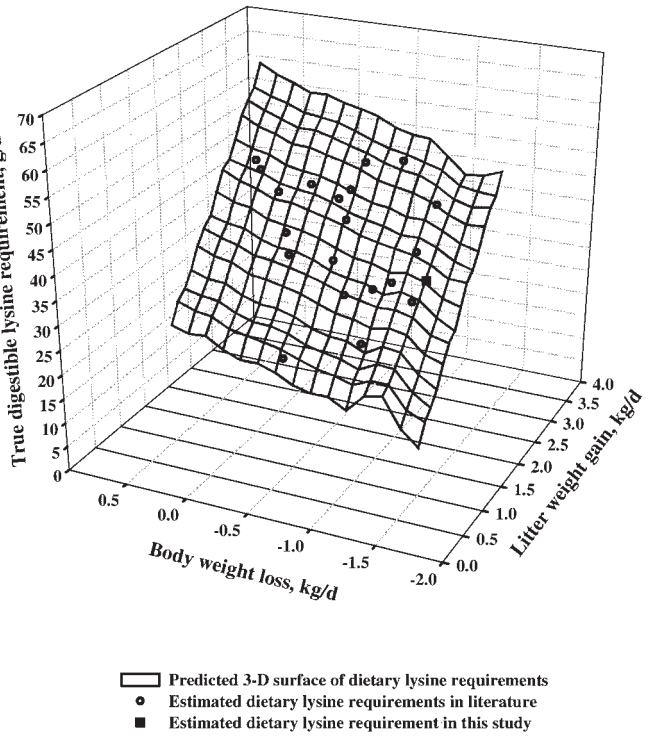

FIGURE 3 A 3-D response surface of dietary true digestible lysine requirements (*Y*) of lactating sows fitted by the multiple regression model: $Y = 0.83 + 20.20$ litter weight gain $- 7.28$ body weight loss ($P < 0.0001$, $R^2 = 0.64$). Each dot represents an individual estimate of dietary true digestible lysine requirement in the literature (2–5,8,35–39). Dietary lysine requirements are expressed on true digestible basis as cited from the literature or calculated from its total basis (1).

dietary true digestible lysine increases with increasing level of lactation performance (i.e., with increasing litter weight gain); but decreases with increasing endogenous contribution of lysine from body protein reserves (i.e., with increasing maternal body weight loss) (**Fig. 3**).

Unfortunately,the dietary IAA need for milk synthesis has not been assessed experimentally. It is impossible to completely compare our estimates with any published values (of the total requirements), which are estimated at certain contributions of endogenous IAA and specific levels of lactation performance. In general, our estimates of dietary IAA need for milk synthesis were higher than those values derived from the AA profile in milk (1). It was established that mammary uptake of the branched-chain AA is much higher than their output in milk (10), which might be attributed to their increased metabolism in the MG during lactation (32). For example, ~30% of valine uptake is oxidized to CO_2 in goat MG (40). In addition to oxidation, valine may also provide the carbon skeleton and/or α-amino nitrogen for synthesis of dispensable AAs in the MG (41). Although it is not fully understood whether this metabolism is essential for higher rates of milk synthesis, dietary valine need for milk synthesis is apparently higher than its output in milk (42,43). Moreover, the ratio (1.10:1) of dietary valine need for milk synthesis (based on the maximal mammary uptake) is very close to a ratio of 1.05:1 (14) or 1.17:1 (44) for its total requirement based on mammary protein synthesis or on milk yield, respectively. Similarly, we found that the dietary arginine need for milk synthesis is much higher than the value recommended by the NRC (1). Besides incorporation into milk, arginine may be converted to nitric oxide and proline (45). As a result, the mammary uptake of arginine greatly exceeds its output in milk (10). It is thus conceivable that an additional amount of arginine may be required for de novo synthesis of nitric oxide (needed for mammary blood flow) and dispensable AAs (e.g., proline and glutamate needed for milk synthesis) (45,46).

Dietary true digestible threonine need for milk synthesis was estimated at 14.1 g/kg litter wt gain at zero body weight loss in the present study, which is comparable to an estimate of 12.08 g/kg litter wt gain at minimal body weight loss (47). The ratio (0.70:1) of dietary threonine need for milk synthesis is in agreement with the study of Cooper et al. (47). Threonine can also be oxidized to CO_2 and converted to dispensable AAs in the MG (48). Therefore, the estimate of its dietary need for milk synthesis may only be a minimum if it is based on its output in milk. The ratio (0.60:1) of dietary phenylalanine need for milk synthesis is very close to a ratio of 0.63:1 for its total requirement when based on milk yield and plasma AA profile (1,49). Dietary phenylalanine need for milk synthesis predicted by the maximal mammary uptake was substantially lower than that derived from the reverse factorial approach. Whole body hydroxylation of phenylalanine to tyrosine is upregulated by increasing systemic phenylalanine availability in lactating goats (17). The hydroxylation would decrease the efficiency of dietary phenylalanine utilization for milk synthesis. Thus, dietary phenylalanine need for milk synthesis might be overestimated by the reverse factorial approach. Dietary true digestible methionine need for milk synthesis was estimated to be 14.9 g/d, which is in agreement with a value of 17 g/d for total requirement of dietary methionine at minimal body weight loss (50). However, the dietary methionine need for milk synthesis is related to the oxidation of methionine and conversion to cysteine in the MG.

In summary, dietary IAA needs for milk synthesis can be determined by their maximal mammary uptake, and these estimates were further validated by the reverse factorial approach. Dietary true digestible lysine need for milk synthesis was estimated to be 20.15 and 20.26 g/kg litter wt gain, respectively, by these 2 methods, which is supported by the multiple regression analysis of empirical estimates of total requirements of dietary lysine. Dietary ratios of true digestible arginine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine to lysine required for milk synthesis were higher than values recommended by the current NRC (1). Dietary IAA needs for milk synthesis defined by their maximal mammary uptake not only include their output into milk, but also cover their mammary metabolic needs. Therefore an estimate of dietary IAA need for milk synthesis might be a minimum if it is solely based on the AA profile in milk, especially for arginine and the branched-chain AA.

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