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AMINO ACID TRANS-MEMBRANE TRANSPORT, NET UPTAKE, AND INTRACELLULAR KINETICS IN THE PORCINE MAMMARY GLAND DURING LACTATION

By

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ABSTRACT

AMINO ACID TRANS-MEMBRANE TRANSPORT, NET UPTAKE, AND INTRACELLULAR KINETICS IN THE PORCINE MAMMARY GLAND DURING LACTATION

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The lactating porcine mammary gland (MG) has a large demand for indispensable amino acids (IAA) to meet a high rate of milk protein synthesis. Mammary trans-membrane transport and net uptake of IAA can be rate-limiting for the synthesis of milk protein. Endogenous contributions of IAA mobilized from body protein, their dietary needs for milk synthesis by the MG, and ideal dietary IAA pattern of the lactating sow have not been quantified. In the first study, 16 lactating sows were fed one of four isocaloric diets varying in protein concentration (7.8, 13.0, 18.2, and 23.5 %). The sows were fitted with catheters in the carotid artery and the main mammary vein. On d 10, 14, 18, and 22 of lactation, arterial and venous blood samples were simultaneously obtained every 30 min over 6 h. Milk yield on d 11 and 21 of lactation was estimated by the D_2O dilution method. Results were summarized as follows. (1) Dietary protein concentration affected mammary IAA uptake pattern, but did not affect the IAA profile in milk. Uptake of plasma Arg and the branched-chain AA by the MG exceeded their output in milk. (2) Endogenous IAA contributions, defined by mammary uptake when intake was extrapolated to zero, were 10.5, 2.1, 5.6, 6.4, and 4.8 g/kg body weight loss, respectively, for Lys, Met, Phe, Thr, and Val. (3) Dietary needs of truly digestible IAA for milk

synthesis, defined by their maximal mammary uptake, were 28.8, 10.0, 20.2, 6.9, 12.1, 13.9, 4.5, and 22.4 g/kg litter weight gain, respectively, for Arg, His, Lys, Met, Phe, Thr, Trp, and Val. In the second study, 9 lactating sows were fed ad libitum one of three diets varying in Lys and Val concentrations (4.93, 9.87; 9.71,10.15; and 9.76, 13.37 g/kg diet as-fed, respectively, in the Lys-deficient diet (LD), the positive control diet (PC), and the Val-excess diet (VE)). The jugular vein, the anterior main mammary vein, and the carotid artery were cannulated. A mixture of the labeled IAA ([2-^{15}N]-L-Lys•HCl, [S-methyl-$^{2}H_3$]-L-Met, and [1-^{13}C]-L-Val) was infused continuously via the jugular vein for 20.5 h on d 18 of lactation; meanwhile, milk, carotid arterial, and mammary venous blood samples were obtained. Milk yield, milk protein content, and litter weight gain were decreased in the LD, but were not affected in the VE. In the LD, inward trans-membrane transport (Fmg,a) and outward trans-membrane transport (Fv,mg) across the MG were decreased for Lys, and tended to be decreased for Met, resulting in decreased net uptake of plasma Lys and Met by the MG. In the VE, Fmg,a for Val was decreased numerically and Fv,mg for Val was completely blocked, resulting in numerically increased net uptake of plasma Val by the MG. However, Fv,mg for Lys was numerically increased to a greater extent than Fmg,a for Lys, resulting in a decreased net uptake of plasma Lys by the MG. Protein synthesis and breakdown were decreased in the MG in the LD, resulting in decreased net balance of mammary protein. Protein breakdown was decreased to a greater extent compared to protein synthesis in the VE. In conclusion, endogenous IAA contributions and dietary IAA needs for milk synthesis can be defined by their mammary uptake. Moreover, trans-membrane transport, net uptake, and the intracellular kinetics of IAA in the MG are tightly regulated by dietary AA regime.

To my wife, daughter, and family

for their love, support, and trust throughout my life

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LIST OF ABBREVIATIONS

Arginine.....Arg

Hstidine.....His

Isoleucine.....Ile

Leucine.....Leu

Lysine.....Lys

Methionine.....Met

Phenylalanine.....Phe

Threonine.....Thr

Tryptophan.....Trp

Tyrosine.....Tyr

Valine.....Val

Nitrogen.....N

Crude protein.....CP

Indispensable amino acids.....IAA

Branched-chain amino acids.....BCAA

Mammary gland.....MG

Arterio-venous.....A-V

Nitrogen.....N

Low protein diet.....LP

Appropriate protein diet.....AP

Median protein diet.....MP

High protein diet.....HP

Truly digestible indispensable amino acids.....TDIAA

Contributions of endogenous indispensable amino acids.....IAA_c

Mammary arterio-venous difference model.....MAVD

Body composition change approach.....BCCA

Lysine-deficient diet.....LD

Positive-control diet.....PC

Valine-excess diet.....VE

INTRODUCTION

The porcine mammary gland has a large demand for indispensable amino acids (IAA) to meet a high rate of milk protein synthesis. For example, a sow nursing a litter of 10 or more piglets has daily output of protein and lysine in milk of 550 and 42 g/d, respectively. The lactating sow must be provided with adequate amounts of IAA in optimal proportions to maximize productive performance and utilization efficiency of dietary protein. The total dietary requirement of an IAA defined by the factorial approach is comprised of dietary needs for three components: maintenance need, milk synthesis, and body protein gain (or loss). However, none of these individual component needs have been defined experimentally. The total dietary requirement of an IAA estimated by the empircal method is affected by the endogenous contributions of this IAA mobilized from body protein. Since both dietary and endogenous IAA can be used to meet the high rate of milk protein synthesis, low intake of an IAA with high endogenous contribution may maintain a similar level of milk yield as high intake of this IAA with low (or zero) endogenous contribution. Dietary needs of IAA other than lysine have been derived by multiplying dietary lysine needs and ideal dietary IAA patterns. The IAA profile in milk is considered an ideal dietary IAA pattern for milk synthesis (ARC 1981, NRC 1998). However, this may be inappropriate for arginine and branched-chain AA because their uptake by the mammary gland exceeds their output in milk (Trottier et al. 1997). Mammary IAA uptake pattern has been proposed as a reference for an ideal dietary IAA pattern for milk synthesis by the mammary gland (Trottier and Guan 2000). The uptake of lysine by mammary tissue is inhibited by cationic and branched-

chain AA in vitro (Hurley et al. 2000, Shennan et al. 1994), suggesting that intake of protein or dietary AA may regulate the uptake of IAA by the mammary gland in vivo. Moreover, the synthesis of milk protein may be restricted by the intracellular free IAA availability, which is mainly controlled by their mammary net uptake (Mepham 1982). Net uptake of an individual IAA is determined by its inward and outward trans-membrane transport across the mammary epithelium. There is substantial protein turnover in the lactating goat mammary gland (Oddy et al. 1988). Obviously, increased protein synthesis and/or decreased protein breakdown would result in an increase in net balance of protein. Protein turnover in the porcine mammary gland has not been investigated. Therefore, the objectives in this study were to: (1) evaluate the effects of dietary protein concentration on the AA profile in milk and mammary uptake pattern of plasma IAA; (2) quantify the contributions of endogenous IAA mobilized from body protein in lactating sows; (3) estimate dietary needs of truly digestible IAA by the mammary gland for milk production in the lactating sow; and (4) evaluate if dietary lysine deficiency or valine excess affect trans-membrane transport of IAA (lysine, methionine, and valine) and intracellular protein synthesis and breakdown in the lactating porcine mammary gland.

The following chapters of this dissertation describe two studies that address these objectives. In the first study, lactating sows were provided ad libitum access to one of four diets varying in dietary protein concentration to establish a regression of mammary IAA arterio-venous difference and intake of dietary IAA. The results are summarized in

Chapters 1, 2, and 3. In the second study, lactating sows were provided ad libitum access to one of three diets varying only in Lys and Val (i.e., Lysine-deficient, positive control, and valine-excess). The results are summarized in Chapter 4. In Chapter 1, the effects of dietary protein concentration on the IAA profile in milk and the mammary IAA uptake pattern are described. In Chapter 2, the endogenous IAA contributions from body protein loss are quantified using a mammary IAA arterio-venous model, and are validated based on both nitrogen balance and the AA composition of body protein, and a factorial approach. In Chapter 3, dietary needs of truly digestible IAA for milk synthesis by the mammary gland are determined using their maximal mammary IAA uptake, and further validated by a factorial approach. In Chapter 4, inward and outward trans-membrane transport, net uptake, and intracellular kinetics of IAA (lysine, methionine, and valine) across the mammary epithelium are defined using a three-compartmental model. Chapter 5 contains the summary and conclusions of this dissertation.

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CHAPTER 1

Effects of Dietary Protein Concentration on the Amino Acid Profile in Milk and the Amino Acid Uptake Pattern by the Lactating Porcine Mammary Gland

ABSTRACT Objectives of this study were to evaluate the effects of dietary protein concentration on the amino acid (AA) profile in milk and on mammary AA uptake pattern to define an ideal pattern of indispensable amino acids (IAA) for milk synthesis by the lactating sow. Sixteen Landrace x Yorkshire lactating sows were provided ad libitum access to one of four isocaloric diets varying in protein concentration (7.8, 13.0, 18.2, and 23.5 % CP) with a similar pattern of IAA. The sows were fitted with catheters in the carotid artery and the main mammary vein on d 4 of lactation. On d 10, 14, 18, and 22 of lactation, arterial and venous blood samples were obtained simultaneously every 30 min over 6 h. Milk yield on d 11 and 21 of lactation was estimated by the D_2O dilution method. Sows fed the diet containing 7.8% CP (the low protein diet, LP) had decreased loin eye area, milk yield, and litter weight gain over a 21-d lactation period. Dietary protein concentration and stage of lactation did not affect milk composition except protein content. The content of true protein in milk decreased in the sows fed the LP and on d 18 of lactation, which decreased concentrations of IAA in defatted milk. The AA profile in milk, expressed either as the proportion of individual IAA to true protein or to Lys in milk, was not affected by dietary protein concentration or stage of lactation. Arterial concentrations and mammary arterio-venous (A-V) differences of plasma IAA increased linearly with increasing dietary protein concentration and increasing day of lactation ($P < .05$). Mammary A-V differences and extraction rates of plasma IAA

tended ($P < 0.10$) to be greatest in sows fed the diet containing 18.2% CP and d 18 of lactation. Mammary uptake pattern, defined either as the proportions of individual IAA to the total IAA or to Lys taken up by the mammary gland, was affected by dietary protein concentration ($P < .05$). Mammary uptake proportions of branched-chain AA (Ile, Leu, and Val) increased with increasing dietary protein concentration, while those of Lys, Met, Phe, and Thr decreased. The mammary uptake pattern was not altered across stage of lactation. In conclusion, mammary uptake ratios for Arg and the branched-chain AA were greater than their ratios in milk. The mammary uptake pattern of plasma IAA under appropriate intake of protein would represent an ideal pattern of IAA for milk synthesis by the mammary gland because they are presumably required for incorporation into milk protein and other metabolic pathways. The ideal dietary AA pattern for milk synthesis based only on the AA profile in milk seems inappropriate, especially for Arg and the branched-chain AA.

KEY WORDS: •amino acids •milk •mammary glands •uptake •lactation •sows

INTRODUCTION

Swine must be provided with adequate amounts of indispensable amino acids (IAA) in optimal proportions for maximal growth, reproduction, and lactation. The concept of an ideal protein has been employed to establish dietary requirements for IAA and to balance dietary IAA in diets for swine (ARC 1981, NRC 1988, NRC 1998). An ideal protein, i.e., the ideal pattern of dietary IAA, is defined as the optimal balance among dietary IAA and the relative proportion of IAA-nitrogen (N) to dispensable amino acid (AA)-N, which corresponds to dietary requirements of IAA by the animal (ARC 1981, NRC 1998). Four sets of ideal patterns of IAA for maintenance, protein accretion, milk synthesis, and body tissue, respectively, have been recommended by the NRC (1998) to constitute the entire ideal pattern of dietary IAA for swine for particular productive purposes. The ideal pattern of IAA for milk synthesis by the lactating sow has been derived from the AA profile in milk (NRC 1998), but this approach may be inappropriate.

The AA profile in milk is presumably the ideal pattern of dietary IAA required for milk synthesis based on the fact that dietary IAA absorbed by the lactating sow are mainly used for milk synthesis (ARC 1981). The AA in porcine milk is comprised of two forms: protein-bound AA (predominant) and free AA (negligible) (Wu and Knabe 1994a). The protein in porcine milk is quantified by multiplying 6.38 and the total N % in milk (Elliott et al. 1971, King et al. 1993a). Approximately 15.0% of the total N in porcine milk is attributed to non-protein N (Klobasa et al. 1987). Thus, the AA profile in milk, expressed as the percentage of total N % x 6.38 in milk, may not accurately represent the AA composition of true proteins in milk.

The AA profile in milk depends mainly on the AA composition of milk proteins. Casein and whey proteins are the predominant milk proteins. The ratio of casein to whey proteins in rat milk is positively related to dietary protein intake (Ronayne-De-Ferrer and Sambucetti 1993). The proportion of casein-N to the total N in porcine milk increases approximately 10% as dietary Ile concentration increases; in contrast, the proportion of whey protein-N and non-protein N decreases (Richert et al. 1997). The proportion of casein-N to the total N in milk from sows fed Lys-adequate diet appears to be higher than that from sows fed Lys-deficient diet (53.3% vs. 49.0% on d 18 of lactation) (Guan et al. unpublished data). However, whether the ratio of casein to whey proteins in porcine milk is affected by dietary protein intake is not known, though the concentration of the total N in milk is decreased in sows fed a diet low in protein content (King et al. 1993a, Kusina et al. 1999a). The ratio of whey protein-N to casein-N in porcine milk decreases from .40 to .31:1.00, respectively, on d 7 and d 21 of lactation (Brent et al. 1973). Changes in the ratio of caseins to whey proteins in milk may affect the AA profile in milk given the differences in the AA composition between caseins and whey proteins (Swaisgood 1995). Therefore, the AA profile in milk may be affected by dietary protein intake and the stage of lactation.

Plasma free AA are taken up by the mammary gland through specific AA transport systems situated on the basolateral membranes of the mammary epithelium (Shennan et al. 1997). Mammary uptake of plasma free AA can be quantified by the technique of their arterio-venous (A-V) differences across the mammary glands of the lactating sow

(Trottier et al. 1995). The amount of branched-chain amino acids (Ile, Leu, and Val, BCAA) and Arg taken up by porcine mammary glands exceeds their output in milk by approximately 25 to 30% (Nielsen et al. 2000, Trottier et al. 1997). The IAA taken up in excess of their output in milk may be required for metabolism and protein synthesis in the mammary gland. The rate of mammary uptake for plasma Arg, the precursor for de novo synthesis of nitric oxide, may be involved in regulation of mammary blood flow (Lacasse et al. 1996, Wu and Morris 1998). In addition to incorporation into milk protein, the BCAA taken up by the mammary gland can provide α-amino N and carbon for dispensable AA synthesis and provide energy for synthesis of other compounds (e.g., lactose and fatty acids) in the gland (Wohlt et al. 1977). Therefore, the ideal pattern of IAA required for milk synthesis in the lactating sow, based only on the AA profile in milk, may be inappropriate.

Nutritional regulation of plasma free AA uptake by the mammary gland is poorly understood. In the lactating porcine mammary tissue, Lys uptake is inhibited in vitro by cationic AA and neutral AA as shown in the lactating rat mammary tissue in vitro (Calvert and Shennan 1996, Hurley et al. 2000, Shennan et al. 1994). Methionine uptake by the mammary glands from the starved lactating mice is increased in vitro compared to fed lactating mice (Verma and Kansal 1995). Taken together, we hypothesize that the mammary uptake pattern of plasma IAA, defined as the proportion of an individual IAA to the total IAA taken up by the mammary glands, is affected by dietary protein concentration. Though the mammary AA uptake pattern has been proposed as a reference for an ideal dietary AA pattern for milk synthesis (Trottier and Guan 2000), it is

not known if intake of dietary protein regulates the uptake pattern. It is imperative to use the best dietary AA pattern for milk synthesis to derive dietary needs and balance dietary AA so that the lactating sow can reach milk synthesis potential and utilize dietary protein efficiently. The objectives of this study were to evaluate effects of dietary protein concentration on the AA profile in milk and the mammary uptake pattern of plasma IAA to derive an ideal pattern of IAA for milk synthesis by the lactating sow.

MATERIALS AND METHODS

Michigan State University All-University Committee on Animal Use and Care approved all procedures in this study.

Experimental Design and Dietary Treatments. Sixteen Landrace x Yorkshire lactating sows (parity 2 or 3) were allocated to dietary treatments according to a randomized block design. Each block consisted of four sows. Each sow in one block was provided ad libitum access to one of four diets. Diets contained different protein (CP) concentrations (7.8, 13.0, 18.2, and 23.5% as-fed basis) and were balanced to contain a similar pattern of indispensable amino acids (IAA) recommended by the NRC (1988). All diets were isocaloric with a ME of 14.3 MJ/kg. The composition of diets is given in **TABLE 1-1**.

Animals. Litters were adjusted to 11 piglets per sow by cross-fostering within 48 h after birth. Sows were housed individually in farrowing crates in a mechanically ventilated, thermally controlled room (21 °C). Sows were provided free access to water. Rations were offered in a stair step manner during the first 3 d post-surgery and sows were fed to

appetite thereafter. Sow feed intake was recorded daily. Sow body weight, backfat depth, and loin eye area were recorded after farrowing (d 1) and at weaning (d 21). Backfat depth and loin eye area were scanned 5 cm lateral from the spine and centered over the 10th rib (Ultrasound Scanner 200 Vet with ASP-18 linear probe, Pie Medical Equipment B.V., Maastricht, the Netherlands). Piglets were weighed individually weekly.

Cannulation. For the catheter, microbore Tygon® tubing (1.0 mm i.d., 1.8 mm o.d., Norton Performance Plastics Co., Akron, OH) was used and the lumen was coated with triododecylmethylammonium chloride - heparin complex (7% (wt/wt), Polysciences, Inc., Warrington, PA). The anterior main mammary vein and the carotid artery were cannulated on d 4 ± 1 of lactation following the surgical procedure described by Trottier et al. (1995). Antibiotic (Naxcel®, Pharmacia and Upjohn Co., Kalamazoo, MI) and anti-inflammatory (Banamine®, Schering-Plough Animal Health Corp., Kenilmorth, NJ) were administered i.v. for 3 d following surgery. The catheters were flushed once daily with heparinized saline (20 IU heparin/ml).

Milk Yield Estimation. Milk intake by piglets (i.e., milk yield) 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. with a dose (1 mg/kg BW) of D_2O (Cambridge Isotope Laboratories, Andover, MA). At 1 h after the dose, piglets were bled from the jugular vein using vacutainers containing SST® gel and clot-activator (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ).

At 24 h after the dose, the piglets were fasted for 1 h, weighed, and bled as before. During this 24-h period, piglets were not provided access to water. Blood samples were centrifuged at 1500 x g for 15 min at 4 °C, and then serum samples were separated and stored at -20 °C until assayed for D_2O. Serum D_2O concentration was determined by an infrared spectrophotometer (The Foxboro Co., East Bridgewater, MA) using a fixed-filter length of 4 μm. Milk intake was computed on the basis of the D_2O dilution principle (Pettigrew et al. 1987).

Blood and Milk Sampling Protocol. Blood samples from sows were collected on d 9 ± 1, 13 ± 1, 17 ± 1, and 21 ± 1 (referred to as d 10, 14, 18, and 22 of lactation, respectively). Sows were fed 1 h prior to blood sampling and provided ad libitum access to feed and water as usual. Matched sets of carotid arterial and main mammary venous blood samples (10 ml each) were collected into sterile syringes from 0800 every 30 min over 6 h. Thus, a total of 13 blood samples were collected from each sow per sampling day. Blood was transferred into EDTA-coated tubes and centrifuged at 1500 x g for 15 min at 4 °C. Plasma was removed and stored at -20 °C. On the same day as blood sampling, milk was collected and pooled from all functional teats on each sow following an i.v. dose of oxytocin (10 IU). Approximately 20 ml pooled milk was transferred into a vial containing a preserver (Broad Spectrum Microtales^{tm}, D & F Control System, Inc., San Ramon, CA) and stored at 4 °C until assayed for milk composition. Another portion of pooled milk was stored at -20 °C until assayed for nitrogen. The remainder of pooled milk was defatted by centrifugation at 1500 x g for 15 min at 4 °C and stored at -20 °C until assayed for amino acids (AA).

Laboratory measurements.

(1) Milk Composition and Amino Acid Analysis. Milk lipid, lactose, and protein contents were assayed using a mid-infrared spectroscope (Multispec M, Berwind Instrument Ltd., York, England, U.K.)(AOAC 1990). Frozen milk samples were thawed at 37 °C and mixed by a vortex. Milk nitrogen was determined using a LECO Nitrogen Analyzer FP-2000 (LECO Corp., St. Joseph, MI) with EDTA (Sigma Chemical Co., St. Louis, MO) as a calibration standard. The energy content of milk was estimated by multiplying the percentage of milk protein, lipid, and lactose by 5.70, 9.40, and 4.15 kcal, respectively (Jenness 1974). Amino acid concentrations in the defatted milk were quantitated by reverse-phase, high-performance liquid chromatography using phenylisothiocyanate precolumn derivatization (Pico•Tag®, Waters Corp., Milford, MA). In brief, 200 μL of defatted milk was hydrolyzed with 6 mol/L HCl for 24 h at 110 °C. Amino acids in the hydrolysate were derivatized with phenylisothiocyanate and separated by a Pico•Tag® column (3.9 mm x 150 mm) and detected at 254 nm by Waters™ 486 Tenable Absorbance Detector (Waters Corp., Milford, MA). Norleucine as an internal standard was added into defatted milk samples before hydrolysis. Amino acid standard H (Pierce, Rockford, IL) was used as a calibration standard. The method was validated with certified AA standard (NIST, Gaithersburg, MD).

(2) Plasma Amino Acid Analysis. Frozen plasma samples were thawed at 4 °C overnight and mixed using a vortex. Plasma samples were pooled from the 13 samples taken from each sow on each sampling day. Glucosaminic acid was added to the pooled samples as

an internal standard. Amino acid concentrations were determined using a Beckman 6300 Amino Acid Analyzer following the method described by Lee and Slocum (1987). In brief, plasma samples were deproteinized by 35% sulfosalicyclic acid precipitation. Amino acids in the supernatant were separated by a Beckman cation-ion exchange column charged in lithium citrate buffer. The eluted AA were measured spectrophotometrically following postcolumn derivatization with ninhydrin.

(3) Dietary Nitrogen and Amino Acid Analysis. Feed samples were finely ground using a Cyclotec® 1093 Sample Mill (Foss Tecator, Sweden). Feed nitrogen was analyzed by the micro-Kjeldahl method (AOAC 1990). Hydrolysis of feed samples and determination of AA in the hydrolysate were performed as described in *(1)*. The content of Trp in the diets was calculated using its content in feed ingredients (NRC 1988).

Calculations.

(1) Milk Output of Indispensable Amino Acids. Milk output of IAA (g/d) = milk yield (L/d) x the concentrations (μmol/L) of IAA in defatted milk x (1 – the content of lipid in whole milk) x molecular weight (g/mol) x 10^{-6}. The concentrations of IAA in defatted milk were corrected by the content of lipid in whole milk to obtain the concentrations of IAA in whole milk. To quantify milk output of IAA, milk yield on d 11 was multiplied with an average concentration of IAA in whole milk from d 10 and 14 of lactation, and milk yield on d 21 with that from d 18 and 22 of lactation.

(2) Mammary Extraction Rates of Plasma Indispensable Amino Acids. Mammary extraction rate (%) = 100 x mammary A-V difference of plasma IAA / arterial concentration of the plasma IAA.

(3) Mammary Uptake Pattern of Plasma Indispensable Amino Acids. Mammary uptake pattern of plasma IAA was defined either as the proportion (%, mol/mol) of individual IAA to total IAA taken up by the glands or the ratios (wt/wt) of other IAA to Lys taken up by the glands. Note that mammary plasma flow rate as a variable was cancelled out in the following calculations:

Mammary uptake proportions (%, mol/mol) = 100 x mammary arterio-venous (A-V) difference (μmol/L) of an individual IAA x mammary plasma flow rate (L/d) / (mammary A-V difference (μmol/L) of the total IAA x mammary plasma flow rate (L/d)); i.e.,

Mammary uptake proportions (%, mol/mol) = 100 x mammary A-V difference (μmol/L) of an individual IAA / mammary A-V difference (μmol/L) of the total IAA. **(Eq.** 1)

Mammary uptake ratios (wt/wt) = mammary A-V difference (μmol/L) of IAA x mammary plasma flow rate (L/d) x its molecular weight (μg/μmol) / ((mammary A-V difference (μmol/L) of plasma Lys x mammary plasma flow rate (L/d) x Lys molecular weight (μg/μmol)); thus,

Mammary uptake ratios (wt/wt) = mammary A-V difference (μmol/L) of IAA x its molecular weight (μg/μmol) / (mammary A-V difference (μmol/L) of plasma Lys x Lys molecular weight (μg/μmol)). **(Eq.** 2)

Statistical Analyses. Data were analyzed by the Mixed Procedure with a repeated statement of SAS/STAT (Version 6.12, SAS Institute Inc., Cary, NC). The first-order autoregressive was assumed in the covariance structure. Individual sow was considered the experimental unit (the random effect) and day of lactation as the repeated effect. The model for milk and plasma variables included block, dietary treatment, day of lactation, and all two-way interactions with day of lactation in a repeated statement. The interaction between dietary treatment and day of lactation was not significant ($P > .10$). Therefore, only main effects (dietary treatment or day of lactation) are presented. The model for performance variables included block and dietary treatment; sow body weight, backfat depth, and loin eye area on d 1 of lactation were used as covariates, respectively, to adjust their changes over a 21-d lactation. Least-squares means are presented because of missing values in the data. Differences are considered significant at $P < .05$ or .01.

RESULTS

Sow and Litter Performance

Sows fed the 13% CP diet (the appropriate protein diet, AP) had higher feed intake over a 21-d lactation period than sows fed other diets (**TABLE 1-2**). Intake of protein was lower and higher, respectively, for sows fed the 7.8% CP diet (the low protein diet, LP) and the 23.5% CP (the high protein diet, HP) compared with that of sows fed the AP and the 18.2% CP diet (the median protein diet, MP). Dietary treatments did not affect sow body weight change and backfat depth change over the 21-d lactation period. However, sows fed the LP lost two-fold loin eye area as sows fed other diets. Daily litter weight

gain over the 21-d lactation period was 1.88, 2.43, 2.07, and 2.23 kg/d, respectively, for sows fed the LP, AP, MP, and HP. Sows fed the LP had the lowest litter growth rate and milk yield on d 21, though sows fed other diets were not different in litter growth rate and milk yield.

Milk Composition and Output

Dietary treatments and stage of lactation did not affect milk composition except protein concentration (**TABLE 1-3**). Milk protein concentration increased linearly with increasing dietary protein concentrations ($P < .01$). In contrast, the content of milk lactose tended to decrease with increased dietary protein concentrations ($P < .10$). The content of milk protein decreased approximately 10% in sows fed the LP and AP compared to that in sows fed the MP and HP. Effect of stage of lactation on milk protein concentration was quadratic ($P < .05$). It decreased from d 10 to 18 and then increased by d 22 of lactation. The content of nitrogen in milk changed as the content of milk protein did (data not shown). The concentrations of indispensable amino acids (IAA) in defatted milk reflected the content of milk protein (**TABLE 1-4**). The concentrations of IAA (except His and Met) in defatted milk increased linearly with increasing dietary protein concentrations ($P < .05$). Effect of stage of lactation on concentrations was quadratic ($P < .05$) for His, Thr, and Val, and tended to be quadratic for other IAA ($P < .10$).

Dietary treatments affected output of protein in milk ($P < .05$) and tended to affect output of dry matter in milk ($P < .10$), but did not affect output of lipid, lactose, or energy in

milk (data not shown). Sows fed the LP had the lowest output of macro-nutrients and energy due to their lowest milk yield. Milk yield and output of macro-nutrients and energy were not different between d 11 and 21 of lactation (11.67 ± .62 vs 11.41 ± .62, 2.18 ± .11 vs 2.09 ± .11, .55 ± .03 vs .55 ± .03, and .66 ± .04 vs .63 ± .04 kg/d, respectively, for milk yield, dry matter, protein, and lactose, and 13.19 ± .69 vs 12.11 ± .67 Mcal/d for energy), though output of lipid in milk tended to be higher on d 11 than that on d 21 of lactation (.78 ± .04 vs .68 ± .04 kg/d, $P < .10$). Output of individual IAA in milk increased linearly with increasing dietary protein concentrations ($P < .05$) (**TABLE 1-5**). The output of individual IAA in milk decreased approximately 25 to 30% from sows fed the LP compared to that fed the AP. As reflected in the output of protein in milk, output of individual IAA in milk was not different between d 11 and 21 of lactation.

The Amino Acid Profile in Milk

The AA profile in milk was expressed as the proportion of individual IAA to milk protein or the ratios of other IAA to Lys in milk. Dietary treatments did not affect the AA profile in milk (**TABLE 1-6**). There were slight alterations in the ratios for Ile, Leu, and Met among dietary treatments. Average ratios of other IAA to Lys in milk across dietary treatments were .79, .45, .65, 1.15, .34, .60, .66, and .84:1.00, respectively, for Arg, His, Ile, Leu, Met, Phe, Thr, and Val. Stage of lactation did not affect the AA profile in milk either (**TABLE 1-7**) except for His. The proportion of Lys and the ratio of Leu to Lys were slightly altered across stage of lactation. Average ratios of other IAA to Lys in milk

across stage of lactation were .78, .45, .65, 1.15, .34, .59, .66, and .83:1.00, respectively, for Arg, His, Ile, Leu, Met, Phe, Thr, and Val.

Arterial Concentrations, Mammary Arterio-venous Differences, and Mammary Extraction Rates of Plasma Indispensable Amino Acids

Arterial concentrations of plasma individual IAA (except His and Ile) and total IAA increased linearly with increasing dietary protein concentration ($P < .05$) (**TABLE 1-8**). Arterial concentrations for the majority of plasma individual IAA (including Arg, Ile, Leu, Lys, Phe, and Val) and total IAA increased as lactation progressed ($P < .05$).

Mammary A-V differences of plasma individual IAA and total IAA were affected linearly by dietary treatments ($P < .05$) (**TABLE 1-9**). Mammary A-V differences of plasma IAA other than branched-chain AA (Ile, Leu, and Val) tended to be greatest in sows fed the MP ($P < .10$). Mammary A-V differences of plasma individual IAA and total IAA tended to increase as lactation progressed ($P < .10$).

Mammary extraction rates of plasma individual IAA (except Arg, Lys, and Trp) were affected by dietary treatments ($P < .05$) (**TABLE 1-10**). Mammary extraction rates for the majority of plasma individual IAA (including Arg, His, Ile, Met, Phe, Thr, Trp, and Val) tended to be the greatest in sows fed the MP ($P < .01$). In contrast, they decreased in sows fed the HP compared to those in sows fed the MP ($P < .05$). Mammary extraction rates of total IAA tended to be affected by dietary treatments ($P < .10$). Mammary

extraction rates of plasma IAA (except Ile, Trp, and Val) and total IAA increased on d 18 compared to those on d 14 of lactation.

Mammary Uptake Patterns of Plasma Indispensable Amino Acids

Mammary uptake pattern of plasma IAA was defined as the proportion of individual IAA to total IAA taken up by the mammary glands or the ratio of other IAA to Lys taken up by the glands. The proportions for Lys, Met, Phe, and Thr decreased with increasing dietary protein concentration (**FIGURE 1-1**). In contrast, the proportions for the branched-chain AA increased with increasing dietary protein concentration. The proportions for Arg, His, and Trp were not affected significantly by dietary protein concentration. Thus, the ratios of other IAA to Lys taken up by the glands for Arg, Met, and the branched-chain AA increased with increasing dietary protein concentration ($P <$ .05) (**TABLE 1-11**).

The proportions of individual IAA to total IAA were not altered across stage of lactation (**TABLE 1-12**). Those proportions were averaged across stage of lactation at 12.15, 3.60, 11.80, 19.33, 13.50, 4.30, 7.30, 9.93, 1.43, and 16.63 % (mol/mol), respectively, for Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val. Based on the average proportions, the ratios (wt/wt) of other IAA to Lys were 1.07, .28, .78, 1.28, 1.00, .33, .61, .60, .15, and .99, respectively, for Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val.

Discussion

Lactation Performance

Sows fed LP had lower milk yield and lower litter growth rate. This is in agreement with the results from other studies (Elliott et al. , King et al. 1993b, Kusina et al. 1999b, Sauber et al. 1998). Dietary protein concentration did not affect sow body weight loss during lactation in the present study, which is in agreement with the results of King et al. (1993). However, body weight loss during lactation increased in sows fed diets deficient in CP and (or) Lys in other studies (Dourmad et al. 1998, Johnston et al. 1993, Jones and Stahly 1999, Kusina et al. 1999b, Stahly 1992). The inconsistent effect of dietary protein concentration on body weight loss may be confounded by intake of dietary energy during lactation. In the present study, dietary protein concentration did not affect sow backfat depth change during lactation as reported in other studies (Dourmad et al. 1998, Johnston et al. 1993, Jones and Stahly 1999, Revell et al. 1998b, Sauber et al. 1998). In contrast, sows fed the LP lost more than twice as much loin eye area compared with sows fed diets higher in CP, indicating that sows mobilized body protein reserves to support milk production when fed diets deficient in CP (Dourmad et al. 1998, Jones and Stahly 1999).

Milk Composition and Output

Dietary protein concentration did not affect milk composition except protein concentration in the present study. Sows fed the LP and AP had low content of true protein in milk and thus lower concentrations of IAA in milk, indicating that 7.8 and 13.0% of dietary protein concentrations were not adequate for milk synthesis. The content of true protein in milk ranged from 4.5 to 5.0% in the present study, which agrees

with values (from 4.0 to 5.0%) of true protein in milk from sows fed diets containing 7.9 and 19.0% CP (Revell et al. 1998a). The content of true protein in milk may be lower by 15% than the content of CP (i.e., 6.38 x N %) in milk because non-protein N contributes approximately 15% of the total N (Klobasa et al. 1987). The content of the CP in milk decreases when sows fed diets low in CP (King et al. 1993b, Kusina et al. 1999b, Sauber et al. 1998). Stage of lactation influenced milk protein concentration, but did not affect the concentrations of dry matter, lipid, lactose, or energy in the present study. Similarly, no changes in milk composition or the content of N-containing fractions in milk (such as casein and whey) were found between d 7 and 21 of lactation (Brent et al. 1973, Dourmad et al. 1998, Klobasa et al. 1987, Pluske et al. 1998).

Output of macro-nutrients and IAA in milk decreased in sows fed the LP because of lower milk yield and (or) the content of true protein and IAA in milk in this study. Because there was neither difference in milk yield between d 11 and 21 of lactation as reported in other studies (King et al. 1993b, Pluske et al. 1998) nor difference in the average content of macro-nutrients and IAA in milk between early lactation (d 10 to 14) and late lactation (d 18 to 22), there was no difference in their output in milk.

The Amino Acid Profile in Milk

Dietary protein concentration did not affect the AA profile in milk in the present study. Elliott et al. (1971) and King et al. (1993) found the concentrations of individual IAA in milk decreased when sows were fed diets low in CP. This evidence suggests that output of individual proteins in milk may be affected proportionally by intake of dietary protein;

and supports the notion that modification of the amount of milk protein, rather than the AA profile of milk protein, may be an evolutionary mechanism used to meet the unique AA needs by mammalian neonates (Davis et al., 1994). The AA profile in milk was not altered between d 10 to 22 of lactation. Similarly, Elliott et al. (1971) found that the AA profile in milk was not altered between d 5 to 21 of lactation. Moreover, the AA profiles in milk are consistent throughout all independent studies (**TABLE 1-13**). The proportions of Lys were based on the true protein (in the present study), on the total individual AA (in which Trp was not included) (Davis et al. 1994), or on the N x 6.38 (Dourmad et al. 1998, Dourmad et al. 1991, Elliott et al. 1971, King et al. 1993a). The average proportion of Lys to true protein (7.83%) in the present study was slightly lower than that in the study of Davis et al. (1994), but was higher than those in the other studies (**TABLE 1-13**). In addition, the average concentration of Lys (including free and protein-bound forms) in defatted milk was 26.5 mmol/L in the present study, which is within the range of 24 to 30 mmol/L of protein-bound Lys in defatted porcine milk reported by Wu and Knabe (1994b).

Dietary pattern of individual IAA required for milk synthesis is presumably dominated by the AA composition of proteins secreted into milk (ARC 1981). Thus, the AA profile in milk has been used to derive the ideal pattern of IAA required for milk synthesis in the lactating sow by the NRC (1998).

Mammary Arterio-venous Difference of Plasma Indispensable Amino Acids

The mammary gland during lactation requires a large amount of AA for metabolism and protein synthesis. Amino acids are extracted from blood by specific AA transport systems situated in the blood-facing aspect of the mammary epithelial cells, as reflected in mammary A-V differences of blood AA (Shennan et al. 1997). The transport of certain AA by the mammary epithelial cells could be rate limiting for the synthesis of milk protein (Mepham 1982). Milk yield is positively related to mammary A-V difference of plasma IAA in the lactating cow and sheep when mammary blood flow rates are constant (Fleet and Mepham 1985, Guinard et al. 1993, Madsen et al. 1999). Thus, mammary A-V difference of plasma IAA may be considered as a physiological response criterion to determine the IAA needs for milk synthesis by the lactating porcine mammary gland (Trottier and Guan 2000).

Mammary A-V differences of plasma IAA in the present study are within ranges reported in the recent studies (Nielsen et al. 2000, Trottier et al. 1997). The mammary A-V differences increased in a quadratic manner with increasing dietary protein concentration and with advancement of lactation in the present study. Similarly, mammary A-V differences of plasma IAA increase in the lactating cow with duodenal infusion of casein or whey protein (Guinard and Rulquin 1994a). Mammary A-V difference of plasma Lys decreased approximately 25% in sows fed the HP compared to that in sows fed the MP, which is possibly due to a decreased mammary extraction rate of plasma Lys. The relationship between mammary A-V difference and arterial concentration of plasma Lys is curvilinear in the lactating sow (Trottier 1997) and in the lactating cow infused duodenally with Lys (Guinard and Rulquin 1994b). Furthermore, Lys uptake by the

lactating rat and porcine mammary tissue is inhibited in vitro by physiological concentrations of cationic AA and high concentrations of neutral AA because these AA inhibit Lys influx into the lactating rat mammary tissue and stimulate Lys efflux from the tissue (Calvert and Shennan 1996, Hurley et al. 2000, Shennan et al. 1994). Thus, Lys uptake by the porcine mammary gland may be inhibited in vivo by physiological concentrations of arterial cationic AA and neutral AA.

Mammary A-V differences and extraction rates of plasma IAA increased by d 18 of lactation, implying that mammary uptake may be up-regulated to meet increased IAA needs for milk synthesis by the mammary glands. Similarly, in lactating sheep mammary A-V differences of plasma IAA are regulated by stage of lactation (further corresponding to milk yield) (Fleet and Mepham 1985). This regulation may be through actions of lactogenic hormones (e.g. prolactin) on the AA transport systems in the mammary gland (Sharma and Kansal 1999, Sharma and Kansal 2000, Shennan et al. 1997, Vina et al. 1981).

Mammary Uptake Pattern of Plasma Indispensable Amino Acids

An important finding in the present study was that the proportions of the branched-chain AA to the total IAA taken by the mammary gland were increased for the lactating sows with high intakes of protein; in contrast, the proportions of Lys, Met, Phe, Thr, and Trp to the total IAA taken by the mammary gland maintained at high levels for the lactating sows at low intake of protein (**FIGURE 1-1**). This may have important physiological implications. On one hand, Lys, Met, Phe, Thr, and Trp might become the limiting AA

for the lactating sow at low intake of protein, leading to up-regulation of their uptake (indicated by their increased proportions to the total IAA taken by the mammary gland) to meet their needs for milk synthesis. On the other hand, the branched-chain AA (e.g., Val) might become the limiting AA for the lactating sow at high intakes of protein, leading to up-regulation of their uptake (indicated by their increased proportions to the total IAA taken by the mammary gland), which might be driven by their increased intracellular removal (e.g., oxidation). Activities of branched-chain aminotransferase and branched-chain α-keto acid dehydrogenase, the first two enzymes in the branched-chain AA catabolic pathway, increase dramatically in rat mammary glands during lactation (DeSantiago et al. 1998). Moreover, a high intake of dietary protein up-regulates the expression of branched-chain aminotransferase and activity of branched-chain α-keto acid dehydrogenase in rat (Torres et al. 1998). This up-regulation might also occur in the lactating mammary gland to increase its capacity to dispose the branched-chain AA at a high intake of protein. Though physiological consequences of their excess uptake by the mammary gland are not known at this moment, we speculate that the BCAA may have two aspects of roles: (1) providing substrates and energy for milk synthesis and (2) participating in regulations of protein turnover, by decreasing protein breakdown (MacLean et al. 1994) (Mitch and Clark 1984, Nair et al. 1992).

Mammary uptake patterns of individual IAA, defined as the ratios of other IAA to Lys taken up by the lactating porcine mammary gland, are relatively constant across five independent studies (**TABLE 1-14**), though the ratios were regulated by dietary protein concentration in the present study. These patterns might be required by the mammary

glands for metabolism and protein synthesis. Thus, this set of average proportions for individual IAA to total IAA may represent an ideal pattern of plasma IAA taken up by the mammary glands. In comparison to the AA profiles in milk (**TABLE 1-13**), the ratios of Arg and the branched-chain AA to Lys are higher in the mammary uptake pattern (**FIGURE 1-2**). Arginine and the branched-chain AA are taken up by the lactating porcine mammary gland in excess of their output into milk (Nielsen et al. 2000, Trottier et al. 1997). Similar results have been found in other lactating species, such as dairy cattle, goats, and sheep (Bequette et al. 1997, Fleet and Mepham 1985, Guinard and Rulquin 1994a).

Besides incorporation into milk protein, the IAA taken up by the mammary gland may play an important role in other metabolic pathways: (1) They can provide α-amino N and carbon for synthesis of dispensable AA (Wohlt et al. 1977). (2) They can meet energy needs for high rates of protein turnover due to substantial synthesis and degradation of protein within the lactating goat mammary gland (Oddy et al. 1988). (3) They can be used for synthesis of structural proteins and enzymes to maintain mammary cell integrity and function (Bequette et al. 1997), as partly accreted in the lactating porcine mammary gland (Kim et al. 1999). (4) They can be oxidized to yield energy for the synthesis of milk non-protein compounds (e.g., fatty acids and lactose) (Davis and Mepham 1976, Roets et al. 1979). (5) They can be used as precursors for the synthesis of important metabolites. For example, Arg is the precursor for de novo synthesis of nitric oxide that is involved in local regulation of mammary blood flow (Lacasse et al. 1996, Vina and Williamson 1981, Wu and Morris 1998, Wu et al. 1999). These IAA taken up in excess

are presumably needed to meet these metabolic needs (other than milk protein synthesis) by the mammary glands. Thus, we hypothesize that the mammary uptake pattern of plasma IAA may be considered as an ideal pattern for milk production required by the lactating porcine mammary gland.

One application of the mammary uptake pattern is to define an ideal ratio of Val to Lys for milk synthesis in the lactating sow. Mammary A-V differences of plasma Val and Lys increased in a quadratic manner with increasing intake of protein during lactation (**FIGURE 1-3**). Note that the mammary A-V difference of plasma Val was greater than that of plasma Lys when their intake was more than 28 g/d, i.e., the mammary uptake ratio of plasma Val to Lys became larger than 1:1 at high intakes of dietary AA during lactation. This finding provides a physiological explanation for the beneficial effect of increasing dietary ratio of Val to Lys (up to 1:1) on litter growth rates in high producing lactating sows (Moser et al. 2000, Richert et al. 1997, Richert et al. 1996). This finding also supports the concept that Val is the co-limiting AA to Lys in corn soybean meal diets for the lactating sow, and even becomes the first limiting AA at high intakes of dietary AA (NRC 1998). Therefore, the ideal ratio of Val to Lys required for milk synthesis is approximately 1:1, which is higher than the value of .85 to 1 recommended by NRC (1998).

Implications

The ideal pattern for milk synthesis by the lactating sow should be defined by the mammary uptake pattern of plasma indispensable amino acids, which represents the physiological needs of indispensable amino acids for metabolism and protein synthesis by the mammary gland. However, the mammary amino acid uptake patterns are regulated by intake of protein, indicating that the ideal dietary amino acid pattern for milk synthesis is dynamic. Specifically, dietary ratios of the branched-chain amino acids in high concentrations of dietary protein should be increased to meet their increased uptake by the mammary gland. Ideal ratios for milk synthesis defined by the mammary uptake pattern are greater for arginine and the branched-chain amino acids than those based only on the amino acid profile in milk. For example, an ideal ratio of valine to lysine for milk synthesis is approximately at 1:1, which is higher than the value of .85:1 recommended by the current NRC (1998). The ideal pattern of indispensable amino acids for milk synthesis established in this study, together with ideal patterns for maintenance, protein accretion, and (or) body protein loss, can be used to constitute the entire ideal pattern of dietary truly digestible indispensable amino acids for the lactating sow.

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TABLE 1-1 *Composition of diets for lactating sows (%, as-fed basis)*

Item	Dietary protein, %			
	7.8	13.0	18.2	23.5
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	.51	.70	.89	1.08
Salt	.25	.25	.25	.25
Trace mineral premix[a]	.23	.23	.23	.23
Vitamin premix[b]	.90	.90	.90	.90
DL-Met	.027	.044	.061	.079
L-Thr	.011	.019	.025	.033
L-Val	.038	.064	.090	.115
ME, MJ/kg[c]	14.3	14.3	14.3	14.3
Calcium[c]	.90	.90	.90	.90
Phosphorus[c]	.72	.72	.72	.72
CP[d]	8.2	13.2	18.2	23.0
Arg[d]	.60	.93	1.28	1.60
His[d]	.30	.45	.58	.72
Ile[d]	.37	.57	.78	1.00
Leu[d]	.64	1.01	1.36	1.73
Lys[d]	.41	.67	.90	1.19
Met[d]	.18	.27	.34	.42
Phe[d]	.42	.66	.89	1.13
Thr[d]	.32	.53	.66	.93
Trp[c]	.11	.18	.25	.33
Val	.46	.71	.96	1.21

TABLE 1-1 *Continued*

[a]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.

[b]Provided the following amounts of vitamins in milligrams per kilogram of diet: retinyl acetate, 8.3; cholecalciferol, 0.0138; 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.

[c]Calculated values (NRC, 1988).

[d]Analyzed values.

TABLE 1-2 *Effects of dietary protein concentration on lactation performance*[a]

Item	Dietary protein, %				SEM
	7.8	13.0	18.2	23.5	
No. of sows	4	4	4	4	-
Intake, kg/d					
Feed[c]	4.62[y]	5.75[x]	4.67[y]	4.62[y]	.20
Protein[c]	.366[z]	.750[y]	.845[y]	1.070[x]	.037
Sow's body weight, kg					
d 1	209.5	216.1	220.0	230.3	8.4
Change	-25.8	-25.4	-16.8	-26.6	4.6
Backfat depth, mm					
d 1	22.5	21.1	24.4	20.8	2.6
Change	-4.6[xy]	-.2[y]	-6.8[x]	-5.8[x]	1.4
Loin eye area, cm^2					
d 1	46.1	47.2	44.8	51.2	2.4
Change[b]	-8.46[x]	-3.72[y]	-1.26[y]	-3.70[y]	1.44
Litter size					
Pigs, d 1	11	11	11	11	-
Pigs, d 21	10.3[xy]	11.0[x]	9.5[y]	11.0[x]	0.4
Litter weight, kg					
d 1	19.1	19.3	19.3	18.5	.8
Gain	37.5[y]	48.7[x]	41.4[xy]	44.5[xy]	2.9
Milk yield, kg/d					
d 11	10.03	13.07	10.57	12.99	1.25
d 21	9.18[y]	13.21[x]	10.82[xy]	12.43[xy]	1.25

TABLE 1-2 *Continued*

[a]Data are least-squares means ± standard error.

[b, c]Effect of dietary treatments ($P < .05$ and .01, respectively).

[x, y, z]Least-squares means within a row lacking a common superscript letter differ ($P < .05$).

TABLE 1-3 *Effects of dietary protein concentration and stage of lactation on milk composition (%, Mcal/kg)*[a]

Item	Dietary protein, %				Stage of lactation, d			
	7.8	13.0	18.2	23.5	10	14	18	22
No.[f]	16	13	14	13	15	13	13	15
Dry matter	18.42 ± .32	18.27 ± .39	18.56 ± .39	18.91 ± .39	18.65 ± .32	18.66 ± .39	18.26 ± .39	18.59 ± .32
Protein[cd]	4.53 ± .07^{y}	4.51 ± .11^{y}	4.94 ± .08^{x}	5.02 ± .11^{x}	4.77 ± .08^{xy}	4.71 ± .09^{xy}	4.61 ± .09^{y}	4.90 ± .08^{x}
Lipid	6.29 ± .22	6.31 ± .34	6.37 ± .27	6.76 ± .34	6.82 ± .24	6.53 ± .27	6.15 ± .27	6.22 ± .24
Lactose	5.83 ± .09^{y}	5.84 ± .14^{xy}	5.61 ± .11^{xy}	5.47 ± .14^{x}	5.62 ± .10	5.72 ± .11	5.81 ± .11	5.61 ± .10
Energy	1.091 ± .021	1.092 ± .032	1.113 ± .026	1.148 ± .032	1.146 ± .023	1.120 ± .026	1.082 ± .026	1.096 ± .023

[a]Data are least-squares means ± standard error.

[c]Effect of dietary treatments ($P < .01$).

[d]Effect of stage of lactation ($P < .05$).

[f]Number of milk samples analyzed.

[x, y]Within a row (either across dietary treatments or stage of lactation), least-squares means lacking a common superscript letter differ ($P < .05$).

TABLE 1-4 *Effects of dietary protein concentration and stage of lactation on the concentrations (mmol/L) of indispensable amino acids in defatted porcine milk*[a]

IAA	Dietary protein, %				Stage of lactation, d			
	7.8	13.0	18.2	23.5	10	14	18	22
No.[f]	16	13	14	13	15	13	13	15
Arg[b]	16.7 ± .5^{y}	16.4 ± .7^{y}	17.7 ± .6^{xy}	18.6 ± .7^{x}	17.6 ± .5^{xy}	17.5 ± .6^{xy}	16.2 ± .6^{y}	18.2 ± .5^{x}
His[e]	11.0 ± .3	11.2 ± .7	11.3 ± .4	11.8 ± .5	11.5 ± .3^{x}	11.6 ± .4^{x}	10.1 ± .4^{y}	12.2 ± .4^{x}
Ile[c]	17.9 ± .4^{y}	18.3 ± .6^{y}	19.7 ± .4^{x}	20.9 ± .6^{x}	19.3 ± .4^{x}	19.3 ± .5^{x}	18.4 ± .5^{y}	19.9 ± .4^{x}
Leu[c]	31.9 ± .6^{y}	32.0 ± .8^{y}	34.7 ± .7^{x}	36.3 ± .8^{x}	33.8 ± .6^{xy}	33.8 ± .7^{xy}	32.4 ± .7^{y}	34.8 ±.6^{x}
Lys[b]	25.2 ± .7^{y}	24.5 ± 1.0^{y}	27.9 ± .8^{x}	28.5 ± 1.0^{x}	26.7 ± .7^{xy}	26.3 ± .8^{xy}	25.0 ± .8^{y}	28.1 ± .7^{x}
Met	9.1 ± .5	9.0 ± .6	8.7 ± .5	9.5 ± .5	9.1 ± .4	9.1 ± .5	8.6 ± .5	9.6 ± .4
Phe[c]	13.3 ± .3^{y}	13.2 ± .4^{y}	14.5 ± .3^{x}	15.0 ± .4^{x}	14.0 ± .3^{xy}	14.0 ± .3^{xy}	13.4 ± .3^{y}	14.6 ± .3^{x}
Thr[bd]	21.0 ± .5^{xy}	19.8 ± .8^{y}	21.8 ± .6^{xy}	22.9 ± .8^{x}	21.8 ± .6^{x}	21.2 ± .7^{xy}	20.0 ± .7^{y}	22.5 ± .6^{x}
Val[cd]	26.4 ± .4^{y}	25.9 ± .7^{y}	28.1 ± .5^{x}	29.5 ± .7^{x}	27.7 ± .5^{x}	27.5 ± .5^{xy}	26.2 ± .5^{y}	28.5 ± .5^{x}

[a]Data are least-squares means ± standard error.

[b, c]Effect of dietary treatments ($P < .05$ and .01, respectively).

[d, e]Effect of stage of lactation ($P < .05$ and .01, respectively).

[f]Number of milk samples analyzed.

[x, y]Within a row (either across dietary treatments or stage of lactation), least-squares means lacking a common superscript letter differ ($P < .05$).

TABLE 1-5 *Effects of dietary protein concentration and stage of lactation on output (g/d) of indispensable amino acids in porcine milk*[a]

IAA	Dietary protein, %				Stage of lactation, d	
	7.8	13.0	18.2	23.5	11	21
Arg[b]	26.0 ± 2.4[y]	36.3 ± 2.6[x]	30.7 ± 2.4[x]	38.6 ± 2.6[x]	33.3 ± 1.6	32.5 ± 1.6
His[c]	15.3 ± 1.7[y]	21.6 ± 2.0[x]	17.9 ± 1.7[x]	22.0 ± 1.8[x]	19.5 ± 1.1	18.9 ± 1.2
Ile[b]	21.2 ± 2.1[y]	29.8 ± 2.28[x]	25.8 ± 2.1[x]	32.6 ± 2.3[x]	27.6 ± 1.4	27.2 ± 1.4
Leu[b]	37.3 ± 3.7[y]	52.2 ± 3.9[x]	45.5 ± 3.7[x]	56.6 ± 3.9[x]	48.3 ± 2.5	47.5 ± 2.5
Lys[b]	33.0 ± 3.6[y]	44.7 ± 3.9[x]	41.1 ± 3.6[x]	50.5 ± 3.9[x]	42.4 ± 2.4	42.3 ± 2.4
Met[c]	11.1 ± 1.4	14.9 ± 1.6	13.4 ± 1.2	17.4 ± 1.2	14.2 ± .9	14.2 ± .9
Phe[b]	19.7 ± 2.0[y]	27.0 ± 2.1[x]	23.8 ± 2.0[x]	29.5 ± 2.1[x]	25.1 ± 1.3	24.9 ± 1.3
Thr[c]	22.6 ± 2.4[y]	30.0 ± 2.5[x]	26.0 ± 2.4[x]	32.6 ± 2.5[x]	28.0 ± 1.5	27.7 ± 1.5
Val[b]	27.7 ± 2.7[y]	38.1 ± 2.9[x]	32.7 ± 2.7[x]	41.2 ± 2.9[x]	35.2 ± 1.8	34.6 ± 1.8

[a]Data are least-squares means ± standard error.

[b, c]Effect of dietary treatments ($P < .05$ and .01, respectively).

[x, y]Within a row (either across dietary treatments or stage of lactation), least-squares means lacking a common superscript letter differ ($P < .05$).

TABLE 1-6 *Effects of dietary protein concentration on the amino acid profile of defatted porcine milk*[a]

IAA	Dietary protein, %			
	7.8	13.0	18.2	23.5
No.[f]	16	14	14	13
	g/100 g milk protein			
Arg	6.12 ± .15	6.10 ± .18	5.95 ± .18	6.13 ± .23
His	3.59 ± .09	3.68 ± .16	3.38 ± .11	3.45 ± .13
Ile	4.95 ± .06	5.15 ± .07	4.99 ± .07	5.18 ± .09
Leu	8.80 ± .10	8.90 ± .10	8.78 ± .10	8.98 ± .13
Lys	7.76 ± .12	7.83 ± .15	7.87 ± .15	7.85 ± .19
Met	2.67 ± .06	2.67 ± .07	2.56 ± .06	2.76 ± .06
Phe	4.61 ± .05	4.63 ± .06	4.61 ± .06	4.68 ± .08
Thr	5.27 ± .10	5.16 ± .12	4.99 ± .12	5.15 ± .15
Val	6.50 ± .07	6.49 ± .09	6.34 ± .09	6.54 ± .11
	Ratios of IAA to Lys, wt/wt			
Arg	.79 ± .02	.80 ± .03	.76 ± .03	.79 ± .03
His	.46 ± .01	.47 ± .03	.43 ± .02	.44 ± .02
Ile	.64 ± .01^{y}	.66 ± .01^{x}	.64 ± .01^{y}	.66 ± .01^{x}
Leu	1.14 ± .01^{xy}	1.17 ± .02^{x}	1.12 ± .01^{y}	1.15 ± .02^{xy}
Lys	1.00	1.00	1.00	1.00
Met	.34 ± .01^{xy}	.36 ± .01^{x}	.33 ± .01^{y}	.34 ± .01^{xy}
Phe	.60 ± .01	.61 ± .01	.59 ± .01	.60 ± .01
Thr	.68 ± .01	.66 ± .02	.63 ± .01	.66 ± .02
Val	.84 ± .01	.85 ± .02	.81 ± .01	.84 ± .02

[a]Data are least-squares means ± standard error.

[f]Number of milk samples analyzed.

[x, y]Least-squares means within a row lacking a common superscript letter differ ($P < .05$).

TABLE 1-7 *Effects of stage of lactation on the amino acid profile of defatted porcine milk*[a]

IAA	Day of lactation			
	10	14	18	22
No.[f]	15	13	13	16
	g/100 g milk protein			
Arg	6.10 ± .17	6.13 ± .19	5.82 ± .19	6.15 ± .17
His[d]	3.56 ± .10[x]	3.62 ± .13[x]	3.24 ± .13[y]	3.69 ± .12[x]
Ile	5.02 ± .06	5.10 ± .07	4.99 ± .07	5.05 ± .06
Leu	8.82 ± .10	8.93 ± .11	8.80 ± .11	8.86 ± .10
Lys	7.76 ± .12[xy]	7.74 ± .13[xy]	7.57 ± .13[y]	7.93 ± .12[x]
Met	2.69 ± .05	2.67 ± .07	2.69 ± .07	2.64 ± .06
Phe	4.59 ± .05	4.65 ± .06	4.57 ± .06	4.66 ± .05
Thr	5.17 ± .10	5.09 ± .11	4.94 ± .11	5.21 ± .10
Val	6.45 ± .08	6.49 ± .09	6.34 ± .09	6.48 ± .08
	Ratios of IAA to Lys, wt/wt			
Arg	.79 ± .02	.79 ± .03	.77 ± .03	.77 ± .02
His	.46 ± .01	.46 ± .02	.42 ± .02	.46 ± .02
Ile	.65 ± .01	.66 ± .01	.66 ± .01	.64 ± .01
Leu	1.14 ± .01[xy]	1.16 ± .01[x]	1.16 ± .01[x]	1.12 ± .01[y]
Lys	1.00	1.00	1.00	1.00
Met	.35 ± .01	.34 ± .01	.35 ± .01	.33 ± .01
Phe	.59 ± .01	.60 ± .01	.61 ± .01	.59 ± .01
Thr	.67 ± .01	.66 ± .01	.65 ± .01	.66 ± .01
Val	.83 ± .01	.84 ± .01	.84 ± .01	.82 ± .01

[a]Data are least-squares means ± standard error.

[d]Effect of stage of lactation ($P < .05$).

[f]Number of milk samples analyzed.

[x, y]Least-squares means within a row lacking a common superscript letter differ ($P < .05$).

TABLE 1-8 *Effects of dietary protein concentration and stage of lactation on arterial concentrations (μmol/L) of plasma indispensable amino acids in lactating sows*[a]

IAA	Dietary protein, %				Stage of lactation, d			
	7.8	13.0	18.2	23.5	10	14	18	22
No.[f]	12	9	12	12	12	11	11	11
Arg[c]	109.0 ± 12.7[y]	185.5 ± 22.0[x]	219.2 ± 12.7[x]	198.7 ± 12.7[x]	148.9 ± 12.7[y]	190.5 ± 14.7[x]	194.1± 14.7[x]	178.9 ± 14.7[xy]
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
Ile[d]	117.0 ± 6.7[y]	107.0 ± 11.5[y]	122.0 ± 6.7[xy]	139.3 ± 6.7[x]	99.7 ± 6.7[y]	121.7 ± 7.7[x]	136.9 ± 7.7[x]	127.0 ± 7.7[x]
Leu[c]	83.6 ± 9.2[z]	130.3 ± 15.9[y]	161.2 ± 9.2[y]	202.9 ± 9.2[x]	120.4 ± 9.2[y]	151.9 ± 10.6[x]	156.1 ± 10.6[x]	149.5 ± 10.6[xy]
Lys[cd]	126.8 ± 8.6[y]	126.4 ± 14.9[y]	184.0 ± 8.6[x]	162.0 ± 8.6[xy]	127.0 ± 8.6[y]	171.4 ± 9.9[x]	156.0 ± 9.9[x]	144.8 ± 9.9[xy]
Met[b]	36.6 ± 2.6[y]	48.4 ± 4.5[x]	48.6 ± 2.6[x]	46.3 ± 2.6[x]	41.2 ± 2.6	48.7 ± 3.0	46.4 ± 3.0	43.5 ± 3.0
Phe[cd]	52.9 ± 4.1[z]	74.3 ± 7.1[y]	80.3 ± 4.1[y]	96.2 ± 4.1[x]	61.2 ± 4.1[y]	78.5 ± 4.7[x]	83.0 ± 4.7[x]	81.0 ± 4.7[x]
Thr[c]	85.3 ± 8.6[y]	134.8 ± 15.0[xy]	160.1 ± 8.6[x]	160.0 ± 8.6[x]	126.3 ± 8.6	142.6 ± 10.0	136.4 ± 10.0	134.8 ± 10.0
Trp[c]	36.4 ± 2.5[y]	53.9 ± 4.3[xy]	56.5 ± 2.50[x]	56.5 ± 2.5[x]	48.5 ± 2.5	51.7 ± 2.9	53.8 ± 2.9	49.4 ± 2.9
Val[ce]	244.5 ± 14.5[z]	288.3 ± 25.2[yz]	313.1 ± 14.5[xy]	354.6 ± 14.5[x]	245.7 ± 14.5[y]	314.5 ± 16.8[x]	328.4 ± 16.8[x]	311.8 ± 16.8[x]

TABLE 1-8 *Continued*

Total IAA[cd]	985.0 ± 63.4[y]	1243.8 ± 109.7[xy]	1439.6 ± 63.4[x]	1505.1 ± 63.4[x]	1106.4 ± 63.4[y]	1368.3 ± 73.2[x]	1386.9 ± 73.2[x]	1311.8 ± 73.2[x]

[a]Data are least-squares means ± standard error.

[b, c]Effect of dietary treatments ($P < .05$ and .01, respectively).

[d, e]Effect of stage of lactation ($P < .05$ and .01, respectively).

[f]Number of plasma samples analyzed.

[x, y, z]Within a row (either across dietary treatments or stage of lactation), least-squares means lacking a common superscript letter differ ($P < .05$).

TABLE 1-9 *Effects of dietary protein concentration and stage of lactation on mammary arterio-venous (μmol/L) differences of plasma indispensable amino acids in lactating sows*[a]

IAA	Dietary protein, %				Stage of lactation, d			
	7.8	13.0	18.2	23.5	10	14	18	22
No.[f]	12	9	9	12	12	10	10	10
Arg[c]	26.56 ± 3.44^{z}	31.15 ± 6.01^{yz}	62.81 ± 6.01^{x}	38.77 ± 3.44^{y}	34.22 ± 3.43^{y}	35.43 ± 4.31^{xy}	46.89 ± 4.31^{x}	42.77 ± 4.31^{y}
His[b]	9.22 ± 1.58^{y}	7.58 ± 2.77^{y}	21.99 ± 2.77^{x}	12.03 ± 1.58^{y}	12.66 ± 1.58^{xy}	9.88 ± 1.99^{y}	15.56 ± 1.99^{x}	12.72 ± 1.99^{xy}
Ile[c]	24.27 ± 2.76^{y}	33.32 ± 4.83^{xy}	44.99 ± 4.83^{x}	43.81 ± 2.76^{x}	32.69 ± 2.76^{y}	33.27 ± 3.46^{y}	43.69 ± 3.46^{x}	36.74 ± 3.46^{xy}
Leu[cd]	41.63 ± 3.30^{y}	53.73 ± 5.78^{y}	67.06 ± 5.78^{xy}	72.01 ± 3.30^{x}	53.00 ± 3.30^{y}	53.67 ± 4.14^{y}	68.20 ± 4.14^{x}	59.56 ± 4.14^{y}
Lys[c]	33.23 ± 2.59^{z}	36.99 ± 4.53^{yz}	57.00 ± 4.53^{x}	41.98 ± 2.59^{y}	39.93 ± 2.59^{xy}	36.13 ± 3.25^{y}	48.61 ± 3.25^{x}	44.53 ± 3.25^{xy}
Met[c]	10.04 ± .91^{z}	11.85 ± 1.59^{yz}	17.74 ± 1.59^{x}	13.91 ± .91^{xy}	12.47 ± .91^{xy}	12.22 ± 1.14^{y}	15.42 ± 1.14^{x}	13.42 ± 1.14^{xy}
Phe[bd]	17.76 ± 1.41^{z}	20.78 ± 2.47^{xyz}	28.07 ± 2.47^{x}	23.47 ± 1.41^{xy}	20.80 ± 1.41^{y}	19.87 ± 1.77^{y}	26.59 ± 1.77^{x}	22.81 ± 1.77^{y}
Thr[c]	24.68 ± 2.27^{z}	25.35 ± 3.97^{yz}	46.19 ± 3.97^{x}	31.90 ± 2.27^{y}	29.14 ± 2.27^{y}	27.42 ± 2.84^{y}	37.80 ± 2.84^{x}	33.77 ± 2.84^{xy}
Trp[b]	3.54 ± 1.07^{z}	2.65 ± 1.86^{yz}	10.14 ± 1.86^{x}	4.23 ± 1.07^{y}	5.01 ± 1.07	4.48 ± 1.34	5.98 ± 1.34	5.09 ± 1.34

TABLE 1-9 *Continued*

IAA	Dietary protein, %				Stage of lactation, d			
	7.8	13.0	18.2	23.5	10	14	18	22
Val[b]	35.13 ± 5.64[y]	47.99 ± 9.88[xy]	77.02 ± 9.88[x]	58.02 ± 5.64[x]	47.16 ± 5.64[y]	47.51 ± 7.07[y]	68.41 ± 7.07[x]	55.08 ± 7.07[xy]
Total IAA[c]	226.05 ± 23.55[y]	271.38 ± 41.18[y]	433.00 ± 41.18[x]	340.13 ± 23.55[x]	287.08 ± 23.55[y]	279.86 ± 29.53[y]	377.14 ± 29.53[x]	326.48 ± 29.53[xy]

[a]Data are least-squares means ± standard error.

[b, c]Effect of dietary treatments ($P < .05$ and .01, respectively).

[d, e]Effect of stage of lactation ($P < .05$ and .01, respectively).

[f]Number of plasma samples analyzed.

[x, y, z]Within a row (either across dietary treatments or stage of lactation), least-squares means lacking a common superscript letter differ ($P < .05$).

TABLE 1-10 *Effects of dietary protein concentration and stage of lactation on mammary extraction rates (%) of plasma indispensable amino acids in lactating sows*[a]

IAA	Dietary protein, %				Stage of lactation, d			
	7.8	13.0	18.2	23.5	10	14	18	22
No.[f]	12	9	9	12	12	10	10	10
Arg	24.7 ± 1.6^{xy}	18.9 ± 2.8^{y}	29.1 ± 2.8^{x}	20.3 ± 1.6^{y}	23.4 ± 1.6^{xy}	18.6 ± 2.0^{y}	24.9 ± 2.0^{x}	26.1 ± 2.0^{x}
His[c]	9.9 ± 1.4^{y}	8.3 ± 2.5^{y}	23.2 ± 2.5^{x}	13.6 ± 1.4^{y}	14.1 ± 1.4^{xy}	10.0 ± 1.8^{y}	16.3 ± 1.8^{x}	14.6 ± 1.8^{xy}
Ile[c]	21.5 ± 1.8^{z}	32.1 ± 3.1^{xy}	39.8 ± 3.1^{x}	31.7 ± 1.8^{y}	34.4 ± 1.8^{x}	28.2 ± 2.2^{y}	32.1 ± 2.2^{xy}	30.5 ± 2.2^{xy}
Leu[c]	52.5 ± 2.1^{x}	43.1 ± 3.7^{y}	44.3 ± 3.7^{xy}	36.2 ± 2.1^{y}	47.3 ± 2.1^{x}	38.6 ± 2.6^{y}	46.4 ± 2.6^{x}	43.9 ± 2.6^{xy}
Lys[e]	29 ± 2.2	34.1 ± 3.8	31.5 ± 3.8	29.1 ± 2.2	33.9 ± 2.2^{x}	21.8 ± 2.7^{y}	32.8 ± 2.7^{x}	35.2 ± 2.7^{x}
Met[be]	27.6 ± 1.3^{y}	25.2 ± 2.2^{y}	35.4 ± 2.2^{x}	29.9 ± 1.3^{xy}	30.0 ± 1.3^{x}	23.9 ± 1.6^{y}	32.9 ± 1.6^{x}	31.3 ± 1.6^{x}
Phe[b]	34.7 ± 1.8^{x}	27.8 ± 3.1^{xy}	36.6 ± 3.1^{x}	26.3 ± 1.8^{y}	34.9 ± 1.8^{x}	26.8 ± 2.2^{y}	33.5 ± 2.2^{x}	30.1 ± 2.2^{xy}
Thr[bd]	30.6 ± 2.0^{x}	22.0 ± 3.5^{xy}	29.5 ± 3.5^{x}	20.9 ± 2.0^{y}	23.7 ± 2.0^{xy}	21.0 ± 2.5^{y}	30.4 ± 2.5^{x}	27.8 ± 2.5^{xy}
Trp	9.4 ± 2.2^{xy}	7.2 ± 3.8^{xy}	18.7 ± 3.8^{x}	8.0 ± 2.2^{y}	9.6 ± 2.2	10.1 ± 2.7	12.3 ± 2.7	11.3 ± 2.7
Val[b]	14.0 ± 1.7^{y}	16.9 ± 2.9^{xy}	26.1 ± 2.9^{x}	16.8 ± 1.7^{y}	19.2 ± 1.7	15.7 ± 2.0	20.7 ± 2.0	18.2 ± 2.0
Total IAA	23.0 ± 1.4^{y}	22.3 ± 2.4^{y}	31.3 ± 2.4^{x}	23.2 ± 1.4^{y}	26.1 ± 1.4^{x}	20.7 ± 1.7^{y}	27.2 ± 1.7^{x}	25.7 ± 1.7^{x}

TABLE 1-10 *Contined*

[a]Data are least-squares means ± standard error.

[b, c]Effect of dietary treatments ($P < .05$ and .01, respectively).

[d, e]Effect of stage of lactation ($P < .05$ and .01, respectively).

[f]Number of plasma samples analyzed.

[x, y, z]Within a row (either across dietary treatments or stage of lactation), least-squares means lacking a common superscript letter differ ($P < .05$).

TABLE 1-11 *Effects of dietary protein concentration on the mammary uptake patterns for plasma indispensable amino acids*[a]

IAA	Dietary protein, %			
	7.8	13.0	18.2	23.5
No.[f]	12	9	9	12
	Proportions of individual IAA to total IAA, % (mol/mol)			
Arg[b]	$11.7 \pm .4^{y}$	$11.4 \pm .6^{y}$	$14.3 \pm .6^{x}$	$11.3 \pm .4^{y}$
His	$3.8 \pm .4^{xy}$	$2.3 \pm .7^{y}$	$4.9 \pm .7^{x}$	$3.3 \pm .4^{xy}$
Ile[c]	$10.7 \pm .3^{y}$	$12.5 \pm .5^{x}$	$10.8 \pm .5^{y}$	$13.2 \pm .3^{x}$
Leu[b]	$18.9 \pm .8^{y}$	20.2 ± 1.4^{xy}	16.1 ± 1.4^{y}	$22.0 \pm .8^{x}$
Lys[c]	$15.1 \pm .4^{x}$	$13.8 \pm .7^{y}$	$12.9 \pm .7^{yz}$	$12.3 \pm .4^{z}$
Met	$4.5 \pm .1^{x}$	$4.3 \pm .2^{xy}$	$4.2 \pm .2^{xy}$	$4.2 \pm .2^{y}$
Phe[c]	$8.1 \pm .2^{x}$	$7.7 \pm .4^{xy}$	$6.6 \pm .4^{y}$	$6.8 \pm .2^{y}$
Thr[c]	$11.1 \pm .3^{x}$	$8.9 \pm .6^{yz}$	$10.6 \pm .6^{xz}$	$9.2 \pm .3^{y}$
Trp	$1.4 \pm .3$	$1.0 \pm .6$	$2.3 \pm .6$	$1.1 \pm .3$
Val	$14.5 \pm .9$	18.0 ± 1.6	17.4 ± 1.6	$16.6 \pm .9$
	Ratios of IAA to Lys, wt/wt			
Arg[b]	$.94 \pm .05^{z}$	$1.00 \pm .08^{y}$	$1.31 \pm .08^{x}$	$1.09 \pm .05^{y}$
His	$.29 \pm .03^{xy}$	$.20 \pm .06^{y}$	$.39 \pm .06^{x}$	$.28 \pm .03^{xy}$
Ile[c]	$.66 \pm .02^{z}$	$.81 \pm .03^{y}$	$.76 \pm .03^{y}$	$.98 \pm .02^{x}$
Leu[c]	$1.13 \pm .04^{z}$	$1.31 \pm .06^{y}$	$1.14 \pm .06^{yz}$	$1.63 \pm .04^{x}$
Met[b]	$.31 \pm .01^{y}$	$.32 \pm .01^{xy}$	$.33 \pm .01^{x}$	$.35 \pm .01^{x}$
Phe	$.61 \pm .02$	$.63 \pm .03$	$.58 \pm .03$	$.62 \pm .02$
Thr	$.60 \pm .02^{xy}$	$.54 \pm .03^{y}$	$.67 \pm .03^{x}$	$.61 \pm .02^{xy}$
Trp	$.14 \pm .04$	$.10 \pm .06$	$.23 \pm .06$	$.11 \pm .04$
Val[b]	$.81 \pm .07^{y}$	$1.06 \pm .12^{xy}$	$1.07 \pm .12^{xy}$	$1.09 \pm .07^{x}$

TABLE 1-11 *Continued*

[a]Data are least-squares means ± standard error.

[b, c]Effect of dietary treatments ($P < .05$ and .01, respectively).

[f]Number of plasma samples analyzed.

[x, y, z]Least-squares means within a row lacking a common superscript letter.

TABLE 1-12 *Effects of stage of lactation on the mammary uptake patterns for plasma indispensable amino acids*[a]

IAA	Stage of lactation, d			
	10	14	18	22
No.[f]	12	10	10	10
	Proportions of individual IAA to total IAA, % (mol/mol)			
Arg	11.3 ± .4[y]	12.2 ± .5[xy]	12.3 ± .5[xy]	12.8 ± .5[x]
His	4.0 ± .4	3.2 ± .5	3.9 ± .5	3.3 ± .5
Ile	11.7 ± .3	12.3 ± .3	11.9 ± .3	11.3 ± .3
Leu	19.6 ± .8	20.2 ± 1.0	18.4 ± 1.0	19.1 ± 1.0
Lys	14.3 ± .4[x]	12.8 ± .5[y]	13.0 ± .5[y]	13.9 ± .5[xy]
Met	4.4 ± .1	4.4 ± .2	4.2 ± .2	4.2 ± .2
Phe	7.6 ± .2	7.2 ± .3	7.1 ± .3	7.3 ± .3
Thr	10.1 ± .3	9.5 ± .4	9.8 ± .4	10.3 ± .4
Trp	1.3 ± .3	1.4 ± .4	1.6 ± .4	1.4 ± .4
Val	15.7 ± .9	16.7 ± 1.2	17.8 ± 1.2	16.3 ± 1.2
	Ratios of IAA to Lys, wt/wt			
Arg	.96 ± .05[y]	1.14 ± .06[x]	1.13 ± .06[x]	1.11 ± .06[y]
His	.30 ± .03	.27 ± .04	.33 ± .04	.27 ± .04
Ile[e]	.75 ± .02[y]	.88 ± .02[x]	.83 ± .02[x]	.75 ± .02[y]
Leu[d]	1.25 ± .04[y]	1.44 ± .04[x]	1.28 ± .04[y]	1.25 ± .04[y]
Met[d]	.32 ± .01[y]	.35 ± .01[x]	.33 ± .01[xy]	.31 ± .01[y]
Phe	.60 ± .02	.64 ± .02	.62 ± .02	.59 ± .02
Thr	.58 ± .02	.61 ± .02	.62 ± .02	.61 ± .02
Trp	.14 ± .04	.14 ± .04	.17 ± .04	.14 ± .04
Val	.91 ± .07[y]	1.04 ± .09[xy]	1.12 ± .09[x]	.97 ± .09[xy]

TABLE 1-12 *Continued*

[a]Data are least- squares means ± standard error.

[d, e]Effect of stage of lactation ($P < .05$ and .01, respectively).

[f]Number of plasma samples analyzed.

[x, y]Least- squares means within a row lacking a common superscript letter differ ($P < .05$).

TABLE 1-13

Comparison of the ratios (wt/wt) of other indispensable amino acids to lysine in porcine milk

IAA	Davis et al. (1994)[a]	Elliott et al. (1971)[b]	King et al. (1993)[c]	Dourmad et al. (1991)[d]	Dourmad et al. (1998)[e]	Duee and Jung (1973)	This study[f]	Average
Arg	.56	.68	.65	.74	.74	.65	.79	.69
His	.30	.45	.40	.53	.53	.35	.45	.43
Ile	.51	.51	.59	.58	.58	.55	.65	.57
Leu	1.13	1.16	1.14	1.18	1.18	1.15	1.15	1.16
Lys	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Met	.28	.23	.28	.27	.27	.27	.34	.28
Phe	.54	.56	.55	.57	.57	.54	.60	.56
Thr	.47	.61	.59	.59	.59	.55	.66	.58
Trp	-	.17	.19	.15	.15	-	-	.17
Val	.58	.63	.77	.69	.69	.80	.84	.71
	Lys, g/100 g milk protein							
	7.90	7.59	6.95	7.39	7.39	7.75	7.83	7.54

[a]Derived from TABLE 2, 3, and 4 in the paper.

[b]Derived from TABLE 4 in the paper.

[c]Derived from TABLE 3 in the paper.

[d]Derived from TABLE 3 in the paper.

[e]Derived from TABLE 5 in the paper.

[f]Derived from **TABLE 1-7** in this paper.

TABLE 1-14

Comparison of mammary uptake ratios (wt/wt) of indispensable amino acids to lysine in the lactating sow

IAA	Linzell et al. (1969)[a]	Spincer et al. (1969)[b]	Nielsen et al. (2000)[c]	Trottier et al. (1997)[d]	This study[e]	Ave
No.[f]	5	7	10	40	42	-
Arg	1.14	1.07	1.17	1.20	1.09	1.13
His	.58	.56	.30	.37	.29	.42
Ile	.72	1.09	.85	.80	.80	.85
Leu	1.17	1.77	1.58	1.43	1.31	1.45
Lys	1.00	1.00	1.00	1.00	1.00	1.00
Met	.20	.32	.27	.29	.33	.28
Phe	.61	.70	.61	.67	.61	.64
Thr	.52	.68	.64	.67	.61	.62
Trp	-	-	-	.35	.15	.25
Val	1.07	1.44	.88	1.00	1.01	1.08

[a]Derived from TABLE 6 in the paper.

[b]Derived from TABLE 2 in the paper.

[c]Derived from TABLE 3 in the paper.

[d]Derived from TABLE 3 and 4 in the paper.

[e]Derived from **TABLE 1-12** in this paper.

[f]Number of plasma samples analyzed.

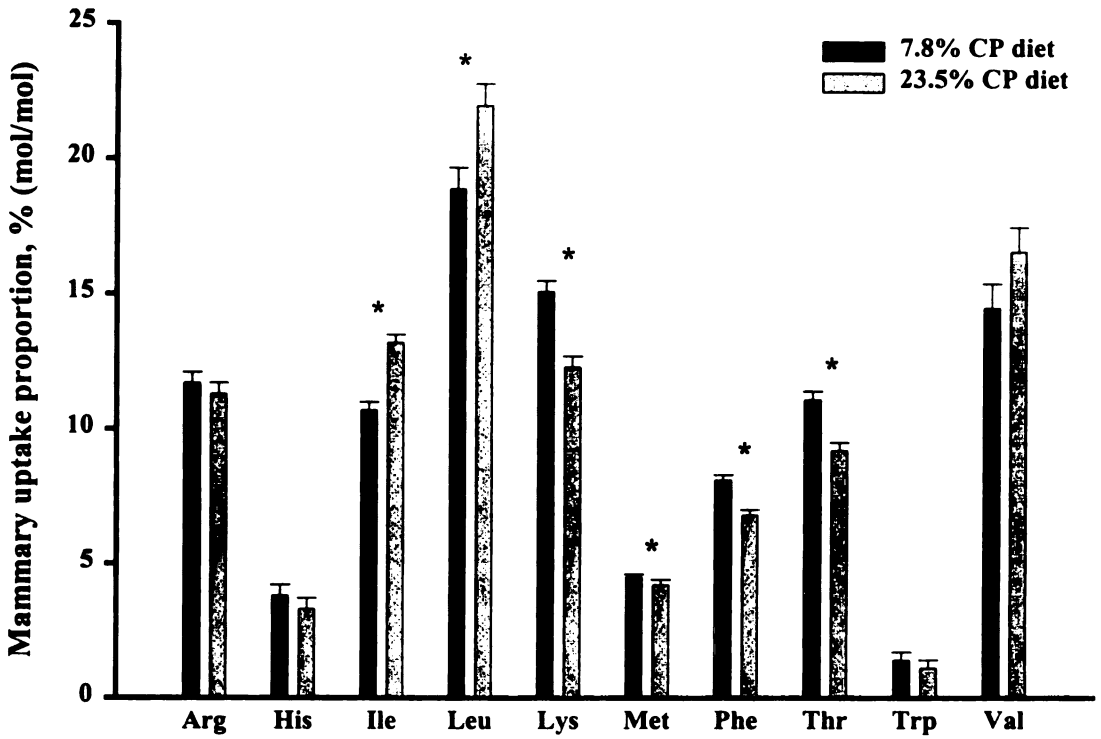

FIGURE 1-1 Effects of dietary protein concentration on the mammary uptake proportions of individual dispensable amino acids to total individual dispensable amino acids. The mammary uptake proportions (%, mol/mol) = 100 x mammary A-V difference of individual IAA / mammary A-V difference of the total IAA (See Materials and Methods). Least-squares means with * differ ($P < .05$) between diets.

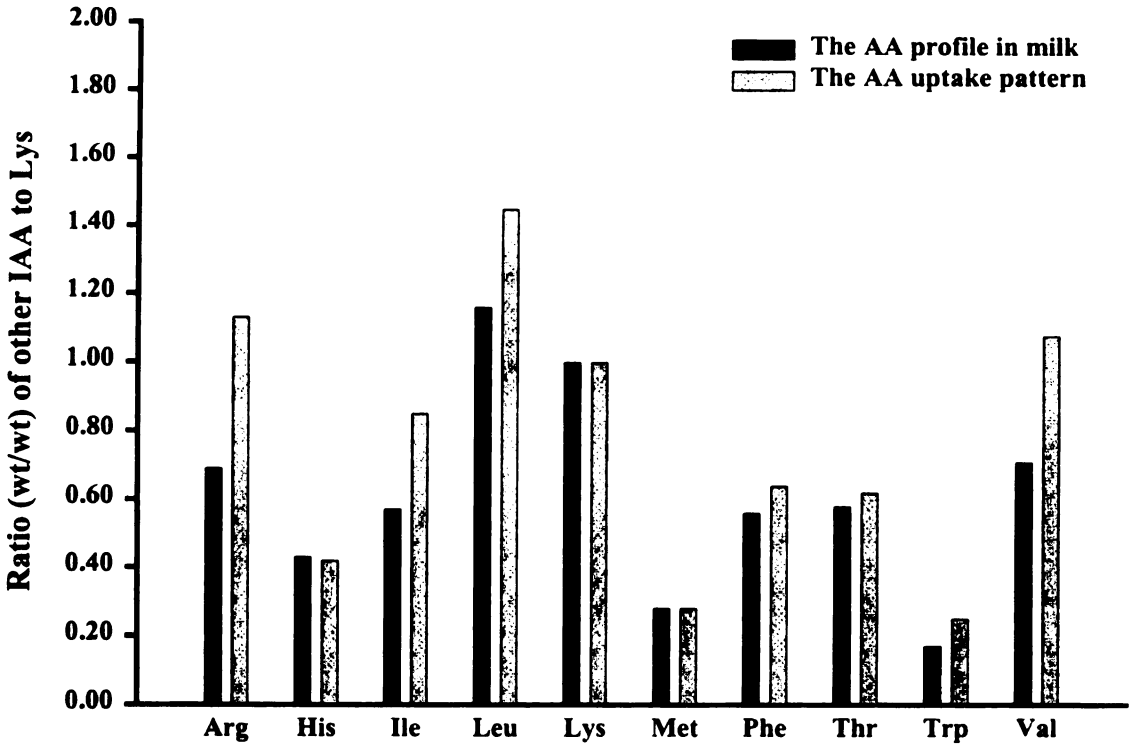

FIGURE 1-2 Comparison of the amino acid profile in porcine milk and the amino acid uptake pattern by the lactating porcine mammary gland. The AA profile in milk was the average ratios (wt/wt) of other IAA to Lys in porcine milk (**TABLE 1-13**). The AA uptake pattern was the average ratios (wt/wt) of other IAA to Lys taken up by the lactating porcine mammary gland (**TABLE 1-14**).

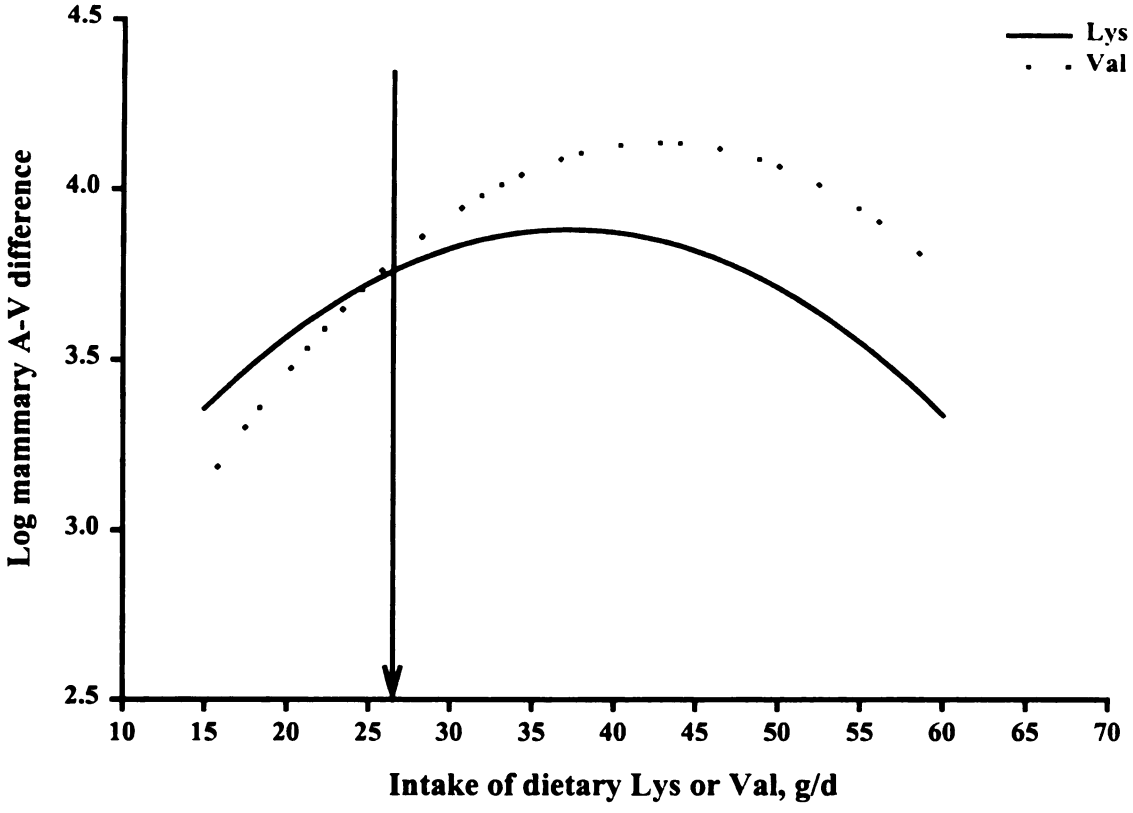

FIGURE 1-3 Mammary arterio-venous difference of plasma lysine or valine affected by their intake. The relationship between log mammary A-V difference of plasma Lys or Val ($\hat{Y}$) and daily intake of dietary total Lys or Val (X) over a 21-d lactation period was best fitted by a quadratic regression model. The solid curve (for Lys) was best fitted by $\hat{Y} = 2.4104 + .07902\ X - .001059\ X^2$ ($P = .036$; $R^2 = .49$) and the dot curve (for Val) by $\hat{Y} = 1.7441 + .1120X - .001308X^2$ ($P = .0066$, $R^2 = .63$). The line with an arrow distinguishes the mammary uptake ratio of plasma Val to Lys less (the left side) and greater (the right side) than 1:1, which corresponds to low and high intake of dietary Lys or Val, respectively.

CHAPTER 2

Quantitation of Indispensable Amino Acids Mobilized from Body Protein Reserves of Extra-mammary Tissues in the Lactating Sow

ABSTRACT Sows mobilize body protein reserves to support milk synthesis when intake of protein is not adequate, which complicates estimates of dietary requirements for indispensable amino acids (IAA). We hypothesize that contributions of IAA mobilized from body protein reserves of extra-mammary tissues to the mammary glands can be predicted by a regression model of mammary arterio-venous (A-V) differences of plasma IAA (MAVD). To quantify the contributions of endogenous IAA to the mammary gland for milk synthesis, 16 Landrace x Yorkshire lactating sows were fed isocaloric (14.3 MJ ME/kg) diets varying in protein concentrations (78, 130, 182, and 235 g/kg). Sows were fitted with carotid arterial and main mammary venous catheters on d 4 of lactation. Beginning at 0800 h on d 10, 14, 18, and 22 of lactation, matched sets of arterial and venous blood samples were obtained every 30 min over 6 h. Milk yield was estimated by the D_2O dilution method. Mammary plasma flow rate was estimated at 6440 L/d by the Fick principle using lysine as an internal indicator. The relationship between log mammary A-V differences of plasma IAA ($\hat{Y}$) and daily intakes of IAA (X) was best fitted by a polynomial regression model: $\hat{Y} = \beta_0 + \beta_1 X + \beta_2 X^2$ ($P < 0.05$). The reverse log intercept (β_0) was defined as the mammary A-V difference of plasma IAA when intake of IAA was extrapolated to zero. The contribution of each IAA was defined as the sum of its

need for maintenance and the amount of mammary uptake of this IAA when intake of this IAA was extrapolated to zero. The contributions of IAA to the mammary gland for milk synthesis predicted by the MAVD were 10.5, 2.1, 5.6, 6.4, and 4.8 g/kg body wt loss, respectively, for lysine, methionine, phenylalanine, threonine, and valine. These predicted values were validated by two other approaches. Firstly, the contributions of IAA were quantified by the algebraic product of body protein loss and the amino acid concentration in body protein. Secondly, the contributions of IAA were estimated by a factorial approach with assumed utilization efficiencies of dietary digestible IAA for milk synthesis. Estimates of the contributions based on body protein loss and the factorial approach were very close to the values obtained by the MAVD. We concluded that in the lactating sow, the contributions of IAA mobilized from body protein to the mammary gland for milk synthesis can be predicted by the MAVD.

KEY WORDS: • body protein mobilization • arterio-venous difference • mammary glands • sows • lactation

INTRODUCTION

Sows lose body weight to support high rates of milk production when their intakes of protein, indispensable amino acids (IAA), and/or energy are not adequate (Clowes et al. 1998, Dourmad et al. 1998, Jones and Stahly 1999, King and Williams 1984, Mullan and Williams 1990, Revell et al. 1998, Williams and Smits 1991). Sow body weight loss varies widely from 0 to 1.35 kg/d during lactation (Jones and Stahly 1999). The protein proportion of body weight loss ranges from 10 to 16% and the lipid proportion of body weight loss from 37 to 50% (Jones and Stahly 1999, Mullan and Williams 1990, Whittemore and Yang 1989, Williams and Smits 1991). The chemical composition of body weight loss during lactation is diet-dependent. Lactating sows lose a large amount of muscle tissue (the major body protein reserve) when fed diets deficient in protein or lysine, and a massive amount of adipose tissue (the major body lipid reserve) when fed diets deficient in energy (Brendemuhl et al. 1989, Dourmad et al. 1998).

Maternal body weight loss may be driven by a homeorhetic mechanism that involves coordinated changes in the metabolism of other body tissues to support high rate of milk synthesis in the mammary glands (Bauman 1999). Thus, sows fed diets low in protein may maintain milk protein production by mobilizing body protein reserves. Inadequate intake of IAA decreases rates of protein synthesis and increases rates of protein degradation in muscle tissue, leading to mobilization of the major body protein reserve in lactating goats, rats, and pigs (Champredon et al. 1990, Clowes et al. 1998, Jones and Stahly 1999, Pine et al. 1994). Mobilization of body protein reserves in sows may occur

throughout the entire period of lactation (Jones and Stahly 1999). This mobilization complicates estimates of dietary requirements for IAA based on the milk production response (Pettigrew 1993).

Dietary requirements for IAA by the lactating sow can be estimated by a factorial approach, i.e., by integrating individual needs of IAA for the various physiological components including body maintenance, milk production, and body protein accretion (or loss) (NRC 1998, Pettigrew 1993). The contribution of endogenous lysine from body protein loss is considered the sum of maintenance need and the intercept in the linear regression of lysine intake against litter weight gain (NRC 1998, Pettigrew 1993). Contributions of endogenous IAA other than lysine from body protein loss are derived by multiplying the contribution of endogenous lysine and ideal ratios of other IAA to lysine in body tissue (NRC 1998, Pettigrew 1993). However, the contributions of endogenous IAA need to be defined experimentally to adjust dietary requirements for IAA at different rates of body protein loss.

We have developed a regression model for mammary arterio-venous (A-V) differences of plasma IAA to predict the contributions of endogenous IAA mobilized from extra-mammary tissues in the lactating sow. Being driven by the homeorhetic mechanism, endogenous IAA mobilized from body protein reserves will be partitioned to the mammary gland for milk synthesis. Nutrients are used first for maintenance and second for milk production in sows (Pomar et al. 1991). Thus, we hypothesize that the contributions of IAA from body protein reserves in the lactating sow can be predicted as

the sum of maintenance needs for IAA and mammary uptakes of plasma IAA when dietary intakes of IAA are extrapolated to zero. Our objective in this study was to quantify the contributions of IAA mobilized from body protein reserves in lactating sows so that these estimates could be used in a factorial approach to define dietary requirements for IAA.

MATERIALS AND METHODS

All procedures in this study were approved by the All-University Committee on Animal Use and Care of Michigan State University.

Experimental design. Sixteen Landrace x Yorkshire multiparous lactating sows (parity 2 or 3) were allocated to treatments according to a randomized block design. Each block consisted of four sows. Each sow in one block was provided ad libitum access to one of four diets. Diets contained different crude protein concentrations (78, 130, 182, and 235 g/kg) and were balanced to contain an ideal pattern of IAA that met recommendations for lactating sows (NRC 1988). Diets were formulated by diluting the highest concentration of crude protein (235 g/kg diet) with starch and sugar. All diets were isocaloric with a metabolizable energy (ME) of 14.3 MJ/kg. The composition of the diets is given in **TABLE 2-1**.

Animals. Litters with 11 piglets per sow were cross-fostered within 48 h after birth. Sow and litter characteristics at the start of the study are given in **TABLE 2-2**. Sows were individually housed in farrowing crates equipped with metal wire floors in a mechanically ventilated, thermally controlled room (21 °C). The sows were fed twice daily to appetite and provided free access to water. Sow food intake was recorded daily. Sow body weight, backfat depth, and loin eye area were recorded after farrowing (d 1) and at weaning (d 21). Backfat depth and loin eye area were scanned (Ultrasound Scanner 200 Vet with ASP-18 linear probe, Pie Medical Equipment B.V., Maastricht, the Netherlands) 5 cm lateral from the spine and centered over the 10th rib. Piglets were individually weighed weekly.

Cannulation. For the catheter, microbore Tygon® tubing (1.0 mm i.d., 1.8 mm o.d., Norton Performance Plastics Co., Akron, OH) was used and the lumen was coated with triododecylmethylammonium chloride - heparin complex (7% w/w, Polysciences, Inc., Warrington, PA). The anterior main mammary vein and the carotid artery were cannulated in sows on d 4 ± 1 of lactation following the surgical procedure described by Trottier et al. (1995). Antibiotic Naxcel® (Pharmacia and Upjohn Co., Kalamazoo, MI) and anti-inflammatory Banamine® (Schering-Plough Animal Health Corp., Kenilmorth, NJ) were administered i.v. for three days following surgery. The catheters were flushed daily with heparinized saline (20 IU heparin/ml). Food was offered in a stair step manner during the first three days post surgery, and was available free access thereafter.

Milk intake 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. with a dose (1 mg/kg BW) of D_2O (Cambridge Isotope Laboratories, Andover, MA). At 1 h after the dose, piglets were bled from the jugular vein using vacutainers containing SST® gel and clot-activator (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). At 24 h after the dose, the piglets were fasted for 1 h, weighed, and bled as before. During this 24-h period, piglets were not provided access to water. Blood samples were centrifuged at 1500 x g for 15 min at 4 °C, and then serum samples were separated and stored at -20 °C until assayed for D_2O. Serum D_2O concentration was determined by an infrared spectrophotometer (The Foxboro Co., East Bridgewater, MA) using a fixed-filter length of 4 μm. Milk intake was calculated on the basis of the D_2O dilution principle (Pettigrew et al. 1987).

Mammary plasma flow estimation. Mammary plasma flow rate was calculated by the Fick principle of Davis et al. (1978) as modified by Trottier et al. (1997) using lysine as an internal indicator. Given that the amount of lysine output in milk contributed from vascular peptides is negligible (Bequette et al. 1999), mammary uptake of plasma lysine can be based on the Fick principle as follows:

Mammary lysine uptake = Plasma lysine A-V difference x plasma flow rate

= Lysine output in milk + lysine's other metabolic fates (1)

Among other metabolic fates, there are two major pathways: lysine oxidation and lysine accretion in the mammary tissue. In the guinea pig mammary tissue, the amount of

lysine oxidation is negligible (Mepham 1982). Because lysine is the first limiting amino acid in a corn-soybean meal diet for lactating sows (NRC 1998) and dietary metabolic energy intake was adequate in the present study, it might be minimally oxidized in the lactating mammary glands. In addition, accretion of lysine in lactating porcine mammary glands was calculated to be around 1 g/d from the study of Kim et al. (1999), which is negligible compared to lysine output in milk (41 g/d) in the present study. Therefore, Equation (1) is simplified as follows:

Mammary lysine uptake = Plasma lysine A-V difference x plasma flow rate

= Lysine output in milk (2)

Thus, mammary plasma flow rate was estimated by dividing lysine output in milk by plasma lysine A-V difference:

Mammary plasma flow rate (L/d) = lysine concentration in milk (mmol/L) x milk yield (L/d) / (lysine concentration in carotid artery - lysine concentration in the main mammary vein) (mmol/L).

Blood and milk sampling protocol. Blood samples were collected on d 9 ± 1, 13 ± 1, 17 ± 1, and 21 ± 1 (referred to as d 10, 14, 18, and 22, respectively). On sampling days, sows were provided ad libitum access to food and water as usual. The sows were fed one h prior to blood sampling. Matched sets of carotid arterial and main mammary venous blood samples (10 ml each) were collected into sterile syringes every 30 min over a 6-h period beginning at 0800 h. Blood was transferred into EDTA-coated tubes and centrifuged at 1500 x g for 15 min at 4 °C. Plasma was removed and stored at -20 °C. On the same day as blood sampling, milk was collected and pooled from all functional

teats on each sow following an i.v. administration of oxytocin (10 IU). Approximately 20 ml pooled milk was transferred into a vial containing a preserver (Broad Spectrum Microtales[tm], D & F Control System, Inc., San Ramon, CA) and stored at 4 °C until assayed for milk composition. Another portion of pooled milk was stored at -20 °C until assayed for nitrogen. The remainder of pooled milk was defatted by centrifugation at 1500 x g for 15 min at 4 °C and stored at -20 °C until assayed for amino acids (AA).

Laboratory measurements

(1) Milk composition and amino acid analysis. Milk fat, lactose, and protein contents were assayed using a mid-infrared spectroscope (Multispec M, Berwind Instrument Ltd., York, England, U.K.) (AOAC 1990). Frozen milk samples were thawed at 37 °C and mixed by vortex. Milk nitrogen was determined using LECO Nitrogen Analyzer FP-2000 (LECO Corp., St. Joseph, MI) and EDTA (Sigma Chemical Co., St. Louis, MO) as a calibration standard. Amino acid concentrations in the defatted milk were analyzed using reversed-phase high-performance liquid chromatography by a precolumn derivatization with phenylisothiocyanate (Pierce, Rockford, IL) following the Pico•Tag® method (Cohen et al. 1989). In brief, 200 μL of defatted milk was hydrolyzed using 6 mol/L HCl for 24 h at 110 °C. Amino acids in the hydrolysate were derivatized with phenylisothiocyanate and separated by a Pico•Tag® column (3.9 mm x 150 mm, Waters Corp., Milford, MA) using Alliance[tm] Waters 2690 Separations Module and detected by Waters[tm] 486 Tenable Absorbance Detector (Waters Corp., Milford, MA). Norleucine (Sigma, St. Louis, MO) as an internal standard was added into defatted milk samples before hydrolysis. Amino acid standard H (Pierce, Rockford, IL) was used as a

calibration standard. The method was validated with certified amino acid standard (NIST, Gaithersburg, MD).

(2) Plasma amino acid analysis. Frozen plasma samples were thawed at 4 °C overnight and mixed by vortex. Plasma samples were pooled from the 13 samples taken from each sow on each sampling day. Glucosaminic acid was added into the pooled samples as an internal standard. Amino acid concentrations were determined using a Beckman 6300 Amino Acid Analyzer following the method described by Lee and Slocum (1987). In brief, plasma samples were deproteinized by 35% sulfosalicyclic acid precipitation. Amino acids in the supernatant were separated by a Beckman cation-ion exchange column charged in lithium citrate buffer. The eluted amino acids (AA) were measured spectrophotometrically following postcolumn derivatization with ninhydrin.

(3) Dietary nitrogen and amino acid analysis. Food samples were finely ground using a Cyclotec® 1093 Sample Mill (Foss Tecator, Sweden). Food nitrogen was analyzed by the micro-Kjeldahl method (AOAC, 1990). Hydrolysis of food samples and determination of AA in hydrolysate were performed as described in *(1)*.

Statistical analyses. The linear regression models were computed by the REG procedure of SAS/STAT (Version 6.12, SAS Institute Inc., Cary, NC). Mammary A-V differences of plasma IAA were log-transformed based on their residual error distribution. The relationship between log mammary A-V differences of plasma IAA and intakes of dietary IAA followed a linear and quadratic pattern ($P < 0.05$), except for isoleucine and leucine.

The best fitting regression model was $\hat{Y} = \beta_0 + \beta_1 X + \beta_2 X^2$, with $\hat{Y}$ being log mammary A-V differences of plasma IAA (the predicted variable), X being daily intake of dietary IAA (the predictor variable), and β_0, β_1, and β_2 being the parameter estimates. Confidence intervals were estimated using the reflection method by the Bootstrapping procedure (Neter et al., 1996). A minimum of 750 bootstrapping samples was set up to compute the confidence intervals. Analysis of variance for milk yield was carried out by the Mixed procedure with a repeated statement of the SAS/STAT.

RESULTS

Average sow and litter performance over a 21-d lactation is shown in **TABLE 2-3**. Dietary ME intake met the level of 70.3 MJ/d recommended by the NRC (1998), although intakes of protein and lysine varied across individual lactating sows. Changes in sow body weight, backfat depth, and loin eye area were within ranges (PigCHAMP 1998). Average litter weight gain across diets met the expected level of 40 kg over a 21-d lactation. Average daily milk yield did not change significantly ($P > 0.05$) between d 11 and 21 of lactation (11.67 ± 0.62 and 11.41 ± 0.62 kg/d, respectively). Therefore, sows in the present study performed at levels comparable to the current U.S. national average performance for lactating sows (PigCHAMP 1998).

Plasma flow rate across the lactating porcine mammary glands was estimated by the Fick principle (**TABLE 2-4**). Lysine was chosen as an internal indicator for evaluating mammary plasma flow rate based on the following reasons: (1) Lysine is the first limiting

amino acid in a corn-soybean meal diet for lactating sows (NRC 1998), implying that its oxidation is minimal when dietary ME intake is adequate; and (2) Kim et al. (1999) have shown that lysine accretion in lactating porcine mammary glands is only about 1 g/d, which only represents approximately 2.5 % of the total lysine output in milk in the present study. Mammary plasma flow rates estimated using phenylalanine plus tyrosine as an internal indicator were not different from those using lysine as an internal indicator (data not shown). The average volume ratio of mammary plasma flow to milk yield was 560:1 over a 21-d lactation period.

Firstly, we have developed a regression model for mammary A-V differences of plasma IAA to predict the contributions of IAA (IAA_c) mobilized from body protein reserves in extra-mammary tissues to the mammary glands in lactating sows. **TABLE 2-5** shows the relationship between log mammary A-V differences of plasma IAA and daily intakes of IAA by the sows over a 21-d period of lactation. The data were best fitted by a polynomial regression model: $\hat{Y} = \beta_0 + \beta_1 X + \beta_2 X^2$, where $\hat{Y}$ was log mammary A-V differences of plasma IAA (the response variable); X was daily intake of IAA (the predictor variable); and β_0, β_1, and $\beta_2{}^2$ were parameter estimates. Probability values were significant ($P < 0.05$) for the model, intercept, and both linear and quadratic terms for arginine, lysine, phenylalanine, threonine, and valine. Except for intercept, *P*-values were significant ($P < 0.05$) for model, the linear terms, and quadratic terms for histidine, methionine, and tryptophan. Although *P*-values were very significant ($P < 0.01$) for the model, they were not significant for the quadratic term for isoleucine or leucine. We focused on lysine, methionine, phenylalanine, threonine, and valine in the present study.

It should be pointed out that our model in the present study did not differentiate two endogenous sources: de novo synthesis and protein breakdown. Because arginine can be synthesized in body (NRC 1998), its endogenous contribution mobilized from body protein can 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 ($P > 0.05$). Thus, their endogenous contributions can not be defined from the models in the present study.

The relationship between log mammary A-V differences of plasma lysine and daily intakes of lysine is shown in **FIGURE 2-1**. The reverse log intercept (β_0) is defined as the mammary A-V difference of plasma lysine when lysine intake is extrapolated to zero. Mammary uptakes of plasma IAA, defined by multiplying mammary A-V differences of plasma IAA and mammary plasma flow rate, were derived from the contributions of endogenous IAA mobilized from body protein reserves in extra-mammary tissues when dietary intakes of IAA were extrapolated to zero. A small proportion of endogenous IAA would be used first for obligatory metabolism. Predominant proportion of endogenous IAA would be taken up second by the mammary glands for milk synthesis. Thus, IAA_c is defined as the sum of these two parts. The amount of IAA_c used for obligatory metabolism was considered as the maintenance need (NRC 1998). The amount of IAA_c used for milk synthesis was predicted by the regressions (**TABLE 2-6**). We named this technique the regression model for the mammary A-V differences of plasma IAA (MAVD).

Secondly, the contributions of endogenous IAA in lactating sows was quantified as the algebraic product of body protein loss during lactation and the respective amino acid concentration in body protein (**TABLE 2-7**). First, nitrogen balance in the lactating sows was predicted from the regression equation: Nitrogen balance (g/d) = -15.8 + 1.22 dietary lysine intake (g/d) - 0.63 nitrogen output in milk (g/d) (Dourmad et al. 1998). Negative nitrogen balance of the whole animal was defined as the amount of nitrogen loss from body protein reserves. Second, amino acid concentrations in body protein were derived from ARC (1981) and NRC (1998). Lysine concentration was estimated at 7 g/100 g body protein (ARC 1981). Methionine, phenylalanine, threonine, and valine concentrations were calculated on the basis of the amino acid profile in body protein (NRC 1998).

Thirdly, the contributions of endogenous IAA were estimated by a factorial approach with assumed utilization efficiencies of digestible IAA for milk synthesis (**TABLE 2-8**). The utilization efficiency of digestible lysine for milk synthesis was assumed to be 100%. This assumption was based on the fact that lysine was the first limiting amino acid and dietary ME intake was adequate in this study. The ratio of valine output in milk (34.9 g/d) to valine uptake by the mammary glands (41.2 g/d) was approximately 85%, which represented an approximate utilization efficiency of digestible valine for milk synthesis by the mammary gland. The dietary ratio of digestible phenylalanine to tyrosine (1.44:1) was higher by 50% compared to their ratio in milk (0.96:1). Phenylalanine might be oxidized considerably and/or be converted to tyrosine in the body in the present study. Thus, the utilization efficiency of digestible phenylalanine for milk synthesis was

arbitrarily assumed at 65%. Methionine and threonine might, to some extent, be oxidized and/or converted to cysteine and glycine, respectively. Their utilization efficiencies for milk synthesis were arbitrarily assumed to be 95%.

DISCUSSION

To quantify mammary uptake of plasma IAA, we estimated mammary A-V differences of plasma IAA and mammary plasma flow rate. Linzell et al. (1969) first developed a mammary A-V difference method to estimate mammary uptake of plasma nutrients in lactating sows. Trottier et al. (1995) further improved this method in lactating sows by introducing a cannulation of the anterior main mammary vein to obtain representative blood samples of the total mammary venous effluent. Mammary plasma flow has been estimated based on the Fick principle using internal indicators such as lysine, methionine, and phenylalanine plus tyrosine (Davis et al. 1978, Trottier et al. 1997). Mammary plasma flow rates estimated using phenylalanine plus tyrosine as an internal indicator were not significantly different from those using lysine as an internal indicator in the present study. Lysine would be minimally oxidized or negligibly accreted in the mammary glands because it is the first liming amino acid for the lactating sow (Trottier et al. 1997). Therefore, mammary plasma flow rate reported herein was calculated using lysine as the internal indicator. An estimate of mammary plasma flow rate was 6440 L/d, equivalent to a volume ratio of plasma flow to milk yield of 560:1. This ratio is in agreement with that of 540:1 in sows (Trottier et al. 1997) and is comparable to the volume ratio of blood flow to milk yield of 500:1 in cows and goats (Linzell 1974).

We have developed a regression model for mammary A-V differences of plasma IAA to predict the contributions of endogenous IAA (IAA_c) mobilized from the body protein reserves of extra-mammary tissues to the lactating porcine mammary glands. When intake of protein is not adequate, sows mobilize body protein reserves to support milk production (Mullan and Williams 1990). Mobilization of body protein reserves may be driven by the homeorhetic mechanism that involves coordinated changes in the metabolism of other body tissues to support milk synthesis (Collier 1999). Sows alter secretion of metabolic hormones to favor this mobilization to support milk synthesis (Quesnel et al. 1998). This mobilization is mainly associated with a decreased rate of protein synthesis and/or an increased rate of protein degradation in the muscle tissue (Baracos et al. 1991, Clowes et al. 1998, Jones and Stahly 1999, Pine et al. 1994). Through this mobilization, IAA from extra-mammary tissues is partitioned to the mammary glands for milk synthesis. Nutrients are used first for maintenance and second for milk production in sows (Pomar et al. 1991). Therefore, we hypothesize that IAA_c mobilized from body protein reserves 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 mammary glands when dietary intake of the IAA is extrapolated to zero.

To validate the MAVD, we quantified the contributions of endogenous IAA by multiplying body protein loss and the concentrations of IAA in body protein. This approach is referred as the body composition change approach (BCCA). In the present

study, body protein loss of the sows was estimated to be 177 g/d or 150 g/kg BW loss using nitrogen balance assuming that negative nitrogen balance of the whole animal represented body protein loss. This estimate of body protein loss is in agreement with previous studies (Jones and Stahly 1999, Mullan and Williams 1990, Whittemore and Yang 1989). Body protein loss can be calculated by multiplying body weight loss and the protein proportion of the body weight loss. The protein proportion of body weight loss is relatively constant compared to the lipid proportion in lactating sows (Whittemore and Yang 1989). The protein proportion of the body weight loss for Large White x Landrace crossbred sows ranges from 122 to 160 g/kg BW loss (Mullan and Williams 1990, Whittemore and Yang 1989). The NRC (1988) assumed the protein proportion to be 130 g/kg BW loss; however, the current NRC (1998) used a value of 94 g/kg BW loss from the study of Beyer et al. (1993a,b). Lysine concentration in body protein was assumed at 70 g/kg body protein in the present study. This assumption was based on estimates of 65 (NRC 1998), 70 (ARC 1981), 70.5 (Kyriazakis and Emmans 1993), and 73 g/kg body protein (Mahan 1998). Moreover, the amino acid composition of the whole body protein was assumed to represent that of the body protein mobilized during lactation. It should be pointed out that protein turnover was not taken into an account for the BCCA. In fact, oxidation rates of the branched-chain, aromatic, and sulfur AA liberated from tissue proteolysis might be increased to maintain their low tissue concentrations, while oxidation rates of lysine and threonine liberated from tissue proteolysis might not be further increased due to their larger pool sizes (Millward 1998). In addition, lactation significantly increases the rate of oxidation for branched-chain AA (DeSantiago et al. 1998), which may decrease the availability of endogenous valine from

extra-mammary tissues to the mammary glands. Therefore, the amount of lysine and threonine mobilized from body protein reserves defined by the BCCA could be close to the estimates of those contributions predicted by the MAVD, while the amount of methionine, phenylalanine, and valine defined by the BCCA are slightly higher than those contributions predicted by the MAVD (**TABLE 2-9**).

Furthermore, the MAVD was validated to some extent by the factorial approach using assumed utilization efficiencies of digestible IAA for milk synthesis. The utilization efficiency of digestible lysine for milk synthesis was assumed at 100% in the lactating sows in the present study. Branched-chain AA are taken up by the mammary glands in excess of their output in milk protein (Davis and Mepham 1976, Trottier et al. 1997, Wohlt et al. 1977). Approximately 30% of valine molecules taken up by goat mammary glands were oxidized to CO_2 and 70% were incorporated into casein (Roets et al. 1979). The ratio of valine output in milk to valine uptake by the mammary glands was about 85% in the present study. This ratio might be defined as the utilization efficiency of digestible valine for milk synthesis by the mammary glands assuming that the amount of milk valine contributed from vascular peptides is negligible. The hydroxylation of phenylalanine to tyrosine in the whole body increased from 10 to 18% with the infusion of phenylalanine in lactating goats (Bequette et al. 1999). Dietary ratio of digestible phenylalanine to tyrosine was higher by 50% compared to their ratio in milk in the present study, implying that hydroxylation of phenylalanine to tyrosine might be considerable. Approximately 3% of threonine molecules taken up by the lactating goat mammary gland were metabolized (Verbeke et al. 1972). To what degree oxidation of

methionine or its conversion to cysteine occurs in the mammary gland is not known. Because of the absence of data, utilization efficiencies of digestible methionine, phenylalanine, and threonine for milk synthesis were assumed to be 95, 65, and 95%, respectively. The contributions of endogenous lysine, methionine, phenylalanine, and threonine derived by the factorial approach are quite comparable to those predicted by the MAVD (**TABLE 2-9**). The contribution of endogenous valine derived by the factorial approach is slightly higher compared to that predicted by the MAVD.

The contribution of endogenous lysine, average of 10.4 g/kg BW loss for the three methods in the present study, is in agreement with the study of (Jones and Stahly 1999) where lactating sows fed diets low in protein lost 10.3 g/kg BW loss. However, it is much higher than the value of 6.2 g/kg BW loss assumed by the current NRC (1998). As discussed above, the current NRC (1998) might underestimate the contribution of endogenous lysine by underestimating the protein proportion of the body weight loss and lysine concentration in body protein for modern genotypes of lactating sows.

In summary, the contributions of IAA mobilized from the body protein reserves to the mammary glands for milk synthesis can be predicted by the MAVD, which is validated to some extent by the BCCA and the factorial approach. The MAVD predicted the amount of IAA from body protein loss to be available to the mammary glands. The BCCA estimated the maximal amount of IAA from body protein loss because it did not take into an account for protein turnover and rates of oxidation for individual IAA. The factorial approach could derive the contributions of IAA if utilization efficiencies of digestible

IAA for milk synthesis were defined. The contribution of endogenous lysine (10.4 g/kg BW loss) estimated in the present study is much higher than the value of 6.2 g/kg BW loss assumed by the current NRC (1998).

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TABLE 2-1 *Composition of diets for lactating sows (g/kg diet, as-fed basis)*

Item	Dietary protein			
	235	182	130	78
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
Salt	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				
Crude 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
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; 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 (NRC, 1988).

TABLE 2-2

Initial characteristics of sows and litters on d 1 of lactation

Item	n	Mean	SD
Sow body wt, kg	16	219.0	25.5
Sow backfat depth, mm	16	22.2	6.0
Sow loin eye area, cm^2	16	47.3	5.7
Litter size	16	11	0
Litter wt , kg	16	18.6	2.3
Piglet body wt, kg	16[1]	1.69	0.21

[1] Piglets in the same litter are considered an experimental unit.

TABLE 2-3

Sow and litter performance over a 21-d lactation period

Item	n	Mean	SD
Sow feed intake, kg/d	16	4.92	0.96
Lysine intake, g/d	16	38.5	14.5
Protein intake, g/d	16	758.0	292.6
Metabolic energy intake, MJ/d	16	70.3	13.7
Average sow body wt during lactation, kg	16	206.25	22.51
Sow body wt change, kg/d	16	-1.18	0.56
Sow backfat depth change, mm/d	16	-0.22	0.18
Sow loin eye area change, mm^2/d	16	-21.4	19.7
Litter size at weaning, No.	16	10.44	1.03
Litter wt gain, kg/21 d	16	43.00	6.72
Daily litter wt gain, kg/d	16	2.15	0.34
Piglet body wt gain, kg	16[1]	4.15	0.69
Milk yield[2] , kg/d	30	11.48	2.19

[1] Piglets in the same litter are considered an experimental unit.

[2] Average of daily milk intakes estimated on d 11 and 21 of lactation.

TABLE 2-4

Estimation of mammary plasma flow rate by the Fick principle

Item	n[1]	Mean
Plasma lysine arteriovenous difference[2], μmol/L	15	43.40
Lysine concentration in skim milk[2], mmol/L	15	26.00
Lipid concentration in milk[2], %	15	6.35
Milk yield, L/d	15	11.48
Plasma flow rate[3], L/d		6440.7

[1] Individual lactating sow is considered an experimental unit.

[2] Averaged four repeated measurements on d 10, 14, 18, and 22 of lactation of individual sows.

[3] Plasma flow rate (L/d) = Lysine output in milk x 10^3 / plasma lysine arteriovenous difference; where Lysine output in milk = Lysine concentration in skim milk x (1 - lipid concentration in milk) x milk yield.

TABLE 2-5

Regressions of log mammary A-V differences of plasma IAA against daily intakes of IAA over a 21-d lactation period[1]

Amino acid	Parameter estimate			R^2	*P* value			
	_0	–1	_2		Model	_0	–1	_2
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	1.7894	0.1284	-0.0022	0.55	0.027	0.007	0.012	0.018
Val	1.7441	0.1120	-0.0013	0.63	0.007	0.017	0.012	0.026

[1] A polynomial regression model is described by: ↗ = $\beta_0 + \beta_1 X + \beta_2 X^2$. With β_0, β_1, and β_2 being parameter estimates, ↗ and X are log mammary arteriovenous differences of plasma IAA (the predicted variable) and daily intakes of dietary IAA (the predictor variable), respectively.

TABLE 2-6 *Contributions of endogenous IAA predicted by the mammary A-V differences of plasma IAA model*[1]

Amino acid	Intercept	A-V difference$_o$[2] μmol/L	Plasma flow rate L/d	Molecular wt g/mol	Uptake$_o$[3] g/d	OM[4] g/d	IAA$_c$ g/d
Lys	2.4104	11.14	6440.7	146.19	10.49	1.96	12.45
Met	0.7216	2.06	6440.7	149.21	1.98	0.55	2.53
Phe	1.6599	5.26	6440.7	165.19	5.60	0.98	6.58
Thr	1.7894	5.99	6440.7	119.12	4.60	2.96	7.56
Val	1.7441	5.72	6440.7	117.15	4.32	1.31	5.63

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

[2] Mammary A-V difference$_o$ of plasma IAA = the reverse log intercept.

[3] Mammary uptake$_o$ of plasma IAA (g/d) = Mammary A-V difference$_o$ of plasma IAA (μmol/L) x mammary plasma flow rate (L/d) x molecular wt of IAA (g/mol) x 10^{-6}.

[4] Obligatory metabolism (OM) is considered as maintenance requirement (NRC 1998). Daily maintenance requirement of the lactating sows (g/d) = Metabolic BW (54.36, kg $BW^{0.75}$) x maintenance requirement (10^{-3} x mg/kg $BW^{0.75} \cdot d^{-1}$).

TABLE 2-7 *Contributions of endogenous IAA estimated by the body composition change approach*

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

[1] Obtained from sows in the present study (n = 16).

[2] This regression equation was established by Dourmad et al. (1998).

[3] Lysine concentration in body tissue was estimated at 7.0 g/100 g protein (ARC 1981).

[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 (NRC 1998).

TABLE 2-8

Contributions of endogenous IAA derived by the factorial approach

Amino acid	Intake of TDIAA[1], g/d	Maintenance need[2], g/d	Accretion[3] g/d	Output in milk, g/d	Efficiency[4]	IAA_c[5] g/d
Lys	33.43	1.96	1.11	42.35	1.00	11.99
Met	13.36	0.55	0.29	14.20	0.95	2.44
Phe	33.66	0.98	0.65	25.00	0.65	6.78
Thr	25.24	2.96	0.63	27.85	0.95	7.70
Val	35.55	1.31	0.84	34.90	0.85	7.81

[1] Based on true ileal digestible AA calculated from regression equations (NRC 1998).

[2] Calculated from NRC (1998) by multiplying metabolic body weight (54.36, kg body $wt^{0.75}$) and maintenance requirement (g/kg body $wt^{0.75}$·d^{-1}).

[3] Calculated from data of Kim et al. (1999) by multiplying daily protein accretion (14.81 g/d) and amino acid composition (7.50, 1.99, 4.40, 4.26, and 5.70 g/100 g protein, respectively, for lysine, methionine, phenylalanine, threonine, and valine) in lactating porcine mammary glands.

[4] Assumed utilization efficiencies of truly digestible IAA for milk synthesis and accretion (See Text).

[5] Contributions of endogenous IAA were derived from the following equation:

Dietary intake of truly digestible IAA (g/d) = maintenance requirement for truly digestible IAA (g/d) + [accretion of IAA in lactating mammary glands (g/d) + output of IAA in milk (g/d)]/ utilization efficiency of truly digestible IAA for milk synthesis – the contribution of endogenous IAA (g/d)

TABLE 2-9

Comparisons of the contributions of endogenous IAA estimated by three methods[1]

AA	MAVD		BCCA		Factorial approach		Mean[4]
	g/d[2]	g/kg[3]	g/d	g/kg[3]	g/d	g/kg[3]	g/kg
Lys	12.45	10.55	12.38	10.49	11.99	10.16	10.40
Met	2.53	2.14	3.36	2.85	2.44	2.07	2.35
Phe	6.58	5.58	7.43	6.30	6.78	5.75	5.88
Thr	7.56	6.41	7.25	6.15	7.70	6.52	6.36
Val	5.63	4.77	8.49	7.20	7.81	6.62	6.20

[1] Based on true ileal digestible IAA.

[2] Adjusted daily contribution of endogenous IAA (g/d) = maintenance requirement for IAA (g/d) + Mammary $uptake_o$ of plasma IAA (g/d).

[3] Daily contribution of endogenous IAA (g/d) divided by daily body wt loss (1.18, kg/d).

[4] Average of estimates by the mammary A-V differences of plasma IAA model, the body composition change approach, and the factorial approach.

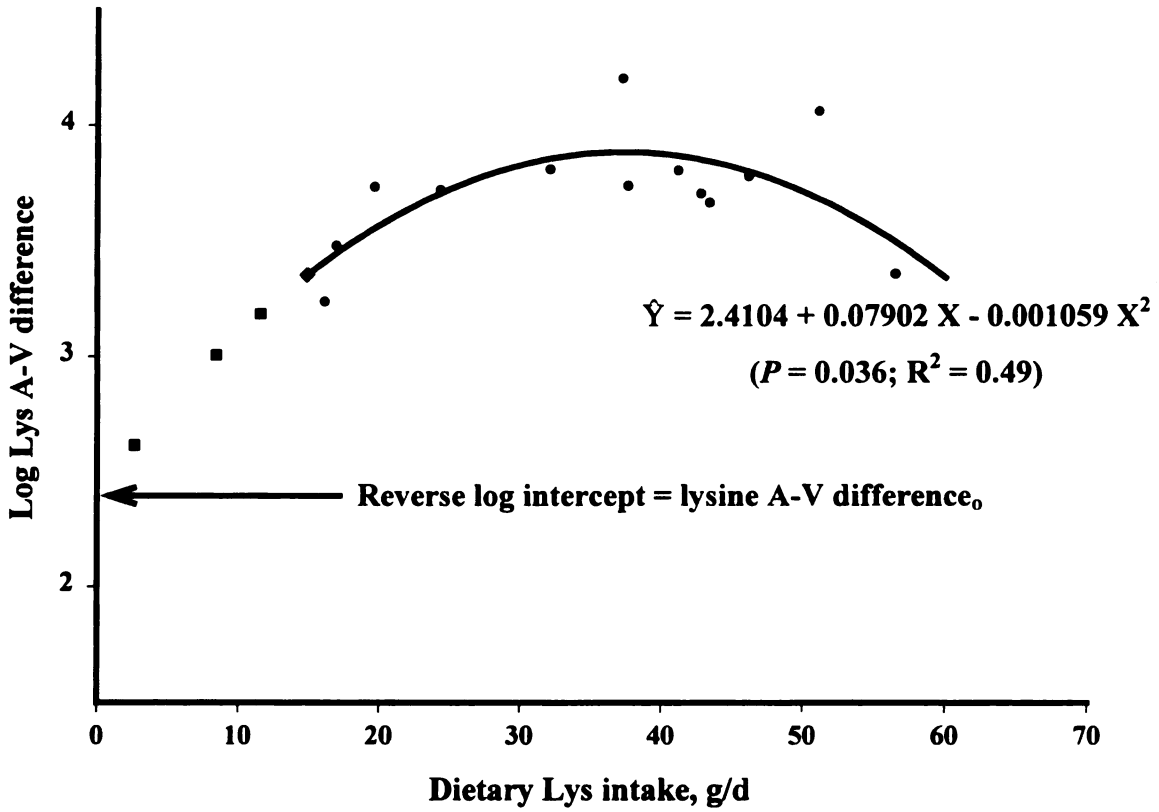

FIGURE 2-1 Relationship between the log mammary A-V difference of plasma lysine and daily intake of dietary lysine over a 21-d lactation period. The solid curve was best fitted by a polynomial regression model: $\hat{Y} = 2.4104 + 0.07902\ X - 0.001059\ X^2$ ($P = 0.036$; $R^2 = 0.49$). Extrapolation of the curve (dotted line) was made on the basis of this model. Each dot represents an individual sow. The reverse log intercept is defined as the plasma lysine A-V difference$_0$ when dietary lysine intake is extrapolated to zero.

CHAPTER 3

Uptake of Plasma Amino Acids by the Lactating Porcine Mammary Glands: A Novel Approach to Define Amino Acid Needs for Milk Synthesis

ABSTRACT We hypothesize that the maximal uptake of plasma indispensable amino acids (IAA) by porcine mammary glands can be defined as dietary needs of truly digestible IAA for milk synthesis in the lactating sow. To determine dietary needs of truly digestible IAA (TDIAA) for milk synthesis, 16 Landrace x Yorkshire lactating sows were used in a randomized block design. Sows were fed isocaloric (14.3 MJ ME/kg) diets varying in protein concentrations (78, 130, 182, and 235 g/kg) with the same pattern of dietary IAA. Sows were fitted with catheters in the carotid artery and the main mammary vein on d 4 of lactation. On d 10, 14, 18, and 22 of lactation, arterial and venous blood samples were simultaneously obtained every 30 min over 6 h. Dietary needs of TDIAA for milk synthesis were determined by a regression model of the mammary arteriovenous (A-V) differences of plasma IAA ($\hat{Y}$) against daily intakes of dietary IAA (X) over a 21-d lactation period. The relationship between $\hat{Y}$ and X was best fitted by a quadratic regression: $\hat{Y} = \beta_0 + \beta_1 X + \beta_2 X^2$ ($P < 0.05$). The vertex (the maximal $\hat{Y}$, X_i) predicted from this regression was employed in two aspects. (1) The maximal mammary uptake of plasma IAA was quantified by multiplying the maximal mammary A-V difference of plasma IAA (= the reverse log maximal $\hat{Y}$) and mammary plasma flow rate. The maximal mammary uptake

was considered as dietary needs of TDIAA for milk synthesis by the mammary glands. Dietary needs of TDIAA for milk synthesis predicted by the maximal mammary uptake were 28.78, 10.04, 20.15, 6.92, 12.10, 13.91, 4.49, and 22.40 g/kg litter wt gain, respectively, for arginine, histidine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. (2) Corresponding to the maximal $\hat{Y}$, dietary intake of TDIAA (derived from X_i) would stand for the total dietary requirement of TDIAA by the lactating sow, which is equal to the sum of maintenance need and milk synthesis need after adjustment by the contribution of endogenous IAA from body protein loss. Estimates of dietary needs of TDIAA for milk synthesis derived by a backward factorial approach were 29.01, 11.68, 20.18, 7.57, 18.40, 13.95, 4.00, and 20.86 g/kg litter wt gain, respectively, for arginine, histidine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Furthermore, dietary need of truly digestible lysine for milk synthesis defined by the maximal mammary uptake was validated by a multiple regression of the total dietary requirements of truly digestible lysine of lactating sows against litter weight gain and maternal body weight loss (data from the literature). We concluded that the maximal mammary uptakes of plasma IAA could be used to determine simultaneously dietary needs of TDIAA for milk synthesis by porcine mammary glands. Dietary need of truly digestible lysine for milk synthesis was estimated at 20.2 g/kg litter wt gain at zero maternal body weight loss. Dietary needs of TDIAA other than lysine for milk synthesis, specifically for arginine and valine, were higher than those recommended by NRC (1998).

KEY WORDS: • amino acid requirement • mammary arterio-venous difference • mammary uptake • sow • lactation

INTRODUCTION

The lactating sow must be provided with adequate amounts of indispensable amino acids (IAA) in optimal proportions for maximal productive performance and utilization efficiency of dietary protein. However, this has been hindered considerably by the paucity of data on standards for optimal proportions of dietary IAA required by the lactating sow. Dietary requirements of IAA by the lactating sow have been estimated using an empirical method and a factorial approach. The empirical method involves exploring response curves of physiological and/or productive criteria by the animal under graded dietary intakes or concentrations of the test amino acid. This method only estimates the total dietary requirement for one amino acid at the specified conditions. It is difficult to extrapolate the total dietary requirement determined by this method to other conditions such as faster or slower litter growth rate. The factorial approach derives the total dietary requirements of the test amino acid by summing up individual needs of the various physiological components (e.g., body maintenance, milk production, and body protein accretion in the lactating sow). The factorial approach has been used to predict dietary requirements of IAA for the lactating sow at various conditions (NRC 1998, Pettigrew 1993). However, application of the factorial approach is limited due to lack of experimental estimates of individual needs of dietary IAA for the specific components and is also limited due to lack of utilization efficiency of dietary IAA for milk synthesis (Trottier and Guan 2000).

Of dietary requirements for IAA, lysine has been the most intensively investigated because it is the first limiting amino acid for the lactating sow fed a corn-soybean meal diet. Estimates of dietary lysine requirements of lactating sows, based on the empirical method, vary widely from 25 to 55 g/d (Boomgaardt et al. 1972, Coma et al. 1996, Lewis and Speer 1973, Johnston et al. 1993, Touchette et al. 1998a, Wilkinson et al. 1982, Yang et al. 2000a). These variations in the estimates might be attributed to changes in maternal body weight, differences in lactational performance, and/or diversity in response criteria selected in individual studies (Guan et al. 2000a, Pettigrew 1993, Trottier and Guan 2000). When dietary intake of protein and/or lysine is inadequate during lactation, sows mobilize body proteins to support milk production (Dourmad et al. 1998, Jones and Stahly 1999, King et al. 1993, Touchette et al. 1998). The contribution of endogenous lysine mobilized from the maternal body protein will compensate for part of the total dietary lysine requirement of the lactating sow (Guan et al. 2000a, King et al. 1993).

Dietary lysine requirement of the lactating sow can also be predicted using the factorial approach (NRC 1998, Pettigrew 1993). The total dietary lysine requirements (depending on litter growth rate and maternal body weight gain or loss) can be estimated by the sum of individual needs of dietary lysine for body maintenance, milk production, and body protein accretion (or adjusted for the contribution from body protein loss). This study will focus on the major component in the factorial approach for predicting dietary IAA requirements of the lactating sow, i.e., dietary IAA need for milk production by the mammary glands. The NRC (1998) and Pettigrew (1993) derived dietary lysine need for milk production from simple linear regression of the total dietary lysine requirements

against litter weight gain. Pettigrew (1993) defined the slope in the linear regression as dietary lysine need for milk production. In contrast, the NRC (1998) used the slope minus the intercept to predict dietary lysine need for milk production. However, dietary lysine need for milk production of the lactating sow has not been defined experimentally.

Dietary requirements of IAA other than lysine have not been extensively estimated in the lactating sow. The NRC (1998) derived dietary requirements for IAA other than lysine by multiplying dietary lysine needs and the ratios of other IAA to lysine in ideal amino acid patterns for maintenance, milk synthesis, and body protein accretion (or body tissue), respectively. The amino acid profile in milk is assumed an ideal amino acid pattern for milk synthesis (NRC 1998, Pettigrew 1993). However, the mammary AA uptake pattern (defined as the relative proportions of other IAA to lysine taken up by the mammary glands) is not identical to the amino acid profile in porcine milk (Nielsen et al. 2000, Trottier et al. 1997), in which lactating sows were fed standard corn-soybean meal diet and attended by different litter size, respectively. The amount of branched-chain amino acids (isoleucine, leucine, and valine) and arginine taken up by porcine mammary glands exceeds their output in milk by approximately 25 to 30% (Nielsen et al. 2000, Trottier et al. 1997). Similar results have been found in lactating dairy cattle, sheep, and goats (Bequette et al. 1997, Davis et al. 1978, Fleet and Mepham 1985, Guinard and Rulquin 1994, Wohlt et al. 1977). In addition to direct incorporation into milk protein, branched-chain amino acids can provide carbon and α-amino nitrogen for dispensable amino acid synthesis and provide energy for synthesis of other compounds (e.g. lactose and fatty acids) in lactating bovine mammary tissue and guinea-pig mammary gland

(Davis and Mepham 1976, Wohlt et al. 1977). The optimal dietary ratio (approximately 1:1) of valine to lysine for the lactating sow, based on litter weight gain, is higher than the value (0.85:1) recommended by the NRC (1998)(Moser et al. 2000, Richert et al. 1997a, Richert et al. 1997c, Rousselow and Speer 1980). Arginine, the precursor for de novo synthesis of nitric oxide (the endothelium-derived relaxing factor), may be involved in regulation of mammary blood flow (Lacasse et al. 1996, Wu and Morris 1998). Therefore, estimates of dietary needs of IAA for milk synthesis based only on the amino acid profile in milk are appropriate in the absence of other data, but they are the estimates of minimal needs.

Milk production is positively related to mammary uptake of plasma lysine in sows (Trottier 1997), which may be extended to mammary uptake of other limiting IAA for milk synthesis. Furthermore, milk production is directly related to mammary arterio-venous (A-V) differences of plasma IAA in dairy cows duodenally infused with casein or whey proteins where mammary blood flow rates are constant (Guinard et al. 1994, Madsen et al. 1999). There is a quadratic relation between mammary A-V differences of plasma lysine and arterial concentrations of plasma lysine in lactating sows and cows (Guinard 1994, Trottier 1997), implying that mammary uptake of plasma lysine will peak at certain concentrations of arterial lysine. However, the relationship between mammary A-V differences of plasma IAA and daily intakes of dietary IAA has not been explored in the lactating sow. Maximal mammary uptake of plasma IAA may provide the intracellular IAA required for milk synthesis potential in the lactating sow. Thus, we hypothesize that the maximal mammary uptake of plasma IAA can be defined as the

mammary need for IAA, and further considered as dietary need of truly digestible IAA for milk synthesis by the mammary glands in the lactating sow. Dietary needs of truly digestible IAA for milk synthesis defined by the maximal mammary uptake of plasma IAA include the amount of direct incorporation into milk and the metabolic need by the mammary gland. Moreover, a set of dietary needs of truly digestible IAA for milk production could be estimated concurrently by maximal mammary uptakes. The objective of this study was to quantify dietary needs of truly digestible IAA by the mammary glands for milk production in the lactating sow at zero change in body protein. This information can be used to constitute an ideal dietary IAA pattern for milk synthesis and predict total dietary IAA requirements of the lactating sow.

MATERIALS AND METHODS

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

Experimental design and dietary treatments. Sixteen Landrace x Yorkshire multiparous lactating sows (parity 2 or 3) were allocated to treatments according to a randomized block design. Each block consisted of four sows. Each sow in one block was provided ad libitum access to one of four diets. Diets contained different crude protein concentrations (78, 130, 182, and 235 g/kg) and were balanced to contain a similar pattern of IAA that met recommendations for lactating sows (NRC 1988). Diets were

formulated by diluting the highest level of crude protein (235 g/kg diet) with starch and sugar. All diets were isocaloric with a metabolizable energy (ME) of 14.3 MJ/kg.

Animals. 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). Sows were provided free access to water. Food was offered in a stair step manner during the first three days post surgery and to appetite thereafter. Sow food intake was recorded daily. Sow body weight was recorded after farrowing (d 1) and at weaning (d 21). Piglets were individually weighed weekly.

Cannulation. For catheter, microbore Tygon® tubing (1.0 mm i.d., 1.8 mm o.d., Norton Performance Plastics Co., Akron, OH) was used and the lumen was coated with triododecylmethylammonium chloride - heparin complex (7% w/w, Polysciences, Inc., Warrington, PA). The anterior main mammary vein and the carotid artery were cannulated on d 4 ± 1 of lactation following the surgical procedure described by Trottier et al. (1995). Antibiotic Naxcel® (Pharmacia and Upjohn Co., Kalamazoo, MI) and anti-inflammatory Banamine® (Schering-Plough Animal Health Corp., Kenilmorth, NJ) were administered i.v. for three days following surgery. The catheters were flushed once daily with heparinized saline (20 IU heparin/ml).

Blood sampling protocol. Blood samples were collected on d 9 ± 1, 13 ± 1, 17 ± 1, and 21 ± 1 (referred to as d 10, 14, 18, and 22, respectively). Sows were fed 1 h prior to blood sampling and provided ad libitum access to food and free access to water as usual.

Carotid arterial and main mammary venous blood samples (10 ml each) were simultaneously collected into sterile syringes from 0800 every 30 min over 6 h. Thus, a total of 13 blood samples were collected from each sow per sampling day. Blood was transferred into EDTA-coated tubes and centrifuged at 1500 x g for 15 min at 4 °C. Plasma was removed and stored at -20 °C.

Laboratory measurements

(1) Plasma amino acid analysis. Frozen plasma samples were thawed at 4 °C overnight and mixed using a vortex. Plasma samples were pooled from the 13 samples taken from each sow on each sampling day. Glucosaminic acid as an internal standard was added to the pooled samples. Amino acid concentrations were determined using a Beckman 6300 Amino Acid Analyzer following the method described by Lee and Slocum (1987). In brief, plasma samples were deproteinized by 35% sulfosalicyclic acid precipitation. Amino acids in the supernatant were separated by a Beckman cation-ion exchange column charged in lithium citrate buffer. The eluted amino acids were measured spectrophotometrically following postcolumn derivatization with ninhydrin.

(2) Dietary nitrogen and amino acid analysis. Food samples were finely ground using a Cyclotec® 1093 Sample Mill (Foss Tecator, Sweden). Concentrations of amino acids in food were analyzed by HPLC following the Pico•Tag® method (Cohen et al. 1989). In brief, food samples were hydrolyzed using 6 mol/L HCl for 24 h at 110 °C. Norleucine as an internal standard was added into food samples before hydrolysis. Amino acids in the hydrolysate were derivatized with phenylisothiocyanate and separated by a Pico•Tag®

column (3.9 mm x 150 mm, Waters Corp., Milford, MA) using Alliance^{tm} Waters 2690 Separations Module and detected by Waters^{tm} 486 Tenable Absorbance Detector (Waters Corp., Milford, MA). Amino acid standard H (Pierce, Rockford, IL) was used as a calibration standard. The method was validated with certified amino acid standard (NIST, Gaithersburg, MD). Tryptophan concentrations in the diets were calculated using the value of tryptophan content in food ingredients (NRC 1988).

Calculations

(1) Milk output of IAA.

Milk output of IAA (g/d) = Milk yield (L/d) x Concentrations (μmol/L) of IAA in defatted milk x (1 - Content of fat in whole milk) x (1 - Proportion of non-mammary synthesized proteins in milk) x molecular weight (g/mol) x 10^{-6}. Milk yield was measured on d 11 and 21 of lactation by the D_2O dilution method (Guan et al. 2000a). Concentrations of IAA in defatted milk (collected on d 10, 14, 18, and 22 of lactation) were analyzed by the Pico•Tag® Method and the content of fat in whole milk by the mid-infrared spectroscopic method (AOAC 1990). Concentrations of IAA in defatted milk were corrected by the content of fat in whole milk to obtain the concentrations of IAA in whole milk. They were further corrected by the proportion of non-mammary synthesized proteins (such as albumin and immunoglobins) in milk, which was calculated to be 7.25 % of the total milk proteins over a 21-d lactation according to the studies (Bourne and Curtis 1973, Klobasa and Butler 1987, Klobasa et al. 1987). To quantify milk output of IAA, milk yield on d 11 and 21 was multiplied by the average concentration of IAA in

whole milk (adjusted by non-mammary synthesized proteins in milk) from d 10 and 14, and from d 18 and 22 of lactation, respectively.

(2) Mammary uptake of plasma IAA.

Mammary uptake of plasma IAA (g/d) = Mammary A-V difference (μmol/L) of plasma IAA x Mammary plasma flow rate (L/d) x molecular weight (g/mol) x 10^{-6}. Mammary plasma flow rate was estimated by the Fick principle using lysine as an internal indicator (Guan et al. 2000a). To quantify mammary uptake of plasma IAA, mammary plasma flow rate (on d 11 and 21) was multiplied by the average mammary A-V difference of plasma IAA from d 10 and 14, and from d 18 and 22 of lactation, respectively.

(3) The vertex of the quadratic regression.

The relationship of log mammary A-V differences of plasma IAA ($\hat{Y}$) and daily intakes of dietary IAA (X) was best fitted by a quadratic regression: $\hat{Y} = \beta_0 + \beta_1 X + \beta_2 X^2$ ($\beta_2 < 0$). The vertex (the maximal $\hat{Y}$, X_i) of this regression was predicted at $d\hat{Y}/dX = 0$, i.e., $X_i = -\beta_1/2\beta_2$, thus the maximal $\hat{Y} = \beta_0 - \beta_1^2/4\beta_2$.

(4) Dietary needs of TDIAA for milk production derived by the factorial approach.

Based on the factorial approach, the total dietary requirement of TDIAA (considered as daily intake of TDIAA at the maximal $\hat{Y}$) = maintenance need of TDIAA + dietary need of TDIAA for milk production – the contribution of endogenous IAA (expressed as an equivalent of TDIAA). Thus, dietary need of TDIAA for milk production = daily intake of TDIAA at the maximal $\hat{Y}$ + the contribution of endogenous IAA - maintenance need of

TDIAA. Daily intake of TDIAA at the maximal $\hat{Y}$ was converted from daily intake (X_i) of IAA at the maximal $\hat{Y}$ based on the regressions of TDIAA against total IAA (NRC 1998). The contribution of endogenous IAA was estimated by (Guan et al. 2000a). Maintenance need of TDIAA was calculated by the NRC (1998).

Statistical analyses. The linear regression models were computed with the REG procedure of SAS/STAT (Version 6.12, SAS Institute Inc., Cary, NC). The relationship between log mammary A-V differences of plasma IAA and daily intakes of dietary IAA followed a linear and quadratic pattern ($P < 0.05$), except for isoleucine and leucine. The best fitting regression model was $\hat{Y} = \beta_0 + \beta_1 X + \beta_2 X^2$, with $\hat{Y}$ being the predicted log mammary A-V differences of plasma IAA (the predicted variable), X being daily intake of dietary IAA (the predictor variable), and β_0, β_1, and β_2 being the parameter estimates. Confidence intervals were estimated using the reflection method by the Bootstrapping procedure (Neter et al., 1996). A minimum of 750 bootstrapping samples was set up for computing the confidence intervals. Comparison between mammary uptake of plasma IAA and their output in milk was analyzed with the TTEST procedure of SAS/STAT.

RESULTS

Mammary uptake vs. milk output. Mammary uptakes of plasma lysine, methionine, phenylalanine, threonine, and tryptophan were not different from their output in milk over a 21-d lactation (**FIGURE 3-1**); mammary uptake of plasma histidine was lower by 18% than its output in milk; and mammary uptakes of plasma arginine, isoleucine,

leucine, and valine were higher by approximately 20 to 50% than their output in milk. This study focused on arginine, histidine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine because they might become limiting IAA for milk production in the lactating sow fed a corn-soybean meal diet.

Mammary needs of IAA for milk production predicted by the maximal mammary uptake of plasma IAA. The mammary need for IAA includes both incorporation of IAA into milk and metabolic need of IAA by the mammary glands (e.g., oxidation, accretion, and conversion to other substrates such as dispensable amino acids, lactose, and fatty acids). The mammary need of IAA was defined by the maximal uptake of plasma IAA across the porcine mammary glands. Relations between log mammary A-V differences of plasma IAA ($\hat{Y}$) and daily intakes of dietary IAA (X) over a 21-d lactation period were best fitted by quadratic regressions (**TABLE 3-1**). Quadratic coefficients in the regressions were negative ($P < 0.05$), indicating that $\hat{Y}$ can be maximized in the vertex. The vertex of the quadratic regression for arginine, histidine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine is represented, respectively, in **FIGURE 3-2a to 4-2h**. Maximal mammary uptakes of plasma IAA were predicted by multiplying maximal A-V differences of plasma IAA (the reverse log maximal $\hat{Y}$) and mammary plasma flow rate of 6440 L/d estimated by the Fick principle using lysine as an internal indicator (**TABLE 3-2**) (Guan et al. 2000a). The sows had an average litter weight gain of 2.15 kg/d and an average milk yield of 11.48 kg/d over a 21-d lactation period in this study. Thus, the maximal mammary uptake was standardized for milk production need by dividing the maximal mammary uptake with the litter weight gain (**TABLE 3-2**). The

maximal mammary uptakes were defined as mammary needs of IAA, and were further considered as dietary needs of truly digestible (absorbed) IAA for milk production (**TABLE 3-2**). This was based on the following assumptions. (1) While the contributions of endogenous IAA from body protein loss are zero, the IAA taken up by the mammary glands are entirely supplied by post-hepatic available IAA of dietary origin. (2) Dietary intake of truly digestible (absorbed) IAA by the lactating sow meets two parts: body maintenance need (including the loss of absorbed IAA metabolized on the first pass by the splanchnic bed) and the mammary need of post-hepatic available IAA (of dietary origin) for milk production. Ratios relative to lysine for the maximal mammary uptake were at 1.43, 0.50, 0.34, 0.60, 0.69, 0.22, and 1.11:1, respectively, for arginine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine for the lactating sows (**TABLE 3-5**).

Mammary needs of IAA for milk production derived from the factorial approach. Corresponding to the maximal A-V differences of plasma IAA (i.e, the reverse log maximal $\hat{Y}$), daily intakes of dietary total IAA (X_i) were estimated from regressions in **TABLE 3-1**. Means of intakes of dietary IAA with 95% confidence intervals were computed by the Bootstrapping reflection method (**TABLE 3-3**). These intakes of dietary IAA, after subtracting maintenance need, were considered as dietary needs of IAA for milk production at given contributions of endogenous IAA. We named this approach as the backward factorial approach. Based on the backward factorial approach, dietary intake of TDIAA at the maximal $\hat{Y}$ = maintenance need of dietary TDIAA + milk production need of dietary TDIAA – the contribution of endogenous IAA (expressed as

an equivalent of dietary TDIAA). Thus, dietary needs of TDIAA for milk production were derived from this equation (**TABLE 3-4**). Ratios of other TDIAA to lysine required for milk production (determined by the backward factorial approach) were 1.44, 0.58, 0.38, 0.91, 0.69, 0.20, and 1.03, respectively, for arginine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine for the lactating sows.

DISCUSSION

Mammary uptake of plasma branched-chain amino acids and arginine exceeded their output in milk by approximately 20 to 50%. Thus, it might be inappropriate to estimate dietary needs of TDIAA other than lysine for milk production based only on the amino acid profile in milk. A novel approach to determine dietary needs of TDIAA for milk production was developed from the relationship between log mammary A-V differences of plasma IAA and daily intakes of IAA in lactating sows. Dietary needs of TDIAA for milk production were determined by maximal mammary uptakes of plasma IAA and further evaluated using the backward factorial approach. Dietary need of truly digestible lysine for milk production was estimated at 20.15 and 20.18 g/kg litter wt gain, respectively, by these two methods. Dietary needs of TDIAA other than lysine for milk production in the present study were higher than the values recommended by the NRC (1998), especially for arginine and valine.

The ratios of mammary uptake to output in milk are approximately at 1:1 for lysine, methionine, phenylalanine, threonine, and tryptophan, while the ratio for arginine and the branched-chain AA exceeds 1:1 (see **FIGURE 3-1**), suggesting that mammary uptake of the plasma free form of these IAA may be sufficient for milk production in lactating sows. However, the ratio of mammary uptake to output for histidine was less than 1:1, possibly indicating that the mammary gland takes up peptides from plasma to meet this deficiency for milk synthesis. In the lactating goat, however, the ratio of mammary uptake to output in milk is less than 1:1 for phenylalanine (Backwell et al. 1996). It is suggested that this shortfall could be met by mammary uptake of the AA derived from plasma peptides. Vascular peptides are hydrolyzed by membrane-bound peptidases at the basolateral surface of the mammary epithelium, which is followed by uptake of the released free AA (Backwell et al. 1994, Backwell et al. 1996, Shennan et al. 1998, Shennan et al. 1999). The present study was the first to provide direct comparison between uptake of plasma free AA and output of AA in milk after corrected for the contribution of protein-bound AA to their output from non-mammary synthesized proteins (e.g., albumin and immunoglobins). In a previous study, this contribution was not taken into account in the comparison (Trottier et al. 1997), leading to no negative differences between mammary uptake of plasma IAA and their output in milk in the lactating sow (Trottier et al. 1997). In fact, any comparison between the mammary IAA uptake and their output is complicated by multiple sources of entrance and outlet. On the entrance end, there are at least (1) uptake of free AA from plasma and blood cells (if nay), (2) direct transport of immune proteins and peptides (if any), and (3) intracellular contribution from protein breakdown and de novo synthesis (if any). On the outlet end,

there are (1) incorporation of peptide-bound and free AA into milk, (2) accretion as synthesized constitutive proteins in the mammary gland, and (3) metabolized loss (including oxidation and conversions to other compounds, if nay, in the mammary gland). In addition to these complications, output of IAA might be not responsive to intake of dietary protein due to lactational homeorhesis (Bauman 1999). For example, sows lose body weight to support high rate of milk production when their intakes of protein, indispensable amino acids (IAA), and/or energy are inadequate (Clowes et al. 1998, Dourmad et al. 1998, Jones and Stahly 1999, King and Williams 1984, Mullan and Williams 1990, Revell et al. 1998, Williams and Smits 1991). Therefore, output of IAA in milk might not be the "gold standard" response to intake of dietary AA. In contrast, mammary uptake of plasma IAA is highly responsive to intake of dietary IAA and might be considered a new response criterion for estimating dietary IAA requirements of the lactating sow (Guan et al. 2000b, Trottier and Guan 2000).

The maximal mammary uptakes of plasma IAA were defined as mammary need of IAA for milk production, and further considered as dietary needs of TDIAA for milk production by the mammary glands in this study. Without any contributions of endogenous IAA, dietary intake of TDIAA would meet both maintenance need and mammary need for milk production. Thus, mammary need of IAA for milk production is equivalent to dietary need of TDIAA for milk production. Dietary needs of TDIAA for milk production predicted by the maximal mammary uptakes are supported by estimates derived from the backward factorial approach except for phenylalanine (see **TABLE 3-2** and **4-4**). Maximal mammary uptakes can quantitatively encompass both direct

incorporation into milk and metabolic needs occurring in mammary glands. The metabolic needs includes oxidation, accretion, de novo synthesis of dispensable AA, and conversions to other substrates, if any, in mammary glands (Mepham 1982, Trottier 1997).

The total requirement of dietary lysine for the lactating sow has been estimated empirically based on productive performance (e.g. milk yield, litter weight gain, and body weight loss) and nutritional status of body nitrogen (e.g. plasma amino acid profile, plasma urea nitrogen, and nitrogen balance) (Trottier and Guan 2000). Considerable variation in empirical estimates may be attributed to differences in lactation performance, high variability in selected response criteria, and changes in body protein reserves (Pettigrew 1993, Trottier and Guan 2000). The total requirements of dietary lysine are strongly related to lactation performance, such as litter weight gain (Dourmad et al. 1998, Pettigrew 1993, Wilkinson et al. 1982). The total requirements of dietary lysine can be met by both exogenous intake of dietary lysine and the contribution of endogenous lysine from the mobilization of liable protein reserves. Sows mobilize body protein reserves to maintain milk production when intake of lysine is not adequate during lactation (Touchette et al. 1998). This contribution of endogenous lysine makes it complicated to empirically estimate the total requirements of dietary lysine for lactating sows (Guan et al. 2000a, King et al. 1993, Pettigrew 1993).

To use these estimates of the total dietary lysine requirements of lactating sows, we developed a response surface model to fit empirical estimates reported in the literature.

The response surface of the total requirements of dietary truly digestible lysine were regressed against litter weight gain and body weight loss during lactation, which corresponds to milk production and the contribution of endogenous lysine, respectively. The total requirements (Y) of dietary truly digestible lysine for 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 as dietary need of truly digestible lysine for milk production 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. Dietary need of apparent digestible lysine for milk production was predicted at 22 g/kg litter wt gain minus 6.39 g (NRC, 1998). As discussed in Chapter 2, the NRC (1998) may have underestimated the contribution of endogenous lysine and consequently had to correct dietary need of apparent digestible lysine for milk production by subtracting the intercept (6.39) from the slope in the simple linear regression. The 3-D response surface is shown in **FIGURE 3-3**. It clearly demonstrates that (1) the total requirement of dietary truly digestible lysine increases with increasing milk production, i.e., with increasing litter weight gain; (2) the total requirement decreases with increasing contribution of endogenous lysine from body protein reserves, i.e., with increasing maternal body weight loss; and (3) the total requirement (considered as the daily intake of lysine at the maximal A-V difference of plasma lysine) in this study is reliable because it fits well on the 3-D response surface. Moreover, the total requirements of truly digestible lysine were predicted at various conditions based on dietary need of truly digestible lysine for milk production (20.15 g/kg litter wt gain, in the present study), the contribution of

endogenous lysine (10.40 g/kg BW loss, Guan et al. 2000), and maintenance need (NRC 1998). The predicted estimates of the total requirements were compared with those derived by the NRC (1998) in **FIGURE 3-4**. It can be seen that two sets of predicted values are very close at zero body weight loss. However, this does not necessarily reflect the identity in estimates used for individual components between the two models. Especially, the differences (in the total dietary lysine requirement between the two models) increase with increased body weight loss, possibly indicating that the value for the endogenous lysine contribution is underestimated in the current NRC (NRC 1998). In the future, the NRC should use estimates of dietary lysine needs for individual components by the lactating sow, which have been generated experimentally to replace totally model-derived values to improve the current model.

Dietary ratio of truly digestible valine to lysine required for milk production was estimated at 1.1:1 based on their maximal mammary uptakes, which is higher than the value of 0.85:1 for milk synthesis recommended by the NRC (1998). The NRC (1998) may underestimate dietary need of truly digestible valine for milk production when based mainly on the ratio of valine to lysine in milk though it had been adjusted up from 0.73 to 0.85:1 (of valine to lysine) according to the study of Richert et al. (1996). The amount of valine taken up by the mammary glands is much higher than its output in milk (Linzell et al. 1969, Roets et al. 1979, Trottier et al. 1997). The branched-chain AA can be catabolized in vitro by bovine, guinea-pig, and porcine mammary tissues (Davis and Mepham 1976, Richert, 1998, Wohlt, 1977). The capacity of rat mammary gland to catabolize the branched-chain AA is increased dramatically during lactation (DeSantiago

et al. 1998). Approximately 30% of valine taken up by the mammary gland was oxidized to CO_2 and 70% was incorporated in casein in isolated goat mammary gland in vitro (Roets et al. 1979), though a small proportion of the branched-chain AA taken up by porcine mammary tissue is oxidized as an energy source (Richert et al. 1998). Moreover, they may provide carbon and α-amino nitrogen for synthesis of dispensable AA in bovine mammary tissue (Wohlt et al. 1977). The ratio for the total requirement of dietary valine to lysine in the lactating sow was estimated to be 0.91:1 and 1.17:1 based on plasma AA profile plus nitrogen balance and litter weight gain plus milk production, respectively (Rousselow and Speer 1980). Effects of high dietary valine on lactational performance are still controversial. Litter weight gain increased with increased dietary ratio of valine to lysine from 0.89:1 to 1.33:1 (Moser et al. 2000) or from 0.80:1 to 1.20:1 for the lactating sow nursing a large size of more than 10 piglets per sow (Richert et al. 1997a, Richert et al. 1997b). However, increasing dietary ratio of valine to lysine (up to 1.20:1) did not improve lactational performance of the lactating sow nursing a large size of piglets of more than 12 piglets per sow (Carter et al. 2000). Differences in these feeding trials might be attributed to uncontrolled treatments (e.g., balance of dietary AA). Note that estimates in the present study represent dietary needs of IAA for milk synthesis by the mammary gland for the lactating sow at zero change in body protein; in contrast, all the estimates in the literature are for the total dietary requirements of IAA for the lactating sow at their given conditions. Ideal dietary ratios of other IAA to lysine for the lactating sow should be expressed on a truly digestible basis and be constituted using three sets of ideal ratios for maintenance, milk production, and body protein accretion or endogenous contribution (**TABLE 5**).

Dietary ratio of threonine to lysine required for milk production was estimated at 0.69:1 in this study, which is higher than the value (0.58:1) recommended by the NRC (1998). Approximately 3% of threonine molecules taken up by perfused goat mammary gland was oxidized to CO_2 and converted to dispensable AA (Verbeke et al. 1972). The ratio for the total dietary requirement of threonine to lysine for the lactating sow was estimated at 0.82:1 and 0.70:1 when based on maximal milk yield plus litter weight gain and on minimal plasma urea nitrogen plus urinary urea nitrogen output, respectively (Lewis and Speer 1975). This ratio was further evaluated at 0.66:1 and 0.68:1 based on maximal litter weight gain and minimal plasma urea N, respectively (Cooper et al. 2000). Dietary need of truly digestible threonine for milk production was estimated at 30 g/d or 13.9 g/kg litter wt gain at zero body weight loss in the present study, which is slightly higher than the estimates of Cooper et al. (2000). The total requirement of dietary truly digestible threonine for lactating sows was estimated at 30 g/d or 11.8 g/kg litter wt gain when based on maximal litter weight gain and minimal maternal body weight loss (Cooper et al. 2000).

Dietary ratio of truly digestible tryptophan to lysine required for milk production was estimated at 0.22:1 in this study, which is slightly higher than the value (0.18:1) for milk synthesis recommended by the NRC (1998) and much higher than an estimate of 0.14:1 for the lactating sow (Lewis and Speer 1974). Lactating sows had higher voluntary feed intake, lower plasma urea nitrogen, and less body weight loss when fed a higher dietary ratio of tryptophan to lysine (0.23:1 vs 0.16:1)(Libal et al. 1997). Increased intake of

dietary tryptophan improved nitrogen utilization by the lactating sows although it did not influence litter weight gain (Libal et al. 1997), implying that the dietary ratio of 0.16:1 might be inadequate in terms of nitrogen balance.

Dietary ratio of truly digestible methionine to lysine required for milk production was estimated at 0.34:1 in this study, which is higher than the value of 0.26:1 recommended by NRC (1998). Dietary need of truly digestible methionine for milk production was estimated at 14.9 and 16.3 g/d, respectively, based on the maximal mammary uptake and the factorial approach, which is in agreement with the study of Schneider et al. (1992). Daily intake of dietary methionine plus cysteine (33.2 g/d) was required by lactating sows based on minimal body weight loss (Schneider et al. 1992). Thus, approximately 17 g/d of the total requirement of dietary methionine might be required by the lactating sows assuming that 50% of S-containing AA could be met by dietary cysteine.

Dietary ratio of truly digestible phenylalanine to lysine required for milk production was estimated at 0.60:1 based on the maximal mammary uptake, which is higher than the value of 0.55:1 for milk synthesis recommended by the NRC (1998). However, it was in agreement with the result of Lellis and Speer (1985). Dietary ratio of phenylalanine to lysine required for lactating sows was calculated at 0.63:1 based on milk yield and plasma amino acid profile and on the assumption that 50% of the total dietary requirement of aromatic AA is met by tyrosine (Lellis and Speer 1985, 1987, NRC 1998). Dietary need of truly digestible phenylalanine for milk production derived from the backward factorial approach is higher than that predicted by the maximal mammary

uptake (39.6 vs 26.0 g/d). Dietary ratio of truly digestible phenylalanine to tyrosine was higher by 50% compared to their ratio in milk in the present study. The hydroxylation of phenylalanine to tyrosine in the whole body increases with increased available phenylalanine in lactating goats (Bequette et al. 1999). The hydroxylation may have occurred in the present study, resulting in a decreased utilization efficiency of dietary truly digestible phenylalanine for milk production. Thus, dietary need of truly digestible phenylalanine for milk synthesis might be overestimated by the backward factorial approach.

The present study was the first to estimate dietary needs of arginine and histidine for milk synthesis by the mammary gland for the lactating sow. We found that dietary needs of truly digestible arginine for milk synthesis is much higher than the value recommended by the current NRC (1998). This is due to the fact that the mammary gland during lactation takes up arginine in excess of its output in milk (Trottier et al. 1997). As discussed previously, arginine may play a very important role involved in local regulation of mammary blood flow (Lacasse et al. 1996). Thus, this additional amount of dietary arginine for this metabolism need should be considered in estimation of its dietary need for milk synthesis. Dietary needs of truly digestible histidine for milk synthesis in the present study is also higher than the value recommended by the current NRC (1998).

In summary, dietary needs of TDIAA for milk production can be determined by their maximal mammary uptake, and their estimates were further validated by the backward factorial approach. Dietary need of truly digestible lysine for milk synthesis by the

mammary gland was estimated at 20.15 and 20.18 g/kg litter wt gain, respectively, by these two methods, which was further supported by the multiple regression analysis of empirical estimates of the total requirements of dietary lysine for lactating sows in the literature. Dietary ratios of truly digestible arginine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine to lysine required for milk synthesis were higher than values recommended by the current NRC (1998). Therefore, it is inappropriate to derive dietary needs of TDIAA for milk synthesis based only on the AA profile in milk, especially for arginine and the branched-chain AA (e.g. valine). Dietary needs of TDIAA defined by their maximal mammary uptake include the amount of direct incorporation into milk and the additional metabolic needs (if any) by the mammary gland as well.

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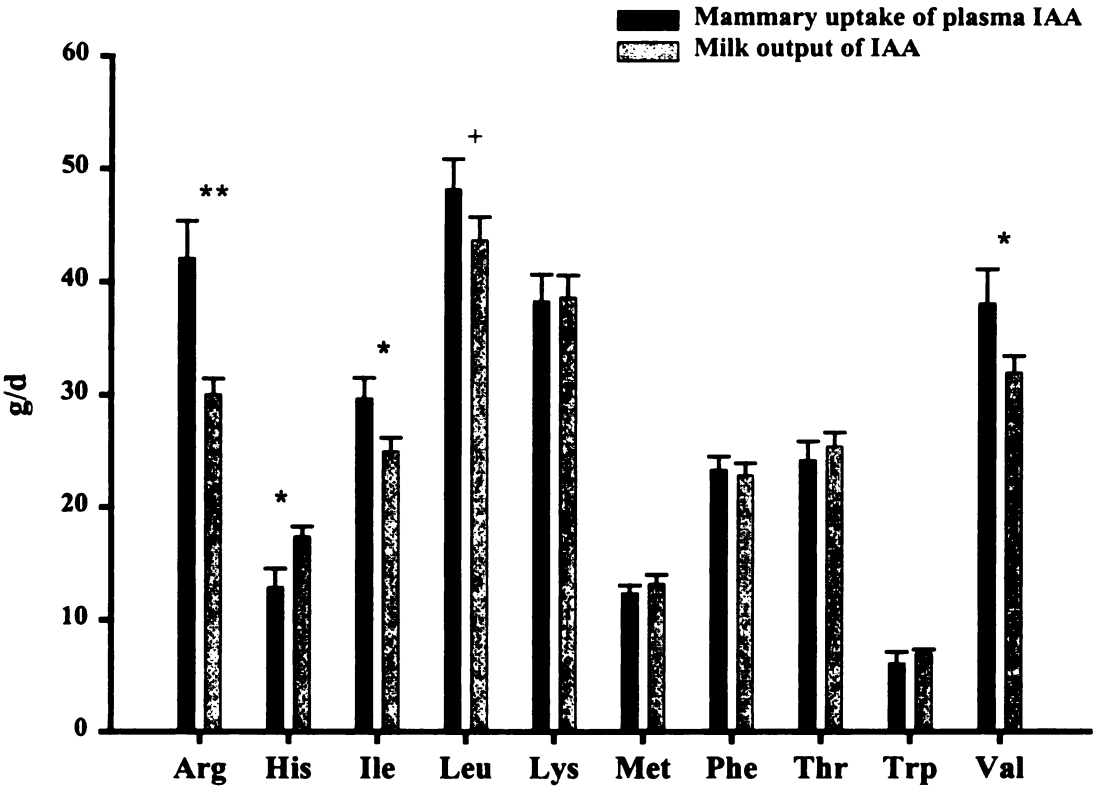


FIGURE 3-1 Comparison between uptake of plasma IAA by the porcine mammary glands and their output in milk over a 21-d lactation. Mammary uptake of plasma IAA was quantified by multiplying mammary plasma flow rate and mammary A-V differences of plasma IAA. Milk output of IAA was estimated by multiplying milk yield and the concentrations of IAA in milk. Calculations in detail are shown in MATERIALS AND METHODS. Values (Mean ± SD, n = 26) between uptake and output with * differ ($P < 0.05$) and with $^{+}$ tend to differ ($P < 0.10$), respectively.

FIGURE 3-2a The relation between log mammary A-V differences of plasma Arg ($\hat{Y}$) and daily intakes of dietary total Arg (X) over a 21-d lactation period. The curve was best fitted by a quadratic regression model: $\hat{Y} = 1.2767 + 0.0992X - 0.0009X^2$ ($P = 0.006$, $R^2 = 0.64$). Each dot represents an individual sow. The vertex (the maximal $\hat{Y}$, X_i) derived from this regression had two implications: (1) The maximal mammary uptake of plasma Arg could be quantified by multiplying the maximal mammary A-V difference of plasma Arg (i.e, the reverse log maximal $\hat{Y}$) and mammary plasma flow rate. The maximal mammary uptake of plasma Arg was defined as mammary need for Arg, and further considered as dietary need of truly digestible Arg for milk production by the mammary glands. (2) Daily intake of dietary total Arg (X_i) would correspond to the entire requirement of dietary total Arg by the lactating sow, i.e., the sum of maintenance need and dietary need for milk production adjusted by the maternal body weight loss of this study. Thus, dietary need of truly digestible Arg for milk production could be derived by the factorial approach. A Bootstrapping method was employed to compute the confidence intervals (95% CI) for daily intake of dietary total Arg (Neter et al. 1996).

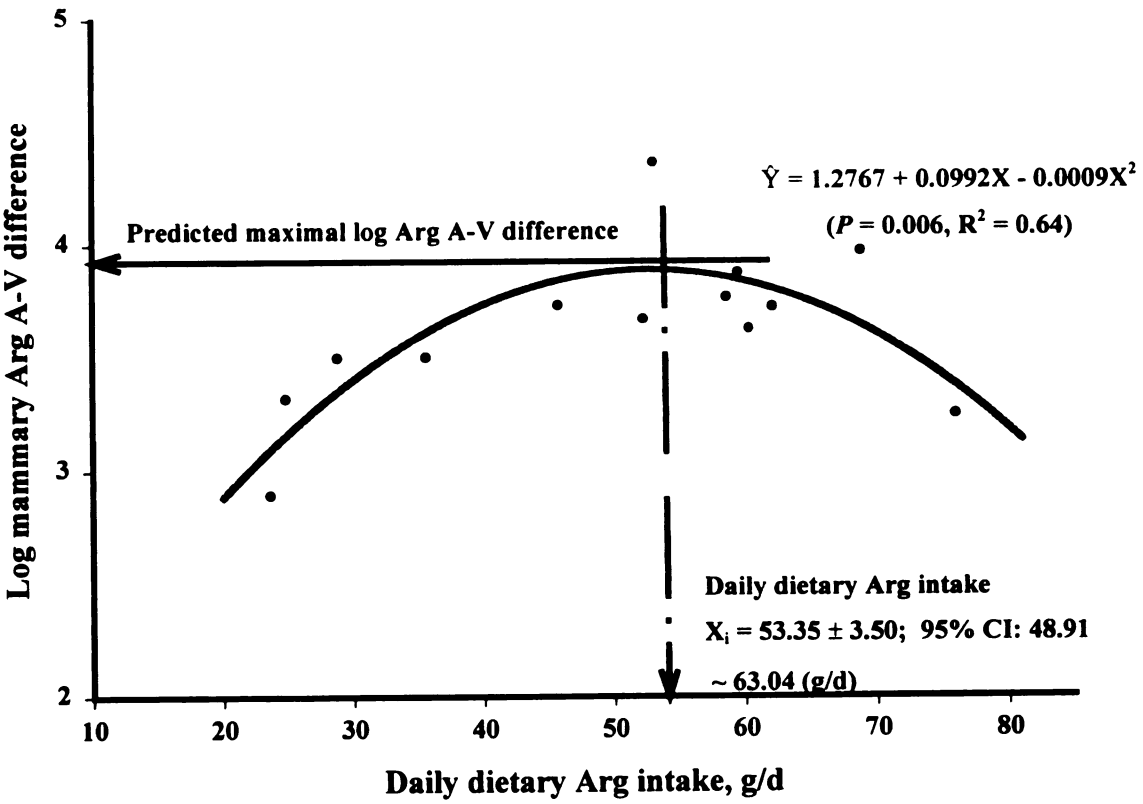

5
4
3
2
Log mammary Arg A-V difference
Predicted maximal log Arg A-V difference
Ŷ = 1.2767 + 0.0992X - 0.0009X²
(P = 0.006, R² = 0.64)
Daily dietary Arg intake
Xi = 53.35 ± 3.50; 95% CI: 48.91
~ 63.04 (g/d)
10
20
30
40
50
60
70
80
Daily dietary Arg intake, g/d

FIGURE 3-2b The relation between log mammary A-V differences of plasma His ($\hat{Y}$) and daily intakes of dietary total His (X) over a 21-d lactation period. The curve was best fitted by a quadratic regression model: $\hat{Y} = -1.4929 + 0.4054X - 0.0090X^2$ ($P = 0.022$, $R^2 = 0.53$). Each dot represents an individual sow. The vertex (the maximal $\hat{Y}$, X_i) derived from this regression had two implications: (1) The maximal mammary uptake of plasma His could be quantified by multiplying the maximal mammary A-V difference of plasma His (i.e, the reverse log maximal $\hat{Y}$) and mammary plasma flow rate. The maximal mammary uptake of plasma His was defined as mammary need for His, and further considered as dietary need of truly digestible His for milk production by the mammary glands. (2) Daily intake of dietary total His (X_i) would correspond to the entire requirement of dietary total His by the lactating sow, i.e., the sum of maintenance need and dietary need for milk production adjusted by the maternal body weight loss of this study. Thus, dietary need of truly digestible His for milk production could be derived by the factorial approach. A Bootstrapping method was employed to compute the confidence intervals (95% CI) for daily intake of dietary total His (Neter et al. 1996).

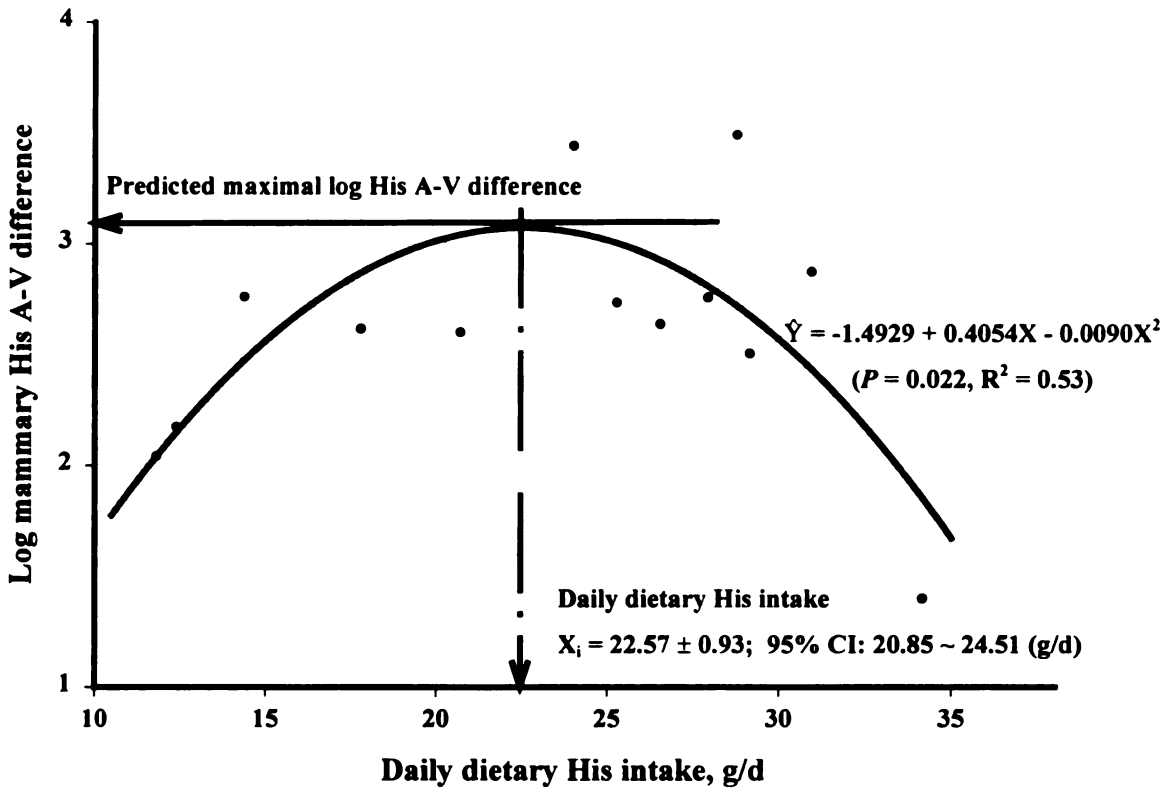

4
3
2
1
Log mammary His A-V difference
Predicted maximal log His A-V difference
Ŷ = -1.4929 + 0.4054X - 0.0090X²
(P = 0.022, R² = 0.53)
Daily dietary His intake
Xi = 22.57 ± 0.93; 95% CI: 20.85 ~ 24.51 (g/d)
10
15
20
25
30
35
Daily dietary His intake, g/d

FIGURE 3-2c The relation between log mammary A-V differences of plasma Lys ($\hat{Y}$) and daily intakes of dietary total Lys (X) over a 21-d lactation period. The curve was best fitted by a quadratic regression model: $\hat{Y} = 2.4104 + 0.0790X - 0.0011X^2$ ($P = 0.036$, $R^2 = 0.49$). Each dot represents an individual sow. The vertex (the maximal $\hat{Y}$, X_i) derived from this regression had two implications: (1) The maximal mammary uptake of plasma Lys could be quantified by multiplying the maximal mammary A-V difference of plasma Lys (i.e, the reverse log maximal $\hat{Y}$) and mammary plasma flow rate. The maximal mammary uptake of plasma Lys was defined as mammary need for Lys, and further considered as dietary need of truly digestible Lys for milk production by the mammary glands. (2) Daily intake of dietary total Lys (X_i) would correspond to the entire requirement of dietary total Lys by the lactating sow, i.e., the sum of maintenance need and dietary need for milk production adjusted by the maternal body weight loss of this study. Thus, dietary need of truly digestible Lys for milk production could be derived by the factorial approach. A Bootstrapping method was employed to compute the confidence intervals (95% CI) for daily intake of dietary total Lys (Neter et al. 1996).

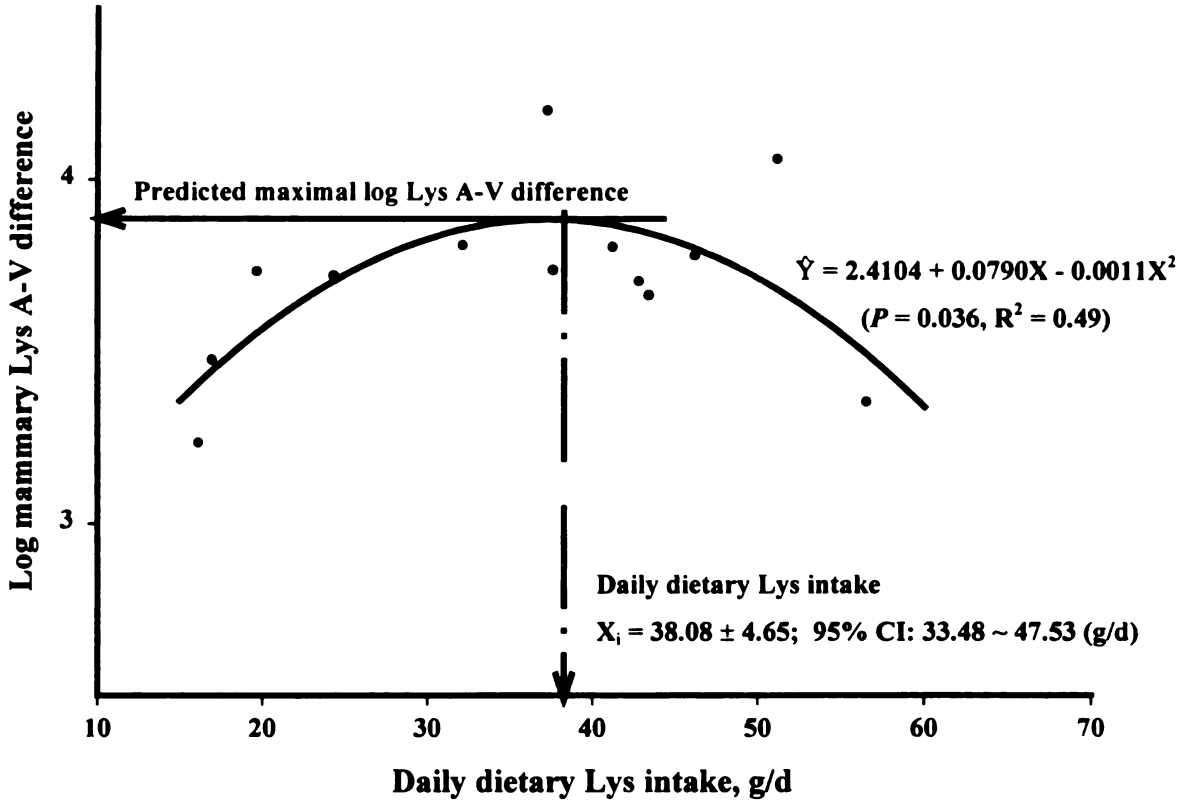

Log mammary Lys A-V difference
4
3
Predicted maximal log Lys A-V difference
$\hat{Y} = 2.4104 + 0.0790X - 0.0011X^2$
($P = 0.036$, $R^2 = 0.49$)
Daily dietary Lys intake
$X_i = 38.08 \pm 4.65$; 95% CI: 33.48 ~ 47.53 (g/d)
10
20
30
40
50
60
70
Daily dietary Lys intake, g/d

FIGURE 3-2d The relation between log mammary A-V differences of plasma Met ($\hat{Y}$) and daily intakes of dietary total Met (X) over a 21-d lactation period. The curve was best fitted by a quadratic regression model: $\hat{Y} = 0.7216 + 0.02710X - 0.0091X^2$ ($P = 0.015$, $R^2 = 0.60$). Each dot represents an individual sow. The vertex (the maximal $\hat{Y}$, X_i) derived from this regression had two implications: (1) The maximal mammary uptake of plasma Met could be quantified by multiplying the maximal mammary A-V difference of plasma Met (i.e, the reverse log maximal $\hat{Y}$) and mammary plasma flow rate. The maximal mammary uptake of plasma Met was defined as mammary need for Met, and further considered as dietary need of truly digestible Met for milk production by the mammary glands. (2) Daily intake of dietary total Met (X_i) would correspond to the entire requirement of dietary total Met by the lactating sow, i.e., the sum of maintenance need and dietary need for milk production adjusted by the maternal body weight loss of this study. Thus, dietary need of truly digestible Met for milk production could be derived by the factorial approach. A Bootstrapping method was employed to compute the confidence intervals (95% CI) for daily intake of dietary total Met (Neter et al. 1996).

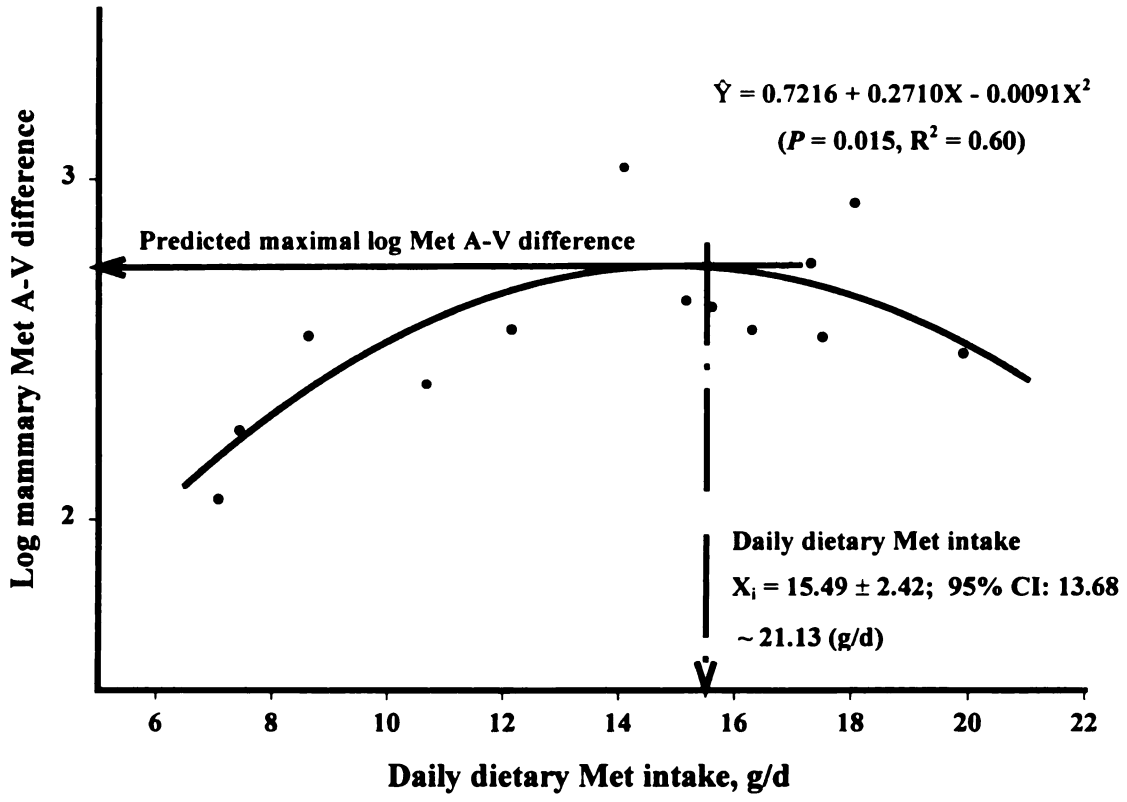

$\hat{Y} = 0.7216 + 0.2710X - 0.0091X^2$
($P = 0.015$, $R^2 = 0.60$)
Predicted maximal log Met A-V difference
Daily dietary Met intake
$X_i = 15.49 \pm 2.42$; 95% CI: 13.68
~ 21.13 (g/d)
Log mammary Met A-V difference
3
2
6
8
10
12
14
16
18
20
22
Daily dietary Met intake, g/d

FIGURE 3-2e The relation between log mammary A-V differences of plasma Phe ($\hat{Y}$) and daily intakes of dietary total Phe (X) over a 21-d lactation period. The curve was best fitted by a quadratic regression model: $\hat{Y} = 1.6599 + 0.0859X - 0.0012X^2$ ($P = 0.020$, $R^2 = 0.54$). Each dot represents an individual sow. The vertex (the maximal $\hat{Y}$, X_i) derived from this regression had two implications: (1) The maximal mammary uptake of plasma Phe could be quantified by multiplying the maximal mammary A-V difference of plasma Phe (i.e, the reverse log maximal $\hat{Y}$) and mammary plasma flow rate. The maximal mammary uptake of plasma Phe was defined as mammary need for Phe, and further considered as dietary need of truly digestible Phe for milk production by the mammary glands. (2) Daily intake of dietary total Phe (X_i) would correspond to the entire requirement of dietary total Phe by the lactating sow, i.e., the sum of maintenance need and dietary need for milk production adjusted by the maternal body weight loss of this study. Thus, dietary need of truly digestible Phe for milk production could be derived by the factorial approach. A Bootstrapping method was employed to compute the confidence intervals (95% CI) for daily intake of dietary total Phe (Neter et al. 1996).

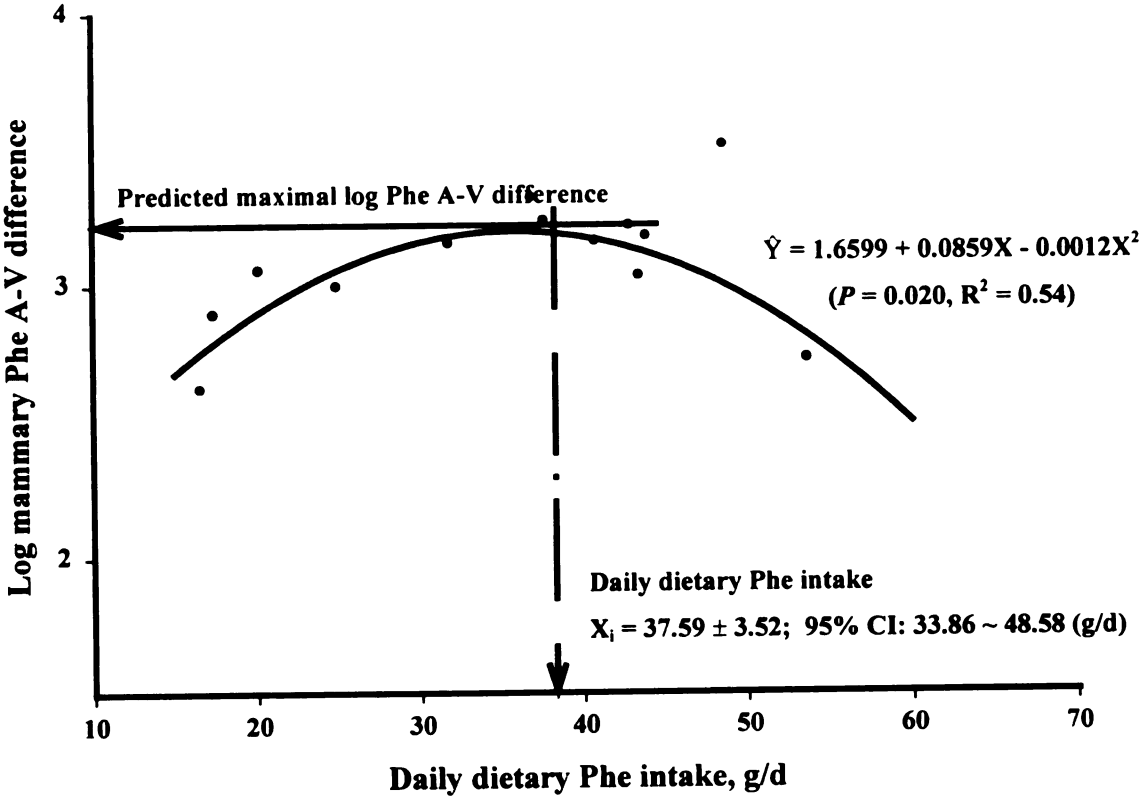

4
3
2
Log mammary Phe A-V difference
Predicted maximal log Phe A-V difference
$\hat{Y} = 1.6599 + 0.0859X - 0.0012X^2$
($P = 0.020$, $R^2 = 0.54$)
Daily dietary Phe intake
$X_i = 37.59 \pm 3.52$; 95% CI: 33.86 ~ 48.58 (g/d)
10
20
30
40
50
60
70
Daily dietary Phe intake, g/d

FIGURE 3-2f The relation between log mammary A-V differences of plasma Thr ($\hat{Y}$) and daily intakes of dietary total Thr (X) over a 21-d lactation period. The curve was best fitted by a quadratic regression model: $\hat{Y} = 0.7894 + 0.1284X - 0.0022X^2$ ($P = 0.027$, $R^2 = 0.55$). Each dot represents an individual sow. The vertex (the maximal $\hat{Y}$, X_i) derived from this regression had two implications: (1) The maximal mammary uptake of plasma Thr could be quantified by multiplying the maximal mammary A-V difference of plasma Thr (i.e, the reverse log maximal $\hat{Y}$) and mammary plasma flow rate. The maximal mammary uptake of plasma Thr was defined as mammary need for Thr, and further considered as dietary need of truly digestible Thr for milk production by the mammary glands. (2) Daily intake of dietary total Thr (X_i) would correspond to the entire requirement of dietary total Thr by the lactating sow, i.e., the sum of maintenance need and dietary need for milk production adjusted by the maternal body weight loss of this study. Thus, dietary need of truly digestible Thr for milk production could be derived by the factorial approach. A Bootstrapping method was employed to compute the confidence intervals (95% CI) for daily intake of dietary total Thr (Neter et al. 1996).

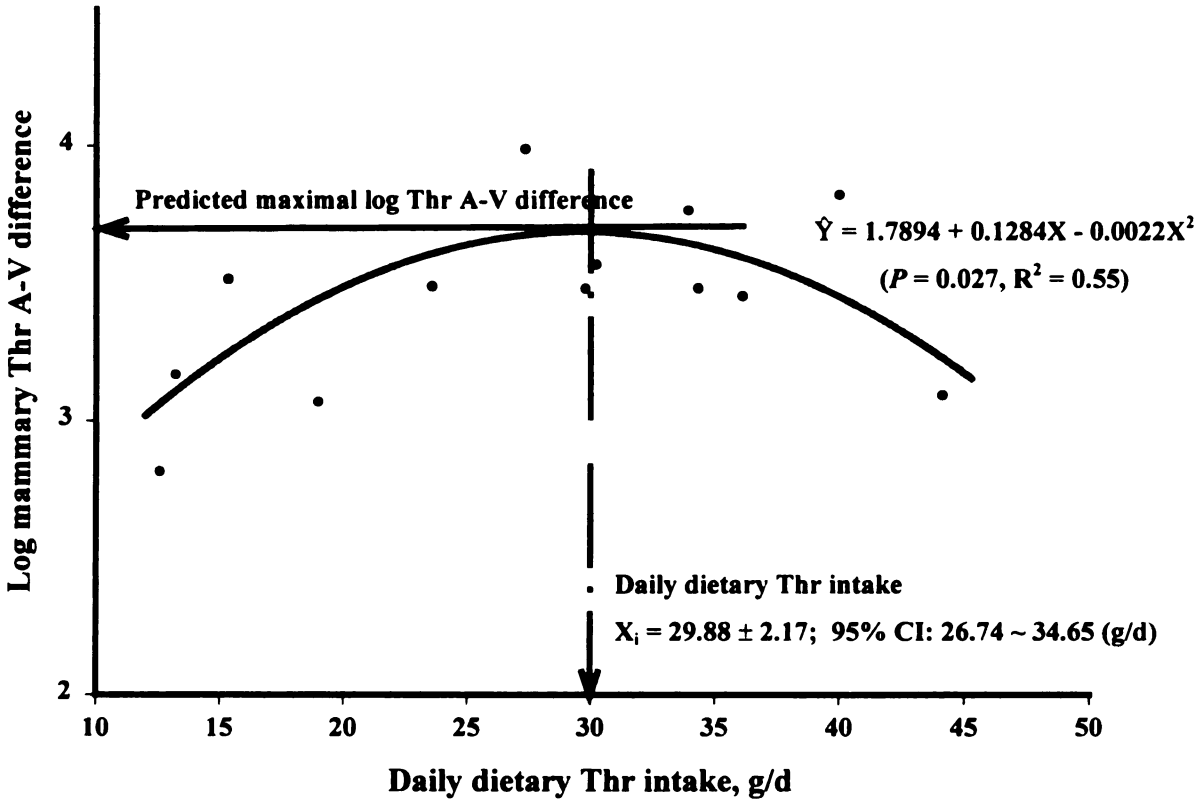

Log mammary Thr A-V difference
4
3
2
Predicted maximal log Thr A-V difference
Ŷ = 1.7894 + 0.1284X - 0.0022X²
(P = 0.027, R² = 0.55)
Daily dietary Thr intake
Xi = 29.88 ± 2.17; 95% CI: 26.74 ~ 34.65 (g/d)
10
15
20
25
30
35
40
45
50
Daily dietary Thr intake, g/d

FIGURE 3-2g The relation between log mammary A-V differences of plasma Trp ($\hat{Y}$) and daily intakes of dietary total Trp (X) over a 21-d lactation period. The curve was best fitted by a quadratic regression model: $\hat{Y} = -0.8173 + 0.6476X - 0.0373X^2$ ($P = 0.007$, $R^2 = 0.63$). Each dot represents an individual sow. The vertex (the maximal $\hat{Y}$, X_i) derived from this regression had two implications: (1) The maximal mammary uptake of plasma Trp could be quantified by multiplying the maximal mammary A-V difference of plasma Trp (i.e, the reverse log maximal $\hat{Y}$) and mammary plasma flow rate. The maximal mammary uptake of plasma Trp was defined as mammary need for Trp, and further considered as dietary need of truly digestible Trp for milk production by the mammary glands. (2) Daily intake of dietary total Trp (X_i) would correspond to the entire requirement of dietary total Trp by the lactating sow, i.e., the sum of maintenance need and dietary need for milk production adjusted by the maternal body weight loss of this study. Thus, dietary need of truly digestible Trp for milk production could be derived by the factorial approach. A Bootstrapping method was employed to compute the confidence intervals (95% CI) for daily intake of dietary total Trp (Neter et al. 1996).

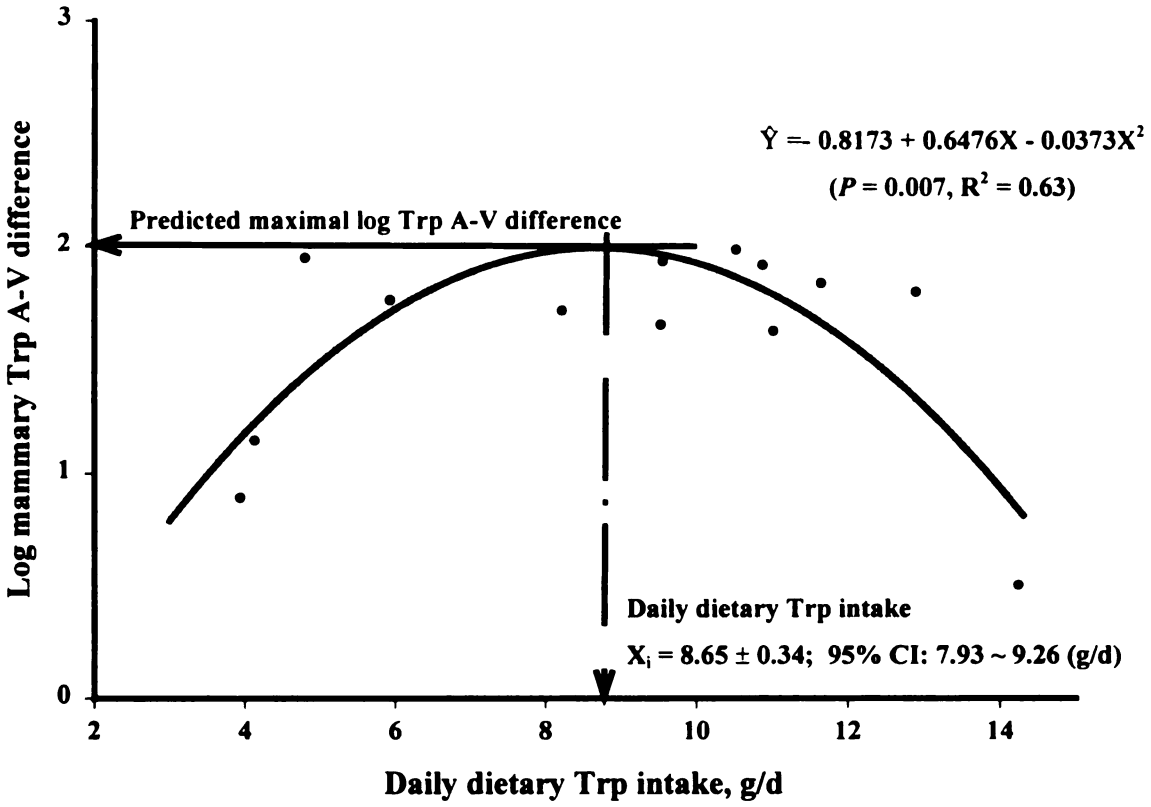

$\hat{Y} = -0.8173 + 0.6476X - 0.0373X^2$
($P = 0.007$, $R^2 = 0.63$)
Predicted maximal log Trp A-V difference
Daily dietary Trp intake
$X_i = 8.65 \pm 0.34$; 95% CI: 7.93 ~ 9.26 (g/d)
Log mammary Trp A-V difference
Daily dietary Trp intake, g/d
0
1
2
3
2
4
6
8
10
12
14

FIGURE 3-2h The relation between log mammary A-V differences of plasma Val ($\hat{Y}$) and daily intakes of dietary total Val (X) 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 (the maximal $\hat{Y}$, X_i) derived from this regression had two implications: (1) The maximal mammary uptake of plasma Val could be quantified by multiplying the maximal mammary A-V difference of plasma Val (i.e, the reverse log maximal $\hat{Y}$) and mammary plasma flow rate. The maximal mammary uptake of plasma Val was defined as mammary need for Val, and further considered as dietary need of truly digestible Val for milk production by the mammary glands. (2) Daily intake of dietary total Val (X_i) would correspond to the entire requirement of dietary total Val by the lactating sow, i.e., the sum of maintenance need and dietary need for milk production adjusted by the maternal body weight loss of this study. Thus, dietary need of truly digestible Val for milk production could be derived by the factorial approach. A Bootstrapping method was employed to compute the confidence intervals (95% CI) for daily intake of dietary total Val (Neter et al. 1996).

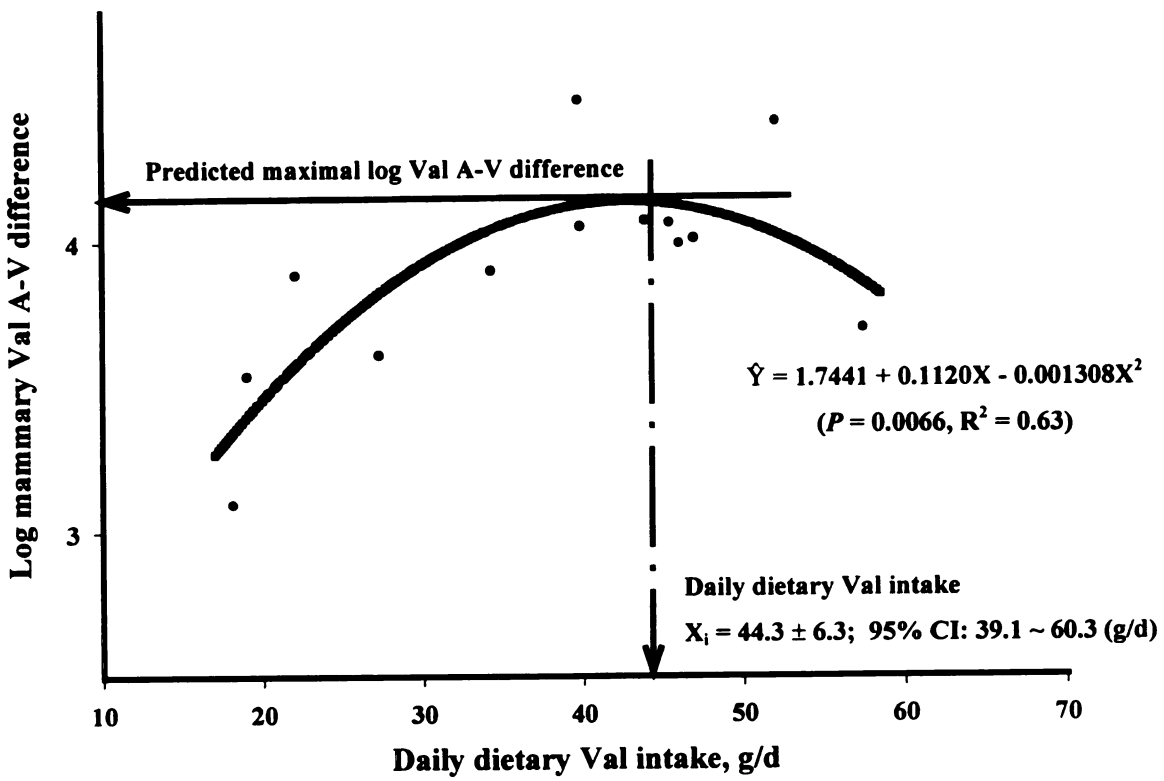
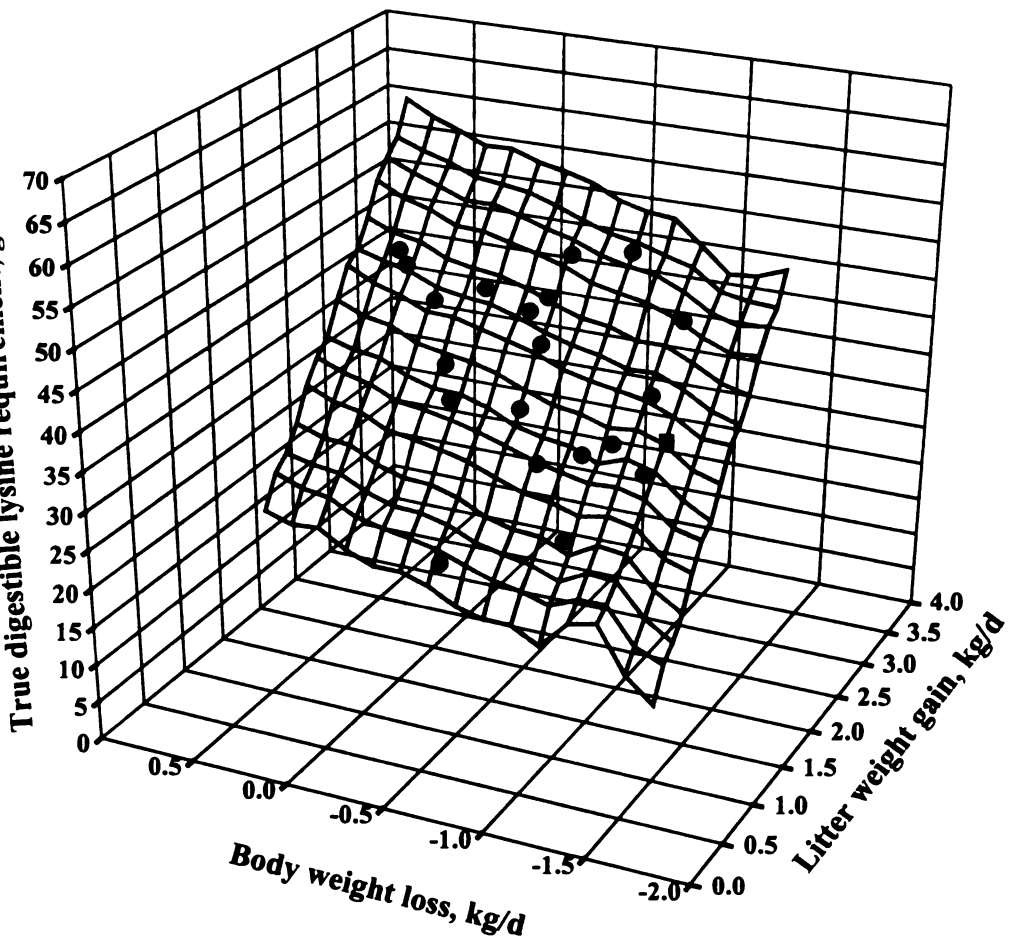

Predicted maximal log Val A-V difference
Log mammary Val A-V difference
4
3
$\hat{Y} = 1.7441 + 0.1120X - 0.001308X^2$
($P = 0.0066$, $R^2 = 0.63$)
Daily dietary Val intake
$X_i = 44.3 \pm 6.3$; 95% CI: 39.1 ~ 60.3 (g/d)
10
20
30
40
50
60
70
Daily dietary Val intake, g/d

FIGURE 3-3 A 3-D response surface of dietary truly digestible lysine requirements (Y) for 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 individual estimate of dietary truly digestible lysine requirement in the literature (Boomgaardt et al. 1972, Chen et al. 1978, Coma et al. 1996, Dourmad et al. 1998, Guan et al. 1998, Johnston et al. 1993, 1999, King and Dunkin 1986, King et al. 1993, Knabe et al. 1996, Kusina et al. 1999, Lewis and Speer 1973, Richert et al. 1997, Sauber et al. 1998, Touchette et al. 1998a, b, Wilkinson et al. 1982, Yang et al. 2000). Two estimates for high and low lean growth genotypes (Sauber et al. 1998) and three estimates for parity 1, 2, and 3 (Yang et al. 2000) are shown, respectively. Dietary lysine requirements are expressed on truly digestible basis as cited from the literature or following conversion from total basis (NRC 1998).



Predicted 3-D surface of dietary true digestible lysine requirements

● Estimated dietary true digestible lysine requirements in literature

■ Estimated dietary true digestible lysine requirement in this study

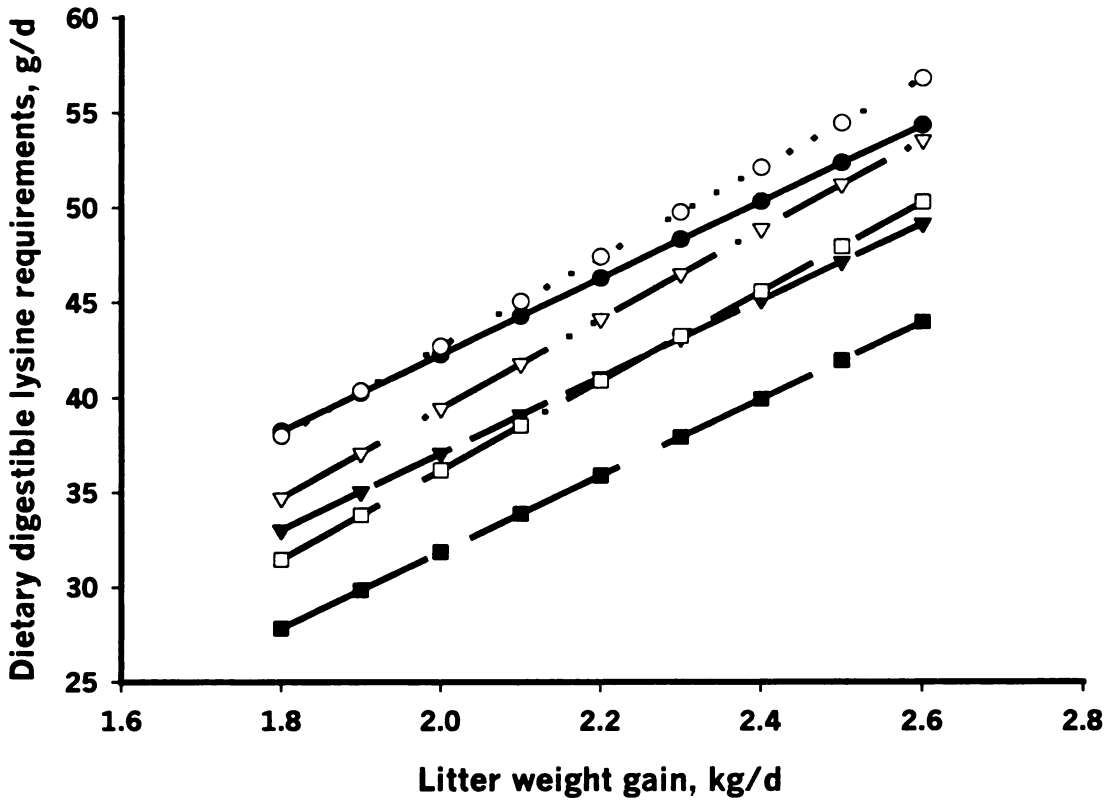


FIGURE 3-4 Dietary truly digestible lysine requirements of lactating sows derived from the maximal mammary uptake model and the NRC model (1998) at different litter weight gains and body weight losses over a 21-d lactation period. Lines with solid symbols were fitted by the maximal mammary uptake model. Lines with empty symbols were fitted by the NRC model. Lines with circles, triangles, and squares represent 0, 0.5, and 1.0 kg/d of body weight losses, respectively.

TABLE 3-1

Regression of log mammary A-V differences of plasma IAA against daily intakes of dietary total IAA over a 21-d lactation period[1]

AA	Parameter estimate			R^2	*P* value			
	_0	−1	_2		Model	_0	−1	_2
Arg	1.2767	0.0992	-0.0009	0.64	0.006	0.055	0.004	0.007
His	-1.4629	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	1.7894	0.1284	-0.0022	0.55	0.027	0.007	0.012	0.018
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: ↗ = $\beta_0 + \beta_1 X + \beta_2 X^2$. Where β_0, β_1, and β_2 are parameter estimates, ↗ and X are the log mammary A-V differences of plasma IAA (the predicted variable) and daily intakes of dietary total IAA (the predictor variable), respectively.

TABLE 3-2

Dietary needs of TDIAA for milk production predicted at the maximal mammary uptake of plasma IAA

Amino acid	Max. $\hat{Y}$[1]	Max. A-V difference[2]	Max. mammary uptake[3]		Milk production need[4]
		μmol/L	mmol/d	g/d	g/kg LWG
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	3.663	39.0	251.0	29.90	13.91
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 maximal $\hat{Y} = \beta_0 - \beta_1^2/4\beta_2$, where β_0, β_1, and β_2 are the intercept, the linear coefficient, and the quadratic coefficient in the regression, respectively (See **TABLE 3-1**).

[2] Maximal mammary A-V difference of plasma IAA (μmol/L) = the reverse log maximal $\hat{Y}$.

[3] Maximal mammary uptake of plasma IAA (mmol/d) = the maximal mammary A-V difference of plasma IAA (μmol/L) x mammary plasma flow rate (6440 L/d) x 10^{-3}(Guan et al. 2000). Maximal mammary uptake in mass (g/d) = maximal mammary uptake (mmol/d) x molecular weight of the respective amino acid (g/mol) x 10^{-3}.

[4] Milk production need is obtained by the maximal mammary uptake (g/d) adjusted for litter weight gain (LWG) of 2.15 kg/d over a 21-d lactation period.

TABLE 3-3

Daily intake of dietary total IAA (g/d) predicted at the maximal log mammary A-V difference of plasma IAA over a 21-d lactation period[1]

Amino acid	Mean ± SD	95% CI[2]
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[3]	8.65 ± 0.34	7.93 ~ 9.26
Val	44.29 ± 6.30	39.05 ~ 60.27

[1] Daily intakes of dietary total IAA (X, g/d) were predicted at the maximal log mammary A-V difference of plasma IAA ($\hat{Y}$) based on the regressions in **TABLE 3-1**. When $d\hat{Y}/dX = 0$, i.e., $X = -\beta_1/\beta_2$, $\hat{Y}$ reaches its maximal value since $\beta_2 < 0$.

[2] Confidence intervals (95% CI) for daily intakes of dietary total IAA were computed using the Bootstrapping reflection method with Bootstrapping sample size of 750.

TABLE 3-4

Dietary needs of TDIAA for milk production derived by the factorial approach

Amino acid	Maintenance need[1] g/d	Contribution of endogenous IAA [2] g/d	Intake of TDIAA [3] g/d	Milk production need[4]	
				g/d	g/kg LWG
Arg	0	10.92	49.48	62.37	29.01
His	0.63	4.68	20.21	25.10	11.68
Lys	1.96	10.40	33.07	43.38	20.18
Met	0.55	2.35	14.04	16.27	7.57
Phe	0.98	5.88	33.60	39.55	18.40
Thr	2.96	6.36	25.45	30.00	13.95
Trp	0.51	1.24	7.65	8.60	4.00
Val	1.31	6.20	38.84	44.84	20.86

[1] Based on average body weight of 206 kg over a 21-d lactation period (NRC 1998).

[2] Estimated by Guan et al. (2000) except for endogenous Arg, His, and Trp contributions which were calculated by multiplying endogenous lysine contribution and the ratio of Arg, His, and Trp to Lys in body tissue, respectively (NRC 1998).

[3] Dietary intakes of TDIAA were converted from dietary intakes of total IAA (in **TABLE 3-3**) based on the regressions of dietary TDIAA against dietary total IAA (NRC 1998).

[4] Dietary needs of TDIAA for milk production were derived by the factorial approach: daily intake of TDIAA = maintenance need + milk production need – the contribution of endogenous IAA. Lactating sows had an average body weight loss of 1.18 kg/d and litter weight gain of 2.15 kg/d over a 21-d lactation period. Milk production needs were adjusted for litter weight gain.

TABLE 3-5

Ideal ratios of other IAA to lysine for maintenance, milk synthesis, and endogenous contribution

Amino acid	Maintenance	Endogenous contribution		Milk synthesis	
	NRC (1998)	Guan et al. (2000)	NRC (1998)	Maximal uptake	NRC (1998)
Lys	1.00	1.00	1.00	1.00	1.00
Arg	0	-[1]	1.05	1.43	0.66
His	0.32	-	0.45	0.50	0.40
Met	0.28	0.23	0.27	0.34	0.26
Phe	0.50	0.56	0.60	0.60	0.55
Thr	1.51	0.61	0.58	0.69	0.58
Trp	0.26	-	0.10	0.22	0.18
Val	0.67	0.60	0.69	1.11	0.85

[1] not estimated.

CHAPTER 4

Effects of Dietary Lysine Deficiency or Valine Excess on Trans-membrane Transport of Indispensable Amino Acids and Intracellular Protein Synthesis and Breakdown in the Lactating Porcine Mammary Gland

ABSTRACT The objectives in the study were to evaluate if dietary lysine deficiency or valine excess affect trans-membrane transport of indispensable amino acids (lysine, methionine, and valine) and intracellular protein synthesis and breakdown in the porcine mammary gland during lactation. From d 1 of lactation, nine Landrace x Yorkshire lactating sows were provided ad libitum access to one of three diets which varied only in lysine and valine contents (g/kg diet): lysine-deficient (LD; 4.93 vs 9.87), positive control (PC; 9.71 vs 10.15), and valine-excess (VE; 9.76 vs 13.37). Dietary methionine content was kept constant (3.26 g/kg diet). Permanent catheters were inserted into a jugular vein, a carotid artery, and the anterior main mammary vein on d 9 ± 1 of lactation. On d 18 of lactation, a solution containing [2-^{15}N]-L-lysine•HCl, [S-methyl-$^{2}H_3$]-L-methionine, and [1-^{13}C]-L-valine was infused continuously into the jugular vein for 20.5 h. Matched sets of carotid arterial and mammary venous blood samples were obtained at 3-h intervals from 0 to 12 h, and at 1-h intervals from 14 to 20 h. An individual anterior gland was milked-out completely by hand at 2-h intervals from 12.5 to 20.5 h for measurement of milk protein content and casein-bound amino acid enrichment. Pre-planned comparisons were made between the PC diet versus LD and VE diets. Milk yield, milk protein

content, and litter weight gain were reduced ($P < 0.05$) by LD whereas the effects of VE were not significant. The whole body plasma flux of lysine was lower ($P < 0.01$) due to LD, and the whole body plasma flux of valine was higher ($P < 0.05$) due to VE. The LD diet decreased ($P < 0.05$) mammary net uptake of lysine and methionine as a result of a decrease ($P < 0.05$) in inward (Fmg,a) and outward (Fv,mg) trans-membrane transport rate for lysine and a decrease ($P < 0.10$) in Fmg,a for methionine. Although Fmg,a and Fv,mg for valine were decreased in the VE, net uptake of valine was not affected. The VE diet reduced the net uptake of lysine. Estimated rates of mammary protein synthesis and breakdown were decreased on the LD diet, which decreased net protein synthesis (secretion plus constitutive protein) by the gland. Protein breakdown was decreased to a greater extent than protein synthesis in the VE, resulting in comparable net protein synthesis compared with the PC. Model-derived estimates of mammary net protein synthesis were not different from the direct measurements of mammary synthesized milk protein output and net accretion. The proportion of protein breakdown to protein synthesis was decreased in the VE, but was not affected in the LD. The partition of valine within the mammary gland toward protein synthesis (66%) compared to oxidation (34%) was not affected by dietary AA regime. In conclusion, trans-membrane transport of lysine, methionine, and valine and the intracellular protein turnover in the porcine mammary gland were regulated by dietary AA regime.

KEY WORDS: • uptake • protein metabolism • mammary gland • pig • lactation • lysine deficiency • valine excess

INTRODUCTION

The lactating mammary gland has a large demand for amino acids (AA) to meet the high rate of milk protein synthesis. Dietary needs of indispensable AA for milk synthesis by the mammary gland account for more than 95% of the total dietary needs of the lactating sow under zero change of body protein (Guan et al. 2000c, NRC 1998). The transport of certain AA by the mammary epithelial cells could be rate-limiting for the synthesis of milk protein (Gomez Angelats et al. 1995, Mepham 1982). The transport of AA from blood into the mammary gland is mediated by AA transport systems across the basolateral membranes of the mammary epithelium (Shennan and Peaker 2000). Most mammalian cells have both ubiquitous AA transport systems (e.g., A, ASC, L, and y^+) and tissue-specific AA transport systems (e.g., $b^{0,+}$ and $B^{0,+}$) (**TABLE 4-1** and **5-2**) (Deves and Boyd 1998, Palacin et al. 1998). Each AA transport system is distinct but exhibits overlapping substrate specificity (Kilberg et al. 1993). This elaborate arrangement of AA transport systems on the plasma membranes permits fine regulation of intracellular availability, cellular influx and efflux, and interorgan flux of AA (Christensen 1990).

Amino acid transport systems in the basolateral membrane are up-regulated by lactogenic hormones (cortisol and prolactin) at the onset of lactation to increase uptake of AA by the mammary gland for high rate of milk protein synthesis (Rillema et al. 1992, Sharma and Kansal 1999, Sharma and Kansal 2000). Furthermore, the transport of AA across plasma membranes is influenced by mass effects (such as substrate saturation, analogue competition, trans-stimulation, and trans-inhibition) (Christensen 1982). The endogenous

$B^{0,+}$ transporter of *Xenopus* oocytes is adaptively regulated (i.e., derepression and repression with deprivation and supplementation of AA) (Taylor et al. 1996). The transport of lysine across the intestinal brush border membrane (epithelium) adapts to intake of dietary protein (Wolffram and Scharrer 1984), suggesting that the uptake of lysine by the mammary epithelium may be regulated by intake of dietary lysine.

The transport of cationic AA (arginine, lysine, and ornithine) across the mammary tissue is carried out at least by two AA transport systems: one is specific for the cationic AA (system y^+) and the other is shared with the cationic and neutral AA (system $b^{0,+}$, $B^{0,+}$, or y^+L). These AA transport systems are situated in the basolateral membrane of the lactating (bovine, mouse, and rat) mammary epithelium (Baumrucker 1984, Calvert and Shennan 1996, Sharma and Kansal 2000). Thus, lysine uptake by the lactating mammary gland can be inhibited in vitro by high concentrations of neutral AA (e.g., leucine) (Hurley et al. 2000, Shennan et al. 1994, Shennan et al. 1997). A Na^+-dependent cationic and neutral AA transporter ($hATB^{0,+}$) has been cloned from the human mammary gland. When expressed in Xenopus oocytes, $hATB^{0,+}$ transports both cationic and neutral AA; and its kinetics characteristics are consistent with system $B^{0,+}$ (Sloan and Mager 1999). The evidence suggests that uptake of plasma lysine by the mammary gland in vivo may be reduced by neutral AA.

The transport of branched-chain AA (isoleucine, leucine, and valine, BCAA) is mediated at least by system L (situated in the basolateral membrane of the mammary epithelium) as demonstrated in lactating bovine, guinea-pig, mouse, porcine, and rat mammary tissue

(Jackson et al. 2000, Kansal and Kansal 1996, Shennan et al. 1997, Shennan and Peaker 2000). The transport system for valine in porcine mammary gland has a K_m of 640 μM (Jackson et al. 2000), implying that the transport of valine by the mammary gland might be saturated under physiological concentrations of plasma valine (~ 600 μM). The uptake of valine by the lactating porcine mammary gland is inhibited in vitro by high concentrations of lysine, suggesting an interaction in the transport between the cationic AA and BCAA (Jackson et al. 2000).

The uptake of methionine by the mammary gland is mediated by multiple AA transport systems A, ASC, and L, having a K_m at ~ 460 μM for mouse mammary tissue (Baumrucker 1985, Shennan and Peaker 2000). Moreover, the transport of L-methionine across the apical membrane of the chicken jejunum is carried out by four systems ($b^{0,+}$, y^+m, L, and B^o) (Soriano Garcia et al. 1998). Thus, transport of methionine across the plasma membranes may be regulated by the cationic and neutral AA because they share AA transport systems (e.g., ASC, L, B^0, and $B^{0,+}$) (Soriano Garcia et al. 1998, Utsunomiya Tate et al. 1996). Dietary excess of L-methionine (4 g/kg diet) down-regulates uptake of L-methionine across the apical membrane of the brush-border membrane to reduce the risk of toxicity of L-methionine (Soriano Garcia et al. 1999). In addition, methionine can be deaminated to α-keto-γ-methiolbutyric acid, which is further decarboxylated by branched-chain α-keto acid dehydrogenase (Dixon and Benevenga 1980, Mitchell and Benevenga 1978). Oxidation of BCAA may be up-regulated by high intake of protein or BCAA through increased expression of branched-chain aminotransferase and/or increased activity of branched-chain α-keto acid dehydrogenase

(Langer et al. 2000, Torres et al. 1998). In this connection, dietary valine may affect metabolism of methionine as suggested by Langer et al. (2000). Effects of dietary AA regime on trans-membrane transport of individual indispensable AA and their intracellular kinetics are not well understood in the lactating porcine mammary gland in vivo.

There is substantial protein synthesis and breakdown in the lactating goat mammary gland (Oddy et al. 1988). Milk protein synthesis accounts for 30 to 80% of the total synthesis in the mammary gland (Bequette et al. 1998). This high rate of protein turnover would help to maintain mammary metabolism and functions by sustaining structural and regulatory proteins (Bequette et al. 1998). Mammary protein mass is determined by net balance between protein synthesis and protein breakdown. Increased protein synthesis and/or decreased protein breakdown would increase net balance of mammary protein, thus leading to an increase in output of protein in milk. However, dietary effects on protein synthesis and breakdown in the lactating porcine mammary gland have not been studied.

Dietary ratio of indispensable AA is important to maximize milk synthesis and efficiency in the lactating sow. The lactating porcine mammary gland takes up branched-chain AA in excess of their output in milk (Guan et al. 2000b, Nielsen et al. 2000, Trottier et al. 1997), questioning whether dietary ratio of valine to lysine required for milk synthesis may be underestimated when only based on their ratio in milk. The data are equivocal with regards to the optimal ratio of valine to lysine required for the lactating sow. For

example, increasing dietary ratio of valine to lysine (up to 1.20:1) increased milk yield and litter growth rate (Moser et al. 2000, Richert et al. 1997, Richert et al. 1996, Rousselow and Speer 1980); where in another study increasing the ratio to 1.22:1 did not improve lactational performance (Carter et al. 2000). Thus, an optimal dietary ratio of valine to lysine for the lactating sow remains to be determined.

Taken together, supplementation of synthetic AA may cause imbalance in their uptake at the tissue level, resulting in a decrease in their utilization. We hypothesize that dietary AA regime affects net uptake of individual AA by the mammary gland through fine regulation of unidirectional trans-membrane transport of individual AA, and further affects kinetics of the mammary intracellular free AA. The objectives of the present study were to evaluate effects of dietary lysine deficiency or valine excess on trans-membrane transport of indispensable AA (lysine, methionine, and valine) and on intracellular protein synthesis, breakdown, and net protein synthesis in the lactating mammary gland. A kinetic model previously developed for the forearm of humans (Biolo et al. 1995), and recently adapted to the goat mammary gland (Bequette et al. 2000) was used to monitor in vivo changes in inward and outward fluxes of AA by the porcine mammary gland. The present study was the first in vivo to measure the rates of inward and outward trans-membrane transport of AA under their deficiency and excess; to estimate protein synthesis, protein breakdown, and oxidation of AA; and to describe quantitatively AA transport by the porcine mammary gland during lactation. This information provides insight into AA interactions on net uptake across the mammary

gland that may further influence the intracellular protein metabolism; and defines the optimal ratio of valine to lysine for milk synthesis by the mammary gland.

MATERIALS AND METHODS

Michigan State University All-University Committee on Animal Use and Care approved all procedures in this study.

Animals and diets. Nine Landrace x Yorkshire lactating sows (parity 2, an average body wt of 212.6 ± 12.0 kg on d 1 of lactation) were allocated to dietary AA regimes according to a randomized block design. Each block consisted of three sows. Each sow in one block was provided ad libitum access to one of three dietary AA regimes from d 1 to 21 of lactation. The three dietary AA regimes contained the same concentrations of metabolizable energy, calcium, available phosphorus, and indispensable AA other than lysine and valine. Dietary ratio of indispensable to dispensable AA-N (1.00:1.00) remained constant. The composition of the diets is given in **TABLE 4-3**. The lysine-deficient diet contained 50% of lysine content in the positive control and valine-excess diets. Dietary ratios of valine to lysine were at 1.05:1.00 and 1.37:1.00, respectively, for the positive control and valine-excess diets. Litters were cross-fostered within 48 h after birth to ensure 12 piglets per sow. Sows were housed in individual farrowing crates in a

thermally controlled room (21 °C). The sows were fed twice daily to appetite and provided free access to water. Sow food intake was recorded daily. Milk yield on d 21 was estimated by the weigh-suckle-weigh method (Pettigrew et al. 1985). Piglets were individually weighed on d 1 and 21.

Cannulation. Catheters were constructed from microbore tubing (Tygon®, ID 1.0 mm, OD 1.8 mm, Norton Performance Plastics Corp., Akron, OH) and the lumen coated with triododecylmethylammonium chloride - heparin complex (Polysciences Inc., Warrington, PA). A jugular vein, the anterior main mammary vein, and A carotid artery were cannulated in sows on d 9 ± 1 of lactation as described by Trottier et al. (1995). Halothane (1.5%) was administered as the anesthetic agent via the nasal/tracheal tube during all surgeries. Antibiotic (Naxcel®, Pharmacia and Upjohn Co., Kalamazoo, MI) and anti-inflammatory medicine (Banamine®, Schering-Plough Animal Health Corp., Kenilmorth, NJ) were administered i.v. for the first three days post surgery. The catheters were flushed daily with heparinized sterile saline (20 IU heparin/ml). Food was offered in a stair step manner during the first three days post surgery, and was available free access thereafter.

Protocol. Sows were provided ad libitum access to food and water as usual on d 18 of lactation when a mixture of stable isotope labeled AA was infused via the jugular vein for measurement of AA transport and metabolism by the mammary gland. Prior to the i.v. infusion, a blood and milk sample were collected to obtain a measurement of ^{13}C, ^{2}H, or ^{15}N natural abundance (background). In a previous study, the enrichment of plasma free

leucine and casein-bound leucine was found to reach a plateau at 12 h of a non-primed, continuous isotope infusion in the lactating sow (Hoffman et al. 1997). So the infusion herein was extended to 20.5 h to allow repeated measurements over the period of plateau. The infusion rates of labeled AA were at 24.2, 5.2, and 17.0 μmol/min, respectively, for [2-^{15}N]-L-lysine•HCl, [S-methyl-$^{2}H_3$]-L-methionine, and [1-^{13}C]-L-valine. All isotopes were purchased from Mass Trace (Woburn, MA) and 99 atoms of ^{13}C, ^{2}H, or ^{15}N. All solutions were prepared in sterile saline (0.9% wt/vol) and cold sterilized through an in-line filter (Nalgene® with surfactant-free cellulose acetate membrane of 0.20 μm pore size, Nalge Nunc Int. Corp., Rochester, NY). The infusion flow rate was controlled with a peristaltic pump (Minipuls 3, Gilson Medical Electronics, Middleton, WI). Matched sets of carotid arterial and mammary venous blood samples were withdrawn simultaneously into sterile syringes at 3-h intervals from 0 to 12 h of infusion and at 1-h intervals from 14 to 20 h of infusion. Blood was transferred into Li-heparin-coated tubes and centrifuged at 1500 x g for 15 min at 4 °C. Plasma was removed and stored at -20 °C. After an i.v. dose of oxytocin (10 IU), a single anterior gland (the 3rd on sow's right lateral side) was milked-out by hand at 2-h intervals from 12.5 to 20.5 of infusion; otherwise, this gland was suckled by its piglet. Milk samples were defatted by centrifugation at 1500 x g for 15 min at 4 °C and stored at -20 °C.

Analytical techniques

(1) Concentrations of nitrogen in food and milk. Food samples were finely ground using a sample mill (Cyclotec® 1093, Foss Tecator, Sweden). Concentrations of total N in food, defatted milk, and casein were determined in a nitrogen analyzer (FP-2000,

LECO Corp., St. Joseph, MI) using EDTA (Sigma, St. Louis, MO) as a calibration standard. The concentration of total N in defatted milk was converted to true protein in milk as follows:

True protein in milk (%) = Total N in defatted milk (%) x (100 - the concentration of lipid in milk) x 6.38 x (1 - 0.15) x 10^{-2}

Where the concentration of total N in defatted milk was corrected by the concentration of lipid in milk to obtain the concentration of total N in milk. The concentration of lipid in milk was assayed using a mid-infrared spectroscope (Multispec M, Berwind Instrument Ltd., York, England, UK). The concentration of crude protein (total N x 6.38) in milk was corrected by the proportion of non-protein N to obtain the concentration of true protein in milk. The proportion of non-protein N was assumed to be 15% of the total N in porcine milk (Bourne and Curtis 1973, Klobasa and Butler 1987, Klobasa et al. 1987).

(2) Concentrations of amino acids in food and milk. Concentrations of AA in food and milk were analyzed by reverse-phase HPLC with pre-column phenylisothiocyanate derivatization (Pico•Tag®, Waters Corp., Milford, MA). In brief, food or milk samples were hydrolyzed in 6 M HCl at 110 °C for 24 h. Amino acids in the hydrolysates were derivatized with phenylisothiocyanate (Pierce, Rockford, IL) and separated on a Pico•Tag® column (3.9 mm x 150 mm) and detected at 254 nm on a tenable absorbance detector (Waters™ 486, Waters Corp., Milford, MA). Norleucine (Sigma, St. Louis, MO) as an internal standard was added into food samples before hydrolysis. Amino acid standard H (Pierce, Rockford, IL) was used as a calibration standard. The method was validated with certified AA standard (NIST, Gaithersburg, MD).

(3) Enrichment of casein-bound and plasma free amino acids. Casein in defatted milk was precipitated at room temperature by adjusting the pH to 4.60 with 1.0 M HCl, and centrifuged at 1500 x g for 15 min at 4 °C. Precipitated casein pellets were washed twice with distilled water, solubilized at pH 7.0, freeze-dried, and stored at -20 °C. Casein was hydrolyzed in 6 M HCl (containing 0.05% (wt/vol) dithiothreitol) at 110 °C for 18 h. The hydrolysate was desalted with cation-ion exchange resin (AG®, 50W-X8, H^+-form, Bio-Rad Laboratories, Hercules, CA) and eluted with 2 M NH_4OH. Frozen plasma were thawed at 4 °C, deproteinized with 5-sulfosalicylic acid by centrifugation at 1500 x g for 15 min at 4 °C, and desalted as above. Isolated AA from casein and plasma were freeze-dried and derivatized with methyl-t-butyldimethylsilyl trifluoroacetamide to form their MTBMS-derivatives. Isotopic enrichments were determined by electron-impact on gas chromatography-mass spectrometry (Trio-1, VG Masslab., Manchester, UK) as described by Bequette et al. (1994). Because of a small (~ 3%) contamination of the labeled L-lysine with D-lysine, chiral separation was necessary (Lobley et al. 1996). All enrichments were expressed as molar percent excess (MPE) with respect to the pre-infusion natural abundance of the casein-bound or free AA.

(4) Concentrations of plasma amino acids. The curves of plasma AA enrichments over the isotope infusion time showed that their plateaus occurred by approximately 12 h post-infusion. Thus, plasma samples from 15 to 20 h of infusion from each sow were pooled for plasma AA assay. Glucosaminic acid as an internal standard was added into the pooled samples. The concentrations of plasma AA were determined using a Beckman

6300 Amino Acid Analyzer. In brief, proteins in plasma were precipitated by 5-sulfosalicylic acid. Amino acids in the deproteinized supernatant were separated by a Beckman cation-ion exchange column charged in Li citrate buffer. The eluted AA were measured spectrophotometrically following post-column derivatization with ninhydrin.

Mammary plasma flow estimation. Mammary plasma flow rate was calculated by the Fick principle based on the assumption that output of phenylalanine and tyrosine (FY) in milk equates to their mammary uptake (Cant et al. 1993). Thus,

Mammary uptake of plasma FY = mammary A-V difference in concentrations of plasma FY x mammary plasma flow

= Output of FY in milk + the amount of FY metabolized in the mammary gland (1)

There might be some factors involved in the balance between mammary uptake of plasma FY and their output in milk. (1) The amount of FY metabolized in the mammary gland is negligible. As demonstrated in the guinea pig mammary tissue, the activity of phenylalanine hydroxylation is insignificant (Davis and Mepham 1976). Phenylalanine is not oxidized in the lactating goat mammary gland (Verbeke et al. 1972). In addition, the amount of FY accretion in the lactating porcine mammary gland is approximately 1 g/d (Kim et al. 1999), which is negligible compared to their output in milk (50 g/d) in the present study. (2) Approximately 5% of the total proteins in milk is derived from non-mammary synthesized proteins (Bourne and Curtis 1973, Klobasa and Butler 1987, Klobasa et al. 1987). (3) The contribution of vascular peptide-bound FY to mammary synthesized proteins might be negligible because 96% of casein-bound phenylalanine

originates from plasma phenylalanine (Verbeke et al. 1972). (4) Losses of FY from the mammary gland via the lymph are negligible as is output of free FY in milk (Linzell 1974). The proportion of free FY is approximately 0.5% of their peptide-bound residues in porcine milk (Wu and Knabe 1994). Therefore, Eq.1 was simplified as follows:

Mammary uptake of plasma FY = mammary A-V difference in concentrations of plasma FY x mammary plasma flow

= Output of FY in milk x (1 – 0.05), i.e.,

Mammary plasma flow rate (L/h) = Concentrations of FY in milk (mmol/L) x milk yield (L/h) x (1 – 0.05) x 10^3 / mammary A-V difference of plasma FY (μmol/L) (2)

Calculations.

The whole body irreversible loss rate (Fwb, See the Appendix) of plasma individual AA was calculated from average arterial enrichment over the last 5 h of the isotope infusion period when the enrichment reached a quasi-isotopic plateau (**FIGURE 4-1a** to **1i**):

$I \times Ei = Fwb \times Ea + I \times Ea$, i.e.,

$$Fwb = (Ei/Ea - 1) \times I \quad (3)$$

Where Ea and Ei are the enrichments of plasma free AA in the carotid artery and AA in the mixture of labeled AA, respectively, and where I is the rate of infusion (see ***Protocol***).

The unidirectional flux (gross) of plasma free AA from mammary artery to the mammary gland (Fmg) was calculated from labeled AA (tracer) and then converted to its tracee:

$$Fmg\ (mmol/h) = (Ca \times Ea - Cv \times Ev) \times MPF \times 10^{-3}/Ea \quad (4)$$

Where Ca and Cv are the concentrations (μmol/L) of plasma AA in the carotid artery and the main mammary vein, respectively. The concentrations of plasma AA in the carotid artery were presumably the same as in the mammary artery. Ev is an average enrichment of plasma free AA in the main mammary vein (over the last 5 h of the infusion). MPF is the plasma flow rate (L/h) across the mammary gland estimated by the Fick principle.

(1) Trans-membrane transport of free amino acids in the mammary gland. Trans-membrane transport and intracellular kinetics of free AA in the lactating porcine mammary gland were derived from a three-compartmental model (**FIGURE 4-2**) originally based on the model of Biolo et al. (1995) for measurements of AA trans-membrane transport across human leg, but modified for the lactating goat mammary gland (Bequette et al. 2000). The model assumes that AA enter and leave the mammary gland via the mammary artery (Fa,o) and the main mammary vein (Fo,v), respectively. Fmg,a and Fv,mg refer to the net movements of free AA from mammary artery to mammary intracellular free AA compartment and from this compartment to mammary vein, i.e., inward and outward trans-membrane transport, respectively. Fv,a refers to direct flow of AA from artery to vein which does not enter intracellular compartment. Fmg,o refers to the rate of mammary intracellular free AA appearance from endogenous sources (i.e., release from protein breakdown (PB) and de novo synthesis (DS), if any). Fo,mg refers to the rate of mammary intracellular free AA disappearance (i.e., the rate of utilization of the intracellular free AA for protein synthesis (PS), oxidation (OX), and other metabolic fates (OM), if any). Fa,o and Fo,v were calculated as follows:

$$Fa,o\ (mmol/h) = Ca \times MPF \times 10^{-3} \quad (5)$$

$$Fo,v\ (mmol/h) = Cv \times MPF \times 10^{-3} \quad (6)$$

Net mass uptake (NB) of AA (including labeled and unlabeled AA) across the mammary gland was determined by the difference between Fa,o and Fo,v:

NB (mmol/h) = Fa,o - Fo,v, i.e.,

$$NB\ (mmol/h) = (Ca - Cv) \times MPF \times 10^{-3} \quad (7)$$

Inward (Fmg,a) and outward (Fv,mg) transport rates can be calculated if the intracellular enrichment of AA is known. This measure is usually obtained by biopsy, for example, the muscle (Biolo et al. 1995), but this is often difficult to obtain, especially for the mammary gland where considerable vascularisation lends itself to excessive bleeding. As an alternative, Bequette et al. (2000) used the enrichment of casein-bound AA in milk, which was assumed to represent the enrichment of the immediate precursor pool within the mammary gland at the site of milk protein synthesis. Thus, the enrichment of casein was also assumed to reflect that of the mammary intracellular free pool at steady state. The average enrichment of casein-bound AA in milk over the last 4 h (16.5 to 20.5 h) of infusion was used in calculations. (Fig. 1a to 1i). The net mass balance and tracer balance of AA across the mammary gland were calculated as follows:

$$(Ca - Cv) \times MPF \times 10^{-3} = Fmg,a - Fv,mg \quad (8)$$

$$(Ca \times Ea - Cv \times Ev) \times MPF \times 10^{-3} = Fmg,a \times Ea - Fv,mg \times Ec \quad (9)$$

Where Ec was the enrichment of casein-bound AA in milk at steady state. The net mass balance and tracer balance across the mammary gland were estimated using the concentrations and the enrichments of plasma AA because of the limited equilibration of unlabeled and labeled AA between plasma and red blood cells and between the latter and the mammary gland. Thus, Fmg,a and Fv,mg could be solved from Eq. 5, 6, 8, and 9:

$$Fmg,a\ (mmol/h) = \{[(Ec - Ev)/(Ea - Ev)] \times Cv + Ca\} \times MPF \times 10^{-3} \quad (10)$$

$$Fv,mg\ (mmol/h) = \{[(Ec - Ev)/(Ea - Ev)] \times Cv + Cv\} \times MPF \times 10^{-3} \quad (11)$$

At steady state, Fa,o = Fmg,a + Fv,a; and Fo,v = Fv,a + Fv,mg. Thus, Fv,a was calculated as follows:

$$Fv,a = Fa,o - Fmg,a \quad (12)$$

Or

$$Fv,a = Fo,v - Fv,mg \quad (13)$$

(2) Kinetics of intracellular free amino acids in the mammary gland. Total rate of mammary intracellular free AA appearance (Ra) was calculated by the intracellular tracer dilution approach. The only source of tracer appearing in the mammary intracellular free AA compartment was transported inward from plasma. Thus, any dilution of tracer AA in casein is assumed to derive from the intracellular free AA from endogenous sources (Fmg,o) (e.g., protein breakdown and de novo synthesis, if any). Therefore,

$$Ra \times Ec = Fmg,a \times Ea, \text{ i.e.,}$$

$$Ra\ (mmol/h) = (Fmg,a \times Ea) / Ec \quad (14)$$

Where Ra is the sum of inward trans-membrane transport (Fmg,a) and the rate of intracellular free AA appearance from the endogenous sources (Fmg,o):

$$Ra = Fmg,a + Fmg,o$$

Thus,

$$Fmg,o\ (mmol/h) = Fmg,a \times (Ea/Ec - 1) \quad (15)$$

At steady state, the total fluxes into the mammary intracellular free AA compartment are equal to the total fluxes out of this compartment, i.e.,

$$Fmg,a + Fmg,o = Fv,mg + Fo,mg$$

Thus,

$$Fo,mg\ (mmol/h) = (Fmg,a - Fv,mg) + Fmg,o \quad (16)$$

Or,

$$Fo,mg\ (mmol/h) = NB + Fmg,o \quad (17)$$

The rate of intracellular free AA disappearance (Fo,mg) could be also directly calculated as the tracer balance divided by the precursor enrichment (Ec):

$$Fo,mg\ (mmol/h) = (Ca \times Ea - Cv \times Ev) \times MPF \times 10^{-3} / Ec \quad (18)$$

(3) Protein synthesis and breakdown in the mammary gland. Fmg,o and Fo,mg may reflect rates of different metabolic pathways for each AA. Fmg,o refers to the rate of intracellular free AA endogenous appearance from protein breakdown and de novo synthesis within the mammary gland. Thus, Fmg,o represents the rate of intracellular protein breakdown because indispensable AA (e.g., lysine, methionine, and valine) can not be synthesized de novo in the mammary gland. Fo,mg refers to the rate of intracellular free AA utilized for protein synthesis, oxidation, and other metabolic pathways, if any, in the mammary gland. Fo,mg for the intracellular methionine may represent the rate of protein synthesis because their oxidation and utilization for other metabolic pathways might be negligible as demonstrated in the guinea-pig mammary gland (Peters et al. 1979). In contrast, Fo,mg for intracellular valine could be subdivided into two fractional fluxes: Fo,mg(p) for intracellular valine utilized for protein synthesis and Fo,mg(o) for the intracellular valine utilized for oxidation and other metabolic pathways, if any. Therefore,

$$PB\ (g\ protein/d) = Fmg,o\ (mmol/h) \times AA\ mol\ wt\ (mg/mmol) \times 24\ (h/d) \times 10^{-1} / AA\ \%\ of\ protein \quad (19)$$

Where AA % of protein in the mammary tissue was averaged at 7.52, 2.05, and 5.53, respectively for lysine, methionine, and valine based on the AA composition in constitutive proteins in the mammary gland (Kim et al. 1999) and that in milk proteins (Guan et al. 2000b).

PS_{met} (g protein/d) = Fo,mg (mmol/h) x methionine mol wt (mg/mmol) x 24 (h/d) x 10^{-1} / methionine % of protein (20)

Where PS_{met} refers to the rate of protein synthesis defined by Fo,mg for the intracellular methionine. On the basis of the equivalent protein mass, the rate of protein synthesis defined by Fo,mg(p) for the intracellular valine would be the same as those defined by Fo,mg for the intracellular methionine, i.e.,

$PS_{val} = PS_{met}$

Where PS_{val} refers to the rate of protein synthesis for intracellular valine. Thus, Fo,mg(p) for intracellular valine utilized for protein synthesis was calculated as follows:

Fo,mg(p) x valine mol wt/valine % of protein = Fo,mg (of methionine) x methionine mol wt/methionine % of protein; i.e.,

Fo,mg(p) (mmol/h) = Fo,mg (of methionine, mmol/h) x [(methionine mol wt/methionine % of protein)/(valine mol wt/valine % of protein)] (21)

Or,

Fo,mg(p) (mmol/h) = PS_{val} (g/d) x valine % of protein x 10 / [24 (h/d) x valine mol wt (mg/mol)] (22)

Thus, Fo,mg for the intracellular valine = Fo,mg(p) + Fo,mg(o), i.e.,

Fo,mg(o) (mmol/h) = Fo,mg - Fo,mg(p) (23)

Where Fo,mg(o) may represent the rate of the intracellular valine utilized for oxidation and other metabolic pathways.

Net production of protein in the mammary gland was derived from the compartmental model, i.e., the difference between protein synthesis and breakdown (Eq. 19 and 20). The values for net production of protein derived from the model could be validated by estimates of accretion of mammary tissue proteins and output of mammary synthesized proteins in milk. Average accretion of mammary tissue proteins was 14.81 g/d over a 21-d lactation period in the lactating sow (Kim et al. 1999). Output of mammary synthesized proteins in milk was calculated as follows:

Output of mammary synthesized proteins in milk (g/d) = Milk yield (kg/d) x 1.15 x the concentration of true protein in milk (%) x (1 - 0.05) x 10 (24)

Where an estimate of milk yield was adjusted by 1.15 because the weigh-suckle-weigh method underestimates milk consumption compared to D_2O dilution method (Guan et al. 2000a, Pettigrew et al. 1987, Pettigrew et al. 1985). The contribution of non-mammary synthesized proteins was assumed at 5.0% of true protein in milk (Bourne and Curtis 1973, Klobasa and Butler 1987, Klobasa et al. 1987).

Statistical Analyses. Data were analyzed by the Mixed Procedure (SAS/STAT Version 6.12, SAS Institute, Cary, NC). The model for the concentration of total N in defatted milk included block, dietary treatment, and sampling time, and all two-way interactions with sampling time in a repeated statement. The model for other variables included block and dietary treatment. Based on the residual distribution, data for Fa,o, Fv,a, Fmg,a,

Fv,mg, Fo,v, and NB were log-transformed prior to their ANOVA. Their least-squares means were then converted to actual values. Least-squares means are presented because of unbalanced data in blocks. Differences between the lysine-deficient diet and positive control diet or between the positive control diet and valine-excess diet are considered significant at $P < 0.05$ or 0.01 and tended to be significant at $P < 0.10$.

RESULTS

Productive performance and mammary plasma flow rate. Productive performance over a 21-d lactation period was affected by the dietary amino acid regime (**TABLE 4-4**). Sows fed the lysine-deficient diet had lower litter weight gain and milk yield than sows fed the positive control diet ($P < 0.05$ and 0.01, respectively). Total nitrogen content in defatted milk and protein content in milk were decreased ($P < 0.05$) in the lysine-deficient diet compared to those fed the positive control diet. The proportion of casein-N to total N in milk was numerically lower in the lysine-deficient diet than that in the positive control diet. No differences were observed in the productive performance between the valine-excess diet and the positive control diet. The dietary amino acid regime did not affect plasma flow rate across the mammary gland (214.4 ± 26.0, 236.8 ± 26.0, and 265.3 ± 26.0 L/h, respectively, for the lysine-deficient diet, the positive control diet, and the valine-excess diet).

Arterial concentrations of plasma amino acids and urea and their extraction rates by the mammary gland. Plasma amino acid and urea profiles are shown in **TABLE 4-5.**

Concentration of plasma lysine in the carotid artery was decreased ($P < 0.01$) in the sows fed the lysine-deficient diet compared to that in the sows fed the positive control diet. In contrast, plasma threonine concentration tended to be increased in the lysine-deficient diet ($P < 0.10$). The concentration of plasma urea was increased ($P < 0.10$) in the lysine-deficient diet, possibly indicating that deamination from indispensable amino acids other than lysine was increased. Taken together, the plasma amino acid and urea profiles in the lysine-deficient diet reflected a deficiency of lysine in the body. The concentration of plasma valine in the artery was increased considerably ($P < 0.01$) in the sows fed the valine-excess diet, indicating the excess of valine in the body. Concentrations of plasma indispensable amino acids other than valine were not altered in the valine-excess diet.

Mammary extraction rates (i.e., the ratios of mammary arteriovenous differences to arterial concentration) of plasma amino acids were affected by the dietary amino acid regime (**TABLE 4-6**). The extraction rate of plasma lysine was increased ($P < 0.05$) in the lysine-deficient diet compared to that in the positive control diet. Moreover, the extraction rates of certain indispensable amino acids (e.g., methionine and leucine) were decreased ($P < 0.05$), possibly due to their increased arterial concentrations in the lysine-deficient diet. In contrast, extraction rates of plasma cationic amino acids (e.g., arginine) and certain neutral amino acids (e.g., leucine, threonine and tryptophan) dended to be decreased ($P < 0.05$) in the valine-excess diet.

Kinetics of plasma lysine across the mammary gland. Kinetics of trans-membrane transport for lysine across the mammary gland was affected by the dietary amino acid

regime (**TABLE 4-7**). The whole body irreversible loss rate (Fwb) and the unidirectional flux from the mammary artery to the mammary gland (Fmg) of lysine were decreased ($P < 0.01$) in the lysine-deficient diet compared to those in the positive control diet. The flux of plasma lysine entrance into the gland from the mammary artery (Fa,o) and the flux of lysine exit from the gland to the mammary vein (Fo,v) were decreased ($P < 0.01$ and 0.05, respectively) in the lysine-deficient diet compared to those in the positive control diet. Inward trans-membrane transport (Fmg,a) of free lysine from the mammary artery to mammary intracellular free lysine compartment and outward trans-membrane transport (Fv,mg) from this compartment to the mammary vein were decreased ($P < 0.05$), resulting in decreased net uptake (NB) of plasma lysine by the mammary gland. The rate of mammary intracellular free lysine appearance from endogenous sources (Fmg,o) was decreased in the lysine-deficient diet, indicating a decreased protein breakdown in the mammary gland. Because of decreased Fmg,a and Fmg,o for lysine, the total rate of mammary intracellular free lysine appearance (Ra) was decreased by approximately 85% in the lysine-deficient diet. Decreased Ra indicated decreased availability of the mammary intracellular free lysine for protein synthesis and other metabolisms, if any, as indicated by decreased rate of the intracellular free lysine disappearance (Fo,mg) ($P < 0.01$) in the lysine-deficient diet.

Though Fwb for lysine was not different between the valine-excess diet and the positive control diet, Fmg for lysine was decreased ($P < 0.01$) in the valine-excess diet. There were no differences in Fa,o and Fo,v for lysine between the valine-excess diet and the positive control diet. Both Fmg,a and Fv,mg for lysine were increased at approximately

35% and 80%, respectively, in the valine-excess diet compared to those in the positive control diet, resulting in decreased NB for lysine in the valine-excess diet. The rate of mammary intracellular free lysine disappearance (Fo,mg) was decreased ($P < 0.01$) in the valine-excess diet.

Fractional proportion of the whole body irreversible loss of plasma lysine partitioned to the mammary gland (Fmg/Fwb) was decreased in the lysine-deficient diet and the valine-excess diet compared to that in the positive control diet ($P < 0.10$ and 0.01, respectively) **(TABLE 4-10)**. The ratio of net uptake by the mammary gland to total entrance of free lysine from the mammary artery (NB/Fa,o), being identical to mammary extraction rate, was increased by 120% in the lysine-deficient diet compared to that in the positive control diet. The dietary amino acid regime did not affect the fractional proportion (Fmg,a/Fa,o) of inward trans-membrane transport to the total entrance of free lysine from the mammary artery, the fractional proportion (Fv,a/Fa,o) of direct flow (from artery to vein) to the total entrance of free lysine from the mammary artery, or the volume of arterial plasma completely cleared of lysine via trans-membrane transport per unit time (Fmg,a/Ca). In the lysine-deficient diet, the contribution of inward trans-membrane transport to Ra (Fmg,a/Ra) was decreased ($P < 0.05$); coincidentally, the contribution from protein breakdown (the only endogenous source in the mammary gland) to Ra (Fmg,o/Ra) was increased ($P < 0.05$). In contrast, the contribution of inward trans-membrane transport was increased ($P < 0.05$) and the contribution from protein breakdown was decreased ($P < 0.05$) in the valine-excess diet. The ratio of the flux of the intracellular free lysine utilized for protein synthesis to the total rate of the

intracellular free lysine appearance (i.e., availability) (Fo,mg/Ra) was increased in the lysine-deficient diet, indicating an increased efficiency of the intracellular lysine for protein synthesis. In contrast, the ratio of Fo,mg to Ra was decreased ($P < 0.10$) in the valine-excess diet.

Kinetics of plasma methionine across the mammary gland. Although concentrations of dietary and arterial plasma methionine were not different among the dietary AA regimes, fluxes of methionine were affected (**TABLE 4-8**). The whole body irreversible loss rate (Fwb) and the unidirectional flux to the mammary gland (Fmg) were decreased in the lysine-deficient diet. In the lysine-deficient diet, inward trans-membrane transport of free methionine (Fmg,a) was decreased by 43% ($P < 0.05$); outward trans-membrane transport (Fo,mg) was decreased by 48%, resulting in decreased net uptake (NB) of plasma methionine by the mammary gland ($P < 0.05$). The rate of mammary intracellular free methionine appearance (Fmg,o) from endogenous sources was decreased ($P < 0.05$) in the lysine-deficient diet, indicating a decreased protein breakdown in the mammary gland. The total rate of the mammary intracellular free methionine appearance (Ra) was decreased ($P < 0.10$) due to decreased Fmg,a and Fmg,o in the lysine-deficient diet. The decreased Ra indicated a decrease in the intracellular methionine availability to protein synthesis and other metabolism (if any) in the mammary gland, as indicated by decreased rate of the intracellular free methionine disappearance (Fo,mg) in the lysine-deficient diet ($P < 0.05$). There were no differences between the lysine-deficient diet and the positive control diet in the flux of methionine entrance into the mammary gland (Fa,o), the direct flow from the artery to the vein (Fv,a), or the flux of methionine exit from the mammary

gland to the vein (Fo,v). Except for Fv,a and Fmg,o, the other fluxes were not different between the valine-excess diet and the positive control diet. Note that decreased Fmg,o implied that mammary protein breakdown was decreased in the valine-excess diet.

The proportion of the whole body irreversible loss of plasma methionine partitioned to the mammary gland (Fmg/Fwb) was decreased in the lysine-deficient diet ($P < 0.05$) compared to that in the positive control diet (**TABLE 4-11**), possibly due to decreased extraction rate (NB/Fa,o). The dietary amino acid regime did not affect the fractional proportion (Fmg,a/Fa,o) of inward trans-membrane transport to the total entrance of free methionine from the mammary artery, the fractional proportion (Fv,a/Fa,o) of direct flow (from the artery to the vein) to the total entrance of free methionine from the mammary artery, or the ratio of the flux of the intracellular free methionine for utilization to the total rate of the intracellular free methionine appearance (availability) (Fo,mg/Ra). More than 50% of the total entrance of free methionine from the mammary artery did not enter the intracellular free methionine compartment, but directly flowed from the artery to the vein, resulting in low extraction efficiency of plasma methionine by the mammary gland. The major proportion (above 70%) of the total appearance of the intracellular free methionine was contributed from inward trans-membrane transport and the minor proportion (below 30%) from endogenous sources (i.e., protein breakdown). In the valine-excess diet, the contribution of inward trans-membrane transport was increased ($P < 0.05$) and that from mammary protein breakdown was decreased ($P < 0.05$).

Kinetics of plasma valine across the mammary gland. Effects of the dietary AA regime

on kinetics of plasma valine across the mammary gland are represented in **TABLE 4-9** and **5-12**. No differences in the whole body irreversible loss rate (Fwb) or the unidirectional flux to the mammary gland (Fmg) were found between the lysine-deficient diet and the positive control diet. The flux of valine entrance into the mammary gland (Fa,o), the direct flow from the artery to the vein (Fv,a), and the flux of valine exit from the mammary gland to the vein were decreased ($P < 0.05$) in the lysine-deficient diet. There were no differences in inward trans-membrane transport (Fmg,a) or outward trans-membrane transport (Fv,mg) between the lysine-deficient diet and the positive control diet. Though the rate of the intracellular free valine disappearance (Fo,mg) was not different between the lysine-deficient diet and the positive control diet, the fractional flux of Fo,mg utilized for protein synthesis (Fo,mg(p)) was decreased ($P < 0.05$) by 40% in the lysine-deficient diet; in contrast, the fractional flux of Fo,mg utilized for oxidation and other metabolism (Fo,mg(o)) was numerically increased by 55% in the lysine-deficient diet.

In the valine-excess diet, the whole body irreversible loss (Fwb), the flux of valine entrance into the mammary gland (Fa,o), the flux of mammary free valine exit to the mammary vein (Fo,v), and the direct flow from the artery to the vein (Fv,a) were increased dramatically ($P < 0.05$ or 0.01). However, inward trans-membrane transport (Fmg,a) was numerically decreased by 35%; and outward trans-membrane transport (Fv,mg) was completely blocked in the valine-excess diet. Therefore, net uptake of plasma valine was slightly increased in the valine-excess diet. The rate of the intracellular valine appearance from endogenous sources (i.e., protein breakdown)

(Fmg,o) was decreased ($P < 0.05$) by 56.8% in the valine-excess diet, indicating a decreased protein breakdown in the mammary gland. The total rate of appearance of the intracellular valine was decreased by approximately 40% due to decreased Fmg,a and Fmg,o. There were no differences in the rate of the intracellular valine disappearance (Fo,mg), the fractional flux of the intracellular valine utilized for protein synthesis (Fo,mg(p)), or the fractional flux of the intracellular valine utilized for oxidation and other metabolism (Fo,mg(o)) between the valine-excess diet and the positive control diet.

In the valine-excess diet, the proportion of inward trans-membrane transport to the total entrance of arterial plasma valine (Fmg,a/Fa,o) was decreased ($P < 0.10$) due to decreased Fmg,a and increased Fa,o; accordingly, the proportion of direct flow (from the artery to the vein) to the total entrance of arterial plasma valine (Fv,a/Fa,o) was increased ($P < 0.10$); thus, mammary extraction rate of plasma valine (NB/Fa,o) was decreased by 50%. The proportion of the whole body irreversible loss of plasma valine partitioned to the mammary gland (Fmg/Fwb) was decreased due to increased Fwb in the valine-excess diet. The dietary amino acid regime did not affect the fractional proportion (Fmg,a/Ra) of inward trans-membrane transport to the total appearance of the intracellular valine, the fractional proportion (Fmg,o/Ra) of the flux of the intracellular valine appearance from endogenous sources (i.e., protein breakdown) to the total appearance of the intracellular valine, the proportion of Fo,mg utilized for protein synthesis (Fo,mg(p)/Fo,mg), or the proportion of Fo,mg utilized for oxidation and/or other metabolic pathways (Fo,mg(o)/Fo,mg). Averaged the ratio of Fo,mg(p) to Fo,mg and the ratio of Fo,mg(o) to Fo,mg were at 0.66 and 0.34, respectively. The volume of arterial plasma completely

cleared of valine through the trans-membrane transport per unit of time was decreased numerically in the valine-excess diet, possibly due to increased size of the extracellular free valine compartment.

Protein synthesis and breakdown in the mammary gland. Protein turnover in the mammary gland was derived from the compartmental model based on methionine fluxes (**TABLE 4-13**). In the lysine-deficient diet, protein synthesis and breakdown were decreased ($P < 0.05$), resulting in decreased net balance of protein in the mammary gland. In the valine-excess diet, protein breakdown was decreased ($P < 0.05$). Moreover, the ratio of protein breakdown to protein synthesis was decreased by approximately 25% in the valine-excess diet compared to that in the positive control diet. Net production of protein, estimated by the sum of output of protein in milk and accretion of protein in the mammary gland, was very comparable to the model-derived values.

DISCUSSION

A three-compartmental model has been developed to quantify the integrated trans-membrane transport of an individual AA across human skeletal muscle (Biolo et al. 1992, Biolo et al. 1995); and has been modified to define the unidirectional influx and efflux of an individual AA across the lactating goat mammary gland by Bequette et al. (2000) using enrichment of casein-bound AA in milk instead of mammary intracellular free AA. In the present study, we demonstrated effects of dietary AA regime on the trans-

membrane transport kinetics and intracellular metabolism of indispensable AA (lysine, methionine, and valine) in the lactating porcine mammary gland.

Milk yield and protein output. Milk yield and milk protein output were significantly decreased in sows fed a lysine-deficient diet as shown in the study of Wilkinson (Wilkinson et al. 1982). However, milk yield was not improved in the valine-excess diet. An optimal ratio of valine to lysine for milk synthesis by the lactating sow was estimated approximately at 1:1 at zero loss of maternal body protein (Guan et al. 2000c). Thus, dietary ratio of valine to lysine (1.05:1) in the positive control diet may be the most optimal for the lactating sow. These results of the present study were supported by a recent feeding trial, in which dietary ratio of valine to lysine at 1.03:1.00 yields the highest litter growth rate, and increased dietary ratio (up to 1.22:1.00) does not improve litter growth rate (Carter et al. 2000).

Trans-membrane transport and net uptake. Net uptake of indispensable AA by the mammary gland is determined by the difference between inward (Fmg,a) and outward trans-membrane transport (Fv,mg). Degrees of asymmetry as to inward versus outward trans-membrane transport are inherent characteristics of AA transport systems of the plasma membranes to favor accumulation or release of the intracellular AA (Christensen 1990, Shennan and Peaker 2000). Trans-membrane transport and net uptake of plasma lysine was decreased in the lysine-deficient diet due to a very low concentration of arterial plasma lysine (~ 30 μM), which was below the K_m value (~ 100 μM) for systems

y^+ and $B^{0,+}$. Transport of L-lysine across the brush-border membrane of the chicken jejunum is regulated by dietary lysine concentration: systems y^+ and $b^{0,+}$ are up-regulated by increasing dietary lysine concentration (from 4.8 to 6.8 g/kg diet)(Torras-Llort et al. 1998). Thus, AA transport systems for lysine in the basolateral membranes of the mammary epithelium might be down-regulated by dietary lysine deficiency.

Dietary valine excess decreased net uptake of plasma lysine, which resulted from increased Fv,mg for lysine to a greater extent than Fmg,a for lysine. Uptake of lysine by the mammary tissue is inhibited in vitro by high concentrations of neutral AA (e.g., leucine) (Baumrucker 1984, Hurley et al. 2000, Shennan et al. 1994, Shennan et al. 1997), by stimulating efflux of lysine from rat mammary tissue (Shennan et al. 1997). In addition, dietary supplementation of valine inhibits uptake of lysine by the brush-border membrane of mouse jejunum (Stein et al. 1987). Recently, a Na^+-dependent AA transporter ($hATB^{0,+}$) cloned from human mammary gland has expressed the same kinetics properties of system $B^{0,+}$ (Sloan and Mager 1999). This AA transport system has high affinity for both lysine and valine (K_m ~ 110 to 140 μM), trans-stimulated, and adaptively regulated. Note that arterial plasma concentrations of lysine and valine were, respectively, approximately at 250 and 650 μM in the lactating sow fed the positive control diet. In this context, uptake of lysine by the mammary gland might be inhibited by physiological (or high) concentrations of plasma valine, through stimulating outward trans-membrane transport of lysine. Decreased net uptake of plasma lysine by the mammary gland in the valine-excess diet was unlikely attributed to mammary plasma

flow rate because it was not decreased. Trans-membrane transport of methionine was not affected in the valine-excess diet possibly due to its multiple AA transport systems in the basolateral membranes.

Inward trans-membrane transport of valine was decreased numerically and its outward trans-membrane transport was completely blocked in the valine-excess diet, resulting in a numerically increased net uptake of plasma valine by the mammary gland. The transport system for valine in the lactating porcine mammary gland has a K_m of 640 μM in vitro (Jackson et al. 2000), indicating that AA transport system for valine may be saturated in vivo. Transport of BCAA into muscle is regulated in vivo mainly by their concentrations in the extracellular fluid and by intracellular metabolic removal (e.g., through catabolism and protein synthesis) (Tovar et al. 1991). Outward trans-membrane transport for valine was blocked possibly due to a very high concentration of free valine in the extracellular fluid. The mammary venous plasma valine concentration (supposed to be lower than that in the extracellular fluid) was as high as 1150 μM in the lactating sow fed the valine-excess diet. Transport of the BCAA into the soleus muscle (presumably through system L) is reduced by their high concentrations in vitro (Tovar et al. 1991). This down-regulation of trans-membrane transport for valine would protect it from the risk of toxicity of the intracellular valine excess since the BCAA may be toxic at high concentrations in the tissue (Millward 1998).

Protein synthesis and breakdown. The present study demonstrated significant protein

synthesis and breakdown in the lactating porcine mammary gland. The proportion of protein breakdown to protein synthesis ranged from 0.31 to 0.41 in the present study, which supports an estimate of 0.33 in the goat mammary gland (Bequette et al. 1998). With the lysine-deficient diet, protein synthesis was inhibited to slightly greater extent than protein breakdown, resulting in decreased net balance of mammary protein. Decreased protein synthesis in the lysine-deficient diet was indicated by decreased fluxes of disappearance of the intracellular free lysine and methionine, the fractional flux of disappearance of the intracellular free valine (Fo,mg(p)). Protein synthesis in the lysine-deficient diet might be restrained by decreased intracellular availability of lysine and methionine, indicated by their decreased Ra.

We found that protein breakdown in the mammary gland was decreased to a greater extent than protein synthesis in the valine-excess diet, resulting in a comparable net balance of mammary protein with the positive control diet. Mammary protein breakdown was significantly inhibited by dietary valine excess, indicating by fluxes (Fmg,o) of appearance for the intracellular lysine, methionine, and valine from intracellular endogenous sources. Moreover, the proportion of protein breakdown to protein synthesis was decreased by 25% in the valine-excess diet. This inhibitory effect on mammary protein breakdown by dietary valine excess, is possibly through its intracellular metabolism (e.g., oxidation) or its extracellular regulatory sites on the basolateral membranes of the mammary epithelial cells. Branched-chain amino acids (e.g., leucine) inhibit muscle protein breakdown, presumably by their increased intracellular

accumulation or their keto-acids (e.g.,α-ketoisocaproate and isovalerate) (MacLean et al. 1994, Mitch and Clark 1984, Nair et al. 1992). We did not measure the mammary intracellular concentrations of BCAA and their keto-acids. Though the total flux of appearance of the intracellular free valine (Ra) was not increased in the valine-excess diet, it can not be ruled out that the intracellular valine was oxidized rapidly in the mammary gland. Oxidation of BCAA in the rat mammary gland is increased significantly during lactation (DeSantiago et al. 1998). Their oxidation may be up-regulated by high intake of protein or BCAA through increased expression of branched-chain aminotransferase and/or increased activity of branched-chain α-keto acid dehydrogenase (Langer et al. 2000, Torres et al. 1998).

However, the inhibitory effect of high valine on mammary protein breakdown was more likely to occur on the plasma membranes since arterial plasma valine concentration almost doubled without significant increase in the intracellular valine appearance in the valine-excess diet. High concentrations of extracellular leucine and α-ketoisocaproate inhibit protein breakdown in vitro, possibly through interactions with their regulatory sites on the plasma membrane in the liver and heart where the activity of branched-chain aminotransferase is minimal (Chua 1994, Miotto et al. 1992, Venerando et al. 1994).

Metabolism other than protein synthesis. In the present study, the proportion of the mammary intracellular free valine utilized for metabolisms other than protein synthesis was not affected by the dietary AA regime and averaged at 0.34, indicating that valine may participate considerately in metabolic pathways (e.g., oxidation) other than protein

synthesis in the mammary gland. As discussed above, the catabolism of BCAA in the rat mammary gland increases significantly during lactation due to increased expression of mitochondrial branched-chain aminotransferase and increased activity of branched-chain α-keto acid dehydrogenase (DeSantiago et al. 1998). The amount of the BCAA taken up by the lactating porcine mammary gland exceeds their output in milk by approximately 25 to 30% (Nielsen et al. 2000, Trottier et al. 1997) (Guan et al. 2000b). Similar results have been found in other lactating species, such as dairy cattle, sheep, and goat (Bequette et al. 1997, Davis et al. 1978, Davis and Mepham 1976, Fleet and Mepham 1985, Guinard 1994, Roets et al. 1979, Vina and Williamson 1981, Wohlt et al. 1977). This positive difference between uptake and output of valine actually reflects its fractional flux utilized for metabolism other than protein synthesis (e.g., oxidation). For example, BCAA can be catabolized to yield carbon and α-amino nitrogen for synthesis of dispensable AA and energy for milk synthesis by the mammary gland (Wohlt et al. 1977).

In summary, trans-membrane transport of indispensable AA (lysine, methionine, and valine) across the basolateral membrane of the mammary epithelium is finely and individually regulated to control their net uptake by dietary AA regime. Inward and outward trans-membrane transport of certain indispensable AA (e.g., lysine) may be the rate-limiting for milk synthesis (as shown in the lysine-deficient diet). Mammary protein synthesis and breakdown are associated with net uptake of plasma individual AA, which determines net balance of mammary protein. Dietary valine excess inhibits protein breakdown in the mammary gland, possibly through its intracellular metabolism (e.g.,

oxidation) and/or its interactions with extracellular regulatory sites on the basolateral membrane. In practice, dietary ratio of valine to lysine higher than 1.05:1 does not show any improvement in milk yield, output of protein in milk, or litter growth rate.

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Appendix A *Glossary*

Term	Unit	Definition
Fwb	mmol/h	The whole body irreversible loss rate of plasma individual AA
Fmg	mmol/h	The unidirectional flux of plasma free AA from mammary artery to the mammary gland
Fa,o	mmol/h	Rate of free AA entrance into the mammary gland from systemic circulation via mammary artery
Fv,a	mmol/h	Direct flow of free AA from mammary artery to mammary vein without entering intracellular fluid
Fo,v	mmol/h	Rate of free AA exit from the mammary gland to systemic circulation via mammary vein
NB	mmol/h	Net mass balance of plasma free AA across the mammary gland
Fmg,a	mmol/h	Inward trans-membrane transport of free AA from mammary artery to mammary intracellular free AA compartment
Fv,mg	mmol/h	Outward trans-membrane transport of free AA from mammary intracellular free AA compartment to mammary vein
Fmg,o	mmol/h	Rate of mammary intracellular free AA appearance from endogenous sources (i.e., from protein breakdown and de novo synthesis, if any)

Fo,mg	mmol/h	Rate of mammary intracellular free AA disappearance (or utilization) (i.e., for protein synthesis, oxidation, and other metabolic fates, if any)
Fo,mg(p)	mmol/h	Fractional rate of mammary intracellular valine disappearance utilized for protein synthesis
Fo,mg(o)	mmol/h	Fractional rate of mammary intracellular valine disappearance utilized for oxidation and other metabolic pathways
Ra	mmol/h	Total rate of mammary intracellular free AA appearance, i.e., the sum of inward trans-membrane transport (Fmg,a) and intracellular appearance from endogenous sources (Fmg,o)
Fmg/Fwb		Fractional proportion of Fwb partitioned to the mammary gland
Fmg,a/Fa,o		Fractional proportion of inward trans-membrane transport to total entrance of free AA from mammary artery, indicating the ability of mammary AA transport systems to take up circulating AA
Fv,a/Fa,o		Fractional proportion of direct flow (from artery to vein) to total entrance of free AA from mammary artery, indicating inability or regulation of mammary AA transport systems to take up circulating AA
NB/Fa,o		Extraction rate of net mass balance to total entrance of free AA from mammary artery

Fmg,a/Ra		Fractional contribution of inward trans-membrane transport to total rate of mammary intracellular free AA appearance
Fmg,o/Ra		Fractional contribution from protein breakdown to total rate of mammary intracellular free AA appearance
Fo,mg/Ra		Ratio of the flux for protein synthesis to total flux of mammary intracellular free AA appearance (availability), an indicator of the utilization efficiency for protein synthesis
Fo,mg(p)/Fo,mg		Ratio of fractional flux for protein synthesis to total flux of mammary intracellular free AA disappearance
Fo,mg(o)/Fo,mg		Ratio of fractional flux for oxidation and other metabolic rates to total flux of mammary intracellular free AA disappearance
Fmg,a/Ca	L/min	The volume of arterial plasma completely cleared of the AA via trans-membrane transport per unit of time, indicating the ability of any particular AA to be transported

TABLE 4-1 *Distinguishing characteristics of cationic amino acid transport systems*

System	Functional expression	Na^+ dependence	Binding specificity (K_m or K_i, μM)	Regulation	Cloned AA transporter
y^+	Ubiquitous	-	Lys, Arg, Orn (89-99) High capacity	Trans-stimulation electrogenic	CAT-1 to -4
y^+L	Ubiquitous: erythrocytes, kidney, placenta, small intestine (BM)	- (CAA) + (NAA)	Lys, Arg, Leu (3-11) Low capacity	Exchange: 1 NAA (influx)/1 CAA (efflux) electroneutral	4F2hc/y^+LAT-1 to -2
$b^{0,+}$	Blastocysts, kidney, small intestine (BBM)	-	Lys, Arg, Leu, Cys (48-135) Low capacity	Exchange: 1 CAA (influx)/1 NAA (efflux)	rBAT/mBAT
$B^{0,+}$	Blastocysts, small intestine, mammary gland	+	Lys, Arg, Val (110-140)	Trans-stimulation adaptive regulation	$hATB^{0,+}$

CAA, cationic AA; NAA, neutral AA; CAT, cationic AA transporters; 4F2hc, heave chain of the surface antigen 4F; y^+LAT, system y^+L AA transporter; r (m) BAT, rat (mouse) broad-scope AA transport proteins; $hATB^{0,+}$, human Na^+-dependent cationic and neutral AA transporter; +, Na^+-dependent; -, Na^+-independent; K_m, Michaelis constant; K_i, inhibition constant; BM, basal membrane; BBM, brush-border membrane. References (Chillaron et al. 1996, Deves and Boyd 1998, Fukasawa et al. 2000, Kamath et al. 1999, Palacin et al. 1998, Segawa et al. 1999, Sharma and Kansal 2000, Sloan and Mager 1999, Torrents et al. 1998).

TABLE 4-2

Distinguishing characteristics of neutral amino acid transport systems

System	Functional expression	Na^+ dependence	Substrate	Regulation	Cloned AA transporter
A	Ubiquitous	+	Ala, Ser, Gln, Met	Trans-inhibition adaptive regulation	unknown
ASC	Ubiquitous	+	Ala, Ser, Thr, Cys, Met	Trans-stimulation electroneutral	4F2hc/ASCT-1 to -2
L	Ubiquitous (BM)	-	Met, branched-chain and aromatic AA	Trans-stimulation electroneutral	4F2hc/LAT-1 to -2
B^0	Kidney, placenta, small intestine (BBM)	+	Met, Thr, branched-chain and aromatic AA		$hATB^0/rATB^0$

4F2hc, heavy chain of the surface antigen 4F; ASCT, system ASC AA transporter; LAT, system L AA transporter; h(r)ATB^O, human (rabbit) Na^+-dependent neutral AA transporter; +, Na^+-dependent; -, Na^+-independent; BM, basal membrane; BBM, brush-border membrane. Trans-inhibition and -stimulation, inhibition and stimulation by substrates in the trans-compartment, respectively. References (Fukasawa et al. 2000, Kanai et al. 1998, Kekuda et al. 1996, Kekuda et al. 1997, Palacin et al. 1998, Pineda et al. 1999, Segawa et al. 1999, Sharma and Kansal 1999, Sloan and Mager 1999, Utsunomiya Tate et al. 1996).

TABLE 4-3 *Composition of experimental diets (as-fed basis)*

Item	Dietary amino acid regime		
	Lysine deficient	Positive control	Valine excess
Ingredient, g/kg diet			
Corn	679.5	679.5	679.5
Soybean meal	125.0	125.0	125.0
Tallow	50.0	50.0	50.0
Solk floc[a]	25.0	25.0	25.0
Calcium phosphate	22.0	22.0	22.0
Calcium carbonate	10.0	10.0	10.0
Salt	2.5	2.5	2.5
Mineral and vitamin premix[b]	11.5	11.5	11.5
Amino acid mixture[c]	14.8	14.8	14.8
Lysine•HCl	0	8.0	8.0
Valine	4.9	4.9	9.3
Glutamic acid	24.1	36.8	42.4
Corn starch	30.7	10.0	0
Analyzed contents of nitrogen and indispensable amino acids, g/kg diet			
N x 6.25	138.1	151.1	158.8
Histidine	4.90	4.94	4.79
Arginine	7.08	7.15	6.83
Threonine	5.17	5.28	5.13
Valine	9.87	10.15	13.37
Methionine	3.15	3.26	3.39
Isoleucine	6.54	6.56	6.52
Leucine	12.90	12.91	12.51
Phenylalanine	8.00	7.87	7.73
Lysine	4.93	9.71	9.76
Tryptophan[d]	2.10	2.10	2.10

[a]The content of cellulose was 98.5%.

[b]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, 8.3; cholecalciferol, 0.0138; 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.

[c]Provided the following amounts of indispensable amino acids in grams per kilogram of diet: histidine•HCl, 1.9; threonine, 3.2; DL-methionine, 1.4; isoleucine, 2.0; leucine, 1.6; phenylalanine, 2.5, tyrosine, 1.3, and tryptophan, 0.9.

[d]Calculated value (NRC, 1998).

TABLE 4-4 *Effects of dietary amino acid regime on productive performance of the lactating sow over a 21-d lactation period*

Item	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Feed intake, kg/d	5.34	5.71	5.83	0.56
Litter size on d 21, n	11.0	11.8	12.0	0.60
Litter growth rate, kg/d	1.69*	2.37	2.50	0.11
Milk yield, kg/d	6.26**	9.20	9.06	0.16
N in defatted milk, %	0.95*	1.01	0.99	0.01
Protein in milk, %	4.88*	5.11	5.03	0.04
Casein-N, % of total N in milk	49.30	53.28	52.69	2.30

Values (least-squares means) with * and ** differ respectively at $P < 0.05$ and 0.01 between the lysine-deficient diet and the positive control.

TABLE 4-5 *Effects of dietary amino acid regime on arterial concentrations of plasma amino acids and urea in the lactating sow*

Amino acid	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Taurine	75.51*	19.89	20.51	9.04
Urea	5514.19†	2794.98	4329.01	816.71
Aspartic acid	34.94	47.51	48.59	6.84
Threonine	470.70†	225.00	224.63	67.48
Serine	111.05	88.88	72.85	7.63
Asparagine	57.58	61.39	67.11	13.45
Glutamic acid	368.08	411.96	398.73	77.45
Glutamine	512.73	559.21	450.32	37.68
Proline	266.28	256.11	250.98	32.75
Glycine	774.64	939.87	785.19	93.02
Alanine	466.62	685.86	666.15	55.19
Citrulline	103.86	86.79	86.62	17.11
Valine	529.96	664.85	1236.68**	60.88
Cystine	6.06†	2.20	4.23	0.91
Methionine	57.15	60.83	51.57	7.38
Isoleucine	109.80	87.38	98.70	11.02
Leucine	192.25	123.52	148.46	24.14
Tyrosine	155.88	88.73	68.31	36.94
Phenylalanine	101.56	61.12	64.27	14.77
Tryptophan	51.69	35.01	34.46	7.83
Ornithine	21.37	30.82	28.33	5.61
Lysine	32.67**	253.98	203.94	27.05
Histidine	108.98	113.21	101.08	9.07
Arginine	102.18	113.84	125.77	15.58

Values (least-squares means, *μmol/L*) with †, *, and ** differ at $P < 0.10$, 0.05, and 0.01, respectively, between the lysine-deficient diet and the positive control or between the valine-excess diet and the positive control.

TABLE 4-6 *Effects of dietary amino acid regime on mammary extraction rates of plasma amino acids and urea in the lactating sow*

Amino acid	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Taurine	12.59	-2.23	-10.40	9.47
Urea	5.93	2.98	-9.91	5.03
Aspartic acid	-2.25	2.57	-10.72	4.40
Threonine	10.60	17.64	4.59†	3.05
Serine	26.18*	46.70	38.18	4.37
Asparagine	26.05	25.43	26.29	11.90
Glutamic acid	16.78	15.27	6.27	7.54
Glutamine	12.52	13.26	5.72	5.30
Proline	12.92	13.74	9.00	2.49
Glycine	9.69	5.94	-2.56	5.62
Alanine	10.71	9.73	-0.83	5.04
Citrulline	14.82	-4.52	-7.71	9.57
Valine	15.30	14.30	6.90	3.39
Cystine	19.53*	41.25	19.11*	2.90
Methionine	18.65*	24.45	24.65	0.45
Isoleucine	28.85	38.28	27.63	4.55
Leucine	28.46*	49.64	35.37†	4.34
Tyrosine	15.76	25.34	20.59	3.32
Phenylalanine	20.75	36.50	30.21	5.17
Tryptophan	6.71	2.40	1.46	7.36
Ornithine	32.06	30.22	15.14†	3.54
Lysine	51.15*	22.75	15.35	4.53
Histidine	12.00	17.12	0.82†	3.63
Arginine	34.79	28.04	14.02*	2.52

Values (least-squares means, %) with † and * differ respectively at $P < 0.10$ and 0.05 between the lysine-deficient diet and the positive control or between the valine-excess diet and the positive control.

TABLE 4-7

Effects of dietary amino acid regime on kinetics parameters of plasma lysine across the lactating porcine mammary gland

Item	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Fwb	14.89**	28.58	29.67	0.48
Fmg	7.71**	15.07	9.58**	0.46
Fa,o	11.22**	55.41	52.16	4.38
Fv,a	2.59	9.13	0.07	7.01
Fmg,a	9.65*	38.47	52.64	7.83
Fv,mg	4.18*	24.87	44.58	7.98
Fo,v	5.57*	42.19	44.02	3.80
Fmg,o	2.57	5.98	3.29	1.07
Fo,mg	9.55**	17.43	10.35**	0.60
Ra	7.32*	48.17	58.23	8.41
NB	6.47*	10.91	7.10*	0.77

Values (least-squares means, mmol/h) with †, *, and ** differ at $P < 0.10$, 0.05, and 0.01, respectively, between the lysine-deficient diet and the positive control or between the valine-excess diet and the positive control.

TABLE 4-8

Effects of dietary amino acid regime on kinetics parameters of plasma methionine across the lactating porcine mammary gland

Item	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Fwb	6.06†	6.93	6.86	0.23
Fmg	2.42*	4.05	3.71	0.37
Fa,o	11.43	13.77	13.37	0.84
Fv,a	7.83	7.55	6.95*	0.15
Fmg,a	3.42†	6.00	6.54	0.94
Fv,mg	1.43	2.73	3.35	0.68
Fo,v	9.20	10.35	10.08	0.59
Fmg,o	1.32*	2.29	1.39*	0.15
Fo,mg	3.34*	5.58	4.51	0.41
Ra	4.74†	8.34	7.94	1.08
NB	1.96*	3.28	3.11	0.26

Values (least-squares means, mmol/h) with † and * differ respectively at $P < 0.10$ and 0.05 between the lysine-deficient diet and the positive control or between the valine-excess diet and the positive control.

TABLE 4-9

Effects of dietary amino acid regime on kinetics parameters of plasma valine across the lactating porcine mammary gland

Item	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Fwb	29.97	34.81	43.17*	1.85
Fmg	20.11	23.08	24.02	4.65
Fa,o	112.57**	150.91	333.99**	4.80
Fv,a	83.61†	111.15	313.68**	8.79
Fmg,a	28.26	36.21	23.47	8.44
Fv,mg	11.82	15.95	0.00*	4.21
Fo,v	95.50*	129.35	310.66**	6.35
Fmg,o	5.79	6.72	2.90*	0.67
Fo,mg	24.02	27.25	27.12	4.99
Fo,mg(p)	11.47*	19.17	15.49	1.41
Fo,mg(o)	12.55	8.08	11.63	4.97
Ra	36.36	44.78	26.34	9.09
NB	15.30	20.19	23.99	4.35

Values (least-squares means, mmol/h) with †, *, and ** differ at $P < 0.10$, 0.05, and 0.01, respectively, between the lysine-deficient diet and the positive control or between the valine-excess diet and the positive control.

TABLE 4-10

Effects of dietary amino acid regime on fractional ratios of plasma lysine kinetics across the lactating porcine mammary gland

Item	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Fmg/Fwb	0.48†	0.54	0.33**	0.02
Fmg,a/Fa,o	0.86	0.74	1.00	0.09
Fv,a/Fa,o	0.14	0.26	0.00	0.09
NB/Fa,o	0.51*	0.23	0.15	0.05
Fmg,a/Ra	0.80*	0.87	0.93*	0.01
Fmg,o/Ra	0.20*	0.13	0.07*	0.01
Fo,mg/Ra	0.67*	0.42	0.20†	0.05
Fmg,a/Ca, L/min	7.42	7.52	10.94	1.39

Values (least-squares means) with †, *, and ** differ at $P < 0.10$, 0.05, and 0.01, respectively, between the lysine-deficient diet and the positive control or between the valine-excess diet and the positive control.

TABLE 4-11

Effects of dietary amino acid regime on fractional ratios of plasma methionine kinetics across the lactating porcine mammary gland

Item	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Fmg/Fwb	0.41†	0.60	0.55	0.06
Fmg,a/Fa,o	0.32	0.45	0.49	0.04
Fv,a/Fa,o	0.68	0.55	0.51	0.04
NB/Fa,o	0.19**	0.24	0.25	0.00
Fmg,a/Ra	0.73	0.72	0.82*	0.02
Fmg,o/Ra	0.27	0.28	0.18*	0.02
Fo,mg/Ra	0.69	0.67	0.57	0.04
Fmg,a/Ca, L/min	2.97	4.60	5.40	0.73

Values (least-squares means) with †, *, and ** differ at $P < 0.10$, 0.05, and 0.01, respectively, between the lysine-deficient diet and the positive control or between the valine-excess diet and the positive control.

TABLE 4-12

Effects of dietary amino acid regime on fractional ratios of plasma valine kinetics across the lactating porcine mammary gland

Item	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Fmg/Fwb	0.63	0.69	0.55	0.13
Fmg,a/Fa,o	0.26	0.26	0.06†	0.06
Fv,a/Fa,o	0.75	0.74	0.94†	0.06
NB/Fa,o	0.15	0.14	0.07	0.03
Fmg,a/Ra	0.84	0.85	0.90	0.02
Fmg,o/Ra	0.16	0.15	0.10	0.02
Fo,mg(p)/Ra	0.33	0.46	0.63	0.12
Fo,mg(p)/Fo,mg	0.58	0.73	0.62	0.18
Fo,mg(o)/Fo,mg	0.42	0.27	0.38	0.18
Fmg,a/Ca, L/min	2.27	2.52	0.74†	0.52

Values (least-squares means) with † differ at $P < 0.10$ between the valine-excess diet and the positive control.

TABLE 4-13

Effects of dietary amino acid regime on mammary protein synthesis and breakdown based upon protein equivalent methionine fluxes

Item	Dietary amino acid regime			SEM
	Lysine deficient	Positive control	Valine excess	
Protein synthesis (PS), g/d[a]	583.3*	975.1	787.1	71.6
Protein breakdown (PB), g/d[b]	229.5*	399.6	243.1*	25.5
PB/PS	0.39	0.41	0.31**	0.01
Net balance of protein, g/d[c]	353.7*	575.5	544.0	46.2
Net production of protein, g/d[d]	355.0**	529.0	514.2	10.1

Values (least-squares means) with * and ** differ at $P < 0.05$ and 0.01, respectively, between the lysine-deficient diet and the positive control or between the valine-excess diet and the positive control.

[a]Protein synthesis is reflected by rate of disappearance of mammary intracellular methionine (Fo,mg, see Figure 2).

[b]Protein breakdown is reflected by rate of appearance of mammary intracellular methionine from endogenous source (Fmg,o, see Figure 2).

[c]Net balance of protein = protein synthesis - protein breakdown.

[d]Estimated from the sum of output of protein in milk and accretion (14.81 g/d) of protein in the lactating porcine mammary gland (Kim et al. 1999).

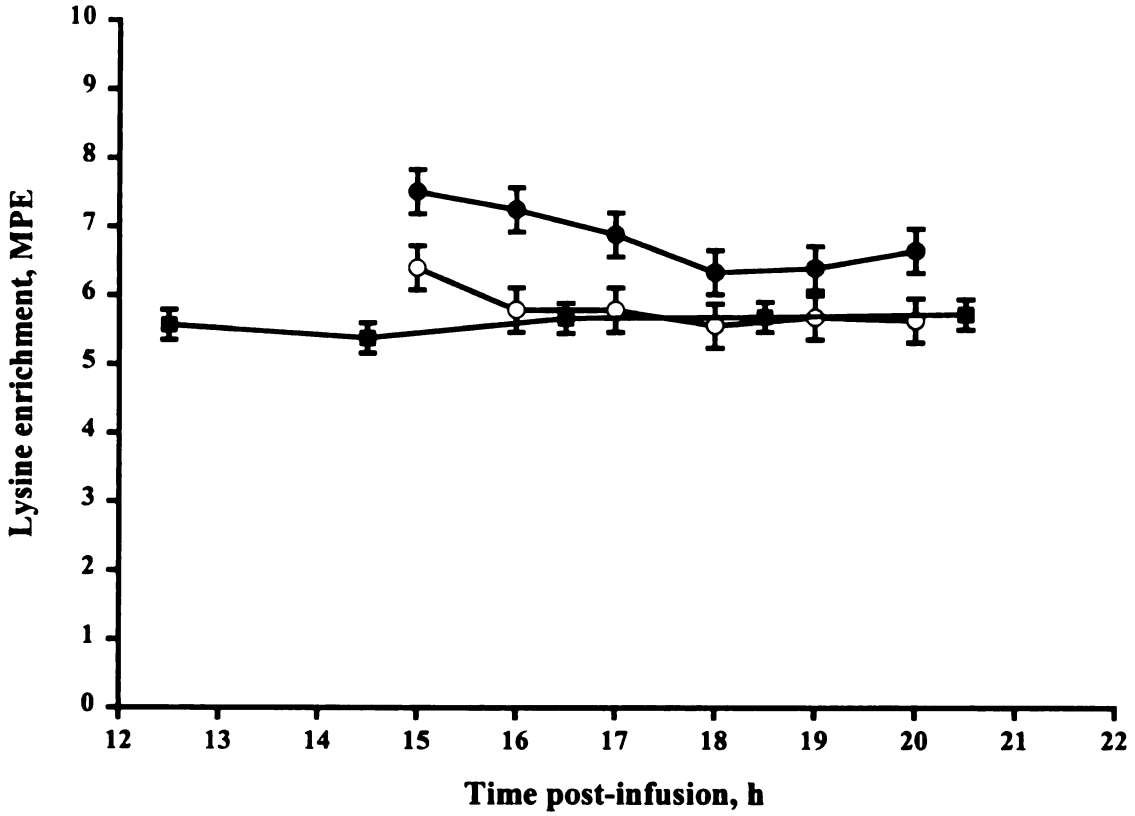

FIGURE 4-1a Enrichment of plasma free- and milk casein-bound lysine in the sows fed the lysine-deficient diet. Lines with closed circles, open circles, and closed squares represent the enrichments of plasma lysine in the carotid artery, plasma lysine in the mammary vein, and casein-bound lysine in milk, respectively.

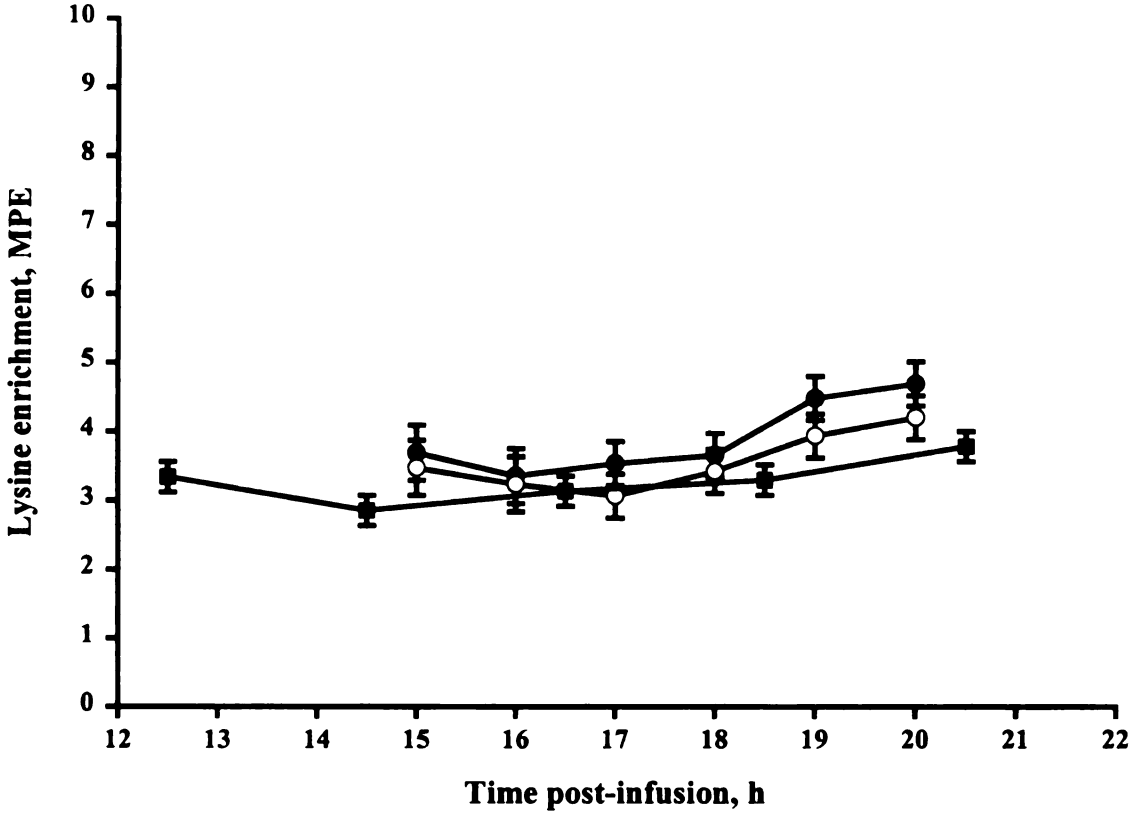

FIGURE 4-1b Enrichment of plasma free- and milk casein-bound lysine in the sows fed the positive control diet. Lines with closed circles, open circles, and closed squares represent the enrichments of plasma lysine in the carotid artery, plasma lysine in the mammary vein, and casein-bound lysine in milk, respectively.

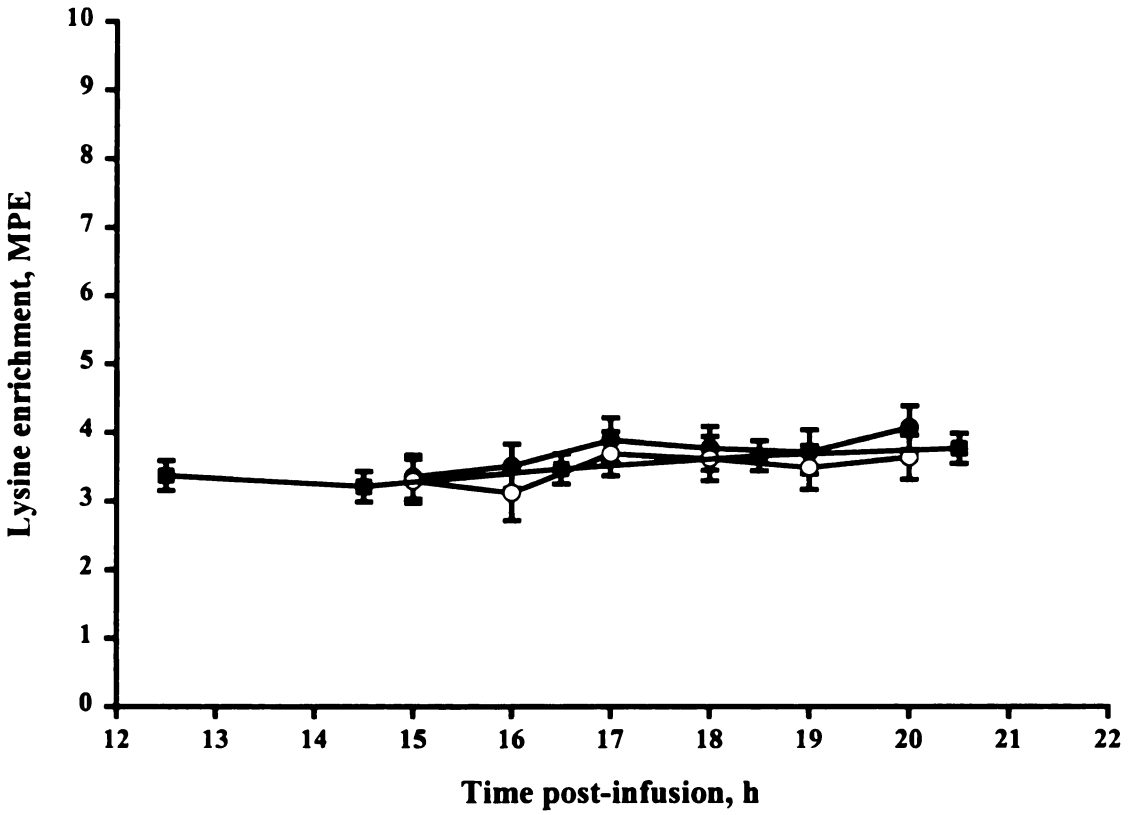

FIGURE 4-1c Enrichment of plasma free- and milk casein-bound lysine in the sows fed the valine-excess diet. Lines with closed circles, open circles, and closed squares represent the enrichments of plasma lysine in the carotid artery, plasma lysine in the mammary vein, and casein-bound lysine in milk, respectively.

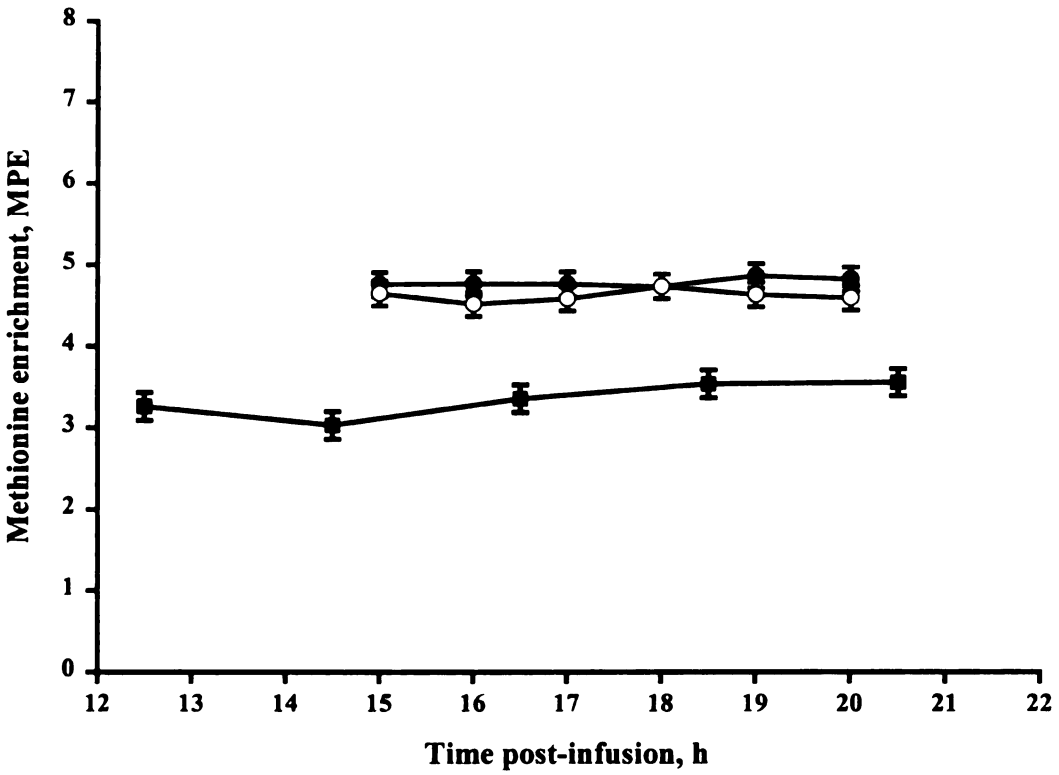

FIGURE 4-1d Enrichment of plasma free- and milk casein-bound methionine in the sows fed the lysine-deficient diet. Lines with closed circles, open circles, and closed squares represent the enrichments of plasma methionine in the carotid artery, plasma methionine in the mammary vein, and casein-bound methionine in milk, respectively.

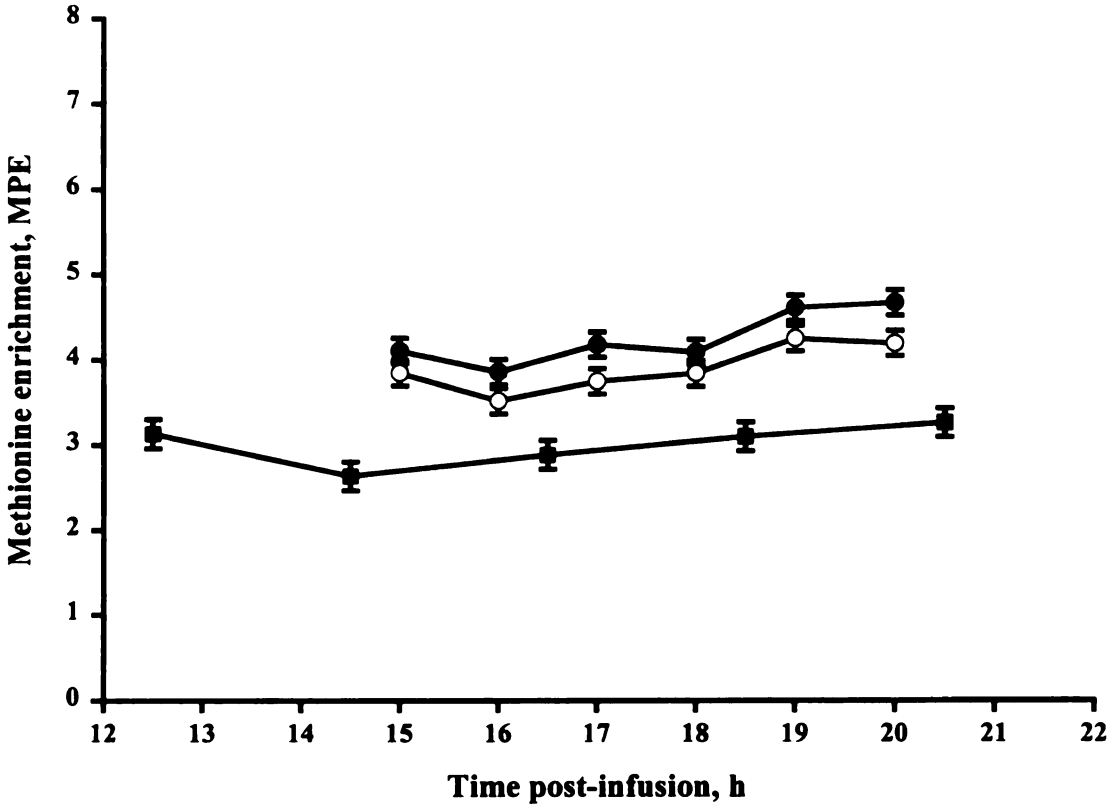

FIGURE 4-1e Enrichment of plasma free- and milk casein-bound methionine in the sows fed the positive control diet. Lines with closed circles, open circles, and closed squares represent the enrichments of plasma methionine in the carotid artery, plasma methionine in the mammary vein, and casein-bound methionine in milk, respectively.

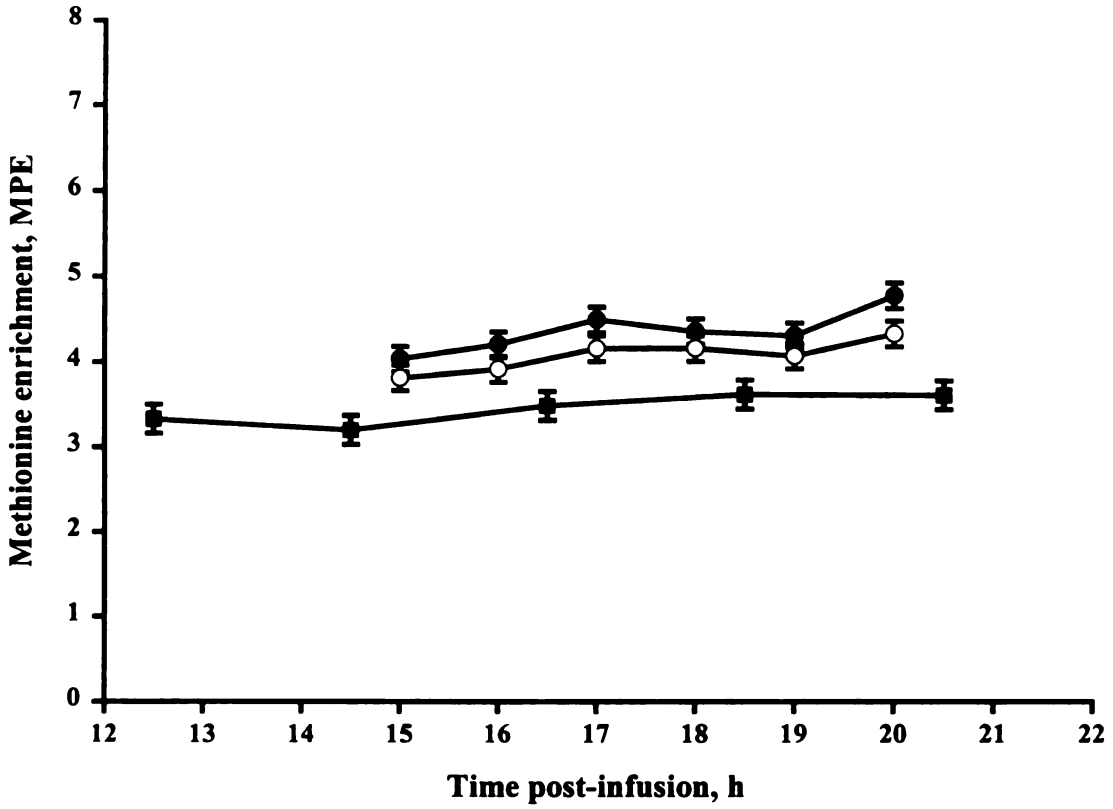

FIGURE 4-1f Enrichment of plasma free- and milk casein-bound methionine in the sows fed the valine-excess diet. Lines with closed circles, open circles, and closed squares represent the enrichments of plasma methionine in the carotid artery, plasma methionine in the mammary vein, and casein-bound methionine in milk, respectively.

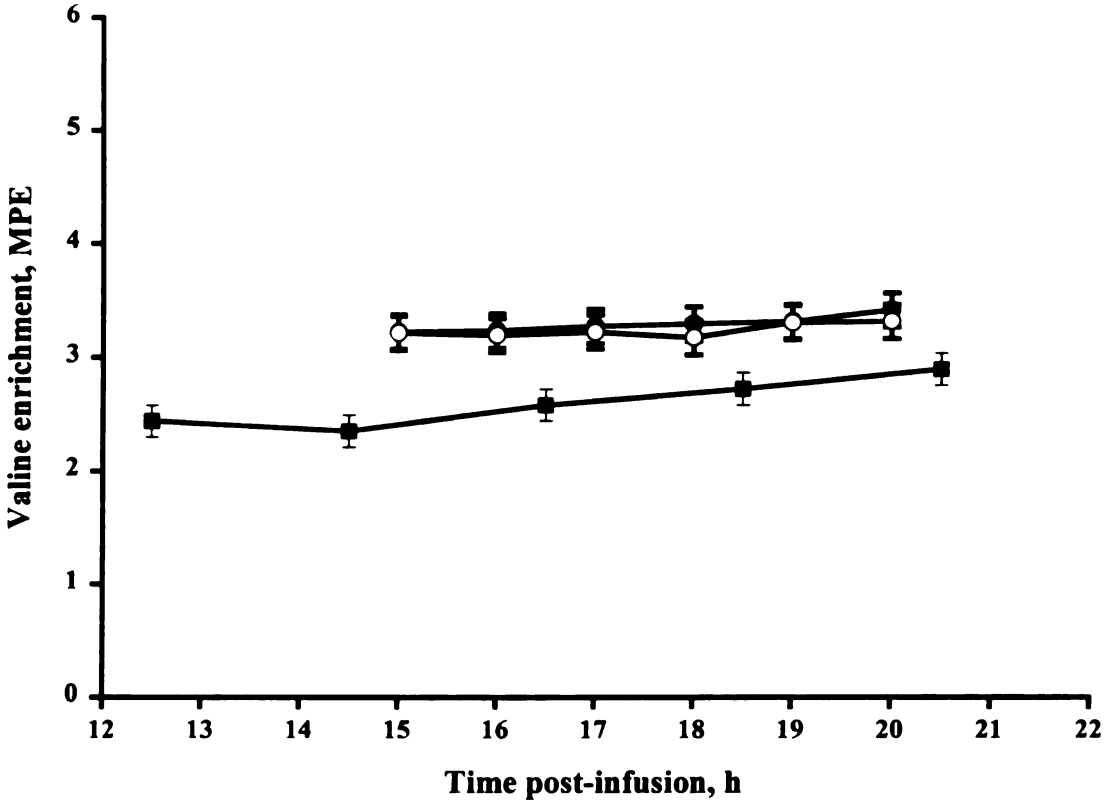

FIGURE 4-1g Enrichment of plasma free- and milk casein-bound valine in the sows fed the lysine-deficient diet. Lines with closed circles, open circles, and closed squares represent the enrichments of plasma valine in the carotid artery, plasma valine in the mammary vein, and casein-bound valine in milk, respectively.

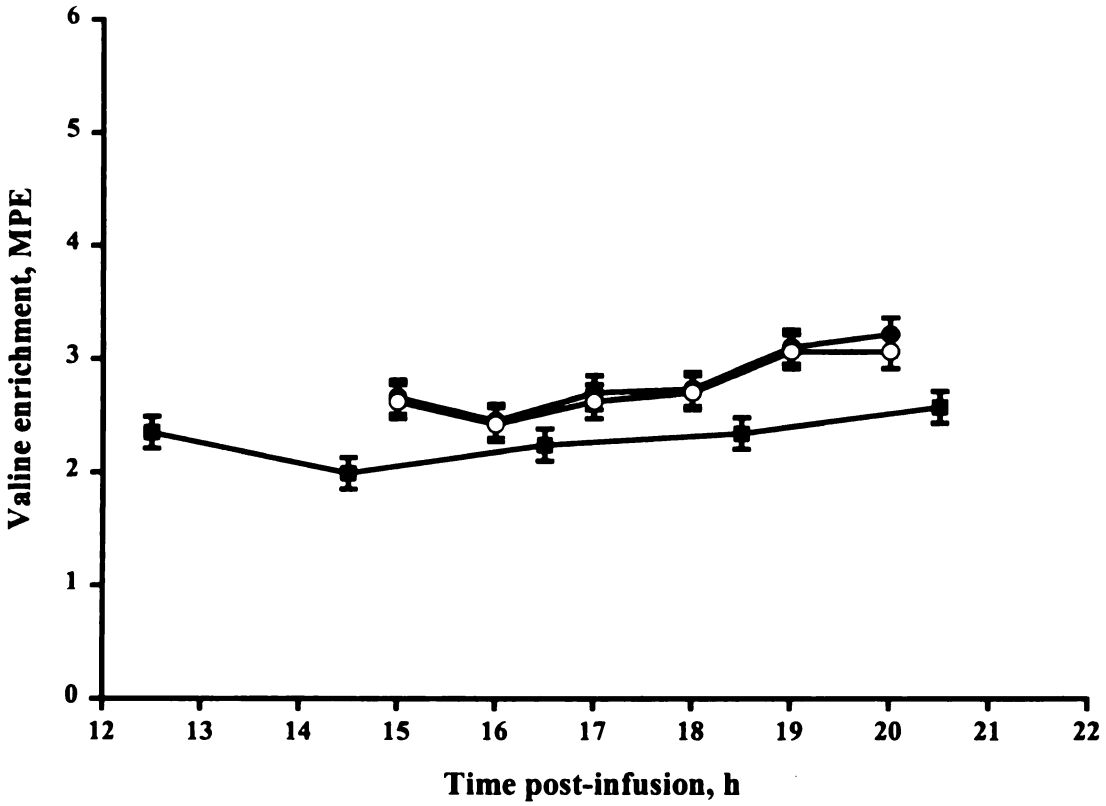

FIGURE 4-1h Enrichment of plasma free- and milk casein-bound valine in the sows fed the positive control diet. Lines with closed circles, open circles, and closed squares represent the enrichments of plasma valine in the carotid artery, plasma valine in the mammary vein, and casein-bound valine in milk, respectively.

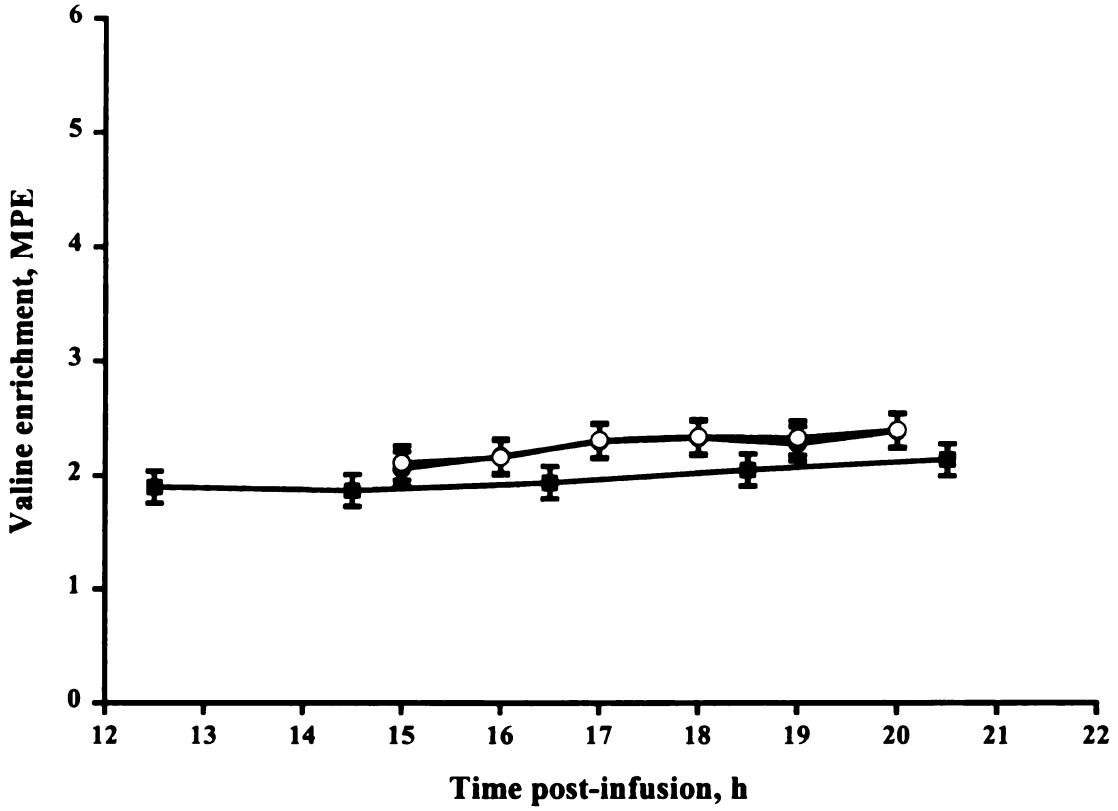

FIGURE 4-1i Enrichment of plasma free- and milk casein-bound valine in the sows fed the valine-excess diet. Lines with closed circles, open circles, and closed squares represent the enrichments of plasma valine in the carotid artery, plasma valine in the mammary vein, and casein-bound valine in milk, respectively.

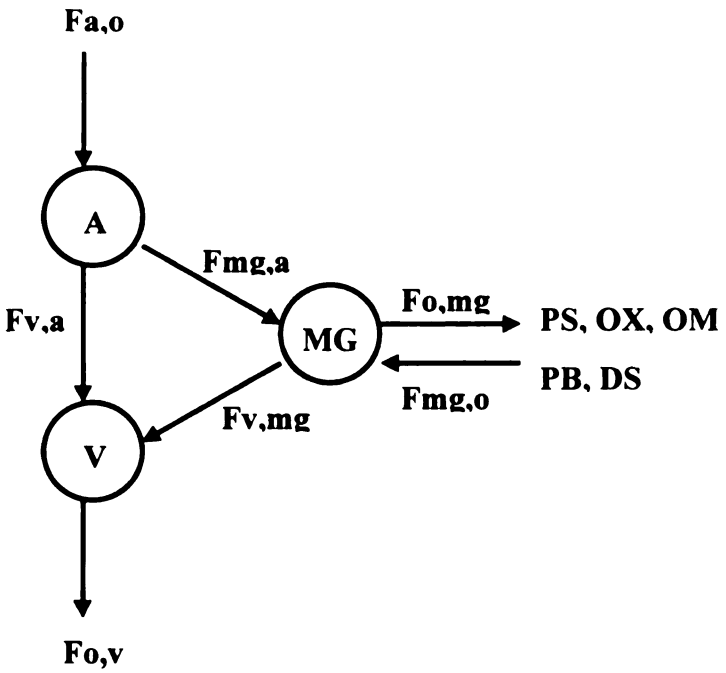

FIGURE 4-2 A three-compartmental model of amino acid kinetics across the lactating porcine mammary gland. Free amino acid compartments in artery (A), main mammary vein (V), and mammary tissue (MG) are connected by arrows indicating unidirectional fluxes of free amino acids between each compartment. Amino acids enter the mammary gland via the mammary artery (Fa,o) and leave the mammary gland via the main mammary vein (Fo,v). Other fluxes are designated as follows: Fv,a, direct flow of amino acids from artery to vein without entering intracellular fluid (by the arterial shunt); Fmg,a and Fv,mg, inward and outward trans-membrane transport of amino acids from artery to the mammary tissue and from the mammary tissue to vein, respectively; Fo,mg, the rate of intracellular amino acid appearance from endogenous sources (i.e., release from protein breakdown (PB) and de novo synthesis (DS), if any); and Fo,mg, the rate of the intracellular amino acids disappearance (i.e., the rate of utilization of intracellular amino acids for protein synthesis (PS), oxidation (OX), and other metabolic fates (OM), if any).

CHAPTER 5

Summary and Conclusions

This dissertation presents data that provide new insight into amino acid nutrition of the porcine mammary gland during lactation. The present study demonstrated that the lactating porcine mammary gland has a large demand for IAA to meet high rate of milk protein synthesis. The lactating sow adapts its physiological needs to the nutritional environment at least on three levels: lactational homeorhesis of the whole body, fine regulation of unidirectional trans-membrane transport across the mammary epithelium, and intracellular control of protein turnover in the mammary gland.

The AA profile in milk is not affected by intake of dietary protein, which supports the notion that modification of the amount of milk protein, rather than the AA profile of milk protein, is the evolutionary mechanism used to meet the unique AA needs of mammalian neonates (Davis et al. 1994a, Davis et al. 1994b). Intake of dietary protein regulates the mammary AA uptake pattern. Interestingly, mammary uptake proportions of the BCAA to the total IAA are up-regulated with increasing intake of dietary protein, implying an increased capacity for the mammary gland to metabolize these AA. This is associated with unique characteristics of intracellular BCAA metabolisms as follows. (1) The BCAA can provide α-amino nitrogen and carbon for the synthesis of dispensable AA (Wohlt et al. 1977); yield energy from their oxidation for high rate of protein turnover and for the synthesis of milk non-protein compounds (e.g., fatty acids and lactose) (Davis and Mepham 1976, Oddy et al. 1988, Roets et al. 1979, Vina and Williamson 1981);

and/or participate in regulation of protein breakdown or in interorgan partitioning of the BCAA (e.g., possibly providing branched-chain α-keto acids for hepatic oxidation) (DeSantiago et al. 1998, MacLean et al. 1994, Mitch and Clark 1984, Nair et al. 1992). (2) The catabolism of the BCAA can be up-regulated to remove their excess (if any) after high intake of dietary protein through increased expression of branched-chain aminotransferase and increased activity of branched-chain α-keto acid dehydrogenase (Langer et al. 2000, Torres et al. 1998). In contrast, mammary uptake proportions of the limiting AA (e.g., Lys, Met, Thr, Phe, and Trp) to the total IAA maintained at a relatively high level under low intake of protein to meet high rate of milk synthesis. Therefore, mammary AA uptake pattern is adaptively regulated by intake of dietary protein to maintain synthesis of milk protein.

Lactational homeorhesis was described in the lactating animal to explain metabolism of the extra-mammary tissues and partitioning of nutrients to the mammary gland to support high rate of milk synthesis (Bauman 1999, Collier 1999). The lactating sow loses body protein and/or lipid to support a high rate of milk production when intake of protein, IAA, and/or energy are inadequate (Clowes et al. 1998, Jones and Stahly 1999, Revell et al. 1998). Thus, we hypothesize that when intake of dietary IAA is extrapolated to zero, the amount of mammary IAA uptake originates from the mobilization of body protein (i.e., endogenous IAA contributions). Endogenous IAA contributions defined by the mammary uptake are supported by two indirect estimations: (1) the algebraic product of body protein loss (defined from nitrogen balance) times the AA composition of body protein, and (2) the backward factorial approach-derived values. These estimates of

endogenous IAA contributions can be used to adjust the total dietary IAA requirements of the lactating sow at different rates of body weight loss.

The factorial approach has been widely used to predict nutrient requirements of domestic animals. The total dietary IAA requirement of the lactating sow encompasses individual components (e.g., maintenance, milk synthesis, and body protein accretion or loss, if any). The present study is the first to estimate dietary IAA needs for milk synthesis by the porcine mammary gland. The estimates are higher than the values recommended by the current NRC (1998), especially for Arg and Val. In the present study, dietary needs of truly digestible IAA for milk synthesis were determined by the maximal mammary uptake. This novel approach has remarkable advantages as follows. (1) Estimates defined by the maximal mammary uptake are independent from any change in body protein, in contrast to estimates of the total dietary IAA requirements determined by conventional approaches. (2) Estimates defined by the maximal uptake account for physiological needs for incorporation into milk protein and metabolism other than protein synthesis (e.g., oxidation) in the mammary gland. In contrast, estimates based only on output of protein in milk might be underestimated, especially for Arg and the BCAA. (3) This novel approach can estimate dietary needs of multiple IAA concurrently; in contrast, the conventional approaches can determine dietary requirement of a single IAA at one time.

Finally, the present study demonstrated that trans-membrane transport, net uptake, and intracellular kinetics of IAA in the porcine mammary gland during lactation are finely regulated by dietary AA regime. Trans-membrane transport and net uptake of an

individual IAA (e.g., Lys) can be rate limiting for the synthesis of milk protein as demonstrated in vitro (Mepham 1982). Moreover, kinetics properties of the intracellular free IAA are coordinated with their cellular transport and intracellular metabolism. For example, net uptake of lysine is inhibited by excess Val, which would result in a decrease in protein synthesis due to decreased availability of the intracellular free Lys. However, intracellular protein breakdown is decreased to a greater degree compared to protein synthesis, which would result in a comparable net balance of protein in the mammary gland.

In conclusion, the present study demonstrated that intake of dietary AA regulates trans-membrane transport, net uptake, and intracellular kinetics of IAA in the porcine mammary gland during lactation to meet high rate of milk protein synthesis. Moreover, trans-membrane transport and net uptake of an IAA (e.g., Lys) is rate limiting for milk synthesis in vivo. Endogenous IAA contributions (mobilized from body protein to the mammary gland for milk synthesis) and dietary IAA needs for milk synthesis by the mammary gland can be estimated by their mammary uptake. Endogenous contributions are 10.40, 2.35, 5.88, 6.36, and 6.20 g/kg body weight loss, respectively, for Lys, Met, Phe, Thr, and Val. Dietary needs of truly digestible indispensable AA for milk synthesis by the mammary gland (at zero change in body protein) are 28.78, 10.04, 20.15, 6.92, 12.10, 13.91, 4.49, and 22.40 g/kg litter weight gain, respectively, for Arg, His, Lys, Met, Phe, Thr, Trp, and Val. While the ideal dietary IAA pattern for milk synthesis based only on the IAA profile of milk protein provides appropriate estimates in absence of

experimental data, it is underestimating for Arg and the BCAA. An optimal dietary ratio of Val to Lys for milk synthesis is approximately at 1:1, which is higher than the ratio of 0.85:1 recommended by the current NRC (1998); however, an increased ratio of dietary Val to Lys above 1:1 will not improve lactational performance.

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