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The effect of litter size and day of lactation on amino acid uptake by the porcine mammary glands[1,2,3]

T. T. Nielsen*, N. L. Trottier†[4], H. H. Stein‡[5], C. Bellaver§, and R. A. Easter‡

*Royal Veterinary and Agricultural University, Copenhagen, Denmark; †Department of Animal Sciences,
Michigan State University, East Lansing 48824; ‡Department of Animal Sciences, University of Illinois,
Urbana 61801; and §EMBRAPA CNPSA, Concordia, Brazil

ABSTRACT: Twelve multiparous sows (PIC Camborough 15; parity >2) were used to investigate the relationship between litter size and day of lactation, and plasma amino acid (AA) arteriovenous differences (A-VD), AA uptake, and plasma flow across the mammary glands. Sows were assigned randomly to one of the following litter sizes: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 pigs per litter by cross fostering on d 2 postpartum. All sows were surgically fitted with catheters in the carotid artery and the main mammary vein. Matched arteriovenous blood samples were obtained on d 9, 12, 15, 18, 21, and 24 postpartum. Daily mammary uptake of AA was based on the product of plasma A-VD and daily mammary plasma flow (MPF). Daily MPF was estimated using the Fick method based on lysine conservation across the gland, and daily milk production. For the majority of AA, as litter size increased, A-VD did not increase, except for alanine ($P < 0.05$, linear and quadratic) and valine ($P < 0.1$; *trend*; linear and quadratic). As day of lactation increased, A-VD for the majority of AA increased ($P < 0.05$, linear and quadratic) except for arginine, lysine, and phenylalanine. As litter size increased, net daily mammary AA uptake increased for all indispensable AA ($P = 0.001$ to $P < 0.05$, linear and quadratic), excepting arginine. Milk production increased with increasing litter size ($P < 0.001$, linear) and with increasing day of lactation ($P < 0.05$, quadratic). Daily MPF increased ($P < 0.05$, linear) with increasing litter size, but did not change during the period measured from d 9 to 24. In conclusion, litter size appears to be a major determinant of net mammary AA uptake with daily mammary plasma flow a driving variable, whereas AA A-VD is a function of day of lactation and a major variable in determining net AA uptake with advancement of lactation.

Key Words: Amino Acids, Lactation, Mammary Glands, Sows, Uptake

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Introduction

The pattern of uptake of amino acids (AA) by the porcine and dairy cow mammary glands differs significantly from that found in secreted milk (Hanigan et al., 1991; Guinard et al., 1994; Trottier, 1997). In consequence, current nutrient models where requirements for milk production are based solely on the AA composition of secreted milk protein may inaccurately predict requirements. Furthermore, empirical approaches such as this provide no means of predicting or maximizing milk production when diet ingredients differ because they lack the mechanistic descriptions necessary to predict how milk production responds to individual nutrients (e.g., AA or energy supply). Modeling AA requirements based on measurement of mammary arteriovenous AA differences (**A-VD**) and blood flow may allow some description of this process. Other important production factors, such as stage of lactation and suckling load (litter size), could be assessed using the net uptake approach to further refine the model. In cows and goats, the technique has been applied for years; recently this information has been used in the development of a mechanistic model of mammary metabolism in the dairy cow (Hanigan et al., 2001). Due to technical limitations, there have been few attempts to monitor net fluxes of AA by the porcine mammary glands. Previous results (Trottier et al., 1997) indicate that daily net mammary AA uptake by the porcine mammary glands may be regulated

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[4] Correspondence: 2209 Anthony Hall (phone: 517-432-5140, fax: 517-432-0190, E-mail: trottier@pilot.msu.edu).

[5] Current address: Department of Animal and Range Sciences, South Dakota State University, Brookings 57007.

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Table 1. Composition of the experimental diet (as fed)

Item	%
Ingredients	
Ground corn	70
Dehulled soybean meal	25
Soybean oil	2
Dicalcium phosphate	1.45
Limestone	1
Mineral mix[a]	0.35
Vitamin mix[b]	0.2
Calculated analysis	
CP	18.1
Arginine	1.22
Histidine	0.49
Lysine	0.96
Tryptophan	0.24
Threonine	0.73
Leucine	1.74
Isoleucine	0.78
Methionine + cysteine	0.63
Valine	0.95
Phenylalanine + tyrosine	1.61

[a] Provided the following per kg of complete diet: Fe, 89.9 mg; Zn, 100.1 mg; Mn, 20.0 mg; Cu, 8.0 mg; I, 0.4 mg; Se 0.3 mg.

[b] Provided the following per kg of complete diet: Vitamin A, 6,667 IU; Vitamin D3, 667 IU; Vitamin E, 88 IU; Vitamin K, 4.4 mg; riboflavin, 8.9 mg; D-pantothenic acid, 24.4 mg; niacin, 33.3 mg; choline, 244 mg.

through A-VD or mammary plasma flow (**MPF**). We hypothesized that net AA uptake by the porcine mammary glands increases as litter size and day of lactation increases via an increase in AA A-VD and MPF. The objectives of the present experiment were to examine relationships between mammary net fluxes of AA and litter size and day of lactation, and to evaluate whether increasing litter size and day of lactation increase AA uptake across the porcine mammary system through change in plasma flow or AA extraction rate.

Materials and Methods

Animals and Experimental Design

The experiment was approved by the University of Illinois Laboratory Animal Care Committee (Protocol No. A3R166).

Twelve multiparous sows (PIC Camborough 15; parity >2) were selected for sound udder conformation and moved to the farrowing facility 1 wk prior to farrowing. Sows were housed in farrowing crates equipped with a plastic-coated expanded metal floor. Drinking water was available at all times from nipple drinkers, and the ambient temperature was kept between 22 and 24°C. Additional heat was provided for nursing pigs by use of heat lamps until 10 d postpartum. After farrowing, sows were given ad libitum access to a common lactation diet (Table 1). This diet was also fed at a rate of 2 kg per day starting 1 wk prior to farrowing. Daily sow feed intake and feed refusal were recorded. On d 1 postpartum, pigs and sows were weighed. On d 2 postpartum, sows were assigned randomly to one of the following litter sizes: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 pigs. All extra pigs were distributed to other sows in the herd to ensure their welfare. To avoid disturbing the established teat order among pigs, no further additions of pigs to the experimental sows were allowed after this day. Therefore, if a pig died during the experiment, it was not replaced. Two sows (litter 8 and 14) lost piglets during the course of the lactation period. They were included in the statistical analysis model with the corresponding number of pigs at any time-point within the lactation period studied.

On d 5 of lactation, all sows were fitted surgically with catheters in the carotid artery and mammary vein following the procedure described by Trottier et al. (1995). Sows were allowed to recover until d 8 of lactation, when the sampling protocol was initiated. Measurements were divided over the lactation period into six 3-d intervals, i.e., d 8 to 10, d 11 to 13, d 14 to 16, d 17 to 19, d 20 to 22, and d 23 to 25. Milk production was measured by the weigh-suckle-weigh method (Speer and Cox, 1984) on the first day of each interval, i.e., d 8, 11, 14, 17, 20, and 23. Arterial and mammary venous blood samples were obtained on the second day of each interval, i.e., d 9, 12, 15, 18, 21, and 24. Milk samples were collected on the third day of each interval, i.e., d 10, 13, 16, 19, 22, and 25.

Sampling Procedures

Arterial and venous blood samples were collected simultaneously via the carotid and mammary venous catheters, respectively, into Luer Monovette syringes containing lithium heparin (Sarstedt, Pennsauken, NJ). Blood samples were obtained every 20 min over a total period of 100 min (6 samples). Plasma was separated after centrifugation, and equal plasma volumes pooled over the 100-min period. Plasma samples were prepared for AA analysis as described by Trottier et al. (1997). Milk samples were obtained from the third and fourth thoracic gland and prepared for AA

Table 2. Sow and litter performance

Item	Mean	SEM[a]
Sows		
Number	12	
Parity no.	4.91	0.38
Daily sow feed intake, kg	4.89	0.12
Body weight on d 1 postpartum, kg	255	3.23
Body weight at weaning, kg	245	5.48
Lactation body weight change, kg	–9.42	3.58
Litter		
Weaning age, d	27.3	0.25
Pig no. at birth	10.8	0.93
Pig weight at birth, kg	1.54	0.03
Pig weight at weaning, kg	6.87	0.16
Avg. daily pig weight gain, g	187	3.11

[a] Standard error of the mean.

Table 3. Amino acid arteriovenous differences (μmol/L plasma) across the porcine mammary glands in relation to litter size[a]

	Litter size											*P*-value	
Amino acid	3	4	5	6	7	9	10	11	12	13	SEM[b]	Linear	Quadratic
Indispensable													
Arginine	32.3	34.3	40.8	37.1	51.4	46.7	36.8	38.9	42.6	46.4	3.89	0.152	0.291
Histidine	15.3	5.5	16	10.1	8.9	12.6	11.8	12	11.3	13.7	1.87	0.777	0.490
Isoleucine	34.5	35.8	41.2	39.7	47.5	44.5	33.4	40	35.5	39	2.66	0.967	0.114
Leucine	64.3	66.2	78.7	70.9	83.1	78.9	62.4	74.8	69.8	74.5	4.87	0.686	0.251
Lysine	38.9	27.4	46.3	38.7	56.6	51.2	34.8	40.5	41	45.1	3.59	0.561	0.322
Methionine	9.3	9.6	11.9	9.6	12.1	12.2	9.4	11.2	10.9	11.5	0.87	0.266	0.444
Phenylalanine	18.6	16.5	23.8	19.5	27.3	25.2	21.9	24	21.6	24	1.84	0.154	0.149
Threonine	29.1	25.5	33.8	28.2	38.6	39	30.2	33.8	31.3	32.3	2.77	0.344	0.144
Valine	38.6	27.7	47.4	49.1	59.8	67.7	46.2	40.4	36.9	50.7	4.82	0.083	0.095
Dispensable													
Alanine	13.3	9.7	38.6	27	44.5	64.1	63	51.8	57.4	52.7	11.50	0.010	0.028
Aspartic acid	1.2	2.1	0.5	1.1	4.2	4.6	3.8	−0.7	−1.1	1.3	0.70	0.654	0.161
Glutamic acid	85.4	89.8	103.2	116.3	144.2	109.6	73.4	61.5	16.5	105.4	11.05	0.252	0.315
Glycine	38	21.4	46.7	40.4	60.6	39.1	77.2	54.9	29.6	43.1	15.69	0.453	0.168
Proline	44.3	40.4	67.9	49.6	65.1	72.3	55.1	60.4	44.2	73.3	7.69	0.247	0.401
Serine	41.5	39.3	41.1	36	49.9	52	39.2	44.7	40.5	43.1	3.27	0.564	0.376
Tyrosine	13.8	13.8	21.2	15.6	18.8	18.3	20	18.7	13.7	18.3	2.73	0.457	0.149

[a] Data represent means pooled across day of lactation (n = 6).

[b] Pooled standard error of the mean.

analysis following the method described by Trottier et al. (1997).

Calculations and Statistical Analysis

Daily MPF rate (L of plasma/d) and plasma flow-to-milk yield ratio (**P:M**) (L plasma:L milk) were estimated for each sow and each interval based upon the conservation of lysine across the gland (Fick principle) as described by Trottier et al., 1997. Net daily mammary uptake of each AA (g/d) was calculated from the product of daily MPF and AA A-VD. Percentage extraction was calculated as: 100 × ((A − V)/V). The proportion of each AA taken up by the gland and secreted into milk (proportion secreted in milk, **PSM**) was calculated as follows:

$$\mathrm{PSM} = (a \times b)/(c \times d)$$

where

a = AA concentration in milk, g/L milk
b = milk yield, L milk/d
c = A-VD, g/L plasma
d = daily MPF, L plasma/d

Normality of data was tested using the Shapiro-Wilks test defined in the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Amino acid A-VD, extraction rate, net uptake, and arterial concentration, PSM, MPF, milk production, and plasma to milk ratio were analyzed using the GLM procedure of SAS. Litter size and day of lactation were included as regression variables. Day of blood sampling was chosen to represent the regression variable "day of lactation." Litter size and day of lactation were regressed against each independent variable listed above using the following model:

$$Y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 z + \beta_4 z^2$$

where Y is the independent variable, β_0 is the y intercept, β_1 and β_2 are the parameter estimates for litter size, and β_3 and β_4 are the parameter estimates for day of lactation. For AA net uptake, parameter estimates for the quadratic term litter size was not significant for any AA, thus it was not included in this

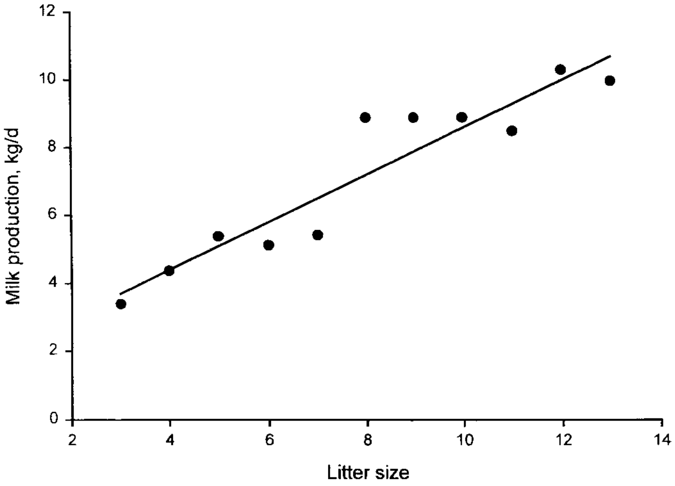

Figure 1. Relationship between litter size and milk production. Form of relationship is linear ($P < 0.001$).

Table 4. Amino acid extraction rates (%) across the porcine mammary glands in relation to litter size[a]

	Litter size											*P*-value	
Amino acid	3	4	5	6	7	9	10	11	12	13	SEM[b]	Linear	Quadratic
Indispensable													
Arginine	21.8	16.6	24.3	30.1	27.2	25.1	22	24.4	24.4	23	3.25	0.635	0.138
Histidine	23.4	11.3	21.1	16.9	6.4	16.9	14.9	16.9	13.5	15.6	2.80	0.503	0.332
Isoleucine	24.9	27.9	34.3	36.8	30.4	38.7	29.4	32.1	38.7	33.6	2.32	0.137	0.266
Leucine	26.3	25.8	33.9	33.8	29.7	38.2	28.8	33	36.1	32.2	2.20	0.123	0.248
Lysine	26.7	20.8	32.8	32.5	37.8	41.8	35.1	29.4	43.7	32.4	4.04	0.097	0.148
Methionine	32.7	25.4	23.1	31.1	32.7	36.3	28.9	29	33.4	35.1	2.90	0.180	0.803
Phenylalanine	21.9	19.5	27.1	25.4	30.2	27.8	24.6	20.2	24.5	24.8	2.19	0.797	0.124
Threonine	18.7	17.6	23	24.3	27.1	27.2	23.1	23.9	25.3	24.2	2.19	0.013	0.024
Valine	12.5	10	17.9	22.5	19.7	26.1	17.1	15	17	19.2	2.08	0.061	0.077
Dispensable													
Alanine	3.9	7.5	7.4	14.1	8.7	14.4	9.2	11.6	12.8	12.1	2.35	0.039	0.152
Aspartic acid	7.6	11	1.2	6.1	15.9	20.2	12.4	−1.1	−3.6	3.2	2.59	0.410	0.164
Glutamic acid	40	32.6	28.8	20.8	36.7	27.1	17.9	20.6	4.3	24	3.04	0.019	0.750
Glycine	5.1	5.2	4.6	6.2	6.5	5	6.1	7.1	3.5	6.1	1.48	0.728	0.488
Proline	12.2	11.3	16.1	22	14.4	21	13.7	16.1	14.1	12.3	3.43	0.051	0.050
Serine	26.6	24.4	25.9	27.1	30.5	34.1	26.8	35.3	26.3	25	2.08	0.460	0.113
Tyrosine	14.9	13.1	17.4	21.9	15.2	17.6	12.6	15.4	9.4	13.7	2.54	0.243	0.192

[a] Data represent means pooled across day of lactation (n = 6). Calculated as arteriovenous difference (μmol/L) × 100/arterial concentration (μmol/L).

[b] Pooled standard error of the mean.

model. Probability values less than 0.05 were considered significant and less than 0.1 were considered indicative of a trend.

Results and Discussion

Sow and litter performance are presented in Table 2. Feed intake and body weight changes of sows during lactation were comparable to similar studies conducted in this facility (Trottier et al., 1997), indicating that the cannulation procedure did not impair performance. For the majority of both indispensable and dispensable AA, no clear relationships (linear or quadratic, $P > 0.05$) were observed between litter size and AA flow across the mammary glands, i.e., A-VD (Table 3) and extraction rate (Table 4). As litter size increased, A-VD for only valine (linear and quadratic trend, $P < 0.1$) and alanine (linear and quadratic, $P < 0.05$) increased, and extraction rates of indispensable AA increased for only lysine (linear trend; $P < 0.1$), threonine (linear and quadratic, $P < 0.05$), and valine (linear and quadratic trend, $P < 0.1$); of the dispensable AA, an increase was noticed for only alanine (linear, $P < 0.05$), glutamic acid (linear, $P < 0.05$), and proline (linear and quadratic, $P = 0.05$). As litter size increased, milk production increased linearly (Figure 1; $P < 0.001$), in agreement with previously reported studies (King, 2000). Because litter size is the main factor driving total milk production, and milk production is directly dependent on the availability of nutrients for milk synthesis, a stronger relationship between litter size and indispensable AA A-VD across the mammary glands would be expected. Availability of nutrients to the mammary glands depends on both blood nutrient concentration and the rate at which these nutrients are delivered to mammary cells. In this study, sows had access to and consumed similar amounts of feed, thus ensuring that AA availability was similar among litter sizes. Except for the BCAA and threonine, most arterial AA concentration remained unchanged with increasing litter size (Table 5). The rate at which the mammary glands extract AA is a reflection of plasma AA concentration and mammary utilization. Lysine, threonine, and valine constitute the main limiting AA in lactating sows diets, which parallel with the increased extraction rate of these AA with increasing litter size. The lack of a strong relationship between litter size and AA A-VD indicates that differences in milk production between varying litter sizes are in great part a product of MPF rate to the entire mammary system, as indicated in Figure 2. Indeed, when daily MPF rate was used to calculate daily net AA uptake by the entire mammary gland system (Table 6), net AA uptake increased linearly ($P < 0.01$) for all indispensable AA, and for the majority of dispensable AA ($P < 0.05$).

It is unknown whether MPF rate to an individual gland or per unit weight varies with different litter sizes. Kim et al. (1999) found that the amount of mammary tissue protein and DNA in individual mammary gland decreased linearly with increasing litter size from 6 to 12 pigs. In this experiment, as litter size increased from 3 to 13, the number of suckled glands increased correspondingly, and average pig daily gain decreased linearly ($P < 0.01$; data not shown). Thus, it may be deducted that litter size and MPF rate per individual gland are inversely related, although the nature and the strength of this relationship are not

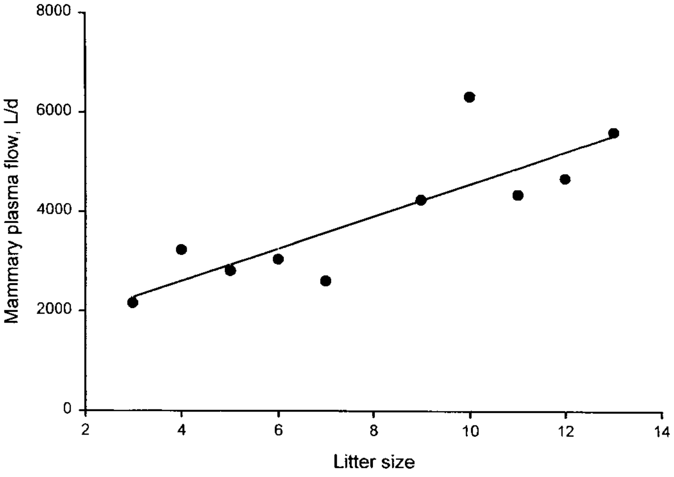

Figure 2. Relationship between litter size and mammary plasma flow. Litter size effect on mammary plasma flow was linear ($P < 0.05$). Parameters estimates and standard error of parameter estimates for the best fit equation are b(0) = 1,297 ± 660.1 ($P = 0.09$); and b(1) = 325.3 ± 76.2 ($P < 0.05$). $r^2 = 0.69$.

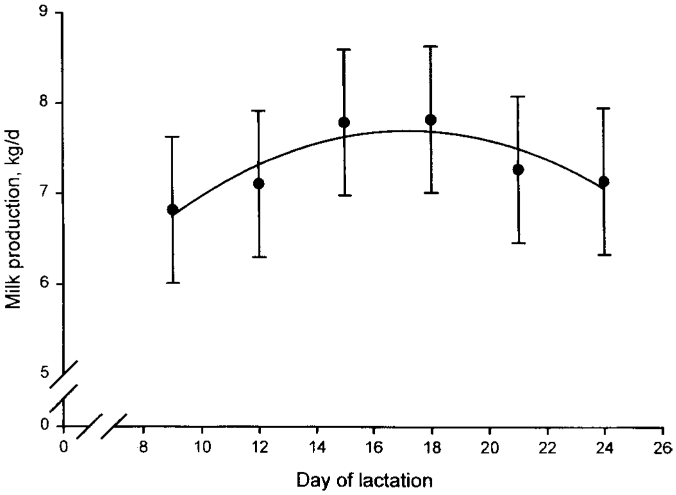

Figure 3. Relationship between day of lactation and daily milk production. Data are least-square means ± SEM pooled across litter sizes. Form of relationship is quadratic at $P < 0.05$.

known. It is well documented in sows that suckling demand is the major determinant of milk yield from an individual gland (Auldist et al., 1995; Hartmann et al., 1997), hence suckling demand per individual gland may decrease with increasing litter size. Nonetheless, the overall suckling demand to the mammary gland system must be increased with increasing litter size, because milk production increases with increasing litter size. Our sampling site was located at the anterior end of the main mammary vein; hence our plasma flow estimate included the entire mammary gland system, hence all suckled mammary glands. Thus, it is possible that the increased milk production resulting from increased overall suckling demand is a function of blood flow.

Milk production increased as day of lactation increased (quadratic, $P < 0.05$) and appeared to reach a maximum between d 15 and d 21 (Figure 3), agreeing with results reported by Jones and Stahly (1999). As day of lactation increased, both A-VD (Table 7) and extraction rates (Table 8) for the majority of indispensable AA increased (linear and quadratic, $P < 0.05$), and appeared to reach a maximum around d 15. This

Table 5. Arterial amino acid concentration (μmol/L plasma) in lactating sows in relation to litter size[a]

	Litter size											*P*-value	
Amino acid	3	4	5	6	7	9	10	11	12	13	SEM[b]	Linear	Quadratic
Indispensable													
Arginine	156.3	225.5	203.6	163.8	227.1	190.0	174.1	165.3	182.7	221.3	52.72	0.942	0.949
Histidine	67.8	62.8	84.1	75.4	81.2	75.8	73.5	71.0	81.3	91.0	13.04	0.105	0.928
Isoleucine	142.6	135.3	140.3	120.1	156.0	116.0	116.5	126.7	94.6	121.8	24.14	0.041	0.889
Leucine	254.1	261.7	278.9	235.7	278.5	208.7	219.7	226.3	194.6	239.3	43.12	0.034	0.862
Lysine	164.9	165.3	163.4	147.0	165.4	122.7	118.5	146.5	92.8	160.0	42.42	0.075	0.454
Methionine	30.9	44.0	39.6	52.0	36.9	33.5	34.5	39.1	31.3	33.1	7.39	0.271	0.296
Phenylalanine	89.2	93.3	106.2	87.6	89.9	92.3	90.3	119.4	89.4	100.4	16.82	0.445	0.873
Threonine	164.5	166.0	171.3	150.9	142.2	146.4	127.3	143.7	125.6	139.5	29.09	0.003	0.345
Valine	334.9	335.9	310.5	268.7	302.0	256.0	258.1	272.5	216.6	262.1	43.20	0.002	0.262
Dispensable													
Alanine	371.0	383.7	485.8	504.9	518.5	450.9	510.2	453.9	458.4	531.4	116.47	0.100	0.183
Aspartic acid	16.2	21.2	31.7	28.6	26.6	23.3	24.6	24.4	31.0	33.8	6.00	0.093	0.873
Glutamic acid	216.2	266.1	492.6	395.1	380.7	407.2	367.6	328.7	396.5	479.9	78.48	0.151	0.443
Glycine	751.3	659.9	961.1	971.0	848.3	828.0	822.6	774.7	819.7	863.5	144.34	0.759	0.327
Proline	395.4	420.8	463.3	422.4	454.5	369.5	379.9	376.4	316.0	410.2	101.12	0.087	0.615
Serine	162.8	169.4	158.4	157.0	164.9	154.7	144.3	127.3	152.5	173.8	25.56	0.340	0.246
Tyrosine	99.9	125.0	130.6	123.1	123.2	114.7	136.6	128.9	149.6	144.7	30.72	0.013	0.788

[a] Data represent means pooled across day of lactation (n = 6).
[b] Pooled standard error of the mean.

Table 6. Amino acid uptake (g/d) by the porcine mammary glands in relation to litter size[a]

	Litter size											
Amino acid	3	4	5	6	7	9	10	11	12	13	SEM[b]	Linear *P*-value
Indispensable												
Arginine	12.3	21.9	16.4	34.6	23.3	33.7	37.8	29.6	34.5	43.6	2.95	0.002
Histidine	5.1	3.6	6.3	6.7	3.8	7.7	9.9	8	7.8	11.6	1.14	0.004
Isoleucine	9.8	16.7	13.5	20.1	16.2	24.5	28.1	22.8	21.7	27.8	1.77	0.002
Leucine	18.2	30	26.1	38.2	28.4	43.4	51.4	42.6	42.5	53.5	3.29	0.001
Lysine	12.3	16	16.5	21.4	21.3	30.9	34.1	25.6	27.5	35.3	1.88	0.001
Methionine	3	4.8	4.4	5.1	4.7	7.4	9.1	7.2	7.6	9.3	0.55	0.001
Phenylalanine	6.7	9.4	9.9	13.4	11.8	17.3	22.7	17.1	16.5	21.4	1.56	0.001
Threonine	7.5	11.3	9.9	15.4	12	19.1	22.2	17.5	17.3	20.5	1.49	0.001
Valine	9.9	12.1	13.8	25.8	18.2	32.4	32.3	20.4	20.2	32.7	2.75	0.021
Dispensable												
Alanine	2.8	8.8	7.4	26	10.2	22	24	19.3	23.9	24.9	4.03	0.007
Aspartic acid	0.3	1.1	–0.1	1.8	1.5	2.5	2.4	–0.5	–0.7	0.8	0.55	0.773
Glutamic acid	26.9	49	39.4	41.7	55.1	67.1	68.8	42	50	85.3	9.09	0.028
Glycine	6.4	8.9	8.1	17.3	12	10.7	20	17	10.6	18.3	4.28	0.046
Proline	11.1	19.6	18.3	19	19.8	33.1	35.2	30.1	23.7	33.7	5.36	0.004
Serine	9.5	14.4	10.9	11.8	13.7	22.6	25.4	20.4	19.8	24.2	1.60	0.001
Tyrosine	5.4	9.1	8.9	18.7	8.9	13.4	16.7	14.4	11.7	18	1.98	0.045

[a] Data represent means pooled across day of lactation (n = 6). Calculated as arteriovenous difference (μmol/L) × daily mammary plasma flow (L/d) × molecular weight (g/μmol).

[b] Pooled standard error of the mean.

parallels well with our observation that P:M decreased (linear and quadratic trend, $P < 0.08$) as day of lactation increased (Figure 4). On the other hand, MPF varied between 3,700 and 4,500 L per day and did not increase with increasing day of lactation from 9 to 24 (Figure 5). All the indispensable AA uptake except arginine (Table 9), a product of AA A-VD and MPF, increased or tended to increase as day of lactation increased (linear and quadratic, $P < 0.05$ to $P < 0.1$). Therefore, our results imply that as lactation advances in the sow, net AA uptake across the gland increases in great part via an increase in A-VD. It is currently unknown whether this increase in A-VD across the porcine mammary glands results from an increase in passive and(or) active transport of AA across the mammary epithelial cells. Amino acid transport from blood into the mammary gland epithelial cells is mediated by AA transport systems (Shennan and Peaker, 2000),

Table 7. Amino acid arteriovenous differences (μmol/L plasma) across the porcine mammary glands in relation to day of lactation[a]

	Day of lactation							*P*-value	
Amino acid	9	12	15	18	21	24	SEM[b]	Linear	Quadratic
Indispensable									
Arginine	35.2	39.9	45.2	45	40.4	44.6	5.37	0.164	0.206
Histidine	10.3	14.3	15.5	13.2	12.5	7.3	2.48	0.010	0.008
Isoleucine	36.3	40	39.1	43.3	38.8	36.4	3.76	0.071	0.070
Leucine	66.6	75.4	72.6	79.7	72.4	66.6	6.61	0.055	0.053
Lysine	37.1	44.5	42.4	45	43.6	43.5	5.23	0.215	0.161
Methionine	9.7	11.6	10.9	11.8	11	9.8	1.16	0.043	0.042
Phenylalanine	21.1	24	22.3	25.6	22.7	21.4	2.49	0.937	0.146
Threonine	29.1	33.8	35.1	34.8	33.4	29.5	3.74	0.001	0.001
Valine	40	48.6	50.7	51	52.3	49.9	7.32	0.014	0.020
Dispensable									
Alanine	32.4	38	70.6	52.7	51.4	42.1	15.80	0.106	0.115
Aspartic acid	1.6	2.5	3.3	2.2	1.8	0.8	1.11	0.035	0.028
Glutamic acid	101.4	105.9	93.2	98.2	87.5	74.3	18.71	0.022	0.592
Glycine	39.5	43.6	91.5	60.7	46.5	26	20.09	0.01	0.022
Proline	51.2	61.1	71.6	69.9	62.3	50.1	10.39	0.002	0.002
Serine	39	44.4	45.1	46	44.4	39.4	4.41	0.003	0.003
Tyrosine	13.5	17.9	25.6	18.9	18.4	15.7	3.43	0.089	0.090

[a] Data represent means pooled across litter sizes (n = 12).

[b] Pooled standard error of the mean.

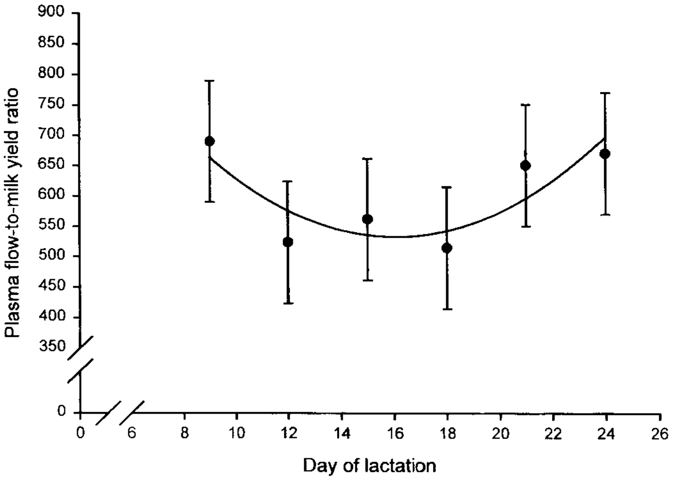

Figure 4. Relationship between day of lactation and plasma flow-to-milk yield ratio (**P:M**) (L plasma:L milk). Data are least-square means ± SEM pooled across litter sizes. Form of relationship is quadratic at $P < 0.076$; *trend*).

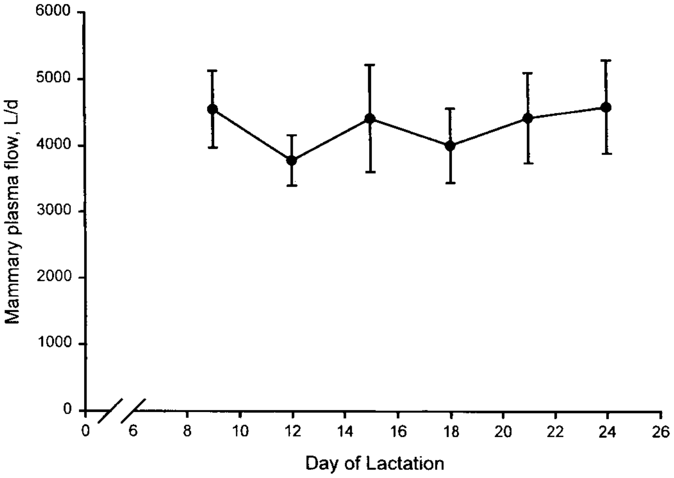

Figure 5. Relationship between day of lactation and mammary plasma flow. Data represent least-square means ± SEM. No relationship found between day of lactation and mammary blood flow.

but the mechanisms involved in their regulation have not been studied in vivo.

It is unclear why A-VD and extraction rate of lysine and arginine in particular did not change with day of lactation. Trottier et al. (1997) reported that with increasing day of lactation, while lysine A-VD did not change, extraction rate increased. In the present study, in contrast to Trottier et al. (1997), arterial lysine concentration did not decrease with day of lactation (Table 10) suggesting that lysine was not limiting. Dietary lysine concentration in the present study was 0.96% as compared with 0.67% in the study by Trottier et al. (1997). Hurley et al. (2000) demonstrated that in vitro lysine uptake in the sow mammary tissue occurred via a sodium-independent transport mechanism with a *K*m of 1.4 m*M*, which was approximately 10-fold higher than the arterial plasma concentration of lysine found in this study. Thus, the lack of variation in lysine A-VD by no means indicates that lysine uptake was saturated during the stage of lactation period studied herein. It is possible that changes in A-VD across the porcine mammary glands, as seen for the

Table 8. Amino acid extraction rates (%) across the porcine mammary glands in relation to day of lactation[a]

	Day of lactation							*P*-value	
Amino acid	9	12	15	18	21	24	SEM[b]	Linear	Quadratic
Indispensable									
Arginine	17.8	22.9	30.9	23.2	23.1	29.3	3.98	0.245	0.592
Histidine	12.7	18.2	19.6	17.6	18.2	6.4	3.50	0.026	0.022
Isoleucine	30.3	33.9	37.1	32.8	31.4	28.3	3.36	0.047	0.039
Leucine	28.6	32.5	36.1	32.1	31.6	29.3	3.19	0.058	0.056
Lysine	26.5	38.9	37.6	32.3	30.9	37.7	5.14	0.569	0.631
Methionine	26.3	31.7	35.6	34.5	31.1	27.3	3.87	0.020	0.002
Phenylalanine	23.5	26.7	29.1	24.7	23.6	21.5	2.98	0.098	0.079
Threonine	19.4	24.7	27.1	25.3	24.5	22.1	2.94	0.014	0.015
Valine	14.8	18.7	21.3	19.3	19.7	16.6	3.04	0.015	0.016
Dispensable									
Alanine	8.2	7.6	12.5	12.1	11.2	11	3.27	0.169	0.227
Aspartic acid	6.7	8.9	9.3	9.7	10	−0.4	4.25	0.073	0.060
Glutamic acid	24.7	25	25	26.9	28	20.7	4.24	0.709	0.212
Glycine	4.9	5.4	5.7	8.2	6.3	3.6	1.90	0.938	0.120
Proline	13	8.3	20.5	17.6	18.6	13.5	4.62	0.476	0.341
Serine	25.8	28.3	28.5	29.8	30	26.2	3.05	0.034	0.036
Tyrosine	10.1	13.6	18.1	17.4	18.3	14.3	3.20	0.010	0.013

[a] Data represent means pooled across litter sizes (n = 12). Calculated as arteriovenous difference (μmol/L) × 100/arterial concentration (μmol/L).

[b] Pooled standard error of the mean.

Table 9. Amino acid uptake (g/d) by the porcine mammary glands as a function of day of lactation[a]

	Day of lactation							*P*-value	
Amino acid	9	12	15	18	21	24	SEM[b]	Linear	Quadratic
Indispensable									
Arginine	26.5	28.5	36.4	36.2	28.8	32.1	5.70	0.471	0.200
Histidine	6.8	9.1	8.3	9.3	8.1	5.4	1.78	0.030	0.023
Isoleucine	20.7	20.6	22.6	24.2	23	19.3	3.46	0.090	0.085
Leucine	37.9	38.8	42.4	45.3	42.4	36.1	6.51	0.050	0.047
Lysine	24.1	26.4	27.8	27.5	27.9	25.2	4.25	0.006	0.007
Methionine	6.4	6.9	7.1	7.3	7.3	6.1	1.18	0.040	0.038
Phenylalanine	15.2	16.1	16.4	18.2	16.9	14.3	2.95	0.070	0.063
Threonine	15.5	16.3	17.3	18.2	17.8	14.2	2.91	0.050	0.044
Valine	20.4	24	24.4	27.2	28.2	23.9	5.08	0.050	0.072
Dispensable									
Alanine	14	17.2	19.8	26.3	17.8	16.3	6.36	0.090	0.098
Aspartic acid	1.1	1.5	1.1	1.3	1.2	0.2	0.70	0.125	0.094
Glutamic acid	70.6	61.6	48	55.3	69	35.1	15.59	0.223	0.913
Glycine	13.4	14.5	13.2	23.5	14.2	6.2	5.96	0.617	0.180
Proline	26.1	16.5	29.2	33.3	31.1	24.2	8.57	0.508	0.508
Serine	18.2	18.4	17.8	19.7	21.2	16.7	3.37	0.856	0.414
Tyrosine	10.9	13.2	14.7	17.7	14.8	11.4	3.31	0.030	0.029

[a] Data represent means pooled across litter sizes (n = 12). Calculated as arteriovenous difference (μmol/L) × daily mammary plasma flow (L/d) × molecular weight (g/μmol).

[b] Pooled standard error of the mean.

other AA, occur much earlier than d 9 of lactation for lysine. Therefore, examination of AA flow across the mammary glands is needed at earlier phases of lactation. Arginine shares a common transport system with lysine, which may explain why no change in arginine A-VD occurred either.

The proportion of milk AA secreted to mammary uptake (PSM) for the dispensable AA were higher than 100% (Table 11) while PSM for the indispensable AA, except for histidine, was less than 100%, suggesting that indispensable AA are used in part for the synthesis of dispensable AA within mammary tissue. This also indicates that the mammary gland system is an active metabolic organ with the capacity to retain, catabolize, and synthesize AA needed in the process of both milk and constitutive mammary protein synthe-

Table 10. Arterial amino acid concentration (μmol/L plasma) in lactating sows in relation to day of lactation[a]

	Day of lactation							*P*-value	
Amino acid	9	12	15	18	21	24	SEM[b]	Linear	Quadratic
Indispensable									
Arginine	201.3	187.5	184.7	197.6	192.9	191.4	57.90	0.737	0.464
Histidine	78.8	83.6	76.5	77.9	74.7	72.3	15.03	0.059	0.473
Isoleucine	124.2	124.3	120.6	132.7	126.0	134.8	27.87	0.135	0.525
Leucine	241.6	243.5	224.0	250.3	236.9	241.3	49.14	0.959	0.740
Lysine	159.7	132.4	144.3	143.8	144.3	148.4	47.01	0.806	0.263
Methionine	39.5	39.4	35.3	35.9	36.4	35.5	9.36	0.070	0.292
Phenylalanine	91.9	94.1	90.0	103.2	96.7	103.2	18.17	0.098	0.811
Threonine	157.4	146.5	142.9	143.6	138.5	143.7	32.31	0.033	0.049
Valine	288.8	282.1	263.5	276.7	276.9	289.1	53.39	0.058	0.057
Dispensable									
Alanine	418.4	524.0	472.7	498.9	496.3	466.9	122.33	0.600	0.214
Aspartic acid	27.9	30.3	25.7	27.6	24.5	23.0	7.67	0.047	0.461
Glutamic acid	423.2	431.0	342.3	387.9	367.9	347.2	108.92	0.091	0.678
Glycine	806.8	866.0	871.1	858.3	841.0	758.9	162.72	0.005	0.004
Proline	414.3	431.3	388.0	431.5	387.2	357.4	103.37	0.136	0.346
Serine	154.9	164.3	156.7	155.2	156.7	152.0	28.44	0.311	0.431
Tyrosine	132.1	141.0	132.4	127.7	120.0	120.1	32.17	0.034	0.443

[a] Data represent means pooled across litter sizes (n = 12).

[b] Pooled standard error of the mean.

Table 11. Proportion of milk amino acid output to mammary amino acid uptake (%)[a]

Amino acid	Mean	SEM[b]
Indispensable		
Arginine	54	1.55
Histidine	120.7	6.47
Isoleucine	62.9	1.47
Leucine	75.3	1.93
Lysine	100	0
Methionine	97.9	3.15
Phenylalanine	92.1	2.38
Threonine	91.8	2.61
Valine	79.4	3.33
Dispensable		
Alanine	102.9	18
Aspartic acid	3,521	1,895
Glutamic acid	128.5	15.1
Glycine	120.9	37.5
Proline	153.2	11.1
Serine	105.8	4.51
Tyrosine	121.3	9.11

[a] Data represent means pooled across litter sizes and day of lactation (n = 72). Calculated as (amino acid concentration in milk (g/L milk) × milk production (L milk/d))/((arteriovenous concentration difference of amino acids (g/L plasma) × daily mammary plasma flow (L plasma/d)).

[b] Pooled standard error of the mean.

sis, and in the synthesis of nonprotein constituents. Lysine PSM is 100% because plasma flow was based on conservation of lysine across the mammary glands. Because lysine shows the highest extraction rate among all AA, and it is the first limiting for milk protein synthesis in lactating sows, its oxidation within the gland is assumed to be minimum. Kim et al. (1999) reported that lysine is accreted by mammary tissue during lactation; however, the daily amount accreted relative to secreted in milk is minimum. Thus, our plasma flow calculation based on lysine conservation may be slightly underestimated. Similar results of mammary plasma flow have been obtained using phenylalanine + tyrosine (Guan, 2000). The lowest PSM were observed for the branched-chain AA (**BCAA**) and arginine. Recently, DeSantiago et al. (1998) have shown that, unlike the liver, the mammary gland of lactating rats contains significant amounts of enzymes involved in the initial step of BCAA catabolism, i.e., branched-chain amino transferase and branched-chain α-keto acid dehydrogenase. The activity of both enzymes was shown to increase during lactation, relative to nonlactating and weaned female rats. Leucine and valine are oxidized by porcine mammary tissue (Richert et al., 1998). Taken together, this could explain in part why BCAA may be needed in diets for lactating sows at concentrations above those needed for milk protein synthesis (Richert et al., 1996, 1997). Arginine PSM was the lowest among all indispensable AA. The biochemical basis for such a high rate of arginine uptake by the porcine mammary glands is currently unknown. Arginine can be metabolized by the cow and rabbit mammary tissues (Clark et al., 1975). Arginine is a precursor for many compounds with recognized biological functions, such as nitric oxide, which plays a critical role in vasodilation (Valance et al., 1989), polyamines, which possess regulatory functions in cellular proliferation (Eremin, 1997), and proline (Clark et al., 1975), which is an indispensable AA for the nursing pig (Ball et al., 1986). Indeed, proline PSM was one of the highest among the dispensable AA measured in the present study, as previously reported (Trottier et al., 1997). The ability of the mammary gland system to redirect indispensable AA toward preferential catabolism and(or) the synthesis of dispensable AA suggests that AA flow measurements in addition to milk AA composition should be taken into consideration in building models to determine AA requirements. However, a better understanding of the nutritional or physiological factors involved in the regulation of AA flow across the mammary in vivo is necessary. The present study shows that AA flow across the porcine mammary glands is a function of litter size and day of lactation. The effect of litter size on AA uptake by the mammary glands seems to be mediated through mammary plasma flow rate, because litter size had minimal effects on AA A-VD. In contrast, day of lactation affects net AA uptake by regulating AA A-VD and extraction rates across the mammary glands, because mammary plasma flow rate did not increase with day of lactation.

Implications

This study indicates that net AA uptake by the porcine mammary glands is regulated in part by two different physiological mechanisms, namely plasma flow and transport systems across the mammary cells. The increase in milk production associated with increased litter size is a result of an increase in mammary plasma flow to the entire mammary system, whereas the increase in milk production associated with day of lactation is a result of an increase in AA arteriovenous differences across the gland. In addition, results of this experiment suggest that the mammary glands have the capacity to catabolize indispensable AA and redirect them for the synthesis of dispensable AA. Therefore, milk AA composition per se may not accurately predict dietary AA needs of the lactating sow. The information obtained in this study will contribute to development of mechanistic models of AA requirements in the lactating sow.

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