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J Anim Sci 1999. 77:3304-3315.

Effect of Nutrient Intake on Mammary Gland Growth in Lactating Sows[1,2]

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ABSTRACT: Sixty-one primiparous sows were used to determine the response of mammary gland growth to different energy and protein intakes during lactation. After birth, litter size was set to 9 or 10 pigs. Sows were slaughtered at selected times up to 30 d of lactation. Individual sows were fed one of four diets that were combinations of different amounts of energy and protein (3.0 Mcal ME and 8.0 g lysine/kg diet; 3.0 Mcal ME and 16.2 g lysine/kg diet; 3.5 Mcal ME and 6.4 g lysine/kg diet; or 3.5 Mcal ME and 13.0 g lysine/kg diet). Mammary glands were collected at slaughter and trimmed of skin and the extraneous fat pad. Each gland was weighed, cut in half to measure cross-sectional area, ground, and stored at –20°C for chemical analysis. Frozen, ground tissue was used to determine dry matter, dry fat-free tissue (DFFT), total tissue protein, ash, and DNA content. Only glands known to have been suckled were included in this data set. Response surface regression was used for statistical analysis. The percentage of protein, fat, ash, and DNA in each suckled mammary gland was affected only by total energy intake ($P < .05$). The percentage of dry tissue and fat decreased as the total energy consumed during lactation increased, whereas the percentage of protein and DFFT increased as total energy intake increased. There were quadratic effects ($P < .05$) of both total energy and protein intake on wet weight, dry weight, protein amount, DFFT amount, and DNA amount of each suckled mammary gland during lactation. This study shows that mammary gland growth is affected by nutrient intake during lactation. The weight of suckled mammary glands and the amount of mammary tissue protein, DFFT, and total DNA were maximal on d 27.5 of lactation when sows had consumed an average of 16.9 Mcal of ME and 55 g of lysine per day during lactation. Provision of adequate amounts of nutrients to sows during lactation is important for achieving maximal growth of mammary glands and maximal milk production.

Key Words: Sows, Lactation, Mammary Glands, Protein Intake, Energy Intake

 J. Anim. Sci. 1999. 77:3304–3315

Introduction

The primary commercial purpose of sows is to produce weanling pigs. Sows have been improved genetically to farrow large litters. However, it is also important to keep pigs alive after birth and to provide for optimal growth of pigs. Postnatal mortality in pigs can be nearly 12% between d 0 and 7 postpartum (Meat and Livestock Commission, 1994). More than 60% of preweaning mortality is caused by maternal factors, such as insufficient nutrient supply and overlying (English et al., 1977; Dyck and Swierstra, 1987; Prime et al., 1987). Pigs are born with low energy reserves; hence, the availability of energy from milk immediately after birth is critical to survival. The importance of nutrition for pig growth and viability cannot be overemphasized.

Newborn pigs achieve only about half of their potential growth rate while nursing the mother in typical management systems (Harrell et al., 1993). Pigs fed milk replacer after d 18 of lactation weighed 20% more by d 25 of lactation than pigs suckled throughout lactation (Zijlstra et al., 1996). The limitation of milk production in sows has led to an increased awareness of the importance of lactation and mammary gland function to pig growth.

The metabolic needs of the mammary gland are substantial. An arteriovenous technique for estimating amino acid uptake into the mammary gland of lactating sows (Trottier et al., 1995) has been used to show that

[1]The authors wish to acknowledge the financial support from National Pork Producers Council, the Pig Improvement Company, the Korean Ministry of Agriculture and Forestry, the helpful assistance from T. M. Parr in care of sows, and S. H. Bae and A. Rivera in collection and dissection of tissue.

[2]Presented in part at the 8th World Conference on Animal Production (Proceedings I:152–153) and ASAS 1998 Annual Meeting (J. Anim. Sci. 76 [Suppl. 1]:163).

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Received December 9, 1998.

Accepted May 22, 1999.

the quantity of amino acids taken into the gland is greater than that secreted in milk proteins (Trottier et al., 1997). That finding provided evidence that the maternal amino acid requirements for lactation must include both an estimate of the quantity needed for milk production, per se, as well as an estimate of the need for growth and maintenance of the glands.

Previous results from Kim et al. (1999) have shown that the porcine mammary gland undergoes hypertrophy and hyperplasia during a 28-d lactation when sows are provided adequate nutrients. Under practical situations, sows often consume different amounts of nutrients, and the effect of nutrient intake on mammary growth is unknown. Goodwill et al. (1996) showed that mammary gland growth is affected by protein intake during lactation in rats. The purpose of this experiment was to determine how different energy and protein intakes affect mammary gland growth in lactating sows.

Materials and Methods

Animals and Experimental Design. Primiparous sows (n = 61; Camborough-15 X line 326, Pig Improvement Co., Franklin, KY) were used for this study. Virgin gilts were selected based on their genetic background and the number of mammary glands observable on the underline. Gilts were bred at around 230 d of age and housed in individual gestation stalls at the University of Illinois Swine Research Center (Champaign, IL). Pregnant gilts were weighed on d 109 of gestation and moved into farrowing crates. Individuals were assigned to heavy or light weight outcome groups according to their body weights at d 109 of gestation. Litter size was set within 2 d after farrowing for each sow to nine or 10 pigs by cross-fostering. Sows were allotted to one of the four dietary treatments and to different slaughter dates between d 4 and 30 of lactation (Table 1). Sow weights and individual pig weights were recorded at birth, weekly, and on the day before slaughter. Feed intake was recorded daily. Teat order was observed on the day before slaughter to determine which mammary glands were suckled during lactation. Pigs were numbered on the back with an indelible marker. Teat order was recorded when milk let-down occurred. Sows were slaughtered at the University of Illinois Meat Science Laboratory (Champaign, IL). The animal care protocol (#A6R267) was approved by Laboratory Animal Care Committee of the University of Illinois at Urbana-Champaign. Animals were available for monitoring by the Institutional Veterinarian or a designated representative.

Diets. The gestation diet contained 77.6% corn as the primary energy source and 16.4% soybean meal (48% CP) and 3% alfalfa (17% CP) as the major protein sources. The gestation diet contained 3.3 Mcal ME/kg, 15% CP, and .72% lysine (Table 2). During the first half of the gestation period, sows were fed 1.9 kg of the gestation diet per day. Around midgestation, backfat thickness was measured ultrasonically. Sows with less

Table 1. Number of sows slaughtered during 30 d of lactation in the dietary groups

	Day of lactation																												
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
LELP[a]				2				2			1	2	1					1	1	1				1	1	1	1		
LEHP[b]		1	1				1		2	1	1						1		1	1				1	2	1			
HELP[c]		1	1					1		3		1							2	2			1		1	2			
HEHP[d]	2	1						2	1		2		1			1		1	2	1			1		1	1			

[a]Group provided low-energy (12.0 Mcal ME/day) and low-protein (32 g lysine/day) diet.
[b]Group provided low-energy (12.0 Mcal ME/day) and high-protein (65 g lysine/day) diet.
[c]Group provided high-energy (17.5 Mcal ME/day) and low-protein (32 g lysine/day) diet.
[d]Group provided high-energy (17.5 Mcal ME/day) and high-protein (65 g lysine/day) diet.

Table 2. Composition of gestation diet

Ingredients	Gestation[a]
Yellow corn, %	77.60
Soybean meal (48%), %	16.40
Alfalfa (17%), %	3.00
Dicalcium phosphate, %	1.60
Limestone, %	.85
Trace mineral mixture[b], %	.35
Vitamin mixture[c], %	.20
Calculated analysis	
Energy, Mcal ME/kg	3.26
Crude protein, %	15.00
Lysine, %	.72
Calcium, %	.75
Phosphorus, %	.60

[a]During the first half of gestation period, sows were fed 1.9 kg/d, and, during the second half, sows were fed based on their backfat thickness.

[b]Trace-mineralized salt providing per kilogram of feed 20 mg of manganese as manganous oxide, 90 mg of iron as iron sulfate, 100 mg of zinc as zinc oxide, 8 mg of copper as copper oxide, .35 mg of iodine, and .3 mg of selenium as sodium selenite.

[c]Vitamin premix providing per kilogram feed 6,613.8 IU of vitamin A as vitamin A acetate, 661.4 IU of vitamin D_3, 88.2 IU of vitamin E, 4.4 IU of vitamin K as menadione sodium bisulfite, 7.3 μg of vitamin B_{12}, 1.8 mg of riboflavin, 5.0 mg of D-pantothenic acid as calcium pantothenate, 6.8 mg of niacin, and 49.9 mg of choline as choline chloride.

than 18 mm of backfat were fed an increased amount of diet, whereas sows with more than 24 mm of backfat were fed less during the remainder of gestation in order to achieve a target backfat thickness between 20 and 24 mm at farrowing. During lactation, sows were provided diets containing corn and soy oil as major energy sources and soybean meal (48% CP) as a major protein source. The four diets were formulated with the combination of different amounts of energy (3.0 Mcal ME and 8.0 g lysine/kg diet, **LELP**; 3.0 Mcal ME and 16.2 g lysine/kg diet, **LEHP**; 3.5 Mcal ME and 6.4 g lysine/kg diet, **HELP**; or 3.5 Mcal ME and 13.0 g lysine/kg diet, **HEHP**) to provide 12.0 or 17.5 Mcal ME per day and 32 g or 65 g lysine per day (Table 3). Sows were provided a maximum of 4 kg of the lactation diet per day for the LELP group to provide a maximum of 12.0 Mcal ME and 32 g of lysine, and 12.0 Mcal ME and 65 g of lysine were provided to the LEHP group in 4 kg daily. Sows in the HELP group were provided a maximum of 5 kg per day to provide 17.5 Mcal ME and 32 g of lysine, and 17.5 Mcal ME and 65 g of lysine were provided to the HEHP group in 5 kg daily. Lactating sows were fed at 0700 and 1600. Feed intake of each sow was recorded on a daily basis.

Sampling. Sows were transported to the University of Illinois Meat Science Laboratory at 0600 prior to the morning feeding for slaughter. Live weights of sows were recorded. The animals were electrically stunned and killed by exsanguination. Functional and nonsuckled teats were identified before glands were removed from the body. Skin and extraneous fat pad were trimmed and each individual gland was weighed and bisected in an approximate midsagittal section to measure cross-sectional area. Cross-sectional area of each gland is an indicator of mammary gland size (Hurley et al., 1991). The other half of each gland parenchymal tissue was ground in a commercial blender (Waring Products, New Hartford, CT) and stored at –20°C for chemical analysis.

Chemical Analysis. Frozen ground tissue was used for measuring dry matter content, dry fat-free tissue (**DFFT**) content, total tissue protein content, individual amino acid contents, ash content, and DNA content. Dry matter content of tissue was measured by dessication at 105°C for 8 h, and DFFT content was obtained by subtracting fat content from dry matter content. Crude fat content was determined with Soxhlet extraction using a chloroform:methanol (87:13) extracting solution with dried tissue, as described by Novakofski et al. (1989). Tissue protein content was obtained by measuring nitrogen content using the Kjeldahl method (AOAC, 1995), and ash content was measured by combustion of dried tissue at 500°C for 8 h. Cross-sectional area was determined by tracing the outline of the parenchymal tissue on the cut face of each gland onto a transparency film, and the area was measured using a digital planimeter (PLANIX 7, Sokkia, Overland Park, KS).

DNA Analysis. The DNA content in mammary tissue was measured using a modification of the method of Labarca and Paigen (1980). Approximately .05 g of triplicate mammary tissue samples were homogenized with 2 mL of homogenization buffer containing 10 m*M* Tris base, 5 m*M* EDTA, .5% cholamidopropyl-dimethylammonio-propane-sulfonate (Sigma Chemical Co., St. Louis, MO), and the pH was adjusted to 7.2. The protease inhibitors, phenylmethylsulfonyl fluoride and ε-amino caproic acid (Sigma Chemical Co.), were added (each at .1 m*M*) to the homogenates. The DNA content was determined as described previously (Buskirk et al., 1996).

Statistical Analysis. Data were obtained only from glands known to have been suckled. Statistical analysis of the data were performed using the response surface regression procedure (PROC RSREG) in SAS/STAT software (version 6.12; SAS, 1989). The mean response was modeled as follows:

$$Y = \beta_0 + \beta_1 E + \beta_2 E^2 + \beta_3 P + \beta_4 P^2 + \beta_5 EP + \varepsilon \quad [1]$$

where Y is the response variable, E is the effect of total energy intake (**TEI**) during lactation, E^2 is the quadratic effect of TEI during lactation, P is the effect of total protein intake (**TPI**) during lactation, P^2 is the quadratic effect of TPI during lactation, EP is the TEI $\times$ TPI interaction during lactation, and ε is the residual error.

The terms TEI and TPI were used to define the cumulative or total amount of energy or protein, respectively, consumed by sows during lactation from farrowing until slaughter. The term "day of lactation" means the number of days from farrowing to slaughter (d 0 is day of

farrowing). The TEI and TPI were used as independent variables. The combined effects of nutrient levels and day of lactation were explained together by using TEI and TPI because each sow, fed different diets with different combinations of energy and protein composition and killed on different day of lactation, has a different amount of total feed intake from farrowing to slaughter.

The x-axis of the three-dimensional graph indicates TEI of sows during lactation until slaughter, the y-axis is for TPI, and the z-axis indicates dependent variables such as wet weight, dry weight, protein amount, ash amount, DFFT amount, and DNA amount of mammary glands (Figure 1a). Total energy and total protein intakes increased as lactation progressed. Sows killed on later days of lactation generally had higher TEI and TPI than sows killed on earlier days of lactation (Figure 1a). Progression across the surface plot from left to right reflects the different diets fed to sows (low vs high energy and low vs high protein). Progression from bottom to top of the surface plot reflects cumulative or total energy and protein, which are functions of day of lactation when sows were slaughtered.

General sequence of data analysis was made in five steps using SAS/STAT software (version 6.12; SAS, 1989). In Step 1, a three-dimensional scattered plot was generated using the PROC G3D. In Step 2, the scatter plot was converted to a three-dimensional response surface plot to show the changing patterns of variables on the z-axis as TEI on the x-axis and TPI on the y-axis changed during lactation. Predicted values for a grid of points was generated using the PREDICT option, and these values were used to create a response surface plot over a three-dimensional grid using the PROC G3D. In Step 3, the predicted value at the maximum, minimum, or saddle stationary point of the response surface (Figure 1b) was obtained with the canonical analysis in SAS/STAT software (version 6.12; SAS, 1989). Canonical analyses are explained by Myers (1971). If a variable has the maximum stationary point, a value of the variable decreases as a point moves away from the maximum stationary point, whereas a value of the variable increases when a variable has the minimum stationary point (Myers, 1971). If a variable has a saddle stationary point, change of a value depends on the direction of moving a point from the saddle point (Myers, 1971). The canonical analysis also provides the response surface shape, the optimum combination of factor values (TEI and TPI) and the sensitivity of the

Table 3. Composition of lactation diets

Ingredients	LELP[a]	LEHP[a]	HELP[a]	HEHP[a]
Yellow corn, %	60.05	36.25	79.70	53.15
Soybean meal (48%), %	14.20	40.00	11.00	37.60
Alfalfa (17%), %	5.00	5.00	—	—
Wheat bran, %	17.50	15.00	—	—
Soy oil, %	—	—	5.50	5.60
Dicalcium phosphate, %	1.30	.90	2.35	1.75
Limestone, %	1.25	1.25	.80	1.00
Trace mineral mixture[b], %	.35	.35	.35	.35
Vitamin mixture[c], %	.20	.20	.20	.20
L-lysine, HCl, %	.10	.20	.15	—
DL-methionine, %	—	.20	—	.05
L-threonine, %	—	.20	—	.05
L-valine, %	.05	.45	—	.25
Chemical composition				
Energy, Mcal ME/kg	3.00	3.00	3.50	3.50
Crude protein, %	15.60	26.50	12.10	22.90
Lysine, %	.80	1.62	.64	1.30
Methionine+cysteine, %	.54	.97	.47	.78
Threonine, %	.57	1.16	.46	.93
Valine, %	.80	1.62	.64	1.30
Calcium, %	.90	.85	.85	.85
Phosphorus, %	.70	.70	.70	.70
Maximum daily nutrients allowance				
Diet, kg/day	4	4	5	5
Energy, Mcal ME/day	12	12	17.5	17.5
Crude protein, g/day	624	1,060	605	1,145
Lysine, g/day	32	64.8	32	65

[a]LE: low energy, HE: high energy, LP: low protein, HP: high protein.
[b]The trace-mineralized salt provided the following per kilogram of complete diet: 20 mg of manganese as manganous oxide, 90 mg of iron as iron sulfate, 100 mg of zinc as zinc oxide, 8 mg of copper as copper oxide, .35 mg of iodine, and .3 mg of selenium as sodium selenite.
[c]The vitamin premix provided the following per kilogram of complete diet: 6,613.8 IU of vitamin A as vitamin A acetate, 661.4 IU of vitamin D_3, 88.2 IU of vitamin E, 4.4 IU of vitamin K as menadione sodium bisulfite, 7.3 μg of vitamin B_{12}, 1.8 mg of riboflavin, 5.0 mg of D-pantothenic acid as calcium pantothenate, 6.8 mg of niacin, and 49.9 mg of choline as choline chloride.

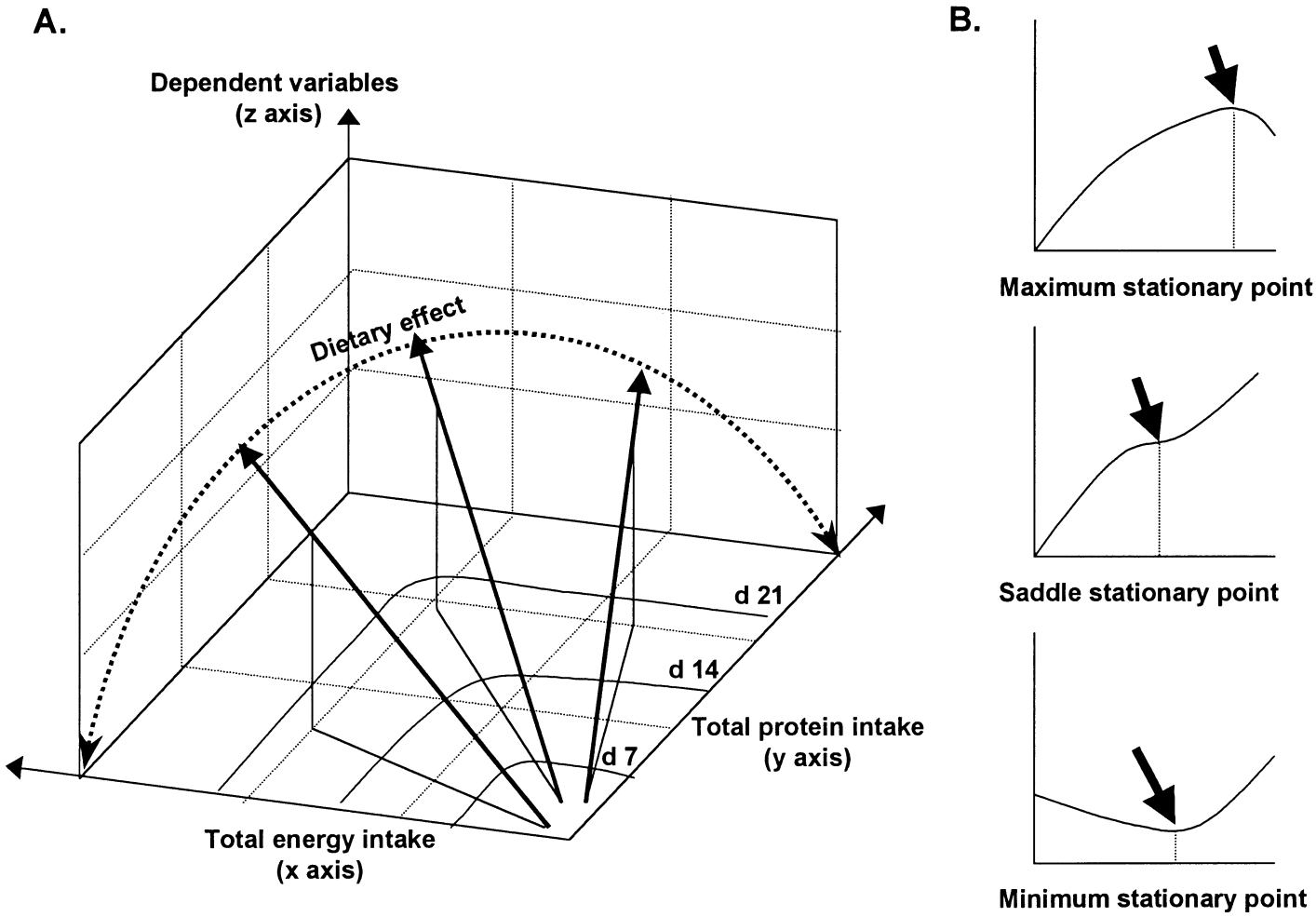

Figure 1. Conceptual explanation of response surface plots and results of canonical analysis. A) Three-dimensional graph with total energy intake (x-axis), total protein axis (y-axis), and dependent variable (z-axis). Total nutrient consumed by d 7 of lactation would be at the lower end of the response surface graph compared with d 21 of lactation, which is further up the response surface graph. Different diets (approximated by the solid arrows) result in a different path up the response surface graph, and the ratio of dietary energy and protein determines the point on the graph from left to right (indicated by the dotted arrow). B) Conceptual explanation of stationary points determined by canonical analysis. The response surface plot is viewed in cross-section with the x-axis as either total energy intake or total protein intake and the y-axis as the dependent variables. Maximum (or minimum) stationary point is the maximum (or minimum) value for the y-axis; the dependent variable is reduced (or increased) as the value on the x-axis decreases or increases relative to maximum (or minimum) stationary point. The saddle stationary point is the inflection point on the response surface graph; in the example here, the dependent variable is reduced as values on the x-axis decrease or the dependent variable is increased as values on the x-axis increase from the saddle stationary point.

predicted responses to factors (TEI and TPI). In Step 4, an equation was obtained from TPI and TEI of each sow vs day of lactation of each sow. This equation was used to obtain a predicted day of lactation from any point on the response surface plot. Thus, it was possible to estimate when the dependent variables were maximal or minimal on the response surface. In Step 5, estimated average daily energy and protein intakes were obtained from dividing TEI and TPI by the estimated day of lactation.

Results

Day of Lactation and Total Feed Intake. Changes of TEI and TPI among days of lactation were modeled as described in Eq. [1]. There was a linear relationship ($P < .01$) between day of lactation and both TEI and TPI. The predicted day of lactation when the maximum or saddle stationary point of a variable is achieved can be calculated from TEI and TPI using the following equation ($R^2 = .84$, $P < .05$):

$$Y = 2.0599 + .0642 \times E + .6335 \times P - .0017 \times E \times P \quad [2]$$

where Y is d of lactation, E is TEI, and P is TPI.

Sow and Litter Performance. The average weight of sows was 201.1 ± 1.79 kg after farrowing and 181.4 ± 2.6 kg (LELP), 198.2 ± 3.6 kg (LEHP), 192.2 ± 5.3 kg (HELP), and 200.8 ± 3.0 kg (HEHP) at slaughter. Backfat thickness at the 10th rib averaged 2.17 ± .13 cm (LELP), 2.39 ± .15 cm (LEHP), 2.66 ± .17 cm (HELP), and 2.62 ± .13 cm (HEHP) at slaughter. Average daily feed intake of sows was 3.0 ± .2 kg (LELP), 3.2 ± .2 kg (LEHP), 4.2 ± .2 kg (HELP), and 3.6 ± .2 kg (HEHP) during lactation until slaughter. Average daily gain of nursing pigs from sows killed between d 20 and d 23 of lactation was 154.0 ± 4.8 g.

Wet and Dry Weight. Wet weight of mammary glands from 61 sows (Table 4) are plotted on a three-dimensional scatter plot graph (Figure 2) based on their TEI and TPI at the time of slaughter. Each observation is the average wet weight of individual suckled mammary glands from each sow. A response surface graph was generated based on the scatter plot (Figure 3). There were quadratic effects ($P < .05$) of TEI and TPI on weight of wet mammary tissue during lactation (Table 5). There was no interaction between TEI and TPI ($P > .05$). The maximum stationary point was 581.7 g of mammary wet weight when TEI was 443.1 Mcal and TPI was 25.7 kg, and this was achieved on the predicted d 27.4 of lactation using Eq. [2] (Table 5).

Dry mammary gland tissue weight was quadratically affected ($P < .05$) by TEI and TPI (Figure 4 and Table 5). There was an interaction ($P < .01$) of TEI and TPI for dry weight. Dry weight of mammary gland tissue reached a maximum stationary point of 134.2 g when TEI was 468.7 Mcal and TPI was 27.3 kg. Predicted day of lactation was 27.6 for the maximum stationary point.

Wet and dry weights of the suckled mammary gland were affected by both energy and protein intakes ($P < .05$) during lactation. Both increased until d 27.5 of lactation. It was predicted that wet and dry weight of the suckled mammary gland tissue was maximized when sows consumed an average of 16.5 Mcal of ME and 950 g of CP per day, which is equivalent to 52.3 g of lysine daily (the average lysine content was 5.55% of crude protein in the experimental diets).

Amount of Tissue Protein, DFFT, and Fat. Protein content was affected by both TEI and TPI (quadratic effects, $P < .05$) during lactation, and there was an interaction between TEI and TPI ($P < .05$). The maximum stationary point was 65.2 g of protein when TEI was 469.4 Mcal and TPI was 28.9 kg and the maximum stationary point was achieved on predicted d 27.4 of lactation (Figure 5 and Table 5). There were quadratic effects ($P < .05$) of TEI and TPI on the amount of DFFT

Table 4. Least-squares means of wet weight in individual suckled mammary glands of sows on different days of lactation from different dietary groups[a,b]

	Wet weight of individual suckled mammary glands, g			
Day of lactation	LELP[c]	LEHP[d]	HELP[e]	HEHP[f]
4	—	—	—	289.6 ± 32.4
5	—	314.4 ± 48.2	441.8 ± 36.5	535.6 ± 50.1
6	—	345.6 ± 45.7	391.7 ± 38.1	—
7	232.9 ± 23.5	—	—	—
10	—	291.4 ± 45.7	—	—
11	378.9 ± 26.4	—	339.4 ± 42.1	402.7 ± 37.4
12	—	465.4 ± 36.1	—	509.1 ± 56.1
13	—	473.4 ± 48.2	458.2 ± 23.4	—
14	392.7 ± 40.8	487.2 ± 45.7	—	426.4 ± 36.4
15	472.7 ± 29.8	—	386.5 ± 39.9	—
16	480.4 ± 51.5	—	—	597.0 ± 52.9
19	—	—	—	620.0 ± 56.1
20	—	409.2 ± 51.1	—	—
21	502.3 ± 43.6	—	—	550.8 ± 59.9
22	587.1 ± 38.4	407.2 ± 48.2	416.3 ± 39.9	514.2 ± 52.9
23	415.4 ± 38.4	534.6 ± 64.6	564.1 ± 30.6	680.7 ± 56.1
26	—	—	494.0 ± 47.7	593.3 ± 52.9
27	522.3 ± 40.5	—	—	—
28	517.6 ± 36.5	500.4 ± 48.2	484.2 ± 39.9	534.3 ± 56.1
29	417.8 ± 40.8	502.1 ± 36.1	554.1 ± 29.8	566.9 ± 56.1
30	396.6 ± 40.8	731.9 ± 72.2	—	—

[a]Data obtained only from mammary glands known to be suckled during lactation.
[b]Least-squares mean ± standard error.
[c]Group provided low-energy (12.0 Mcal ME/day) and low-protein (32 g lysine/day) diet.
[d]Group provided low-energy (12.0 Mcal ME/day) and high-protein (65 g lysine/day) diet.
[e]Group provided high-energy (17.5 Mcal ME/day) and low-protein (32 g lysine/day) diet.
[f]Group provided high-energy (17.5 Mcal ME/day) and high-protein (65 g lysine/day) diet.

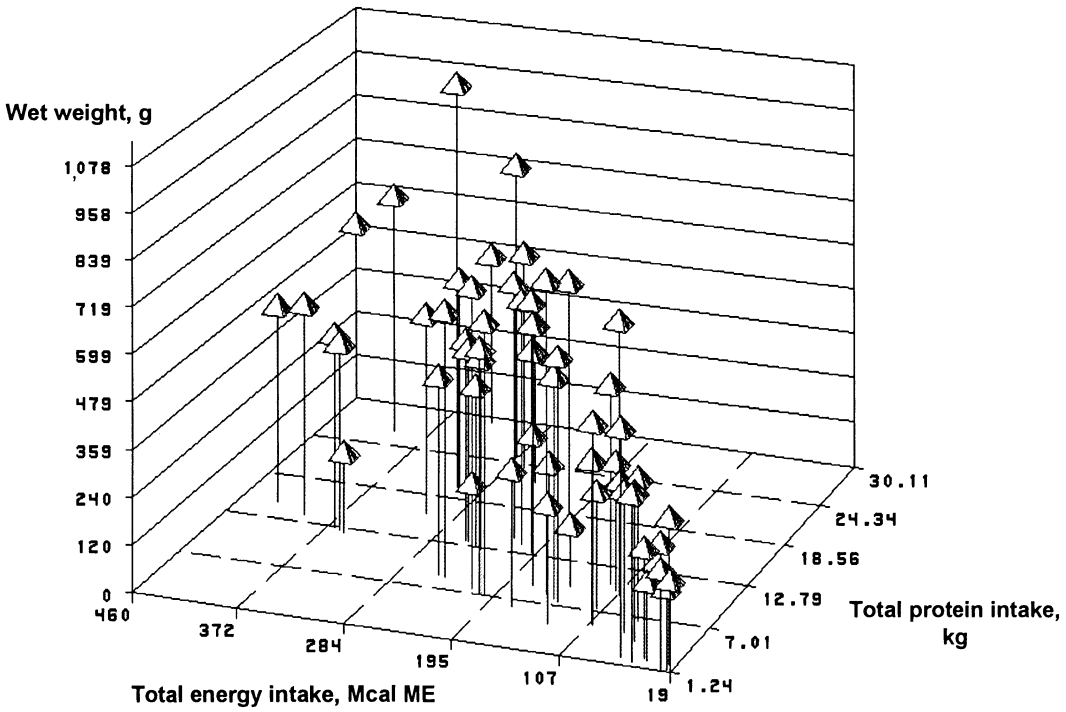

Figure 2. Scatter plot of the wet weight of suckled mammary glands from sows fed different levels of energy and protein. Arrowheads are average wet weight of individual mammary gland from each sow. Sows were slaughtered at different times during lactation accounting for the different total energy intake and total protein intake.

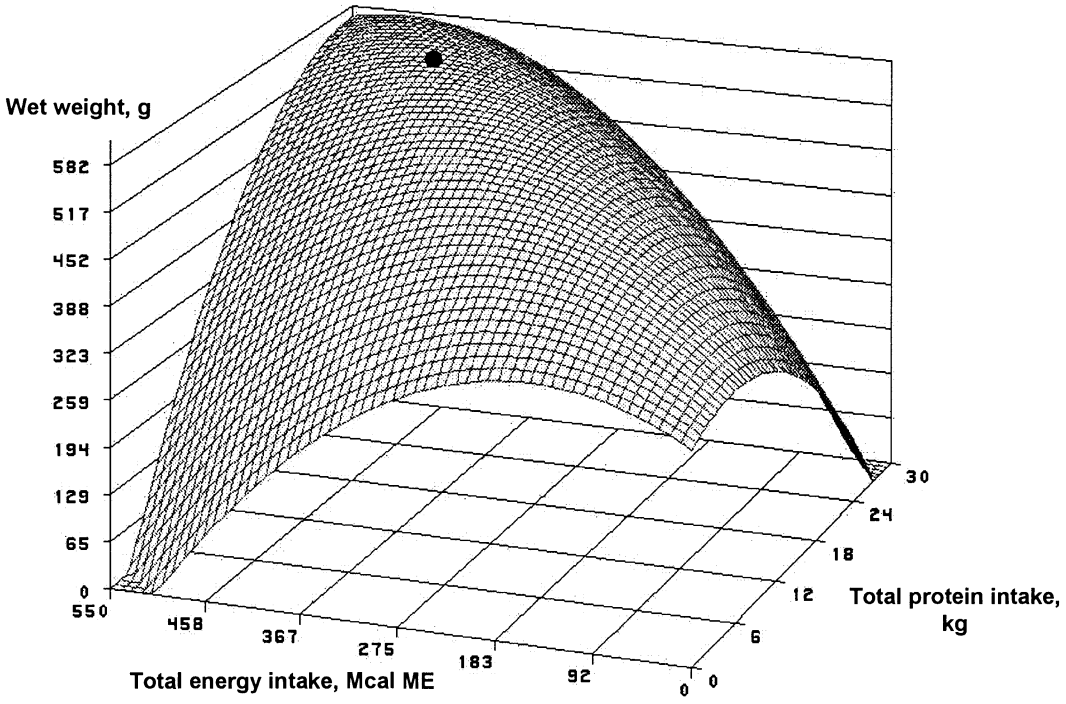

Figure 3. Response surface plot of the wet weight of suckled mammary glands from sows fed different levels of energy and protein. Graph was created from data in Figure 2. ● = Maximum stationary point. Day of lactation when the maximum stationary point occurred was 27.4 (estimated by Eq. [2]).

Table 5. Changes in mammary gland tissue composition in lactating sows consuming different amounts of energy and protein

Item	Maximum point[a]	Total energy intake, Mcal[b]	Total protein intake, kg[b]	Predicted day of lactation, d[c]
Wet weight	581.7 g	443.1*	25.7*	27.4
Dry weight	134.2 g	468.7*	27.3*	27.6
Protein amount	65.2 g	469.4*	28.9*	27.4
DFFT amount	75.2 g	480.5*	29.2*	27.5
Fat amount	59.4 g	411.4*	23.6*	26.9
DNA amount	1,582.6 mg	662.2*	39.0*	25.3

[a]Maximum stationary point estimated from response surface regression.
[b]Total energy or protein intake of sows during lactation until their slaughter.
[c]Predicted day of lactation when maximum stationary point was achieved.
*Quadratic effect of total energy intake or total protein intake ($P < .05$).

during lactation, and there was an interaction ($P < .05$) between TEI and TPI (Figure 6 and Table 5). Dry fat-free tissue reached its maximum value of 75.2 g when TEI was 480.5 Mcal and TPI was 29.2 kg, which was predicted to occur on d 27.5 of lactation (Table 5). There were quadratic effects ($P < .05$) of TEI or TPI on mammary fat amount during lactation. The amount of mammary fat reached was the highest at 59.4 g when TEI was 411.4 Mcal and TPI was 23.6 kg, which was predicted to occur on d 26.9 of lactation (Table 5). The quantity of tissue protein and DFFT was greatest on predicted d 27.5 of lactation when sows had consumed a daily average of 17.3 Mcal ME and 1,058 g of crude protein, which is equivalent to 58.2 g of lysine.

Amount of DNA and Cross-Sectional Area. There were quadratic effects ($P < .05$) of TEI and TPI on the DNA amount of mammary glands during lactation, and there was an interaction ($P < .05$) between TEI and TPI during lactation (Figure 7 and Table 5). The maximum stationary point was 1,582.6 mg when TEI was 662.2 Mcal and TPI was 39.0 kg, which was predicted to occur on d 25.3 of lactation.

The cross-sectional area of the mammary gland was affected by TEI during lactation (quadratic effect, $P < .01$). There was no interaction between TEI and TPI ($P > .05$) during lactation. The cross-sectional area of suckled mammary gland reached the maximum stationary point of 62.9 cm^2 when TEI was 377.2 Mcal and

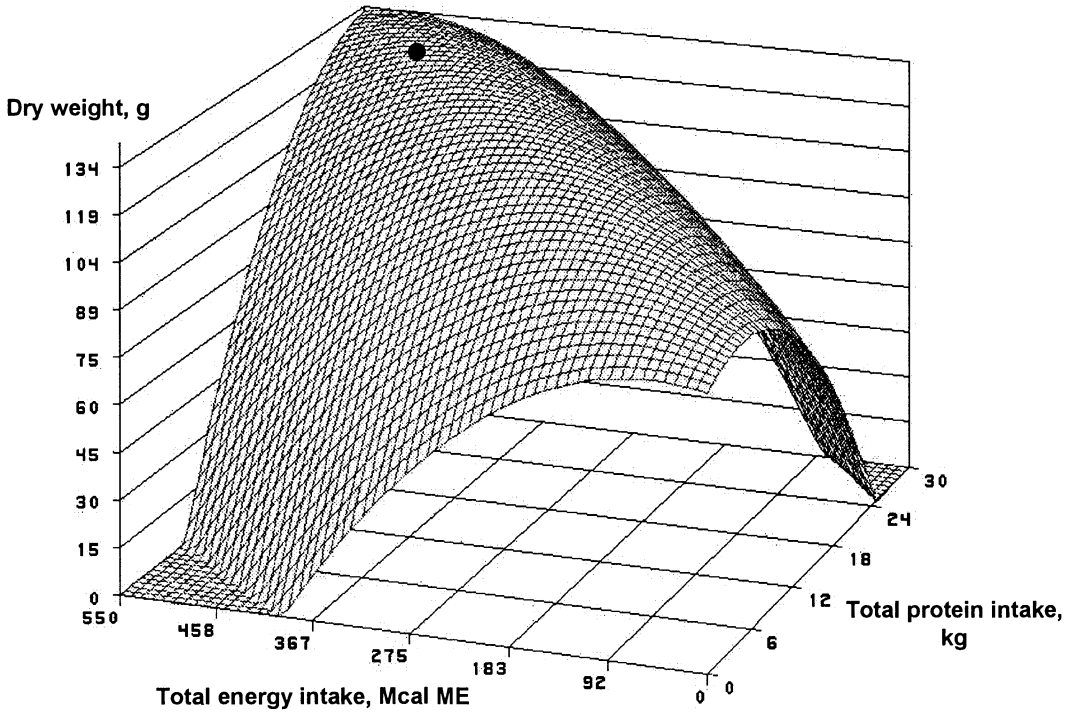

Figure 4. Response surface plot of the dry weight of suckled mammary glands from sows fed different levels of energy and protein. ● = Maximum stationary point. Day of lactation when the maximum stationary point occurred was 27.6 (estimated by Eq. [2]).

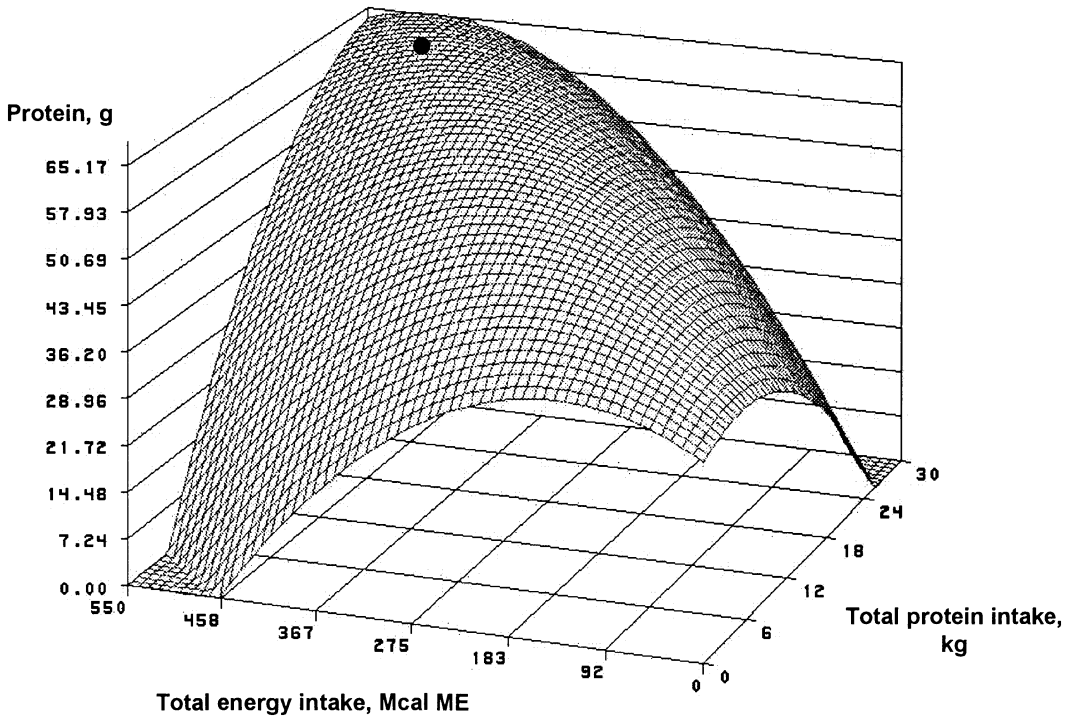

Figure 5. Response surface plot of the protein amount in suckled mammary glands from sows fed different levels of energy and protein. ● = Maximum stationary point. Day of lactation when the maximum stationary point occurred was 27.4 (estimated by Eq. [2]).

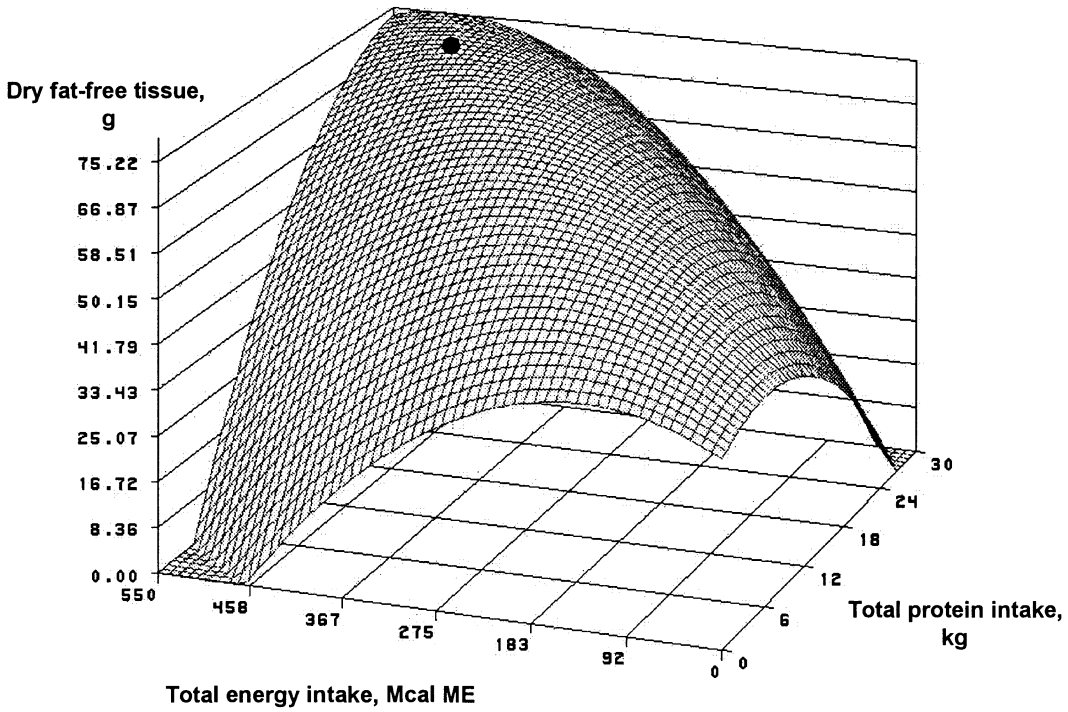

Figure 6. Response surface plot of the dry fat-free tissue amount in suckled mammary glands from sows fed different levels of energy and protein. ● = Maximum stationary point. Day of lactation when the maximum stationary point occurred was 27.5 (estimated by Eq. [2]).

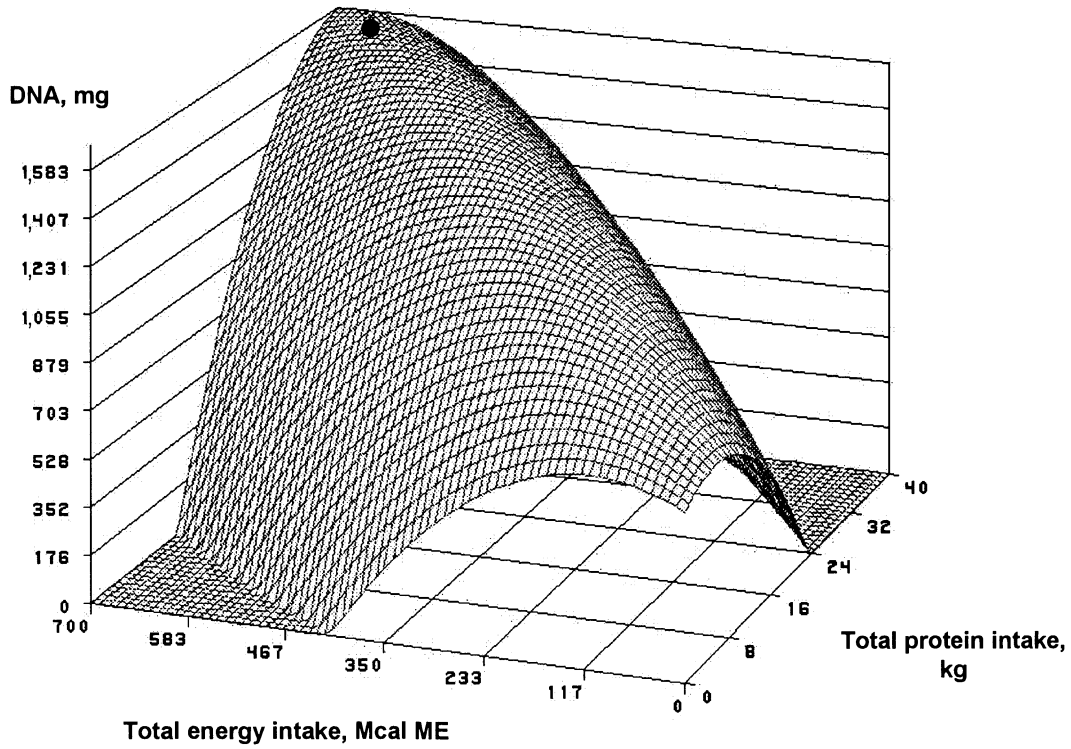

Figure 7. Response surface plot of the DNA amount in suckled mammary glands from sows fed different levels of energy and protein. ● = Maximum stationary point. Day of lactation when the maximum stationary point occurred was 25.3 (estimated by Eq. [2]).

TPI was 44.0 kg, which was predicted to occur on d 25.9 of lactation.

Percentage of Dry Tissue. There was a linear effect ($P < .01$) of TEI on dry tissue as a percentage of wet weight during lactation. The saddle stationary point was 23.0% dry tissue when TEI was 353.2 Mcal and TPI was 22.4 kg, which was predicted to occur on d 25.4 of lactation. The percentage of dry tissue decreased as a point on the surface moved from the saddle stationary point toward later lactation and increased as the point moved toward farrowing.

Composition of Mammary Glands (Dry Tissue Basis). Protein content was affected by TEI (linear effect, $P < .01$) and was not affected by either TPI ($P > .05$) nor by an interaction of TEI and TPI ($P > .05$). The protein content reached a saddle stationary point at 48.6% when TEI was 396.9 Mcal and TPI was 20.5 kg, which was predicted to occur on d 26.7 of lactation. Mammary gland protein content increased as TEI increased ($P < .01$). There was a linear effect ($P < .01$) of TEI on DFFT content during lactation. The saddle stationary point was 55.8% when TEI and TPI were 418.8 Mcal and 21.1 kg, respectively, which was predicted to occur on d 27.2 of lactation. The mammary DFFT content increased as TEI increased ($P < .01$). There was no effect of TEI and TPI on DNA content (%) during lactation.

Mammary fat content was affected only by TEI (linear effect, $P < .01$) during lactation and the saddle stationary point was 44.2% when TEI was 418.9 Mcal and TPI was 21.1 kg, which was predicted to occur on d 27.2 of lactation. Fat content decreased as TEI increased ($P < .01$).

Discussion

Growth of the sow mammary gland is influenced by energy and protein intake during lactation. Total energy and total protein consumed during lactation affect the wet and dry weights, the amount of tissue protein and DFFT, and the amount of DNA of each suckled gland. Statistical analysis using response surface regression allowed for interpretation of results beyond simple comparison between treatment groups. Indices of mammary gland growth were generally maximized between d 25 and 28 of lactation in sows in which litter size was set to nine or 10 pigs and when sows were consuming an average of 16.9 Mcal ME and 55 g of lysine daily during lactation. Cross-sectional area of mammary parenchymal tissue and percentage of dry mammary tissue that was protein, DFFT, or fat were affected by total energy consumed during lactation, but not by total protein consumed. As lactation progressed and as sows consumed increased total amounts of dietary energy, their mammary glands grew more and had more parenchymal tissue, resulting in increased protein and DFFT contents and decreased fat content as a percentage of mammary tissue. Results from the current study illustrate that both increased energy and protein intakes stimulate mammary gland growth.

Only glands known to have been suckled during lactation were included in the results. Milk in the tissues will contribute to total tissue composition; however, this is estimated to be a minor contribution because mean gland weights range from about 300 to nearly 600 g (Table 4), whereas milk content in each gland is only about 20 to 30 g, even during peak lactation (Hughes and Hart, 1935; Allen and Lasley, 1960; Hartman et al., 1962).

Both TEI and TPI affected mass of parenchymal tissue of each suckled mammary gland. Increasing TEI and TPI also resulted in greater total DNA in each gland, indicating hyperplasia of the tissue and accumulation of more mammary cells in each gland. This is consistent with the increased cross-sectional area that occurs during lactation (Kim et al., 1999) in response to increasing TEI and TPI observed in the present study. Mammary tissue grows by elongation and branching of ducts into the mammary fat pad (Tucker, 1987). Even with extensive formation of lobules and alveoli during pregnancy, adipocytes are retained in the interlobular connective tissue. Because the extraneous fat pad was trimmed from the lactating tissues in the present study, the fat content of the tissue reflects this interlobular fat, as well as cellular lipid and milk fat. The decline in tissue fat that occurs during lactation (Kim et al., 1999) suggests a loss of the interlobular fat, which is replaced by secretory parenchymal tissue. In addition to effects on total mammary mass, TEI has an impact on mammary tissue composition. Increasing TEI results in an increase in percentage of dry tissue that is protein or DFFT, and a corresponding decrease in percentage that is fat. Such a shift to a leaner tissue composition suggests a greater proportion of milk-secreting parenchyma in the gland and a lower proportion of the interlobular adipocytes. The loss of interlobular fat cells is consistent with the compression of connective tissue stroma observed during lobuloalveolar development (Tucker, 1987). However, TEI did not affect DNA as a percentage of dry tissue. This may indicate that the effect of TEI on tissue composition occurs primarily as a result of hypertrophy of the parenchymal tissue and not because of greater density of cells within the parenchymal tissue. We have previously provided evidence for both hypertrophy and hyperplasia occurring in sow mammary glands during lactation (Kim et al., 1999).

Increased energy intake during lactation increases milk production (Noblet and Etienne, 1986; Schoenherr et al., 1989) resulting in greater litter weight gain (O'Grady et al., 1973; Nelssen et al., 1985; Brendemuhl et al., 1987; King et al., 1993). Protein or lysine intake during lactation also affects milk production and litter weight gain (Woerman and Speer, 1976; Brendemuhl et al., 1987). Greater litter weight gain during lactation mainly comes from greater milk production from sows (Hartman and Pond, 1960; Williams, 1976; Noblet and Etienne, 1987; Roos, 1989; King et al., 1993). Milk productivity mainly depends on the activity and number of epithelial cells in the mammary gland. The DNA amount in mammary glands, which is an indicator of the number of mammary cells (Hacker and Hill, 1972; Kensinger et al., 1982), is highly correlated (R^2 = .85) with litter weight gain in rats (Tucker, 1966). Similarly, the size of a suckling pig is positively correlated with mass of the gland that is suckled (Nielsen and Sorensen, 1998). In addition, stimulus to the gland, as determined by frequency of milk removal, affects both cellular differentiation and cellular proliferation in mammary tissue (Wilde and Knight, 1989). From the present study, it can be hypothesized that increased energy intake improves milk yield, resulting in heavier and more active pigs. These pigs would further stimulate the mammary glands and elicit more effective milk removal, resulting in a higher rate of mammary tissue growth.

The recent NRC (1998) report on swine nutrient requirements recommends feeding 17.3 Mcal of ME and 47 g of lysine per day for a sow with body condition and litter size similar to those used in the current study. Results from our study suggest that sows need marginally more lysine intake than the amount suggested by NRC (1998) to achieve maximal mammary gland growth during lactation. Nutrient requirements have been established empirically on the basis of sow body condition and litter performance. If a factorial approach is used, then nutrient requirements for mammary gland should be included in the calculation. Trottier et al. (1997) reported that the essential amino acid uptake by mammary glands was 188.5 g/d. Some 49 g of this amount was not secreted as milk. Kim et al. (1999) showed that the amount of essential amino acids accumulated in mammary gland of the sow with 10 pigs during lactation was about 7.0 g/d. Thus, 74% of essential amino acid was secreted as milk, and 26% was retained in mammary glands. Among essential amino acids retained in mammary glands, 14.3% was accumulated in mammary tissue for the growth. The remaining 85.7% of essential amino acids taken up by the gland, but not secreted as milk protein, might be accounted for by oxidation, conversion to nonessential amino acids, or utilization by the cells for other metabolic pathways (Richert et al., 1998). Thus, total nutritional requirement for mammary glands will be greater than the requirement for milk production alone. The additional requirement of lysine for mammary gland growth may explain the apparent differences in requirement estimates between NRC (1998) and the present study. In practical situations, however, litter size varies among sows. Studies on the effect of litter size on mammary gland growth and pig growth are needed to extend observations of the present study to the full range of practical situations.

Implications

Mammary mass and mammary growth during lactation are directly related to milk yield and litter weight gain. Mammary gland growth of sows responds to energy and protein intake during lactation, indicating the

importance of nutrient intake for mammary gland growth. Lactating sows may need slightly more lysine than the currently recommended amount to achieve maximal mammary gland growth during lactation, maximal milk production, and further improvement of litter weight gain.

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