

Feeding Lactating Primiparous Sows to Establish Three Divergent Metabolic States: II. Effect on Nitrogen Partitioning and Skeletal Muscle Composition^{1,2}

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ABSTRACT: We established an experimental model to study nitrogen (N) partitioning in lactating primiparous sows alimeted to three levels of nutrient intake. Thirty-six sows fitted with a gastric cannula and fed a 15.4 MJ DE/kg and 18.6% CP diet were allocated to one of three treatments after farrowing: 1) ad libitum-fed; 2) restricted-fed to 55% of the ad libitum feed intake; and 3) superalimeted to at least 125% of the ad libitum feed intake. These feed intakes were successfully achieved throughout lactation. Nitrogen balance was studied for three 5-d periods starting on d 2, 11, and 19 of lactation, and a triceps muscle biopsy was taken on d 26. For all treatments, N intake increased, milk N production increased, urinary N losses decreased, but fecal N losses increased as the 28-d lactation progressed. Restricted-fed sows had the lowest fecal N and urinary losses and mobilized the most maternal protein (-23.0 vs -7.4 ± 6.5 g N/d for ad libitum-fed sows) during lactation. As a consequence of these economies, and extensive

protein mobilization, restricted-fed sows were able to maintain milk N production similar to that of sows on the other treatments. Superalimeted sows did not mobilize protein, had the poorest protein digestibility, directed the least digestible N toward milk (40.1 vs 78.3% in restricted-fed sows), and produced amounts of milk N similar to those produced by sows on the other treatments. The treatment differences in N retention measured by N balance were reflected in differences in skeletal muscle variables and urinary creatinine. Skeletal muscle cell size (protein:DNA ratio) and protein synthetic capacity (RNA:DNA ratio) increased in response to feed intake. The protein:DNA ratio increased ($P < .01$) linearly and the RNA:DNA ratio increased ($P < .05$) in a curvilinear manner. These data suggest that primiparous sows partition additional retained N toward their maternal reserves rather than milk N. They also suggest that sows fed inadequate N intakes maintain milk production by mobilizing maternal protein reserves. Such sows also conserve maternal N during lactation, possibly by reducing muscle protein synthesis.

Key Words: Sows, Lactation, Feed Intake, Nitrogen Balance

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Introduction

Modern sows generally fail to consume sufficient nitrogen and energy to meet requirements for milk production and growth during lactation (Aherne and Williams, 1992). Thus, sows mobilize protein reserves, primarily composed of skeletal muscle (Swick and Benevenga, 1977), to make up this deficit during

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lactation (King et al., 1993; Everts and Dekker, 1994). Dietary N is partitioned in lactating sows toward losses in feces and urine and retention in milk and maternal body mass. To efficiently utilize dietary N, lactating sows probably limit N losses and prioritize N toward maternal gain and(or) the mammary gland for milk synthesis. Whole-body protein mobilization in lactating sows can be measured with fairly noninvasive techniques. Nitrogen partitioning can be estimated from N balance studies, and muscle protein:DNA and RNA:DNA ratios, measured after muscle biopsy, estimate the extent of skeletal muscle protein mobilization. Because creatinine is synthesized in muscle and excreted in urine in amounts proportional to an animal's muscle mass (Tietz, 1986), modifications in urinary creatinine excretion during lactation reflect changes in the maternal muscle protein mass.

In this experiment, we compared lactating primiparous sows fed to establish three divergent metabolic states (anabolic, slightly catabolic, and extremely catabolic) to evaluate the partitioning of N toward losses and retention. We modified the "superalimentation" model (feeding via a gastric cannula) of Matzat et al. (1990) to overcome the lactational appetite limitation and create anabolic sows during lactation. Multiparous sows were used in this experiment, and the extent of N partitioning was not quantified. Because primiparous sows have smaller appetites than multiparous sows and are still growing, we hypothesized that primiparous lactating sows 1) allocate additional nutrients toward their maternal reserves rather than the mammary gland and 2) minimize N losses when dietary N is limiting.

Materials and Methods

This experiment was approved by the University of Alberta Animal Care Committee to ensure adherence to the Canadian Council of Animal Care Guidelines. The experimental design, housing conditions, and management of gilts during lactation and gestation are described in Zak et al. (1998).

Experimental Design. The experiment was conducted using a randomized complete block design with three treatments in three blocks between March and November 1994. Each block represented a 12 crate farrowing unit, and sow was considered the experimental unit. Camborough × Canabrid gilts (n = 36; PIC, Acme, Alberta) were individually housed and fed 2 to 2.3 kg/d of a conventional gestating sow diet (Table 1), according to their live weight. Between d 65 and 75 of gestation, all gilts underwent surgery for the insertion of a gastric cannula (Pluske et al., 1995). On d 109 of gestation, gilts were moved into individual farrowing crates, and the ration was increased by 1 kg/d and changed to the lactation diet (Table 1). The temperature of the farrowing room was controlled at between 20 and 23°C, and an evaporative

Table 1. Composition of the experimental diets (as-fed basis), as a percentage of the diet

Item	Gestation	Lactation
Ingredient		
Barley	56.3	24.0
Wheat	30.0	24.0
Soybean Meal (44% CP)	7.0	22.0
Fish meal	—	5.5
Sugar	—	16.0
Canola oil	—	5.0
Tallow	2.0	—
Iodized salt	.5	—
Dicalcium phosphate	1.7	2.6
Limestone	1.4	—
Vitamin/mineral supplement ^a	1.0	1.0
Analysis		
Digestible energy, MJ DE/kg	13.4	15.4
Crude protein, %	13.7	18.6
Lysine, %	.56	1.05

^aSupplied the following per kg of complete feed: 10,000 IU vitamin A, 1,000 IU vitamin D, 80 IU vitamin E, 2 mg vitamin K, 30 µg vitamin B₁₂, 12 mg riboflavin, 25 mg niacin, 25 mg calcium pantothenate, 600 mg choline, 200 µg biotin, 200 mg folic acid, 5 mg ethoxyquin, 150 mg iron, 12 mg manganese, 120 mg zinc, 12 mg copper, 200 µg iodine, and 100 µg selenium.

cooling system was automatically switched on if the room temperature increased above 23°C. Water was freely available to sows and pigs through nipple drinkers at all times.

Gilts were randomly allocated within 36 h after parturition to one of three nutritional treatments: 1) ad libitum-fed sows that were encouraged to eat as much as possible during lactation; 2) restricted-fed sows that were fed 55% of their estimated ad libitum feed intake in three equal-sized meals at 0600, 1330, and 2100; and 3) superalimented sows that were infused through their gastric cannula at least 125% of their estimated ad libitum feed intake in seven meals evenly spaced throughout the day between 0600 and 2100. Superalimentation commenced within 2 to 4 d after parturition and continued until the end of lactation on d 28. Fresh feed was available to superalimented sows at all times, and for all treatments any uneaten feed was weighed-back the following morning and feed intake was recorded. To facilitate direct gastric feeding of superalimented sows .5% xanthan gum was added to the lactation diet, which was then mixed with approximately two parts water to one part feed (Pluske et al., 1995).

Litter size was standardized to at least eight pigs within 2 d after parturition, and the pigs had no access to creep feed throughout lactation. Sows and pigs were weighed, and sow backfat was measured ultrasonically (Scanoprobe II, Scano, Ithaca, NY) 65 mm from the midline at the last rib (P₂) at farrowing and every 7 d until weaning.

Nitrogen Balance. A subset of 24 sows were catheterized with an indwelling foley catheter (french size 18 to 22, 30-mL balloon type) for collection of

urine. Catheters were inserted while the sows were fully conscious and standing in their farrowing crates at the time of feeding on d 2, 9, and 17 of lactation. Urine was collected between d 3 and 7 (early lactation), d 11 and 15 (midlactation), and d 19 and 23 (late lactation) postpartum. Total urine was collected daily into 100 mL of 20% HCl in a plastic container and weighed, and a 1% sample by weight was composited for the three 5-d collection periods and stored at -20°C for subsequent N, creatinine, and energy analysis. Chromic oxide was incorporated into the lactation diet at a level of .3% and was fed to sows for at least 5 d before fecal collection. Fecal grab samples were collected daily throughout the collection periods, stored at -20°C , and then oven-dried at 60°C to constant weight. Fecal samples were then pooled by equal weight, within each sow and period. Feed samples were collected and stored at -20°C . Feed and fecal samples were later analyzed for N, amino acids, chromic oxide, and energy. Milk N was calculated in the three stages of lactation for the estimation of N balance using Eq. [1]:

$$\text{Milk N} = \left[\frac{\text{Milk production} \times (\text{milk protein}/100)}{100} \right] \times .158 \quad [1]$$

where Milk production = milk production estimated from litter gain for this population of sows (3.88 g milk:1 g of pig gain; J. R. Pluske, personal communication), g/d; milk protein was measured in this experiment, %; and .158 = inverse of the conversion factor for milk protein to milk N (6.38; McDonald et al., 1988).

Energy Balance. Energy balance (Eq. [2]) for the three stages of lactation was calculated from the measured feed energy intake and fecal and urinary energy output. The maintenance energy requirement (Eq. [3]) and daily milk energy output (Eq. [4]) of the lactating sow were calculated:

$$\text{Energy balance} = \text{Energy intake} - (\text{Maintenance} + \text{Emilk}) \quad [2]$$

$$\text{Maintenance} = .485 \times \text{wt}^{.75} \quad [3]$$

$$\text{Emilk} = [(2.54 \times \text{ADG}) + (78.7 \times \text{BW}) + 153] \times (4.184 \times \text{LS})/k_1 \quad [4]$$

where Maintenance = lactating sow maintenance energy requirement, based on the mean of the values calculated by Burlacu et al. (1983) and Noblet and Etienne (1987), MJ ME/d; $\text{wt}^{.75}$ = sow metabolic body weight at the start of each period, $\text{kg}^{.75}$; Emilk = energy required for milk production, kJ/d; ADG = average daily gain per pig during the different lactational periods, g/d; BW = average pig weight at the beginning of the given period, kg; LS = litter size; and k_1 = efficiency of utilization of ME for milk production (.72) (Noblet et al., 1990).

Muscle Biopsy. On d 24 to 26 of lactation, a biopsy of the triceps muscle was performed on three sows from each treatment to obtain 3 to 4 g of muscle tissue. The tissue was immediately frozen on Dry Ice and stored at -70°C for RNA, DNA, and protein analysis. The surgical technique for the muscle biopsy is described below.

Sows were anesthetized with Pentothal (.17 mL 5% sodium thiopental; Sanofi Animal Health, Victoriaville, Quebec) administered via an ear vein while the animal was restrained with a nose snare. Anesthesia was maintained with a closed-circuit system of halothane (2%), oxygen (2.5 to 3.5 mL/min), and nitrous oxide (.5 to 1.0 L/min), with dosage rate depending on a sow's body weight. Sows were placed in dorsal recumbency with the right foreleg extended to expose the right shoulder. The lateral surface of the right shoulder and forelimb was shaved and scrubbed with Betadine solution (Ayerst, St. Lauren, Quebec), swabbed with ethanol, and sprayed with Betadine solution. A skin incision, approximately 10 to 15 cm in length, was cut midway along the line between the deltoid tubercle of the humerus and the olecranon. The lateral head of the triceps brachii muscle was identified and exposed by blunt dissection along its length to within 2 cm of the olecranon and a little underneath the deltoid muscle. When a section of muscle was exposed, a strip approximately 1 cm wide and 3 to 4 g in weight was cut along its length and immediately frozen on Dry Ice. The incision was closed with three or four sutures through both sides of the cut fascia along the cut edge of the muscle. Two or three sutures closed the connective tissue and interrupted vertical mattress sutures closed the skin. When able to walk, the sows were immediately offered feed and water. Sows were monitored daily for signs of ill-health after surgery.

Effect of Xanthan Gum on Diet Digestibility. The effect of addition of xanthan gum on apparent fecal N digestibility of the diet was tested using the mobile nylon bag technique (Sauer et al., 1983; de Lange et al., 1991). In brief, approximately 1 g of the lactation diet, with or without .5% xanthan gum (ground through a .8-mm mesh screen), was added to nylon bags (25 × 40 mm, pore size 48 μm). The nylon bags were predigested for 2.5 h at 37°C in a predigestion solution (.01 N HCl and 377 IU pepsin/L in double-distilled H_2O). Three 60-kg barrows, fitted with a simple T-cannula in the duodenum, were individually housed in metabolism crates. Four nylon bags per treatment were inserted into the digestive tract, via the cannula, of these barrows in the evening and following morning of the trial. The bags were recovered in the feces 24 to 36 h later. The difference between the amount of N within the bag before and after passage through the digestive tract was used to calculate the apparent fecal digestibility of N in the diet.

Chemical Analyses

Feed and fecal samples were ground in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) through a .8-mm screen, mixed well, and stored at 4°C until analysis. Before analysis, individual muscle samples were pulverized in a mortar and pestle in liquid N₂ and stored at -70°C.

Proximate Analysis. Feed and fecal nitrogen were analyzed using the FP-428 Nitrogen Determinator, System: 601-700-900 (LECO Corp., St. Joseph, MI). Urinary nitrogen was determined on 5 g of urine using the Kjeldahl procedure (AOAC, 1980). Fecal, feed, and urine energy were determined using an adiabatic bomb calorimeter. Urine was prepared for energy determination by freeze-drying 5-mL urine samples in 10- × 5-cm plastic bags. The difference in energy value between the plastic bag and the urine was accounted for. Chromic oxide in feed and feces was determined with the method of Fenton and Fenton (1979).

Creatinine Analysis. Creatinine was determined colorimetrically in urine, on at least 2 d of collection, using a kit (Sigma Chemical, St. Louis, MO; catalog no. 555) following the manufacturer's protocol.

Amino Acid Analysis. Amino acids in feed and fecal samples were determined by HPLC (Sedgewick et al., 1991). Methionine, cysteine, tryptophan, and proline were not determined.

RNA Analysis. Approximately 100 mg of pulverized muscle tissue was homogenized (Polytron, Tekmar TISSUMIZER) in duplicate in 1 mL of TRIzol™ (GibcoBRL/Life Technologies, Gaithersburg, MD) according to the manufacturer's protocol (Chomczynski, 1993).

DNA Analysis. The DNA measurements were conducted according to the fluorometric procedure detailed by Downs and Wilfinger (1983) using approximately 100 mg of pulverized muscle tissue, in duplicate. The fluorescent dye bisbenzimidazole (Sigma Chemical, St. Louis, MO) was used and the excitation and emission wavelengths were set at 359 and 446 nm, respectively, with a 10-nm slit width. The reliable limit of sensitivity of this assay was 20 ng DNA. The accuracy of the estimates were determined by addition, in quadruplicate, of five quantities of DNA standard (calf thymus DNA; 18 to 120 ng DNA/cuvette) to aliquots of tissue homogenate. These DNA-spiked samples ran parallel with the standard curve. The coefficient of variation among the calculated slope values for the three assays was 4.54%.

Protein Analysis. The crude tissue homogenates from the DNA analysis were assayed, in duplicate, for protein using a modification of the Bradford procedure and 2 mL of Bradford dye (Darbre, 1986).

Statistical Analyses

All computations were performed using the GLM procedures of SAS (1990).

Production Data. Weekly lactational feed, energy, N, and lysine intakes, and sow live weight change and backfat changes for 26 sows were analyzed using repeated measures analysis of variance. Sources of variation among sows were block (b = 3), treatment (t = 3), and block × treatment. Variation among the experimental units (sows within block × treatment) was used as the estimate of experimental error and for significance testing of treatments. Significant differences among treatment × week were determined using Fisher's protected least significant difference test.

Nitrogen and Energy Balance Data. Measures of N and energy in feed, feces, and urine and estimates in milk were used to calculate sow N and energy balances. These variables were measured in late lactation of block 2, and in early, mid-, and late lactation in block 3, except for ad libitum-fed sows in early lactation. Because of missing data, two different analyses were computed, one with three treatments in mid- and late lactation only and a second analysis with restricted-fed and superalimentation treatments across all three stages of lactation. Preliminary analyses indicated no significant contribution due to block, and because the majority of sows were in only one of the stages of lactation, sows were considered to be nested in treatment × stage of lactation. Therefore, the data were analyzed as treatment, stage of lactation, treatment × stage of lactation, and error.

Urinary Creatinine Data. Urinary creatinine in all stages of lactation and as a percentage of levels in early lactation were analyzed in block 3 only. Variation among the experimental units (sows within block × treatment) was used as the estimate of experimental error and for significance testing across treatments.

Digestibility and Muscle Data. Feed intake was treated as a continuous variable, and apparent fecal N, energy, and lysine digestibilities and skeletal muscle RNA, DNA, protein, RNA:DNA ratio, protein:DNA ratio, and RNA:protein ratio were regressed against feed intake.

Results

Although 36 gilts were placed on experiment, only data collected from 26 sows were used: one sow gave birth to four pigs; six sows suckled less than eight pigs for at least 1 wk of lactation; one sow's litter developed severe diarrhea; the gastric cannula of one sow leaked; and the final sow had a persistent elevated temperature associated with metritis. Sow production data was analyzed for the first 3 wk of lactation because sows underwent surgery for the implantation of a jugular catheter and for a muscle biopsy between d 24 and 26 of lactation. Nitrogen and energy balance data for all three stages of lactation were examined for restricted-fed and superalimented sows only because

Table 2. Nutrient intake and weight and backfat changes in primiparous sows restricted-fed (R), ad libitum-fed (AL), and superalimented (SA) in the first 3 weeks of lactation

Item	Treatment				Statistical significance ^b	
	R	AL	SA	SEM ^a	T	T × W
No. of sows	9	9	8			
Farrowing weight, kg	174	182	175	4.5	.361	—
Farrowing backfat, mm	18.3	17.9	16.8	.81	.357	—
Feed intake, kg/d	2.96 ^{cx}	5.37 ^y	7.22 ^z	.157	.001	.001
Digestible N, g/d	79.2 ^x	138.6 ^y	172.0 ^z	3.70	.001	.084
Digestible lysine, g/d	29.6 ^x	51.8 ^y	65.0 ^z	1.51	.001	.010
Digestible energy, MJ/d	46.8 ^x	81.8 ^y	104.5 ^z	2.36	.001	.001
Energy intake/maintenance, % ^d	215 ^x	346 ^y	447 ^z	12.0	.001	.010
Weight change, kg/wk	-9.24 ^x	-1.50 ^y	.83 ^y	.217	.001	.129
Backfat change, mm/wk	-2.13 ^x	-.75 ^y	.39 ^z	.283	.001	.724
Litter growth rate, kg/d	1.85	1.79	2.22	.128	.051	.118
Litter size ^e	8.7	8.8	9.2	.36	.618	.098

^aSEM for AL, SEM for R = .85 × SEM for AL, SEM for SA = .91 × SEM for AL.

^bEffects: T, treatment; T × W, interaction between treatment and week of lactation.

^cLeast squares mean.

^dMaintenance energy requirement = .485 × wt^{.75}, MJ DE/d, with wt^{.75} calculated based on animal weight at the start of each week, kg.

^eAverage litter size in the first 3 wk of lactation.

^{x,y,z}Means within a row lacking a common superscript letter differ by value indicated.

there were insufficient ad libitum-fed sows in early lactation.

Treatment Effects. There was no difference in sow weight and backfat thickness at farrowing among the three treatments (Table 2). Ad libitum-fed sows (n =

9) ate intermediate amounts of digestible N, lysine, and energy (Table 2), and excretion of N in urine and feces was intermediate for ad libitum-fed sows compared with the other two treatments (Table 3). Ad libitum-fed sows lost weight (4.5 kg) and backfat (2.3

Table 3. Nitrogen partitioning in primiparous sows restricted-fed (R), ad libitum-fed (AL), and superalimented (SA) in the first 3 weeks of lactation

Item	Treatment				Statistical significance ^b		
	R	AL	SA	SEM ^a	T	S	T × S
N intake, g/d	103.4 ^{cx}	172.5 ^y	252.0 ^z	5.99	.001	.062	.367
Fecal N, g/d	10.9 ^x	24.5 ^y	57.2 ^z	1.82	.001	.001	.003
Urinary N, g/d	43.1 ^x	79.1 ^y	94.5 ^z	3.83	.001	.001	.032
N retention, g/d ^d	49.3 ^x	68.9 ^y	100.2 ^z	5.84	.001	.001	.116
Milk N, g/d ^e	72.3	76.3	77.5	3.58	.633	.370	.470
N balance, g/d	-23.0 ^x	-7.4 ^x	22.8 ^y	5.87	.001	.001	.484
Urinary creatinine, g/d	6.31	8.25	8.04	.61	.147	.955	.410
Protein digestibility, % ^f	89.5 ^x	85.8 ^y	77.3 ^z	.84	.001	.001	.088
Fecal N/N intake %	10.5 ^x	14.2 ^y	22.7 ^z	.85	.001	.001	.088
Urinary N/digestible N, %	46.6	54.5	48.4	2.46	.098	.001	.239
Urinary creatinine, % ^g	78 ^x	93 ^{xy}	104 ^y	6.0	.038	.552	.725
Milk N/digestible N, %	78.3 ^x	52.7 ^y	40.1 ^z	3.67	.001	.285	.632
Milk N/retained N, %	150.9 ^x	126.4 ^x	80.6 ^y	9.64	.001	.001	.557

^aSEM for AL, SEM for R = 1.10 × SEM for AL, SEM for SA = 1.18 × SEM for AL.

^bEffects: T, treatment; S, stage of lactation; T × S, interaction between treatment and stage of lactation.

^cLeast squares mean.

^dN retention = N intake - (fecal N + urinary N).

^eMilk N (g/d) = [(3.88 × daily litter gain) × (protein % in milk/100)] × .158, where % milk protein = milk protein content in the experiment (Pluske et al., 1998).

^fApparent fecal digestibility, %.

^gUrinary creatinine, as a percentage of values in early lactation, %.

^{x,y,z}Means within a row lacking a common superscript letter differ by value indicated.

Table 4. Calculated energy balance (MJ ME/d) in primiparous sows restricted-fed (R), ad libitum-fed (AL), and superalimented (SA) in the first 3 weeks of lactation

Item	Treatment				Statistical significance ^b		
	R	AL	SA	SEM ^a	T	S	T × S
Energy intake, MJ ME/d ^c	50.5 ^{dx}	82.0 ^y	112.4 ^z	2.54	.001	.001	.449
Maintenance, MJ ME/d ^{ef}	20.5 ^x	22.3 ^y	22.5 ^y	.34	.003	.005	.411
Milk energy, MJ ME/d ^g	62.7	66.3	66.1	2.77	.374	.202	.766
Energy balance, MJ ME/d ^h	-32.7 ^x	-6.6 ^y	20.9 ^z	4.13	.001	.109	.952
Energy digestibility, % ⁱ	88.3 ^x	85.9 ^y	80.2 ^z	.49	.001	.002	.030

^aSEM for AL, SEM for R = 1.10 × SEM for AL, SEM for SA = 1.18 × SEM for AL.

^bEffects: T, treatment; S, stage of lactation; T × S, interaction between treatment and stage of lactation.

^cEnergy intake (MJ ME/d) = GE intake - (fecal energy + urinary energy).

^dLeast squares mean.

^eMaintenance energy requirement (MJ DE/d) = .485 × wt^{.75}.

^fCalculated for each period of lactation based on animal weight at the start of each period, kg.

^gMilk energy = [(2.54 × ADG) + (78.7 × BW) + 153] × 4.184 × litter size/k_l, MJ ME/d, where ADG = ADG per pig during the period of lactation, g; BW = weight of the average pig at the beginning of the short period kg; and k_l = efficiency of utilization of ME for milk production (.72) (Noblet et al., 1990).

^hEnergy balance (MJ ME/d) = Energy intake - (Maintenance energy requirement + milk energy).

ⁱApparent fecal digestibility (%).

^{x,y,z}Means within a row lacking a common superscript letter differ by value indicated.

mm) during lactation and were in negative energy balance in midlactation (-12.1 ± 6.4 MJ ME/d) but close to zero energy balance in late lactation (-1.1 ± 5.2 MJ ME/d). They were also in negative N balance in midlactation (-26.2 ± 9.1 g/d) but were in positive N balance in late lactation (11.3 ± 7.42 g/d).

Restricted-fed sows (n = 9) consumed 57% of the digestible N, lysine, and energy intake of ad libitum-fed sows. This level of intake supplied over twice the sow's calculated maintenance energy requirement (Table 2). Restricted-fed sows lost more ($P < .001$) weight and backfat during lactation and excreted less ($P < .001$) fecal and urinary N but secreted quantities of milk N similar ($P = .63$) to those secreted by sows on the two higher intake levels (Table 3). Consequently, restricted-fed sows utilized a greater percentage of their digestible N for milk production ($P < .001$), resulting in a greater loss of maternal N compared to ad libitum-fed sows. Energy intake and energy balance were also lower ($P < .001$) in restricted-fed sows, but there was no difference in milk energy secreted among the three treatments (Table 4).

Superalimented sows (n = 8) consumed between 124 and 128% of the digestible N, lysine, and energy intakes of ad libitum-fed sows. This level of intake supplied nearly 4.5 times the sow's calculated maintenance energy requirements (Table 2). Superalimented sows gained weight (2.5 kg) and backfat (1.2 mm) during lactation (Table 2). They secreted similar quantities of milk N, and hence utilized a smaller percentage of their digestible N for milk production than sows fed the lower intake levels (Table 3). Overall, superalimented sows were in positive N and energy balance during lactation (Tables 3 and 4). In early (-4.1 ± 16.9 g/d) and

midlactation (-1.9 ± 12.6 g/d), superalimented sows were in close to zero N balance but by late lactation (42 ± 9.8 g/d) they were in positive N balance.

Diet Digestibility. From the digestibility trial, using the nylon bag technique, the addition of .5% xanthan gum to the diet had no effect on apparent N digestibility (90.3 ± 3.49 vs $91.5 \pm 3.66\%$ for diets containing and not containing xanthan gum, respectively). Apparent fecal digestibilities for protein, energy, and all amino acids, except histidine, were higher ($P < .001$) in restricted-fed than in ad libitum-fed sows and higher in ad libitum-fed than in superalimented sows (Tables 3, 4, and 5). The apparent fecal digestibilities of protein and lysine decreased ($P < .001$) in a cubic manner with increasing feed intake, and the apparent fecal digestibility of energy decreased in a quadratic fashion with increasing feed intake (Figure 1).

Stage of Lactation. Nitrogen intake and N excreted in feces increased between mid- and late lactation, but N excreted in urine decreased with stage of lactation (Table 6). The percentage of retained N secreted as milk also decreased ($P < .001$) with stage of lactation. Similar effects of stage of lactation were seen when comparing all three stages of lactation in restricted-fed and superalimented sows only. Milk N increased ($P = .039$) between early (57 ± 7.0 g/d) and midlactation (79 ± 5.0 g/d), but it did not differ between mid- and late lactation (70 ± 4.6 g/d). Urinary N as a percentage of digestible N decreased ($P = .076$) between early ($72.3 \pm 8.54\%$) and midlactation ($51.1 \pm 6.86\%$), but there was no difference between mid- and late lactation ($42.7 \pm 5.69\%$). Nitrogen retention increased as lactation progressed for all treatments; however, restricted-fed sows did not achieve a positive N balance at any time.

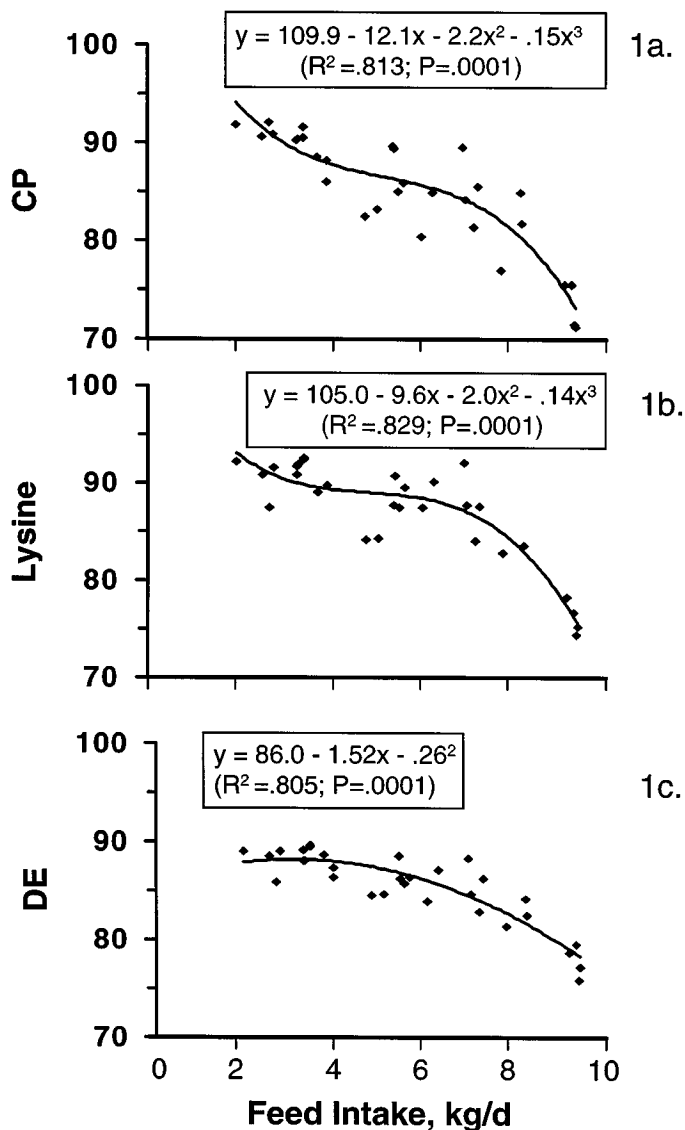


Figure 1. Effect of feed intake on apparent fecal digestibility of crude protein, lysine, and energy of lactating primiparous sows.

Urinary Creatinine. Excretion of urinary creatinine, as a percentage of creatinine in early lactation, was lower ($P = .038$) in restricted-fed than in superalimented sows and intermediate in the ad libitum-fed sows (Table 3).

Skeletal Muscle Protein/RNA/DNA. Sows recovered quickly from the muscle biopsy surgery and, after 24 h of minor stiffness, were not hindered by the incision site. Treatment differences in skeletal muscle variables were observed (Figure 2). This confirmed that the lateral head of the triceps is a good site for observing changes in the skeletal musculature of the lactating sow. In late lactation (d 24 to 26), skeletal muscle RNA content and RNA:DNA ratio increased ($P < .05$) in a curvilinear fashion with increasing feed intake, reaching a plateau at intakes seen in ad libitum-fed and superalimented sows (Figure 2a and

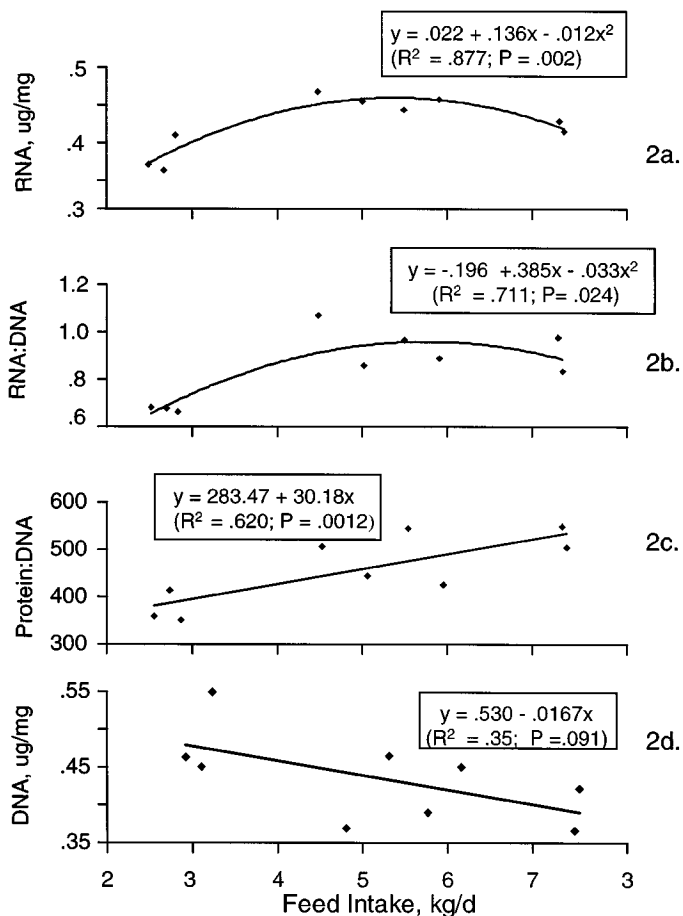


Figure 2. Effect of feed intake on skeletal muscle (a) RNA concentration, (b) RNA:DNA ratio, (c) protein:DNA ratio, and (d) DNA concentration at the end of lactation in primiparous sows.

b). Skeletal muscle DNA content decreased linearly ($P = .091$) with feed intake, and the protein:DNA ratio increased linearly ($P = .001$) with increasing feed intake (Figure 2c and d).

Discussion

When primiparous sows were superalimented during lactation, they partitioned the extra nutrients they received almost exclusively into their own bodies and not into milk production; superalimented and ad libitum-fed sows produced the same estimated amount of milk energy (66 MJ/d) and milk N (76 g/d). Superalimented sows retained an additional 31 g/d of nitrogen over and above the sows fed ad libitum, and almost all of this, 96%, appeared in maternal protein. This supports the hypothesis that primiparous sows allocate any additional nutrients toward their own maternal reserves. It is in contrast to the multiparous sows of Matzat et al. (1990) that, when superalimented to 117% of their ad libitum intake, chan-

Table 5. Apparent fecal digestibility of selected dispensable and indispensable amino acids in primiparous sows restricted-fed (R), ad libitum-fed (AL), and superalimented (SA) in the first 3 weeks of lactation

Amino acid	Treatment			SEM ^a	Statistical significance ^b		
	R	AL	SA		T	S	T × S
Indispensable							
Histidine	94.3 ^{cx}	92.9 ^x	88.5 ^y	.60	.001	.190	.385
Threonine	88.2 ^x	84.7 ^y	77.3 ^z	1.18	.001	.224	.200
Phenylalanine	91.8 ^x	88.8 ^y	82.9 ^z	.72	.001	.046	.196
Tyrosine	88.8 ^x	84.7 ^y	77.0 ^z	1.10	.001	.095	.115
Isoleucine	90.1 ^x	86.1 ^y	78.3 ^z	.90	.001	.045	.217
Leucine	91.2 ^x	87.7 ^y	81.1 ^z	.75	.001	.040	.118
Valine	90.2 ^x	86.4 ^y	78.8 ^z	.86	.001	.031	.110
Arginine	94.5 ^x	92.5 ^y	87.6 ^z	.49	.001	.011	.022
Lysine	91.0 ^x	87.6 ^y	79.7 ^z	.89	.001	.045	.073
Dispensable							
Alanine	87.0 ^x	81.7 ^y	71.6 ^z	1.07	.001	.011	.025
Aspartate	90.7 ^x	87.5 ^y	79.9 ^z	.82	.001	.039	.127
Glutamate	94.8 ^x	92.1 ^y	87.1 ^z	.57	.001	.077	.207
Glycine	90.8 ^x	87.3 ^y	80.6 ^z	.90	.001	.089	.074
Serine	92.3 ^x	89.8 ^y	84.8 ^z	.58	.001	.033	.041

^aSEM for AL, SEM for R = .83 × SEM for AL, SEM for SA = 1.11 × SEM for AL.

^bEffects: T, treatment, S, stage of lactation; T × S, interaction between treatment and stage of lactation.

^cLeast squares means.

^{x,y,z}Means within a row lacking a common superscript letter differ by value indicated.

neled extra nutrients into maternal tissue accretion and milk production. Why the difference between primiparous and multiparous sows?

The simple explanation is that our primiparous sows were younger and physiologically less mature than the multiparous sows used by Matzat et al. (1990). Everts (1994) suggested that sows have a biological “need” to achieve a predetermined protein

body mass (approximately 35 kg) during their lifetime, and the closer an animal is to this protein mass the smaller the “drive” to achieve this mass. Therefore, primiparous sows have a greater “drive” to grow (accrete maternal protein) during lactation than more mature animals of a larger maternal protein mass. In contrast, multiparous sows are closer to their mature protein mass, have a smaller “drive” to grow, and

Table 6. Nitrogen partitioning in midlactation and late lactation in primiparous sows restricted-fed, ad libitum-fed, and superalimented in the first 3 weeks of lactation

Item	Stage of lactation		SEM ^a	Statistical significance
	Mid (d 11–15)	Late (d 19–23)		
N intake, g/d	168.4 ^b	183.0	5.65	.062
Fecal N, g/d	25.1	36.7	1.71	.001
Urinary N, g/d	84.0	60.5	3.61	.001
N retention, g/d ^c	59.3	86.3	5.00	.001
Milk N, g/d	77.4	73.3	3.37	.370
N balance, g/d	-18.1	13.0	5.53	.001
Protein digestibility, % ^d	86.2	82.2	.80	.001
Energy digestibility, % ^d	85.9	83.7	.47	.002
Fecal N/N intake, %	13.8	17.8	.80	.001
Urinary N/digestible N, %	57.9	40.9	2.32	.001
Milk N/digestible N, %	59.6	54.5	3.46	.285
Milk N/retained N, %	142.8	95.8	9.08	.001

^aSEM for Period 2, SEM for Period 3 = .89 × SEM for Period 2.

^bLeast squares mean.

^cN retention = N intake - (fecal N + urinary N).

^dApparent fecal digestibility (%).

therefore partitioned more nutrients toward the mammary gland for milk production than primiparous sows.

If accretion of maternal protein has priority over milk production in primiparous sows, then it follows that as feed intake is restricted, maternal growth should have priority over milk production, and milk production in turn should fall. This was not the case in this experiment, because milk production did not differ among the three treatments (Pluske et al., 1998). When sows were severely restricted in feed intake, milk output was maintained and sows extensively mobilized their protein reserves to maintain their milk production; restricted-fed sows mobilized 20 g/d of nitrogen more than ad libitum-fed sows.

From our N balance studies, we estimated that restricted-fed sows mobilized 3.6 kg of protein in the first 3 wk of lactation. This value is in agreement with the predicted loss of 3.9 kg of maternal protein from restricted-fed sows, calculated using the equation of Whittemore and Yang (1989). The physiological implications of this maternal protein loss were evident by the end of lactation, when restricted-fed sows showed reduced litter gains; pig growth rates in wk 3 and 4 of lactation were lower ($P = .001$) in restricted-fed than in ad libitum-fed sows. This observation agrees with that of others that feeding protein-inadequate diets during lactation causes reduced litter growth rates in mid- to late lactation in pigs (Mahan and Mangan, 1975; Verstegen et al., 1985; Kusina et al., 1995) and rats (Friggens et al., 1993; Pine et al., 1994a).

Up to 25 to 30% of maternal protein can be mobilized by lactating dairy cows (Botts et al., 1979), rats (Pine et al., 1994b), and sows (Kotarbinska, 1983; Mullan and Williams, 1990), and lactational performance was reduced in dairy cows when more than half this protein reserve (10 to 15% of maternal protein) was mobilized (Botts et al., 1979). Assuming that skeletal muscle accounts for 45% of maternal protein (Young, 1970) and that muscle is the main contributor to mobilizable protein (Swick and Benevenga, 1977), the reduction in pig growth rate seen in restricted-fed sows by wk 3 of lactation suggests that these sows had mobilized 10 to 15% of their maternal protein, or 25 to 30% of their skeletal muscle. Furthermore, assuming that excretion of urinary creatinine is proportional to an animal's muscle mass (Tietz, 1986), the 24% reduction in urinary creatinine observed in restricted-fed sows in late lactation gives an indication of the amount of skeletal muscle protein mobilized during lactation. This agrees fairly well with the estimation (25 to 30% of muscle) based on the reduction in pig growth rate.

Milk production is a function of mammary gland nutrient uptake and biosynthetic capacity, which are determined by a number of factors including precursor availability and uptake by the mammary gland (Boyd

et al., 1995). In this experiment, we increased primiparous sow nutrient intake during lactation to the level that sows accreted maternal tissues without observing an increase in milk production. Thus, even though the growth potential of suckling pigs is greater than that observed in this experiment (Boyd et al., 1995; Williams, 1995), superalimented sows did not mobilize their protein reserves and partition additional nutrients toward the mammary gland to increase milk production. This suggests that sow milk production limits litter growth and that other factors, such as endocrine control, may suppress milk production.

Skeletal Muscle. Skeletal muscle is the main source of mobilizable protein (Swick and Benevenga, 1977). Therefore, changes in skeletal muscle composition and N balance reflect changes in whole-body protein mobilization, and in turn changes in urinary creatinine excretion reflect changes in maternal muscle mass. Together, the N balance studies, percentage changes in urinary creatinine excretion, and the observed changes in skeletal muscle composition indicated that sows provided with increasing levels of nutrient intake mobilized progressively less of their maternal protein reserves and, when feed intake was high enough, even accreted protein during lactation. The lower skeletal muscle protein:DNA ratio (amount of protein per cell unit) observed in restricted-fed sows is indicative of a reduction in cell "size" and protein stores. Similarly, Brendemuhl et al. (1989) observed that primiparous sows fed low protein levels (61 g N/d) during lactation mobilized more protein from their shoulder muscle (left supraspinatus muscle) than sows fed twice this amount.

Lactating women (Motil et al., 1990) and pigs use adaptive mechanisms to promote conservation of skeletal muscle protein stores. By relating tissue RNA (indicator of capacity for protein synthesis) to DNA concentration, the protein synthetic capacity "per muscle cell unit" can be estimated. Muscle DNA concentrations can be used as an index of cell number in lactating sows, even though these cells are multicellular, because porcine muscle DNA concentrations remain fairly constant after 5 mo of age (Powell and Aberle, 1975). Lower skeletal muscle RNA:DNA ratios in restricted-fed lactating sows, compared with sows fed higher intakes, suggest that a reduction in the rate of muscle protein synthesis is one of the mechanisms of protein conservation in such sows. This is supported by data showing reduced muscle protein synthesis in dairy goats (Champredon et al., 1990; Baracos et al., 1991). Because ad libitum-fed and superalimented sows were in zero or positive N balance in late lactation, no N-conserving mechanisms would have been implemented in these animals. This was reflected in their RNA:DNA ratios, which were greater than those of restricted-fed sows, suggesting that the protein synthetic capacity in muscle was maximal in

these animals at the end of lactation. This difference among treatments in late lactation may explain why plasma IGF-I levels did not differ between super-alimented and ad libitum-fed animals but were lower ($P < .01$) in restricted-fed animals at this time (Zak et al., 1998).

Stage of Lactation. All lactating sows in this experiment adapted to the increasing requirement for N in milk by excreting less N in feces (reflecting an increased digestibility) and urine (reflecting decreased hepatic amino acid metabolism) as lactation progressed, in agreement with Noblet and Etienne (1987). This was especially true for restricted-fed sows that were fed an extremely limiting intake during lactation yet maintained their milk production at a level similar to that maintained by ad libitum-fed sows for the majority of lactation. The deficit in nutrient requirements for milk production was supplied by extensive mobilization of maternal protein and lipid reserves.

Diet Digestibility. The reduction in apparent digestibility with increase in feed intake observed in our experiment agrees with the findings of Parker and Clawson (1967). In their study, increasing the feed intake of multiparous, lactating sows from 2.7 to 8.1 kg/d decreased the coefficient of apparent DM digestibility from 89 to 85 due to an increase in the rate of passage of ingesta through the gastrointestinal tract. But, despite the reduced apparent digestibility, the intake of digestible N, lysine, and energy by super-alimented sows was still greater than that by sows on the other treatments. The quadratic relationship between protein digestibility and feed intake suggests that when sows were fed more than their voluntary feed intake (approximately 5 to 6 kg/d) apparent digestibility decreased. Thus, the gastrointestinal tract of lactating sows may not be capable of efficiently digesting feed after intake exceeds a sow's voluntary intake. The additional feed probably increased peristalsis and, therefore, passage rate of feed through the gastrointestinal tract. This would result in less time being available for enzymes to digest the feed and cause the reduction in apparent fecal digestibility observed.

In conclusion, primiparous sows prioritize additional nutrients administered during lactation toward their maternal protein reserves rather than the mammary gland. This suggests that primiparous sows limit the nutrient uptake of suckling pigs. Although we saw no increase in milk production by administering feed intakes considerably above ad libitum intake to young sows, if the pigs had provided a larger nutrient drain (i.e., larger litter size and greater suckling stimulus) on the sow, partitioning of N to the mammary gland may have increased in super-alimented compared to ad libitum-fed animals. Furthermore, when N is limiting during lactation, sows mobilize their protein reserves and may even imple-

ment adaptive mechanisms, such as a reduction in muscle protein synthesis, to conserve maternal protein stores.

Implications

These data support the well-established conclusions that underfed sows mobilize body protein and fat reserves to maintain milk production. Thus, feed intake is the key to preventing weight loss in lactation and the consequent reduction in lactational and reproductive performance. Nitrogen balance studies and the observed changes in skeletal muscle composition indicated that sows provided with increasing levels of nutrient intake mobilized progressively less of their own protein reserves, and when feed intake was high enough actually accreted protein during lactation.

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