Selective protein loss in lactating sows is associated with reduced litter growth and ovarian function

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J Anim Sci 2003. 81:753-764.

Selective protein loss in lactating sows is associated with reduced litter growth and ovarian function[1]

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ABSTRACT: This study was designed to test the degree of protein loss that may be sustained by lactating sows before milk biosynthesis and ovarian function will be impaired. First-parity Camborough × Canabrid sows were allocated to receive isocaloric diets (61 ± 2.0 MJ of ME/d) and one of three levels of protein intake in lactation: 1) 878 g of CP and 50 g of lysine/d (n = 8), 2) 647 g of CP and 35 g of lysine/d (n = 7), or 3) 491 g of CP and 24 g of lysine/d (n = 10). Every 5 d during a 23-d lactation, sow live weight, backfat depth, and litter weight were recorded, and a preprandial blood sample was collected. Milk samples were collected on d 10 and 20 of lactation. Sows were slaughtered on the day of weaning, and liver and ovarian variables were measured. Lower dietary protein intakes elicited progressively larger live weight losses during lactation (–13, –17, and –28 ± 2.3 kg; $P < 0.001$), but similar and minimal backfat losses (–1.3 ± 0.29 mm). Approximately 7, 9, and 16% of the calculated body protein mass at parturition was mobilized by d 23. Lactation performance did not differ among treatments until d 20, at which time approximately 5, 6, and 12% of the calculated protein mass at parturition had been lost. The milk protein concentration on d 20 of lactation reflected the amount of body protein lost, and was lowest ($P < 0.05$) in sows that lost the most protein. After d 20, piglet growth rate decreased ($P < 0.05$) in a manner related to the amount of body protein lost. At weaning, ovarian function was suppressed in sows that had mobilized the most body protein; they had fewer medium-sized follicles (>4mm; $P < 0.05$), their follicles contained less ($P < 0.01$) follicular fluid, and had lower estradiol ($P < 0.05$) and IGF-I ($P < 0.10$) contents. Culture media containing 10% pooled follicular fluid (vol/vol) from high-protein-loss sows were less able to support nuclear and cytoplasmic maturation of oocytes in vitro, evidenced by more oocytes arrested at metaphase I ($P < 0.05$) and showing limited cumulus cell expansion ($P < 0.06$). Plasma insulin and IGF-I concentrations did not seem to be related to the observed differences in animal performance. Our data suggest that no decline in lactational performance or ovarian function when a sow loses approximately 9 to 12% of its parturition protein mass. However, progressively larger decreases in animal performance are associated with a loss of larger amounts of body protein mass at parturition.

Key Words: Lactation, Litter Performance, Protein Intake, Protein Loss, Reproductive Performance, Sows

 J. Anim. Sci. 2003. 81:753–764

Introduction

Sows often lose large amounts of live weight in lactation as a result of their high milk yields and relatively small appetites (Aherne and Williams, 1992). A loss of 10 to 15% of the sow's body weight in lactation reduces milk production (King and Dunkin, 1985; Verstegen et al., 1985) and subsequent reproductive performance (Aherne and Kirkwood, 1985; Prunier et al., 1993). Maternal live weight loss is composed of adipose and protein-containing tissue, and it is unclear to what degree

[1]The authors gratefully acknowledge the Alberta Agric. Res. Inst., Farming for the Future Matching Funding Programme, and Alberta Pork for their financial support; Pig Improvement Co. (Canada) Ltd. for the provision of experimental animals; L. Underwood and J. J. Van Wyk from the University of North Carolina at Chapel Hill for the production of the hIGF-I antiserum (anti-somatomedin-C, USDA-UB2-495) kindly provided by NIDDK's National Hormone and Pituitary Program and NICHD; Alberta Swine Genetics Corp., Nisku, AB, and Alberta Milk Testing Laboratory, Edmonton, AB, for their gifts in kind; the staff of the Swine Research Unit, especially J. Goller and J. Willis for care of the animals and assistance with experimental procedures; the statistical assistance of R. T. Hardin, and the technical assistance of R. O'Donoghue, S. Shostak, G. Sedgewick, and other staff in the Department of Agricultural, Food and Nutritional Science; and the statistical assistance L. Goonewardene, K. Toogood, and Z. Wang at Alberta Agriculture, Food, and Rural Development.

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Received December 7, 2001.

Accepted November 18, 2002.

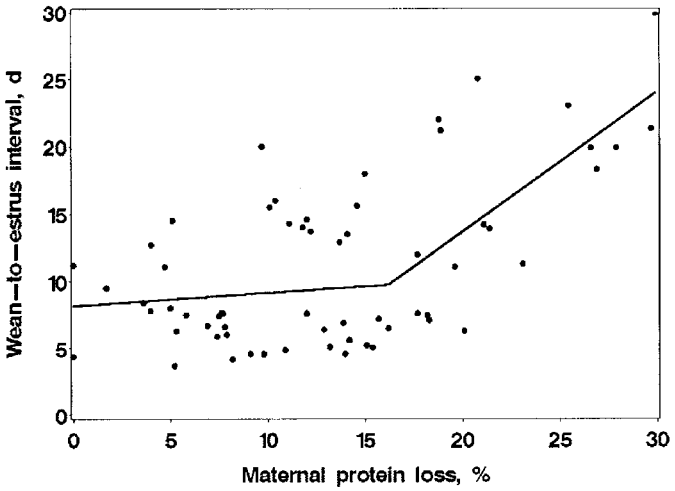

Figure 1. Break-point analysis of maternal protein loss vs. wean-to-estrus interval (WEI). The effect of lactational protein loss on WEI was analyzed on retrospective data from 16 published experiments with two-phase regression using the NLIN procedure of SAS (1998); the WEI increased once sows had mobilized >16% of their protein mass. Sow protein loss was calculated using the equation of Whittemore and Yang (1989).

depletion of maternal fat reserves, protein reserves, or both are responsible for the reduction in sow performance.

Large losses of fat tissue in lactation are clearly associated with a decline in lactational and reproductive performance (Yang et al., 1989). However, the simultaneous depletion of energy and protein reserves has made it difficult to isolate the role of protein depletion in altered animal performance (King and Dunkin, 1985; Jones and Stahly, 1999; Yang et al., 2000b). Retrospective data analysis suggests that with a loss of >16% of a sow's body protein mass, a progressive decline in subsequent reproductive performance occurs (Figure 1). Thus, we hypothesized that the lactating sow might sustain a degree of protein loss without functional loss of milk biosynthesis or reproductive function. We also conjectured that progressive mobilization of body protein would at some point fail to produce a total delivery of amino acids adequate to maintain lactational and reproductive function.

To test the hypothesis that protein loss might be specifically related to reductions in animal performance, we fed first-parity sows divergent protein/lysine levels in lactation to elicit three progressively larger maternal protein losses. We provided similar energy intakes to prevent differential losses of body fat. Lactational performance and ovarian function were assessed and related to body protein loss calculated according to the equations of Whittemore and Yang (1989).

Materials and Methods

This experiment was conducted in accordance with the Canadian Council of Animal Care Guidelines and was approved by the Institutional Animal Policy and Welfare Committee.

Table 1. Composition of the lactating and gestating sow diets (%, as-fed basis)

Ingredient	Lactation protein loss[a]			Gestation[b] diet
	Low	Moderate	High	
Wheat	27.0	30.0	30.0	—
Barley	—	—	—	45.8
Hulless barley	39.1	42.4	42.3	40.0
Soybean meal (46 CP %)	20.0	10.0	7.0	4.4
Canola meal	—	—	—	4.3
Fishmeal (herring)	4.5	3.0	—	—
Sugar	—	5.0	10.0	—
Tallow	5.0	5.0	5.0	1.0
Iodized salt	0.6	0.6	0.6	1.5
Dicalcium phosphate	1.4	1.7	1.9	1.4
Limestone	1.2	1.2	1.1	0.6
Supplement[c]	1.0	1.0	1.0	1.0
Lysine HCl	—	0.07	0.07	—
Valine	0.16	0.08	0.01	—
Calculated analysis				
ME, MJ/kg	14.21	14.10	14.10	12.53
Chemical analysis				
CP, %	19.77	15.06	11.85	11.79
Lysine, %	1.06	0.75	0.58	0.53
Valine, %	1.19	0.87	0.64	0.69

[a]Lactation diet formulated to 0.91% Ca, 0.75% P, and to induce sows to lose a low, moderate, or high degree of body protein in lactation.
[b]Gestation diet formulated to 0.93% Ca, 0.70% P.
[c]The vitamin/mineral supplement supplied the following per kilogram of complete feed: 10,000 IU of vitamin A, 1,000 IU of vitamin D3, 80 IU of vitamin E, 2 mg of vitamin K, 30 μg of vitamin B_{12}, 12 mg of riboflavin, 40 mg of niacin, 25 mg of D-pantothenic acid, 1,000 mg of choline, 250 μg of biotin, 1.6 mg of folic acid, 5 mg of ethoxyquin, 150 mg of Fe (ferrous sulfate), 12 mg of Mn (manganese oxide), 120 mg of Zn (zinc oxide), 15 mg of Cu (copper sulfate), 200 μg of I (calcium iodate) and 300 μg of Se (sodium selenite).

Experimental Treatments and Measurements

Thirty-six Camborough × Canabrid gilts (PIC, Acme, Alberta) were allocated to the study. Gilts were bred on at least their second estrus, and were then housed in an environmentally controlled room, in groups of five to seven animals. Gilts were individually fed 2.5 kg/d of a conventional dry sow diet (Table 1) once daily for the first 30 d of gestation. There was some heterogeneity in the initial live weights of available animals (96 to 143 kg) due to age differences (168 to 261 d). To achieve a similar target live weight and backfat depth at parturition gilts were fed, based on live weight, 2.8, 2.5, or 2.3 kg/d from d 30 of gestation. This feeding regimen in gestation produced animals at parturition that had a uniform backfat depth (15.7 ± 1.1mm), calculated fat mass (44 ± 1.9 kg), live weight (196 ± 3.4 kg), and calculated protein mass (31.5 ± 0.7 kg). From d 109 of gestation until farrowing, gilts were offered an additional 0.5 kg of feed/d and were moved into individual farrowing crates in an environmentally controlled room containing 12 crates. At parturition, sows from the three gestational feeding treatments were randomly

and equally allocated, based on live weight and protein mass, to be fed three divergent protein intakes in lactation (Table 1). Sows were fed a mash diet formulated to provide about 60 MJ of ME/d and either 1,000 g of CP and 60 g of lysine, 800 g of CP and 45 g of lysine, or 600 g of CP and 34 g of lysine/d to elicit a low (~5%), moderate (~10%), or high (>15%) amount of body protein loss in lactation, respectively. These intakes were calculated based on the sow's maintenance and milk production requirements, assuming additional protein would be provided by the sow's mobilized body protein reserves. We assumed litter growth rates in wk 1, 2, 3, and 4 of lactation of 1.28, 1.84, 2.16, and 2.16 kg/d respectively.

To reduce the variance in feed intake between animals, feed was offered three times daily, at approximately 85% of the ad libitum intake of lactating first-parity sows in our herd. Daily feed disappearance was measured by weigh-back of feed refusals. Water was freely available to sows and pigs through nipple drinkers throughout gestation and lactation.

Sow backfat depth (65mm from the midline at the last rib) was measured ultrasonically (Scanoprobe II, Scano, Ithaca, NY) on d 30, 70, and 109 of gestation, soon after farrowing, and on d 5, 10, 15, and 20 of lactation. Sow live weight was measured on the same days as backfat depth and on the day of breeding and weaning. Litter size was standardized to at least nine piglets within 36 h after parturition by cross fostering. Routine procedures on piglets, such as teeth clipping, tail docking, ear notching, and iron injection were conducted 2 d postpartum. No creep feed was available to the piglets during lactation. Litters were weighed on d 0, 3, 5, 10, 15, and 20 of lactation, and at weaning on d 23. Milk samples (10 to 20 mL) were obtained twice from sows after an i.m. injection of 10 IU of oxytocin on d 10 and 20 of lactation, or 3 d prior to weaning, whichever came first. Milk samples were immediately stored at –20°C and later analyzed for protein, fat, and lactose content. A single 10-mL blood sample was collected from an ear vein prior to the morning feeding on d 107 of gestation and on d 1, 5, 10, 15, and 20 of lactation. To ensure that sows were fasted for at least 16 h prior to all blood sampling, feed was removed from the sows at 1800 h the previous evening. Blood was collected into heparinized tubes, centrifuged at 1,500 × *g* for 15 min, the plasma poured off, and stored at –20°C for later insulin and IGF-I analysis.

Sows were slaughtered within 2 to 4 h of weaning, and the liver and reproductive tract were collected. The liver was weighed after removal of the gall bladder, and approximately 200 g of hepatic tissue was collected from the same lobe and stored at –20°C for DM and N analysis. The uterus was trimmed of mesenteric tissue, sectioned immediately below the cervix, and weighed. Both ovaries were collected and washed twice in sterile saline containing kanamycin (0.1 mg/mL; Sigma, St Louis, MO). The external diameter of the largest eight follicles on each ovary from each sow were determined as the mean of two calliper measurements taken at 90° to one another. Treatment effects were established by comparing the proportion of the largest 16 follicles from each sow categorized as having an external diameter of either ≤ or > 4 mm. Follicular fluid from these follicles was then aspirated individually with a 250-μL Hamilton syringe and collected. The syringe weight before and after aspiration was recorded, and the difference between these two weights was calculated as the follicular fluid weight. Follicular fluid volume was then calculated assuming a density of 1 g/mL. Individual follicular fluid samples were diluted to 10% (vol/vol) with tissue culture media (TCM 199 containing Earle's salts, L-glutamine, and no sodium bicarbonate; GibcoBRL/Life Technologies, Grand Island, NY) and stored at –30°C in 1.5-mL microcentrifuge tubes. An equal volume of diluted follicular fluid from the 12 largest follicles from each sow was pooled and filtered with a 0.2-μm filter (Millipore, Millipore SA, France) under a sterile hood. The diluted, sterile, filtered follicular fluid was stored at –20°C until required for in vitro culture of generic oocytes. The remaining follicular fluid samples were used for E_2 and IGF-I analysis.

Analyses and Calculations

Calculated Lysine and Energy Balance, Milk Production, and Sow Protein and Fat Mass. Energy and lysine balances were calculated in lactation based on the recorded values of sow energy and lysine intake minus the calculated requirements for sow maintenance and milk production. The sow's maintenance requirements were assumed to be 106 kcal of ME/kg $BW^{0.75}$ (444 kJ of ME/kg $BW^{0.75}$; NRC, 1998) and 0.039 g of digestible Lys/kg $BW^{0.75}$ (Fuller et al., 1989). The digestibility of dietary lysine was assumed to be 86% (Mullan et al., 1989). The ME requirement for milk production (**$Energy_{milk}$**) was calculated from the equation of Noblet and Etienne (1989) modified by NRC (1998). The dietary efficiency of ME use for milk production was taken as 72% (Noblet and Etienne, 1987). The total lysine requirement for milk production (**$Lysine_{milk}$**) was calculated from the equation of Pettigrew (1993). Milk production was calculated, based on litter growth rate, using the equation of J. R. Pluske (personal communication; 3.88 g of milk/g of litter gain) that was derived from the same genotype of first-parity sow in our herd:

$$\text{Energy}_{\text{milk}}, \text{ kJ ME/d} = \{[(4.92 \times \text{litter gain, g/d}) - (90 \times \text{No. pigs})]/0.72\} \times 4.184$$

$$\text{Lysine}_{\text{Milk}}, \text{ g/d} = 26 \times \text{litter gain (kg/d)}$$

Because of the difficulties of directly determining body composition changes in large domestic animals, sow body protein (**$Body_{protein}$**) and fat (**$Body_{fat}$**) masses were estimated with the equations of Whittemore and Yang (1989):

$$\text{Body}_{\text{fat}}, \text{ kg} = -20.4 + (0.21 \times \text{live weight, kg})$$

$$\text{Body}_{\text{protein}}, \text{kg} = -2.3 + (0.19 \times \text{live weight, kg}) - [0.22 \times \text{backfat (P2; mm)}] + [1.5 \times \text{backfat (P2; mm)}]$$

Liver, Feed and Milk Analyses. Liver samples were freeze-dried to constant weight, weighed, and the tissue was pulverized into powder. Subsamples were used for DM and N analysis. Feed samples were ground in a Wiley mill through a 0.8-mm screen, mixed well, and stored at 4°C until DM, N, and amino acid analysis. Feed and liver N was analyzed using the FP-428 Nitrogen Determinator, System, model 601-700-900 (LECO Corp., St. Joseph, MI). Liver protein was calculated as 6.25 × N. The lysine and valine concentrations in the feed were determined by HPLC (Sedgewick et al., 1991). The concentrations of milk fat, protein and lactose were determined by infrared analysis using a MilkoScan Analyzer (Foss Electrics, Hillerød, Denmark).

Plasma Insulin, and Plasma and Follicular Fluid Insulin-Like Growth Factor-I Analyses. Plasma insulin concentrations were analyzed by the double-antibody RIA as described by Cosgrove et al. (1992), with modifications as described by Patterson et al. (2002). The mean sensitivity of the two insulin assays was 0.019 ng/tube and the mean intra- and interassay CV was 5.6 and 11.9%. The double-antibody RIA of Glimm et al. (1990), as modified by Cosgrove et al. (1992), was used to measure plasma and follicular fluid IGF-I after acid-ethanol extraction. Based on an estimate of cold recovery of IGF-I added to standard plasma and follicular fluid pools, extraction efficiency was 100 and 91%, respectively, and sample potencies were not corrected for extraction efficiency. The IGF-I assay sensitivity was defined as 92% of the total binding, and the intraassay CV was 11.2%. Individual follicular fluid IGF-I concentrations from each sow were evaluated from diluted follicular fluid (10% in TCM 199) from the eight highest follicular fluid volumes, and 300 μL of the neutralized sample was assayed. Parallelism was tested on 300, 150, 75, 37.5, 18.85, and 9.375 μL of diluted control follicular fluid. Volumes less than 300 μL were made up to 300 μL with assay buffer. The slope of the control curve and standard curve did not differ significantly.

Follicular Fluid E_2 Analysis. Diluted follicular fluid (10% in TCM 199) from the eight highest follicular fluid volumes from each sow were further individually diluted 1:50 with PBS gelatin assay buffer. This assay buffer contained $NaH_2PO_4 \cdot H_2O$ (2.77 m*M*), NaH_2PO_4 (7.22 m*M*), NaCl (139.7 m*M*), NaN_3 (15.38 m*M*), and 0.1% (wt/vol) gelatin. Diluted follicular fluid (final dilution 1:500) from the four highest and the next four highest follicular fluid volumes was pooled and used to measure E_2 concentrations using a double-antibody estradiol RIA kit (Diagnostic Product Co., Los Angeles, CA; catalog No. KE2D1) with a minor modification. The volumes and additions were: sample (0.2 mL), assay buffer (0.1 mL), and Ab1 (0.2 mL). To improve assay sensitivity, the kit Ab1 was diluted threefold in distilled deionzed H_2O. Assay sensitivity, defined as 95% of total binding, was 0.03 ng/mL, the intraassay CV for the two assays averaged 4.5%, and the interassay CV was 6.9%.

In Vitro Maturation of Generic Oocytes. Oocyte-cumulus cell complexes were matured in vitro using modifications (Zak et al., 1997b; Yang et al., 2000a) of a system first described by Ding and Foxcroft (1994). The volume of follicular fluid available allowed duplicate batches of 20 (selected) oocytes to be matured in vitro in 1 mL of sterile diluted follicular fluid (10% in TCM 199), from each experimental sow. Culture was carried out under an atmosphere of 5% CO_2 in air at 39°C for 46 ± 1 h. The degree of oocyte nuclear maturation and cumulus cell expansion was recorded as a percentage of the oocytes present. The usual additions of 100 μg/mL of glutamine, 70 μg/mL of L-ascorbic acid, and 35 μg/mL of insulin were not included in the culture medium. This ensured that the ability of these factors within follicular fluid to support oocyte maturation were not masked. Oocyte cytoplasmic maturation was determined indirectly from the degree of cumulus cell expansion, which was classified into four categories, from no or limited expansion (category 1) to the highest degree of expansion (category 4) (Sirard et al., 1988; Vanderhyden and Armstrong, 1989). Oocyte nuclear maturation was classified according to Hunter and Polge (1966) as germinal vesicle, germinal vesicle breakdown, metaphase I, or metaphase II.

Statistical Analyses. Analyses involving continuous variables were computed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Variables were tested for normality using the Shapiro-Wilk statistic. Any abnormally distributed variables were normalized using a log transformation. The effect of feeding level in gestation (2.8, 2.5, and 2.3 kg/d), the degree of body protein lost in lactation (protein loss: low, moderate, and high), and their interaction were analyzed over time on sow live weight and backfat depth, feed intake, calculated balances, plasma hormone levels, and litter and milk variables using repeated-measures ANOVA. Pig birth weight was included as a covariate in the analysis of pig growth rate in lactation. Feeding level and the feeding level × protein loss interaction were not significant ($P > 0.05$) for some of these traits. Hence, these main effects were removed from these models. In the event of a significant ($P < 0.05$) time × protein loss interaction, the differences among time within each protein loss treatment were computed using *a priori* orthogonal contrasts. Ovarian, uterine, and liver variables at weaning were analyzed with two-way analysis with interaction. Follicular fluid E_2 values for the four largest and next four largest volumes for each sow, and individual follicular IGF-I content for the largest eight follicular fluid volumes for each sow were analyzed using the main effect of protein loss in lactation and sow nested in protein loss. Follicle diameter was included as a covariate in the follicular IGF-I content analysis. In the event of a significant effect of protein loss, the differences among protein loss treatments were compared using

Table 2. Litter variables in first-parity sows that lost a low, moderate, or high amount of body protein in lactation[a]

	Protein loss in lactation		
	Low	Moderate	High
n	8	7	10
Litter size, d 0	9.4 ± 0.37	9.5 ± 0.38	9.3 ± 0.36
Litter size, d 23	9.1 ± 0.24	9.0 ± 0.25	9.0 ± 0.23
Pig birth weight, kg	1.65 ± 0.16	1.37 ± 0.16	1.63 ± 0.15
Pig wean weight, kg	6.04 ± 0.28	5.42 ± 0.29	5.90 ± 0.27
Pig growth rate (GR), g/d			
d 0 to 23	257 ± 9.9	247 ± 10.6	256 ± 9.2
d 0 to 20, Period 1	251 ± 8.9	244 ± 9.7	261 ± 8.6
d 20 to wean (d 23; Period 2)	287 ± 17.3	259 ± 18.2	228 ± 16.0
GR change, % (Period 1 to Period 2)[b]	13 ± 5.7^{y}	7 ± 6.2^{xy}	-11 ± 5.4^{x}

[a]Least squares means ± standard error of the mean.
[b]Percentage of change in pig growth rate between the first 20 d and the end (d 20 to wean) of lactation.
^{x,y}Within a row, means that do not have a common superscript differ ($P < 0.02$).

orthogonal contrasts. The categorical response model was used to determine differences in the proportion of follicles between protein loss treatments, the two size categories (≤ or > 4 mm external diameter), and the interaction.

Results

Of the 36 bred gilts allocated to treatments, five were not pregnant and six were taken off test due to illness, lameness, or diarrhea in the litters. Thus, 8 sows were evaluated on the low-, 7 on the moderate-, and 10 on the high-protein-loss treatments. Litter size born (10.1 ± 1.2) and born alive (9.5 ± 1.3), piglet birth weight, and the number of pigs (~9 pigs) suckling sows throughout lactation were similar among treatments (Table 2).

There were no treatment differences in feed intake or energy intake in lactation overall (Table 3). Protein intakes for the three treatments were 878, 647, and 491 g/d, and lysine intakes varied correspondingly (Table 3). The dietary treatments elicited a progressively larger ($P < 0.001$) degree of live weight loss in lactation (Table 3). Backfat losses were minimal in lactation (–1.3 ± 0.29mm) and did not differ among treatments, but calculated fat losses in lactation did increase with weight loss ($P < 0.01$; Table 4). The difference in live weight loss among treatments was also attributable to protein loss, and was estimated to represent 7, 9, and 16% of the sow's body protein mass at parturition (Table 4). At weaning, liver weight and protein mass ($P < 0.01$) also reflected the dietary treatments in lactation, and were highest in sows that lost the least amount of protein (Table 3). Liver DM did not differ among treatments and averaged 27.3 ± 0.2%.

Litter Performance. There was a significant ($P < 0.001$) protein loss × time interaction for piglet growth rate in

Table 3. Sow and nutrient variables in first-parity sows that lost a low, moderate, or high amount of body protein in lactation[a]

	Protein loss in lactation			
	Low	Moderate	High	*P*-value
Sow lactation intake				
Feed, kg/d	4.49 ± 0.14	4.33 ± 0.14	4.03 ± 0.13	0.103
ME, MJ/d	63.7 ± 1.93	61.0 ± 2.01	56.9 ± 1.91	0.089
CP, g/d	878 ± 19^{z}	647 ± 19^{y}	491 ± 18^{x}	0.001
Lysine, g/d	50.2 ± 1.06^{z}	34.6 ± 1.10^{y}	24.2 ± 1.04^{x}	0.001
Weight, kg				
Farrow	195 ± 3.6	197 ± 3.8	200 ± 3.4	0.516
Loss in lactation	12.9 ± 2.3^{x}	16.9 ± 2.4^{x}	28.4 ± 2.1^{y}	0.001
Backfat, mm				
Farrow	15.4 ± 1.18	15.0 ± 1.24	16.3 ± 1.10	0.712
Loss in lactation	0.89 ± 0.32	1.45 ± 0.34	1.57 ± 0.30	0.340
Liver[b]				
Weight, kg	2.83 ± 0.10^{y}	2.72 ± 0.11^{y}	2.31 ± 0.10^{x}	0.009
Protein, g[c]	588 ± 15.9^{z}	533 ± 16.9^{y}	428 ± 15.4^{x}	0.001

[a]Least-square means ± standard error of the mean.
[b]Tissues were collected 2 to 4 h after weaning, on d 23 of lactation, and at the time of slaughter.
[c]Liver protein calculated as 6.25 × N (%).
^{x,y,z}Within a row, means that do not have a common superscript differ by the *P*-value in that row.

Table 4. Calculated lysine and energy balance and protein and fat body mass of first-parity sows that lost a high, moderate, or low amount of protein in lactation[a]

Item	Protein loss in lactation			*P*-value
	Low	Moderate	High	
Calculated loss in lactation[b]				
Body protein, kg	2.1 ± 0.47[x]	2.9 ± 0.49[x]	5.0 ± 0.49[y]	0.002
Body protein, % of parturition mass	6.9 ± 1.55[x]	9.2 ± 1.62[x]	15.8 ± 1.44[y]	0.003
Body fat, kg	3.9 ± 0.86[x]	5.7 ± 0.89[x]	8.3 ± 0.82[y]	0.010
Body fat, % of parturition mass	9.4 ± 2.09[x]	12.9 ± 2.19[x]	18.0 ± 1.95[y]	0.035
Cumulative loss to d 20[b]				
Body protein, % of parturition mass	5.1 ± 1.42[x]	6.1 ± 1.49[x]	12.3 ± 1.32[y]	0.006
Body fat, % of parturition mass	7.9 ± 2.00	10.4 ± 2.08	15.2 ± 1.86	0.060
Lysine balance, g/d				
d 0 to 20, Period 1	–16.1 ± 1.8[z]	–27.2 ± 1.9[y]	–41.5 ± 1.7[x]	0.001
d 20 to wean, Period 2[c]	–20.9 ± 2.8	–29.4 ± 2.9	–28.2 ± 2.8	0.134
Change from Period 1 to Period 2[d]	–4.8 ± 3.0[x]	–2.1 ± 3.1[x]	13.0 ± 3.0[y]	0.003
Energy balance, MJ of ME/d				
d 0 to 20, Period 1	–22.6 ± 2.3[y]	–20.0 ± 2.4[y]	–29.7 ± 2.1[x]	0.020
d 20 to wean, Period 2	–25.1 ± 3.7	–22.1 ± 3.9	–16.0 ± 3.7	0.273
Change from Period 1 to Period 2[d]	–2.5 ± 5.2	–2.1 ± 5.4	14.0 ± 5.1	0.075

[a]Least squares means ± standard error of the mean.
[b]Body protein and fat mass predicted from the equations of Whittemore and Yang (1989).
[c]Calculated lysine balance adjusted for varying milk protein concentrations among treatments at d 20 of lactation.
[d]Change in energy and lysine balance between the first 20 d and the end (d 20 to wean) of lactation.
[x,y,z]Within a row, means that do not have a common superscript differ by the *P*-value in that row.

lactation. Piglet growth rate rose progressively over the first 20 d of lactation, peaked between d 15 and 20, and declined thereafter; data not shown. There was no treatment difference in piglet growth rate over the first 20 d of lactation or any interval thereof. Piglet growth rate diverged by treatment after d 20 of lactation, such that larger amounts of protein loss were associated with reduced piglet growth (Table 2). Sows that lost the most protein showed the largest ($P < 0.02$) decline in piglet growth. The change in piglet growth rate at the end of lactation (d 20 to weaning) in low-, moderate-, and high-protein-loss sows was +13, +7 and –11% of the average growth rate during the first 20 d of lactation, respectively, and differed ($P < 0.02$) among treatments. The decrease in piglet growth rate at the end of lactation (d 20 to weaning) was –8, –13, and –26% of peak piglet growth (d 15 to 20), respectively, and also differed ($P < 0.06$) among treatments. Furthermore, calculated milk production at the end of lactation was lowest ($P < 0.08$) in sows that lost the most body protein and was 10.1, 9.0, and 8.0 kg/d in sows that lost a low, moderate and high amount of protein, respectively.

Milk protein concentration did not differ among treatments on d 10 of lactation, but by the end of lactation (d 20) also reflected the amount of body protein mobilized and was lowest ($P < 0.05$) in sows that had mobilized the most protein (Figure 2). Milk lactose (5.44 ± 0.10%) and fat concentration did not differ among treatments at this or at any time in lactation. However, milk fat concentration declined ($P < 0.001$) between d 10 and 20 of lactation (7.81 vs. 6.80 ± 0.18).

Indices of Maternal Tissue Change in Lactation. The calculated lysine and energy balances reflected the sow's dietary intake and the fact that piglet growth rate was essentially identical among treatments for the first 20 d of lactation (Table 4). There was a significant protein loss × time interaction ($P < 0.001$) for lysine and energy balance in lactation. Both balances decreased

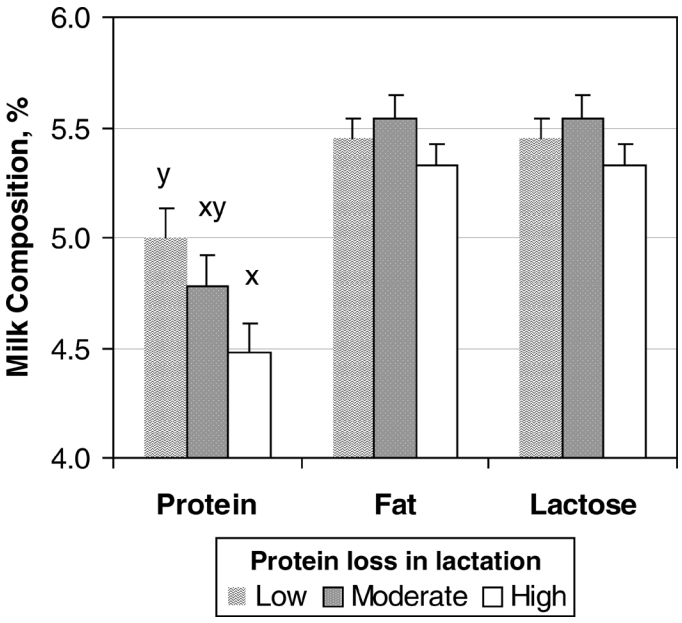

Figure 2. Milk protein, fat, and lactose composition (%) on d 20 of lactation in first-parity sows that lost a high, moderate, or low amount of protein in lactation.
[x,y]Within each variable, values that do not have a common superscript differ ($P < 0.05$).

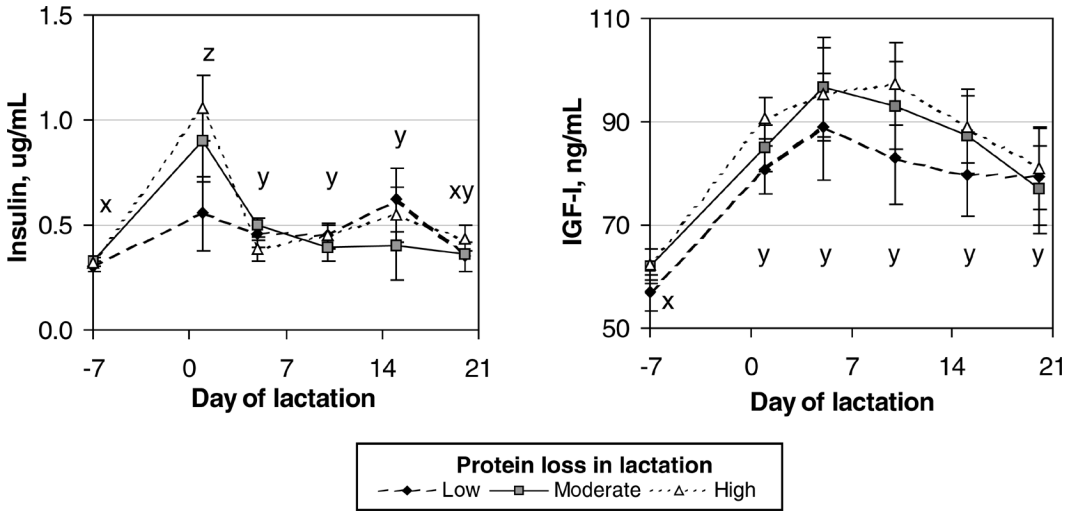

Figure 3. Change in preprandial plasma insulin and IGF-I concentrations from late gestation (d 107) until weaning in first-parity sows that lost a high, moderate, or low amount of protein in lactation.
[x,y,z]Time points that do not have a common superscript differ ($P < 0.001$).

($P < 0.001$) from early- (d 5) to mid-lactation (d 10) and remained at this level until d 20; data not shown. After d 20, the pattern of lysine and energy balance among treatments diverged (Table 4). Neither balances changed between d 20 and the end of lactation in sows fed to lose a low or moderate amount of protein, indicating that these sows appeared to lose body protein at a relatively constant rate from d 10 of lactation until weaning. However, in sows that lost the most protein, both balances became more positive after d 20; this was significant ($P < 0.01$) for lysine balance and tended ($P = 0.08$) to be different for energy balance (Table 4). The increase in energy and lysine balance in these sows was achieved mainly through a reduction in milk production and milk protein content. At the onset of the shift towards maternal retention, the sows that lost the most protein were estimated to have lost approximately 12% of their initial (parturition) protein mass (Table 4). Sows in the other two treatments had only lost an estimated 5 and 6% of their initial protein mass by d 20.

Neither preprandial plasma insulin nor IGF-I concentrations differed among protein loss treatments. Both hormones differed ($P < 0.001$) with time, but there were no gestation feeding level × time or protein loss × time interactions (Figure 3a,b). Both hormones increased sharply ($P < 0.001$) between late gestation and the start of lactation, IGF-I remained higher than prepartum levels throughout lactation, but insulin concentrations declined between d 1 and 5 of lactation to levels that were still above prepartum concentrations. Plasma insulin concentrations remained above prepartum levels until d 20 when they declined further to levels that could not be differentiated from those prepartum.

Ovarian and Uterine Variables. Differences in the measured ovarian variables between sows that lost approximately 7 and 9% of their estimated body protein mass in lactation were small and generally not significant (Figure 4b,c; Table 5). There was no difference in the proportion of follicles in each size category or in follicular fluid volume in the largest 16 or largest 8 follicles between these two treatments. Furthermore, there were no differences between these two treatments in follicular IGF-I content and concentration, follicle E_2 content, or in the ability of follicular fluid from these sows to advance in vitro oocyte nuclear maturation. However, follicular fluid E_2 concentrations were lower ($P < 0.05$) in sows that lost approximately 9 vs. 7% of their calculated body protein mass in lactation (Table 5). Also, a larger proportion ($P = 0.06$) of oocytes cultured in follicular fluid from these sows showed moderate cumulus cell expansion (Figure 4c).

By contrast, there was sharp demarcation in ovarian measures between sows that lost approximately 16% of their calculated body protein mass and those that lost approximately 7 and 9%. Sows that lost the most protein had fewer ($P < 0.001$) follicles >4 mm compared to the other two treatments (Table 5). Also both the largest 16 and the largest 8 follicles from these sows had about half the volume ($P < 0.01$) of follicular fluid compared to the other two treatments (Table 5). Only 6 of the 10 sows that lost the most protein in lactation could be evaluated for the in vitro ovarian measures. The four sows were excluded because the follicular fluid collected from them was insufficient to conduct determinations of hormone content and in vitro maturation; the ovaries of these sows had less than five follicles with an external diameter of >3.5mm at weaning. Nevertheless, follicular fluid from sows that lost the most protein was less able to advance in vitro oocyte nuclear maturation (Figure 4b), as indicated by a larger proportion ($P < 0.05$) of oocytes cultured in follicular fluid from these sows arresting at the metaphase I stage of nuclear development. Follicular fluid from these sows also showed the poorest ability ($P < 0.06$) to support cumulus

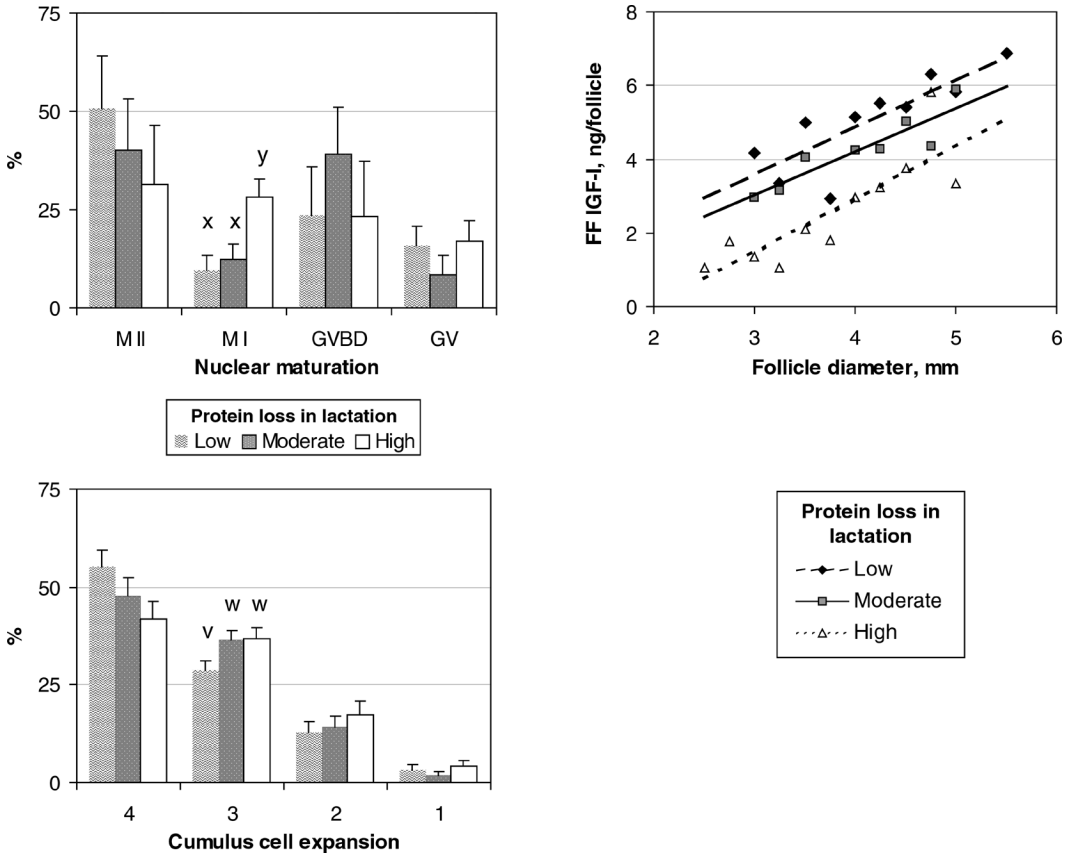

Figure 4. Ovarian variables at weaning in first-parity sows that lost a low, moderate, or high amount of protein in lactation. The ability of follicular fluid from ovaries recovered at weaning to advance oocyte nuclear maturation (a) and cumulus cell expansion (b), and follicular fluid (FF) IGF-I content (ng/follicle; c). The number of animals evaluated for follicular IGF-I content and the ability of follicular fluid to advance oocyte maturation were seven, seven, and six for sows that lost a low, moderate, and high amount of protein, respectively. Follicular IGF-I content tended (P = 0.10) to be lower in high than low and moderate protein loss sows. Classification of cumulus cell expansion ranged from no or minimal expansion (1) to extensive expansion (4). Classification of nuclear maturation: M II = metaphase II; M I = metaphase I; GVBD = germinal vesicle breakdown; and GV = germinal vesicle.

[v,w]Within each variable, values that do not have a common superscript differ ($P < 0.06$).

[x,y]Within each variable, values that do not have a common superscript differ ($P < 0.05$).

cell expansion (Figure 4c) and the lowest overall E_2 concentration (Table 5). Treatment also affected wet uterine weight at slaughter ($P < 0.05$; Table 5) and reflected the gradation in protein loss across the three treatment groups.

Discussion

Our data reveal that sows can sustain a loss of 9 to 12% of their previously existing body protein mass during lactation without any detriment to piglet growth, or the determined indices of ovarian function. Beyond this amount of protein loss, milk protein concentration and piglet growth rates started to decline. More extensive protein loss was associated with marked reductions in many indices of ovarian function. A few slight reductions in ovarian variables were observed in the animals that had sustained approximately a 9% protein loss by the end of lactation. This suggests that the threshold for initiation of these alterations in animal performance was in the vicinity of 9 to 12% protein depletion.

Study Design. Ideally, in an experiment of this type, the lactating sow should not lose any fat tissue to ensure that the effects of maternal protein loss are not confounded by fat loss. Due to the limitation of sow feed intake, it is difficult to design an experiment of this nature with protein loss exclusively and no maternal fat loss (Zak et al., 1998). Therefore, it may be difficult to exclude the possibility that the animal's system perceives the composite loss of both protein and fat. The majority of body weight loss from our lactating sows consisted of tissue of the lean body mass. For example, of the 28.4 kg live weight loss in sows fed in the most restricted fashion during lactation, only 8.3 kg was adipose tissue (Table 4). We calculated that the extent of fat loss in our sows during lactation ranged from 3.9 to 8.3 kg (Table 4). Fat loss of this order is not thought

Table 5. Ovarian and uterine characteristics at weaning in first-parity sows that lost a high, moderate, or low amount of body protein in lactation[a]

	Protein loss in lactation			
	Low	Moderate	High	*P*-value
Largest 16 follicles [b]				
Follicular fluid volume, μL[c]	47 ± 5.0[y]	45 ± 4.1[y]	24 ± 3.9[x]	0.003
Follicles ≤ 4 mm diameter, %	44.6	44.6	74.4	0.001[d]
Follicles > 4 mm diameter, %	55.4	55.4	23.6	
Follicular fluid[e]				
Volume, μL	68 ± 7.7[y]	55 ± 7.2[y]	32 ± 6.8[x]	0.006
IGF-I, ng/mL	92 ± 13.5	84 ± 23.8	73.3 ± 16.8	0.683
E_2, ng/mL	3.82 ± 0.69[y]	1.80 ± 0.64[x]	1.47 ± 0.60[x]	0.045
Uterine weight, g[f]	280 ± 16.9[y]	231 ± 16.5[xy]	216 ± 14.1[x]	0.027

[a]Least squares means ± standard error of the mean.
[b]Variables evaluated from the eight largest follicular fluid volumes from each ovary. There were seven, seven, and six sows evaluated for low-, moderate-, and high-protein-loss groups, respectively. One low-protein-loss sow had polycystic ovaries at weaning and was not evaluated.
[c]Average follicular fluid volume from the largest 16 follicles (largest eight from each ovary) measured at weaning.
[d]The *P*-value for the χ^2 value of 72.60 (with two degrees of freedom) involves the comparison of both follicle group sizes (≤ and > 4 mm diameter).
[e]Follicular fluid variables were measured on the largest eight follicular fluid volumes collected.
[f]Tissues were collected 2 to 4 h after weaning on d 23 of lactation and at the time of slaughter.
[x,y]Within a row, means that do not have a common superscript differ by the *P*-value in that row.

to have adverse effects on animal performance during lactation (Yang et al., 2000a,b). The animals in our study may be compared with those in several published reports. Protein loss was accompanied by substantial fat loss (–6 to –8 mm of backfat depth; –18 to –26 kg of calculated body fat) in some studies, making it more difficult to differentiate between the roles of fat and lean tissue loss on animal performance (King and Dunkin, 1985; Mullan and Williams, 1989; 1990). Some researchers employed insufficient protein restriction to impact on animal performance (Dourmad et al., 1998; Touchette et al., 1998). Other studies used very severe protein restrictions, such that milk production and litter growth rate were impaired from the onset of lactation (King et al., 1993; Jones and Stahly, 1999; Kusina et al., 1999b). Our study design allows for a strong association between the observed alterations in lactation and ovarian variables and protein mobilization. This is because the limited difference in backfat loss observed in the animals on the progressively lower protein intakes likely had a minimal effect on animal performance.

Direct determinations of body composition and energy and protein balance are technically difficult to achieve in lactating sows. We therefore used a number of indirect estimates of these variables. We estimated the relative change in the sow's maternal protein and fat mass during lactation using prediction equations (Whittemore and Yang, 1989). These equations were derived from animals similar to those used in our study with respect to parity and initial body composition, albeit of a different genotype. The sows' lysine and energy balances were calculated factorially. The sows' lysine balance represents its actual protein balance because lysine is a limiting essential amino acid and invariably is not in excess in the lactation diet. Also, unlike other essential amino acids (e.g., branched-chain amino acids), lysine is not retained to any great degree in the lactating mammary gland (Trottier et al., 1997).

Litter Performance

The sow's milk yield is a function of her age and genotype and the nutrient uptake by, and biosynthetic capacity of, the lactating mammary gland (Boyd et al., 1995). All animals in this experiment were of the same age and genotype, and we controlled for the potential differences in mammary gland biosynthetic capacity by establishing similar litter sizes (~9 pigs) within 36 h after parturition. We also fed gilts in gestation in a manner that would not be expected to alter fetal or mammary growth and development (Shields et al., 1985; Kusina et al., 1999a). The similarity in litter growth among treatments for the majority of lactation supports this claim. A reduction in the supply of nutrients and energy to, and uptake by, the mammary gland was therefore likely to be the main factor that could affect milk production in our experimental sows.

Sows fed the lowest protein intake drew increasingly upon their protein reserves to meet the discrepancy between the demands of the lactating mammary gland and their dietary intake. A decrease in litter growth—and as a consequence, an increase in energy and lysine balance—was observed in sows that had lost more than approximately 12% of their protein mass. Because feed intake was not altered during this period, a reduction in the amount of body reserves mobilized and a parallel reduction in the amount of substrates delivered to the mammary gland would clearly have been necessary to cause a positive change in lysine and energy balance.

It would therefore be expected that protein metabolism in nonmammary tissues would alter at this time to conserve the sow's remaining body protein reserves.

Ovarian Function

The literature supports the concept that ovarian variables are sensitive to nutrient intakes during lactation. Restricted feeding (~50% of ad libitum intake) of sows for a 14- (Miller, 1996) and 28-d (Quesnel et al., 1998) lactation was associated with fewer moderate (>3 to 4 mm diameter) ovarian follicles at weaning and lower LH pulsatility in lactation (Zak et al., 1997a). However, only a marginal (1 to 2 d), if any, extension of the wean-to-estrus interval may be observed as a response to feed restriction in sows similar to those used in this experiment (Zak et al., 1997a; 1998;Yang et al., 2000b).

At weaning, the larger follicles visible on the ovary are likely to be those from which ovulatory follicles are selected. These follicles are also probably affected by the sow's nutritional state in lactation because they likely underwent antral formation in early lactation. This may be concluded because antral follicles (>400 μm diameter) may be recruited into the preovulatory pool in the span of 19 to 21 d (Morbeck et al., 1992), and once recruited into this pool they require a further 5 d to reach ovulatory size (≥8mm diameter). This is consistent with a lactation length of about 20 d and the typical wean-to-estrus interval of first-parity sows in our herd of 3 to 6 d (Zak et al., 1997a; 1998).

Our choice of measured ovarian variables was intended to clarify key elements that could explain the changes in reproductive status observed under similar conditions of feeding and protein depletion to other experiments. In our experiment, sows that lost the most body protein in lactation had fewer larger-sized follicles on their ovaries at weaning and, therefore, likely had a smaller preovulatory follicle pool size. This may have implications for reproductive performance because these changes could underlie a lowered ovulation rate, and ultimately contribute towards a reduced subsequent litter size.

A sow's relative fertility can also be addressed by measuring indices of ovarian function at weaning because the degree to which follicular fluid is able to advance oocyte maturation reflects the ability of the oocytes within those follicles to undergo maturation and fertilization in vitro (Zak et al., 1997b). This is probably associated with a lower reproductive status, including lower embryo survival (Zak et al., 1997a) and ultimately a smaller subsequent litter size. In the present study, follicular fluid from sows that sustained the largest degree of protein mobilization was less able to advance oocyte maturation. In agreement with our results, follicular fluid taken at proestrus from first-parity sows fed 16 vs. 36 and 56 g of lysine/d was less able to advance oocyte nuclear maturation in vitro (Yang et al., 2000a). Sows in the experiment of Yang et al. (2000a) were fed approximately 25% less energy (about 46 MJ of ME/d), but lost a similar amount of backfat (–1.4 to –2.2mm) and live weight (-22 vs. -19 and -15 kg) in lactation compared to sows in our experiment. Thus, these sows also likely lost a large amount of protein.

Ovarian follicular fluid contains many growth factors, such as IGF-I, epidermal growth factor, and transforming growth factor-β, as well as steroid hormones such as E_2, and these factors influence the oocyte's ability to be fertilized, develop into an embryo, and survive (Driancourt and Thuel, 1998). A lower follicular IGF-I content may be implicated in the reduced follicular quality of moderate-sized follicles in sows that lost the most body protein in our experiment. Follicular IGF-I content was also lower in sows that lost more than 20% of their parturient live weight in lactation (Quesnel et al., 1998). Certainly, elevated ovarian follicular fluid IGF-I concentrations are associated with enhanced follicular development (Hammond et al., 1988) and lower follicular atresia (Matamoros et al., 1991) in the pig. Although no specific measure of follicular atresia was included in our study, treatment effects on follicular fluid IGF-I and E_2 concentrations may partly reflect the incidence of follicular atresia among the group of follicles analyzed.

Differences in uterine weight at slaughter were consistent with the concept that increased protein loss had a negative impact on follicular development and ovarian estrogen production, and this has consequences for uterine development. As uterine weight appears to be an effective measure of cumulative estrogenic activity in the pig (Foxcroft et al., 1984), our data suggest that a poor uterine environment, interacting with embryos developing from poorly matured oocytes, will be a critical factor for embryonic survival in sows with excessive protein loss in lactation.

Regulation of Animal Performance. Elucidation of the signal(s) that connects muscle mobilization, milk production, and ovarian variables would require further detailed studies. The key controls that mediate mammary and ovarian changes may be hormonal (Schams et al., 1994). Although fasting plasma insulin and IGF-I concentrations did not appear to be related to the observed differences in animal performance, changes in tissue sensitivity to these hormones (e.g., tissue receptor number and postreceptor signal transmission) and the number and activity IGF-binding proteins cannot be discounted. It would also be appropriate to assess prandial differences in the concentrations of these factors that would likely have been affected by the diets used here (Prunier et al., 1993; Messias de Bragança and Prunier, 1999).

The key controls that mediate mammary and ovarian changes may alternatively be related to the overall availability of substrate derived from protein mobilization. As protein reserves shrink, the rate of mobilization must increase proportionally to maintain the same total supply of amino acids. At some point, the reserve becomes so depleted and/or the rate of mobilization reaches a physiological maximum such that the total

supply of amino acids will fall below the thresholds necessary to maintain milk production or to provide amino acid precursors for biosynthesis of proteins associated with ovarian functions. The effective or net amino acid supply at the mammary and at the ovary and the influence of key individual amino acids on function of these cells remains to be determined.

Implications

First-parity sows can maintain milk production and reproductive function during lactation, even when they lose maternal protein tissue. However, if the sow mobilizes too much body protein—in this case, approximately 9 to 12% of its calculated protein mass—a decrease in litter growth and ovarian function, and by inference, reproductive performance, will occur. The relative decrease in animal performance depends on the amount of body protein lost. Thus, to ensure maximal sow performance, management and/or dietary methods should be practiced to limit sow protein loss during lactation.

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