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Brian K. Whitlock
University of Tennessee-Knoxville

M J. VandeHaar

L F. Silva

H A. Tucker

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Effect of Dietary Protein on Prepubertal Mammary Development in Rapidly Growing Dairy Heifers[1]

B. K. Whitlock, M. J. VandeHaar, L. F. P. Silva, and H. A. Tucker
Department of Animal Science
Michigan State University, East Lansing 48824

ABSTRACT

The objective was to determine whether increased dietary protein would enhance mammary development in prepubertal heifers fed for rapid body growth (1.2 kg/d). Fifty-four Holstein heifers (weighing ~134 kg) were assigned to one of three treatments. Heifers were fed a total mixed ration with metabolizable energy at 2.85 Mcal/kg and metabolizable protein at low, standard, or high concentrations (37, 41, or 44 g/Mcal of metabolizable energy, respectively) from 3.5 mo of age until slaughter at ~46 d after puberty. Heifers fed low, standard, and high protein gained 1130, 1170, and 1180 g/d, respectively. Dietary protein did not affect age or weight of heifers at puberty or slaughter, withers height gain, or carcass composition. Average mammary parenchymal DNA content for heifers on diets of low, standard, and high protein was 595, 619, and 670 mg/100 kg of body weight, respectively, and was not significantly different. However, for heifers that attained puberty early, those fed low protein had 33% less parenchymal DNA than those fed high protein, even though their body growth and carcass composition were not compromised. We conclude that dietary protein does not have a major effect on mammary development of rapidly grown prepubertal heifers, provided the diet contains adequate protein for normal body growth. But we suggest that feeding low-protein diets increases the risk of impaired mammary development when heifers are fed for rapid growth and attain puberty early and that the new National Research Council guidelines for protein relative to energy seem adequate for optimal mammary development.
(**Key words:** heifer, growth, protein, mammary development)

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Corresponding author: M. J. VandeHaar; e-mail: mikevh@msu.edu.
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Abbreviation key: HP = high protein, **LP** = low protein, **ME** = metabolizable energy, **MP** = metabolizable protein, **SP** = standard protein.

INTRODUCTION

Fifteen to twenty percent of the overall expense of milk production is incurred by heifer replacement programs. One way to lower the costs of raising heifers is to reduce their age at first calving, but, unless heifers grow faster, earlier calving will result in a smaller body size at calving. The optimal BW just before first calving is ~640 kg for US Holsteins; lighter BW reduce subsequent milk production (Keown and Everett, 1986; Heinrichs and Hargrove, 1987; Hoffman, 1997). To achieve 640 kg at 24 mo, a heifer must gain an average of 820 g of BW/d. Gains of ~1000 g/d or more are required between 3 and 10 mo of age if calving as early as 20 mo at the recommended BW is to be achieved.

Most studies have shown that feeding prepubertal heifers high-energy diets to promote gains faster than 900 g/d decreases mammogenesis and subsequent milk production. However, the magnitude of the feeding-induced decrease in mammary development in the literature varies considerably (VandeHaar, 1997). Whereas some studies have reported a 50% reduction in subsequent milk yield, others have reported almost no reduction. One possible reason for this response variation is that the protein-to-energy ratio varied among diets that were implemented to promote rapid growth.VandeHaar (1997) examined the relationship between mammary development or milk yield and the protein-to-energy ratio from 11 studies, in which gains of heifers exceeded 900 g of BW/d. The diets for rapidly grown heifers varied from 43 to 83 g of CP/Mcal of metabolizable energy (**ME**). The CP:ME ratio accounted for 51% of the variation in mammary parenchyma responses and 78% of the variation in milk yield responses to rapid growth rate. One limitation with this literature analysis is that protein was evaluated as CP rather than as metabolizable protein (**MP**).

Four of these 11 studies (five rapid growth groups) examined effects of energy intake on mammary paren-

chymal DNA at puberty (Sejrsen et al., 1982; Petitclerc et al., 1984; Capuco et al., 1995; Radcliff et al., 1997). The estimated MP:ME ratio accounts for 88% of the variation in parenchymal DNA responses to high-energy intake across these studies. Estimates were based on reported feed ingredients and energy and protein relationships defined in NRC (1989), with diet %MP calculated as 0.8 × %CP × %RUP + 0.64 × 0.038 × Mcal of ME/kg DMI. The resulting regression suggested that a high-energy diet supplying 44 g of MP/Mcal of ME would not impair mammary development, but one with 37 g of MP/Mcal of ME would decrease mammary parenchymal DNA by 40%. The standard protein-to-energy ratio for prepubertal dairy heifers is ~40 g of MP/Mcal of ME, which is equivalent to ~55 g of CP/Mcal of ME if the CP were 36% RUP.

Thus, we hypothesized that high-energy diets containing 44 g of MP/Mcal of ME, compared with 37 g/Mcal, would increase mammary development in prepubertal dairy heifers fed to gain >1.1 kg/d.

MATERIALS AND METHODS

Animals and Treatments

All procedures were approved by the Animal Care and Use Committee of Michigan State University. Sixty-four Holstein heifers (approximate age = 11 wk and mean BW [±SEM] = 101 ± 1 kg) were purchased within three consecutive weeks in late spring (~21 heifers/wk) with each week classified as a separate age group. Within each age group, heifers were allowed 30 d to adjust to new surroundings. On the first adjustment day, all heifers were injected with 10 mg/kg BW of Micotil (Elanco Animal Health, Indianapolis, IN) as a prophylactic for diseases related to shipping stress. Rectal temperatures were measured daily for the first 5 d, and a heifer was injected with Micotil a second time if her temperature exceeded 39.7°C. During the first 2 wk, heifers were fed ad libitum a 15% CP complete feed and alfalfa-orchard grass hay. During wk 3, they were gradually adjusted to a TMR that was similar to our standard protein diet and that was fed for all of wk 4 of the adjustment period.

Following the adjustment period, the 18 heifers within each age group that had the greatest rate of BW gain during the acclimation period were ranked by similar BW into groups of three and randomly assigned to one of three treatments. All heifers for a given treatment within each age group were housed in the same pen. Thus, three pens of six heifers each (one pen per age group) were used in each of the three treatments. Treatments began at ~106 d of age and continued until the early luteal phase of the fourth estrous cycle.

Table 1. Composition of diets with low, standard, and high protein.

	Protein		
	Low	Standard	High
Ingredients, % of DM[1]			
Alfalfa-grass haylage	40.0	40.0	40.0
Ground corn	54.0	48.1	42.2
Solvent-extracted soybean meal	5.0	5.0	5.0
Expeller soybean meal	0.0	5.9	11.8
Minerals and vitamins	1.0	1.0	1.0
Nutrient composition (DM basis)			
NDF, %	25.1	25.4	25.6
ME,[2] Mcal/kg	2.85	2.85	2.85
NEm,[3] Mcal/kg	1.90	1.91	1.91
NEg,[4] Mcal/kg	1.26	1.27	1.27
CP, %	13.7	16.2	18.8
RUP,[5] % of CP	33.4	36.0	37.9
MP,[6] %	10.6	11.6	12.6
CP:ME (g CP/Mcal ME)	48.1	56.8	66.0
MP:ME (g MP/Mcal ME)	37.2	40.7	44.3

[1]The alfalfa haylage contained 15.3% CP (±0.8 SD) and 47% NDF; its metabolizable energy (ME) value was estimated to be 2.43 Mcal/kg. The corn contained 9.5% CP and 11% NDF with an estimated ME value of 3.18 Mcal/kg. The solvent extracted soybean meal was dehulled and contained 53% CP and 7% NDF with 3.26 Mcal of ME/kg. The expeller soybean meal was SoyPlus (West Central Cooperative, Ralston, IA), which contained 53% CP and 8.0% NDF, with 3.21 Mcal of ME/kg. The mineral and vitamin mix contained 0.5% decoquinate and was formulated so the diet provided 100% of mineral and vitamin requirements.

[2]Metabolizable energy.

[3]Net energy for maintenance.

[4]Net energy for gain.

[5]RUP using common values of 21, 50, 30, and 50% of CP for alfalfa-grass haylage, ground corn, soybean meal, and expeller soybean meal.

[6]Metabolizable protein (%) = 0.64 × microbial protein + 0.8 × %RUP of CP × %CP, where microbial protein = 3.8 × Mcal of ME/kg DM.

The basal treatment diet was 40% alfalfa-grass haylage and 60% grain and contained ~2.85 Mcal ME/kg. This energy density was expected to produce >1.1 kg of BW gain/d when diets were fed ad libitum, so the likelihood of impaired development in heifers fed low protein would be high, and the effect of protein would be most pronounced. Diets were low (13.7% CP [**LP**]), standard (16.2% CP [**SP**]), or high (18.8% CP [**HP**]) protein. Composition of diets based on actual analyses is described in Table 1. The LP, SP, and HP diets were calculated to contain 48, 57, and 66 g of CP/Mcal of ME, respectively, and 37, 41, and 44 g of MP/Mcal of ME, respectively. This range in MP:ME covers that in the literature and also includes the standard MP:ME used in commercial dairy heifer raising. The alfalfa-grass haylage was the first cutting from a single field, harvested during the early bloom period and stored in a bag. Haylage samples were collected twice a week to assess DM content, and haylage was collected every other week to assess protein and fiber content. Samples of ground corn, soybean meal-48, and expeller soybean meal (SoyPlus, West Central Coop, Ralston, IA) were

collected upon purchase to assess protein and energy content, and the same batch of each was used throughout the study.

Diets were fed as a TMR fresh every day between 0900 and 0930 h, and heifers had free access to water and the respective diet. Orts for each pen were collected at 0700 h and weighed daily. Mean DMI for a pen was recorded. Heifers were housed in an open-sided barn at the Michigan State University Beef Cattle and Teaching Center and exposed to ambient temperatures and photoperiod from the time of purchase until slaughter, which occurred in fall and winter.

All heifers were weighed at ~0800 h before feeding on two consecutive days each week to monitor BW gain. The mean of the two weights was then assigned as a heifer's weekly weight and used to calculate average daily BW gain. The height at the withers was measured every 2 wk. BCS was assessed using a five-point scale (1 = thin, 5 = fat) every 4 wk by three experienced examiners. The three scores for each heifer were averaged and assigned to that heifer as her monthly score.

Puberty and Slaughter Age

To examine the pubertal status of heifers, weekly reproductive exams (rectal palpation) began once a heifer weighed 215 kg or was 7 mo old (whichever was first) to determine whether either ovary had a corpus luteum. On average, heifers weighed 215 kg by 5.9 mo of age, so most heifers were being examined for pubertal status before 6 mo of age. Our original design was to kill all heifers at 7.5 mo of age; we expected that most heifers would be prepubertal at that time. However, the heifers in this study attained puberty at an unusually early age, with 11 heifers pubertal already at 7 mo. Thus, all heifers were killed instead at a similar physiological age relative to puberty—during diestrus of their fourth estrous cycle, similar to the protocol used in Radcliff et al. (1997). At this time (46 d after first corpus luteum), mammary gland development in these heifers likely had returned to isometric growth (Sejrsen and Purup, 1997).

Weekly reproductive exams continued after detection of the first corpus luteum, and, if a corpus luteum was present again 21 d following the first one, a heifer was injected with 25 mg of $PGF_2\alpha$ (Lutalyse, Pharmacia and Upjohn, Inc.) 3 d later. Eleven days after this injection of $PGF_2\alpha$, the heifer was examined again. If a corpus luteum was present, she received another injection of $PGF_2\alpha$. Eleven days after the second injection of $PGF_2\alpha$, the heifer was examined again, and, if a corpus luteum was detected, she was killed on the following day, which was ~47 d after detection of the first corpus luteum. The above synchronization schedule was altered slightly to enable slaughter on only 1 or 2 d per week. If no corpus luteum was detected, slaughter was postponed until one was detected. This protocol of $PGF_2\alpha$ injections enabled us to schedule slaughter dates in advance and to ensure that heifers were killed during diestrus.

On the day of slaughter, a heifer was weighed, stunned by captive bolt, and killed by exsanguination between 0700 and 1000 h. The number of heifers killed each week depended on the date for detection of the first corpus luteum and ranged from one to six heifers.

Blood Collection and Analysis

Blood samples (~10 ml) were collected every 4 wk at ~0800 h via jugular venipuncture with Vacutainers (Becton Dickinson and Co., Rutherford, NJ). All samples were stored at room temperature (~21°C) for ~6 h and then at 4°C for ~15 h; serum was harvested and frozen at –20°C. After acid/ethanol extraction, serum IGF-I concentration was measured by radioimmunoassay with IGF-I standard, primary antibody, and methods of GroPep Pty. Ltd. (Adelaide, Australia) modified as in Sharma et al., (1994), with *Staphylococcus aureus* used in place of the secondary antibody.

For slaughter dates in which each treatment was represented by at least one heifer, the profile of somatotropin concentration in blood was assessed 4 d before expected slaughter. The resulting dataset included 21 heifers with 7 LP, 6 SP, and 8 HP. Heifers were fitted with sterile indwelling jugular catheters (18 gauge; Ico-Rally, Palo Alto, CA) 5 d before expected slaughter. On the following day, serial blood samples were collected at 20-min intervals for 12 h (0700 to 1900 h). If a heifer did not have a corpus luteum 3 d later (the day before expected slaughter), her blood samples were discarded. Concentrations of somatotropin in serum were quantified using a double-antibody radioimmunoassay (Gaynor et al., 1995).

Tissue Collection and Carcass Composition

The udder was quickly removed from the carcass and placed with the ventral side up. Distance from the base of each teat to its tip was measured, and the udder was bisected along the median suspensory ligament into right and left halves. The left half was weighed, placed in a plastic bag, and frozen by submersion in a tub of dry ice and 95% ethanol. Frozen hemiglands were stored at –20°C until analyzed.

The digestive tract was removed from the carcass, the gallbladder was removed from the liver, and the liver was weighed. The intestines (with omental fat) were separated from the upper gastrointestinal tract

at the pylorus, flushed with water, and drained. Greater than 90% of the fat was removed from the upper gastrointestinal tract, combined with the intestines, and stored at –20°C until grinding and analysis of lipid content.

After the hide was removed, the carcass was split into halves along the vertebral column, and each half was weighed. Perirenal fat was removed from the left half beginning at the fourth lumbar vertebra and proceeding forward to the adrenal gland and then weighed. The carcass was washed and stored at 2°C.

About 24 h after slaughter, the pelvic area was calculated from two linear measurements of the left half of the carcass, one from the ventral edge of the third coccygeal vertebrae to the symphysis pubis and a second at 90° from the midsagittal plane of the carcass to the middle of the pelvic wall. The second measurement was multiplied by two to represent the total width of the pelvic opening and then multiplied by the first measurement to estimate total pelvic area (Radcliff et al., 1997).

The left half of each carcass was cut between the seventh and eighth ribs and between the twelfth and thirteenth ribs. The rib section, including ribs 8 through 12, was removed. The section containing ribs 9, 10, and 11 was then dissected (Hankins, 1946), weighed, and deboned. Bone and soft tissue were weighed. Soft tissue was ground, mixed, and subsampled for analyses of protein, fat, and water content. Crude protein content was determined in fresh samples by combustion with a LECO FP-2000 (Leco Corporation, St. Joseph, MI). Fat was determined by Soxhlet ether extraction of fresh samples (Association of Official Analytical Chemists International, 1990). Water was determined as the difference in weight after drying fresh samples in an oven at 110°C for 24 h. Carcass protein, fat, and water contents were estimated using equations based on the ninth-tenth-eleventh-rib cut (Hankins, 1946). Equations were $Y = 5.64 + 0.69X$, $Y = 2.73 + 0.78X$, and $Y = 14.28 + 0.78X$, for the protein, fat, and water, respectively, where Y was the edible portion of the dressed heifer carcass, and X was the edible portion of the heifer three-rib cut. The gastrointestinal fat with intestines was weighed and ground. The ground tissue was mixed and subsampled for analyses of fat content. Fat was determined by Soxhlet ether extraction (Association of Official Analytical Chemists International, 1990).

Mammary Tissue Analysis

The frozen left half of the udder was cut transversely with a band saw into 5- to 10-mm thick slices. All slices from both the anterior and posterior ends of the gland that did not contain parenchymal tissue were discarded. Skin, teats, and supramammary lymph nodes were dissected from the remaining slices while frozen. Then fat located beyond the border of the parenchyma (in those slices that contained parenchyma) was dissected and weighed. This fat was defined as extra parenchymal fat. The remaining tissue was referred to as parenchymal tissue. Frozen parenchymal tissue was weighed and ground in a Waring blender with liquid nitrogen into a powder. The powder was mixed and subsampled for subsequent analysis of DM, protein, and fat—using the same methods used for the carcass—and DNA and RNA content (Tucker, 1964).

Statistical Analysis

Eight heifers (3 LP, 3 SP, and 2 HP) were removed from the experiment. Four were removed because they were freemartins (1 LP, 2 SP, and 1 HP). Two LP heifers were removed due to complications from rectal palpation. Two heifers (1 SP and 1 HP) were removed because of late onset of puberty (> 9.5 mo). Forty-six heifers completed the study (15 LP, 15 SP, and 16 HP).

Data for mean live body growth from start of treatments until slaughter, carcass composition, mammary composition, and mammary nucleic acid content were analyzed using the model:

$$Y = \text{treatment} + \text{pen(treatment)} + \text{residual},$$

where treatment = LP, SP, and HP diet.

Pen within treatment was used to test treatment. Differences were determined using orthogonal contrasts for the linear (LP vs. HP) and quadratic (LP + HP vs. SP) effects of treatment. Analysis was done using the GLM procedure of SAS (1996).

The log transformation of plasma concentrations for somatotropin and IGF-I were analyzed with the model:

$$Y = \text{treatment} + \text{group} + \text{group*treatment} + \text{animal(group*treatment)} + \text{time} + \text{time*treatment} + \text{residual},$$

where treatment = LP, SP, and HP diet and group = A, B, and C age group.

Treatment was tested for significance with animal within group by treatment. Analysis was done using the GLM procedure of SAS (1996).

The age at which heifers reached puberty, and thus the number of days on treatment, varied considerably in this study. Therefore, we also analyzed treatment effects using age at slaughter within treatment as a covariate to determine if heifers that reached puberty early responded differently to treatment than those

Table 2. Least squares means for body growth.

	Protein-to-energy ratio[1]				*P* for contrast	
	Low	Standard	High	SEM[2]	Linear	Quadratic
Initial BW, kg	134	135	134	3	0.86	0.85
Initial BCS	2.7	2.7	2.7	0.2	0.97	0.98
Initial withers height, cm	96.9	96.9	96.8	0.6	0.95	0.98
Age at first corpus luteum, mo	7.5	7.5	7.4	0.3	0.94	0.85
BW at first corpus luteum, kg	266	274	271	9	0.71	0.63
BW at slaughter, kg	320	326	320	8	0.97	0.61
Final BCS	3.6	3.7	3.6	0.1	0.43	0.10
Final withers height, cm	118	119	118	2	0.92	0.74
Age at slaughter, mo	8.9	8.9	8.8	0.4	0.78	0.89
Time on treatment, d	165	165	160	12	0.78	0.89
DMI, kg/d	6.19	6.17	6.11	0.11	0.90	0.62
Overall BW gain, kg/d	1.13	1.17	1.18	0.05	0.54	0.79
Overall BCS gain	1.0	1.0	0.9	0.2	0.84	0.69
Withers height gain, cm/d	0.15	0.15	0.16	0.01	0.41	0.78
Final pelvic area, cm^2	206	218	204	6	0.71	0.09

[1]The low-protein diet contained 48 g of CP and 37 g of metabolizable protein (MP) per Mcal of metabolizable energy (ME). The standard-protein diet contained 57 g of CP and 41 g of MP per Mcal of ME, and the high protein diet contained 66 g of CP and 44 g of MP per Mcal of ME.

[2]Pooled SEM using pen within treatment as the error term with three pens per treatment.

that reached puberty late. Data were analyzed using the model:

$$Y = \text{treatment} + \text{group} + \text{pen(treatment)} + \text{age at slaughter(treatment)} + \text{residual},$$

where treatment = LP, SP, and HP diet, and group = A, B, and C age group.

Group and pen within treatment were treated as random variables. Age at slaughter within treatment was treated as a covariate. Treatment least square means were calculated at three different values for age at slaughter: 250, 280, and 310 d of age (corresponding to pubertal ages of ~200, 230, and 260 d, respectively). Analysis was done using the Mixed procedure of SAS (1996).

RESULTS

Body Growth and Hormones

Initial measures of age, BW, height at the withers, and BCS were not different among treatment groups (Table 2). Dietary treatment did not alter rate of BW gain, rate of withers height gain, change in body condition, or age at the time of observed first corpus luteum. Consequently, dietary protein also did not alter age at slaughter, BW at slaughter, or final height at the withers. Dietary protein also did not alter DMI or feed efficiency ($P > 0.6$). Average DMI was 6.2 kg/d (~2.7% of BW), and average feed efficiency was 0.19 kg BW gain/kg DMI.

Carcass weights and carcass weights as a percentage of live BW were similar among treatment groups (Table 3). Dietary protein also did not alter carcass fat and protein composition, mass of internal fat, or liver mass.

The average somatotropin concentration 4 d before slaughter was 2.2 ng/ml. Increasing the ratio of dietary protein to energy did not alter the profile of somatotropin concentration in blood or the average somatotropin concentration ($P > 0.6$). Plasma IGF-I concentration increased with age but was not altered ($P > 0.6$) by treatment (Figure 1).

Mammary Development

Dietary protein did not significantly alter the mass of dissected mammary parenchymal tissue, and it did not alter the concentration or mass of fat-free DM, lipid, protein, DNA, or RNA in mammary parenchyma (Tables 4 and 5). Diet did not alter the mass of dissected extraparenchymal adipose tissue. Correcting these values for BW did not significantly change the results. Although not statistically significant, there was a trend (P = ~0.2) for heifers fed HP to have ~10% more parenchymal DNA and RNA per 100 kg of BW than those fed LP. Diet did not influence the length of the front or rear teats (Table 4). Moreover, the variation in teat length accounted for none of the variation in mammary parenchymal mass or DNA among individual heifers ($r^2 < 0.02$; data not shown).

Day of Puberty and Interaction with Treatments

Average age at the first detected corpus luteum was 7.5 mo (range 6 to 9.3 mo), average age at slaughter

Table 3. Least squares means for carcass composition.

	Protein-to-energy ratio[1]				*P* for contrast	
	Low	Standard	High	SEM[2]	Linear	Quadratic
Carcass weight, kg	175	180	175	6	0.99	0.53
Carcass weight as a % of live BW	54.6	55.0	54.5	0.6	0.86	0.51
Liver weight, kg	5.3	5.6	5.6	0.1	0.17	0.40
Liver weight, kg/100 kg BW	1.68	1.72	1.74	0.04	0.29	0.92
Carcass protein, % of carcass	17.7	18.0	18.0	0.3	0.55	0.68
Carcass fat, % of carcass	21.5	18.9	20.4	1.1	0.51	0.17
Carcass water, % of carcass	59.5	61.5	60.7	0.9	0.38	0.27
Carcass protein, kg	25.8	27.4	26.5	1.0	0.65	0.38
Carcass fat, kg	31.7	28.8	30.1	2.0	0.60	0.42
Perirenal fat, kg	5.1	5.4	4.9	0.5	0.68	0.56
Omental-intestinal fat, kg	13.3	13.9	11.7	1.0	0.33	0.32
Internal fat, kg/100 kg BW	5.5	5.8	5.1	0.3	0.46	0.21

[1]The low-protein diet contained 48 g of CP and 37 g of metabolizable protein (MP) per Mcal of metabolizable energy (ME). The standard-protein diet contained 57 g of CP and 41 g of MP per Mcal of ME, and the high-protein diet contained 66 g of CP and 44 g of MP per Mcal of ME.

[2]Pooled SEM using pen within treatment as the error term with three pens per treatment.

was 8.9 mo, and average time on treatment was 164 d (Table 2). Treatment did not alter ages at puberty or slaughter ($P = 0.9$), and mean values were nearly identical for each treatment. Age at slaughter, was, however, linearly related to BW at slaughter and average daily BW gain ($P < 0.05$); heifers that attained puberty earlier grew faster during the study but weighed less at slaughter than those that attained puberty later. Dietary treatment did not alter this relationship, as indicated by no difference in the slopes for BW at slaughter or average BW gain versus age at slaughter ($P > 0.2$; Table 6).

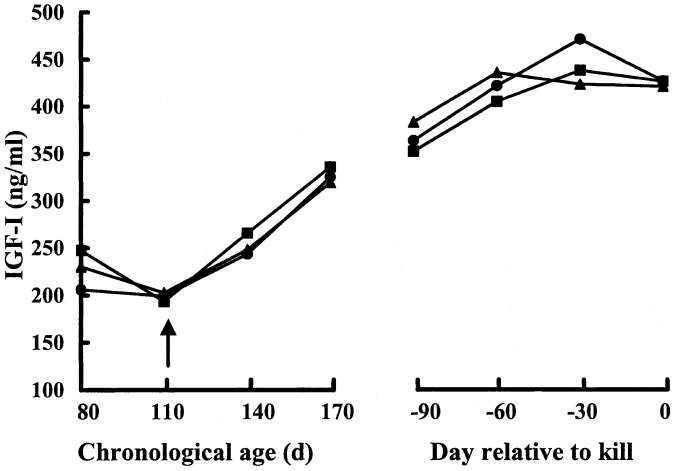

Figure 1. Concentrations of serum IGF-I in heifers fed low protein (LP, n = 15, ●), standard protein (SP, n = 15, ■), or high protein (HP, n = 16, ▲). SEM = 15. The left half of the plot is by chronological age, with the arrow representing the time treatments started; the right half is by day relative to kill, with –90 d being the treatment averages for –110 to –87 d, –60 d being the treatment averages for –82 to –52 d, –30 d being the treatment averages for –54 to –31 d, and 0 d being the treatment averages for –26 to –3 d.

Age at slaughter was not related to mammary parenchymal DNA overall. However, the heifers that attained puberty earlier responded to dietary protein differently than those that attained puberty later. The slopes of parenchymal DNA (Figure 2) and parenchymal DNA per 100 kg of BW regressed over age at slaughter were greater for heifers fed the LP diet than those fed the HP diet ($P = 0.07$ and $P = 0.05$, respectively; Table 6). Based on the predicted least squares means (Table 6), heifers that reached puberty early and were slaughtered at 250 d would have had 33% less mammary parenchyma ($P = 0.03$) if they were fed LP than if they were fed HP. However, dietary protein had no effect on mammary development in heifers that reached puberty later and were slaughtered at 310 d ($P = 0.5$).

DISCUSSION

Although we expected 40% less mammary development in heifers fed LP than those fed HP based on our review of the literature, we observed only a 10% decrease, and it was not statistically significant. The lack of a significant treatment effect was true for all of our measures of mammary development: parenchymal mass, dry fat free tissue, protein, DNA, and RNA, with and without corrections for BW at slaughter.

This lack of a major effect of protein occurred despite the fact that our diets were energy-dense and promoted BW gains of 1160 g/d during the time that the mammary gland is sensitive to rapid growth and despite the fact that our dietary treatments covered the range of protein-to-energy ratios used in previous studies on mammogenesis. Our LP diet contained 48 g of CP and 37 g of MP per Mcal of ME; this protein level is similar to or less than that of rapid-growth diets that decreased the amount of mammary parenchymal DNA (Sejrsen

Table 4. Least squares means for mammary gland composition.

	Protein-to-energy ratio[1]				*P* for contrast	
	Low	Standard	High	SEM[2]	Linear	Quadratic
Parenchyma,[3] g	604	616	662	48	0.42	0.77
Parenchyma,[3] g/100 kg BW	190	188	208	12	0.29	0.49
Extraparenchymal fat,[3] g	1430	1590	1470	60	0.70	0.09
Extraparenchymal fat,[3] g/100 kg BW	444[a]	488[b]	460[a]	12	0.42	0.05
Parenchymal lipid, g	284	274	294	30	0.81	0.69
Parenchymal fat-free dry matter, g	48	51	53.6	3.6	0.32	0.96
Parenchymal protein, g	48	49.6	51.2	3.4	0.54	0.98
Teat length						
Front, mm	33	30	33	2	0.91	0.23
Rear, mm	30	28	32	2	0.58	0.34

[a,b]Least squares means in rows with different superscripts differ quadratically ($P < 0.05$).

[1]The low-protein diet contained 48 g of CP and 37 g of metabolizable protein (MP) per Mcal of metabolizable energy (ME). The standard-protein diet contained 57 g of CP and 41 g of MP per Mcal of ME, and the high-protein diet contained 66 g of CP and 44 g of MP per Mcal of ME.

[2]Pooled SEM using pen within treatment as the error term with three pens per treatment.

[3]Based on wet matter.

et al., 1982; Petitclerc et al., 1984; Capuco et al., 1995). Furthermore, our HP diet contained expeller soybean meal (high in RUP) and had 66 g of CP and 44 g of MP per Mcal of ME; this protein level is similar to that of published rapid-growth diets that did not decrease the amount of parenchymal DNA (Capuco et al., 1995; Radcliff et al., 1997).

Perhaps lower protein would have decreased mammary development, but lower protein may also have altered body growth. The lack of a major effect in our study implies that the discrepancies in the effects of high-energy intake on prepubertal mammogenesis in previous studies, with MP:ME ranging from 37 to 44 g/Mcal, are not due to the differences in dietary protein, but instead must be related to other dietary factors, environmental conditions, or genetic influences.

One potential problem with our design is that we used the same diets throughout the study, but as an animal ages, the required ratio of protein to energy in its diet decreases (National Research Council, 2001). In our original protocol, we intended to kill heifers at 7.5 mo, so diets were designed for feeding from 3.5 to 7.5 mo. Because heifers attained puberty early, we fed heifers their respective diets until ~8.9 mo of age. Perhaps the LP diet was limiting for mammary development early in the treatment period but not later in the treatment period as animals grew older. Based on this idea, we included age at slaughter (which was ~46 d after puberty) as a covariate within treatment in our statistical model. Treatment did not affect day of puberty or slaughter, so adding this covariate to the analysis allowed for an accurate comparison of treatment effects within different maturity groups. Based on our predicted least squares means for heifers that were pubertal at ~6.7 mo and slaughtered at 250 d, those fed LP had 33% less mammary parenchymal DNA than those fed HP (Table 6). In contrast, the treatments did not affect mammary development in heifers that achieved puberty after 7 mo, suggesting that the slower-growing heifers may have obtained sufficient protein

Table 5. Least squares means for mammary gland nucleic acid content.

	Protein-to-energy ratio[1]				*P* for contrast	
	Low	Standard	High	SEM[2]	Linear	Quadratic
Parenchymal DNA, mg	1890	2010	2110	340	0.40	0.96
DNA, mg/100 kg BW	595	619	670	78	0.22	0.78
Concentration DNA, mg/g	6.22	6.5	6.44	0.2	0.47	0.54
Parenchymal RNA, mg	1110	1230	1300	220	0.28	0.89
RNA, mg/100 kg BW	350	379	413	56	0.16	0.93
Concentration RNA, mg/g	3.66	3.94	3.92	0.14	0.29	0.44
RNA:DNA	1.18	1.22	1.22	0.02	0.37	0.67

[1]The low-protein diet contained 48 g of CP and 37 g of metabolizable protein (MP) per Mcal of metabolizable energy (ME). The standard-protein diet contained 57 g of CP and 41 g of MP per Mcal of ME, and the high-protein diet contained 66 g of CP and 44 g of MP per Mcal of ME.

[2]Pooled SEM using pen within treatment as the error term with three pens per treatment.

Table 6. Predicted least squares means for body growth and mammary gland composition at three different ages at slaughter.

	Protein-to-energy ratio[1]				*P* for contrast
	Low	Standard	High	SEM[2]	Low vs. high
Age 250 d[3]					
BW at slaughter, kg	295	299	291	8	0.7
BW gain, g/d	1210	1250	1220	50	0.8
Parenchymal DNA, mg	1580	1820	2340	260	0.04
Parenchymal DNA, mg/100 kg BW	542	616	808	100	0.03
Age 280 d					
BW at slaughter, kg	318	324	322	6	0.6
BW gain, g/d	1140	1180	1180	40	0.4
Parenchymal DNA, mg	1850	2010	2150	180	0.2
Parenchymal DNA, mg/100 kg BW	584	620	684	58	0.2
Age 310 d					
BW at slaughter, kg	341	349	352	7	0.2
BW gain, g/d	1080	1110	1140	50	0.3
Parenchymal DNA, mg	2120	2190	1970	260	0.7
Parenchymal DNA, mg/100 kg BW	628	626	558	100	0.5
Slope[4]					
BW at slaughter, kg	0.78	0.85	1.00	0.20	0.2
BW gain, g/d	–2.13	–2.35	–1.42	1.1	0.5
Parenchymal DNA, mg	9.00	6.04	–6.24	7.2	0.07
Parenchymal DNA, mg/100 kg BW	1.44	0.20	–4.16	2.6	0.05

[1]The low-protein diet contained 48 g of CP and 37 g of metabolizable protein (MP) per Mcal of metabolizable energy (ME). The standard-protein diet contained 57 g of CP and 41 g of MP per Mcal of ME, and the high-protein diet contained 66 g of CP and 44 g of MP per Mcal of ME.

[2]Pooled SEM using pen within treatment as the error term with three pens per treatment.

[3]Ages at slaughter of 250, 280, and 310 d are equal to ages at puberty of 204, 234, and 264 d, respectively.

[4]Slope of partial regression lines of independent variables with age at slaughter.

even from the LP diet. The most likely reason that the heifers achieving puberty early were sensitive to low protein is that the requirement for protein relative to energy decreases with age.

This analysis does not imply that differences in dietary protein are responsible for the discrepancies in the effects of high-energy intake on parenchymal DNA of previous studies, because heifers in those studies achieved puberty on average after 260 d, so the comparable group in our study would be the heifers that attained puberty last.

An external measure for assessing mammary development would be a useful tool for developing optimal heifer diets. Lammers and Heinrichs (2000) used teat elongation as an indicator of mammary development. Although we did not measure teat elongation during our study, we did measure teat length at slaughter (Table 4). We found no treatment differences or correlations between mammary parenchymal mass and teat length, even though both measures varied considerably (parenchyma ranged from 400 to 1200 g, and average teat length ranged from 20 to 50 mm). We expect that if elongation from 3.5 mo of age to 1.5 mo after puberty were related to parenchymal growth, teat length would be correlated with parenchymal mass at slaughter. Thus, we suggest that teat length and elongation are not valid external indicators of mammary development.

Possible mechanisms for an effect of dietary protein on mammogenesis include somatotropin and IGF-I. Capuco et al. (1995) reported that somatotropin concentrations were reduced 25% and mammary development 48% when rapid BW gains were achieved from high intake of a corn-silage-based diet (54 g of CP/Mcal of ME) but neither was reduced from high intake of an alfalfa-based high-protein diet. In the current experiment, all heifers had ad libitum access to their respective high-energy diets, and there was no difference in serum somatotropin concentrations. Most evidence suggests that somatotropin acts indirectly on the mammary gland through other factors such as IGF-I (Akers, 1985). However, we found no effect of dietary protein on serum IGF-I overall or in heifers that attained puberty early.

Although this was the first study to examine effects of dietary protein in rapidly growing heifers, other studies have been published on the effects of prepubertal dietary protein in heifers growing less than 900 g/d or in lambs. Pirlo et al. (1997) fed high-energy prepubertal diets with high or low protein (62 or 50 g of CP/Mcal of ME from 100 to 200 kg of BW and 49 or 40 from 200

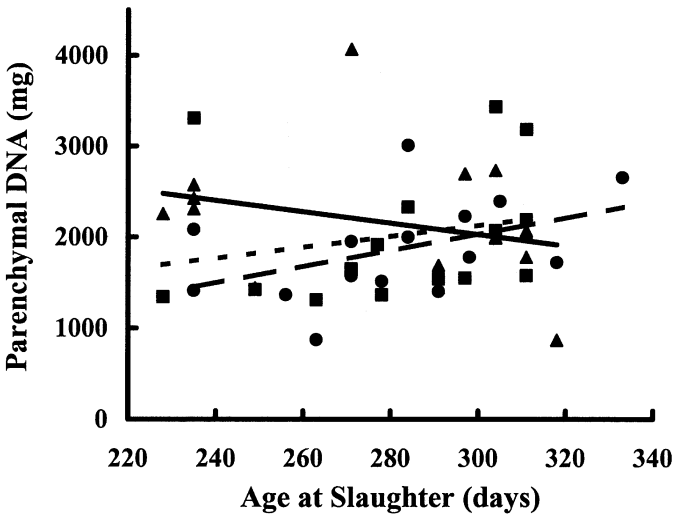

Figure 2. Partial regression lines of total mammary parenchymal DNA against age at slaughter for each dietary treatment: low protein (– – –, slope = 9.0, $P = 0.17$), standard protein (·····, slope = 6.0, $P = 0.36$), and high protein (——, slope = –6.2, $P = 0.24$). Also plotted are individual heifer values for heifers fed low protein (LP, n = 15, ●), standard protein (SP, n = 15, ■), and high protein (HP, n = 16, ▲).

to 300 kg, respectively) to Friesian heifers. Heifers grew ~820 g/d. Compared to a control group fed low-energy diets, heifers fed high energy with low protein tended to produce 15% less milk protein as cows, but those fed high energy with high protein produced as much as controls. Murphy et al. (1991) fed Holstein heifers to grow at ~800 g/d and found that feeding low compared with standard protein (~45 compared with ~55 g of CP/Mcal of ME) decreased subsequent milk yield 10%. In addition, rapidly grown lambs fed ~56 instead of ~75 g of CP/Mcal of ME tended to produce 15% less milk as ewes (Zhang et al., 1995).

Adequate protein nutrition depends on age or weight of a heifer and her expected growth rate. In the NRC's *Nutrient Requirements for Dairy Cattle* (2001), the calculated protein-to-energy recommendations for heifers at 150, 250, and 350 kg of BW are 67, 56, and 50 g of CP/Mcal of ME for gains of 700 g/d, and 72, 58, and 52 g/Mcal for gains of 900 g/d, respectively (National Research Council, 2001). In light of our results, and those of previous studies, these recommendations seem reasonable for optimizing growth and mammary development. The major goal for a dairy replacement program should be to produce a heifer with a well-developed mammary gland. We suggest that feeding low protein, on a CP or MP basis, may not always impair mammary development, but it increases the risk of suboptimal mammary development in heifers that are fed high-energy diets. Adequate protein may be especially important for those heifers within a group that grow the fastest and attain puberty the earliest.

CONCLUSIONS

The major objection to rearing dairy heifers at a high growth rate is compromised mammary development and decreased subsequent milk production. Increasing the protein-to-energy ratio fed to rapidly grown prepubertal heifers from 37 to 44 g of MP/Mcal of ME produced 10% more mammary parenchymal DNA, but this was not statistically significant. Therefore, we conclude that dietary protein does not have a major effect on mammary development of rapidly grown prepubertal heifers, provided the diet contains adequate protein for normal body growth. However, the low protein diet did impair mammary development in animals that achieved puberty early, even though their body growth and carcass composition were not compromised. The new NRC guidelines for protein relative to energy in diets for heifers should be followed to reduce the risk of impaired mammary development when heifers are fed for rapid growth.

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