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Effect of Increasing Energy and Protein Intake on Mammary Development in Heifer Calves*

E. G. Brown,[1] M. J. VandeHaar,[1] K. M. Daniels,[1] J. S. Liesman,[1] L. T. Chapin,[1] J. W. Forrest,[2] R. M. Akers,[2] R. E. Pearson,[2] and M. S. Weber Nielsen[1]
[1]Department of Animal Science, Michigan State University, East Lansing, 48824
[2]Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg, 24061

ABSTRACT

The objective of this study was to determine if increased energy and protein intake from 2 to 14 wk of age would affect mammary development in heifer calves. At 2 wk of age, Holstein heifer calves were assigned to 1 of 4 treatments in a 2 × 2 factorial arrangement with 2 levels of protein and energy intake (moderate, M; high, H) in period 1 (2 to 8 wk of age) and 2 levels of protein and energy intake (low, L; high, H) in period 2 (8 to 14 wk of age), so that mean initial body weights were approximately equal for all 4 treatments (ML, MH, HL, and HH). The M diet in period 1 consisted of a standard milk replacer (21.3% CP, 21.3% fat) fed at 1.1% of BW on a DM basis and a 16.5% CP grain mix fed at restricted intake to promote 400 g of daily gain, whereas the L diet in period 2 consisted only of the grain mix. The H diet in period 1 consisted of a high-protein milk replacer (30.3% CP, 15.9% fat) fed at 2.0% of body weight on a DM basis and a 21.3% CP grain mix available ad libitum. In period 2, the H diet consisted of just the 21.3% grain mix. Calves were gradually weaned from milk replacer by 7 wk and slaughtered at 8 (n = 11) or 14 wk of age (n = 41). Parenchyma from the distal region, midgland, and proximal region relative to the teat from one half of the udder was collected, fixed, and embedded in paraffin. The other half of the gland was used to determine parenchymal mass, protein, fat, DNA, RNA, and extraparenchymal mass. Total parenchymal tissue, parenchymal DNA, parenchymal RNA, and concentrations of DNA and RNA were higher for calves on the H diet during period 1, but were not affected by diet during period 2. Parenchymal fat percentage was increased by the H diet during period 2. The H diet increased extraparenchymal fat during both periods. The area of parenchyma occupied by epithelium was not affected by treatment, but at the end of period 2, the percentage of proliferating epithelial cells as indicated by Ki67, an marker of cell proliferation, expression was greater for calves on the M diet in period 1 compared with calves on the H diet in period 1. Diets did not influence parenchymal protein percentage or the ratio of RNA to DNA. Higher energy and protein intake from 2 to 8 wk of age increased parenchymal mass and parenchymal DNA and RNA in mammary glands of heifer calves without increasing deposition of parenchymal fat. Diet also influenced histological development of mammary parenchyma and subsequent proliferation of ductal epithelial cells. Implications of these effects for future milk production potential are unknown.
(**Key words:** calf, heifer, mammary development, nutrition)

Abbreviation key: D = distal, **ER** = estrogen receptor, **H** = high protein and energy intake, **M** = moderate protein and energy intake, **MID** = mid-gland, **L** = low protein and energy intake, **P** = proximal, **SUB** = subtending ducts, **TDU** = terminal ductular units.

INTRODUCTION

Little is known about body growth rates during the period between birth and approximately 4 mo of age that encourage maximal mammary development and future milk production potential. Before approximately 9 mo of age, a critical period of heifer mammary growth exists during which mammary growth is allometric (Sinha and Tucker, 1969), and can be negatively affected by high energy intake (Sejrsen and Purup, 1997). However, in calves before weaning, 2 studies have reported that increased intake of whole milk was related to higher future milk production. For example, heifer calves consuming whole milk for BW gains of 1.1 kg/d tended to produce more milk as cows than did heifers fed a restricted amount of whole milk before weaning (Foldager and Krohn, 1994). Bar-Peled et al. (1997) showed that calves suckling cows twice daily tended to produce more milk in their first lactations compared with similar heifers fed a defined amount of milk replacer, although it must

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Corresponding author: M. S. Weber Nielsen; e-mail: msw@msu.edu.
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be noted that the importance of the quantity of whole milk consumed relative to differences in diet composition and feeding method in this effect is unknown. Further evidence of the potential to increase the growth rates of young calves without negatively impacting mammary growth was provided by Sejrsen et al. (1998). This group demonstrated that heifer calves fed increased amounts of whole milk for a high rate of gain from birth until 6 wk of age had amounts of mammary parenchyma mass similar to those of heifers fed for moderate rates of gain when slaughtered at 12 wk of age.

Only 1 study has been reported that addresses the potential for dietary manipulation of mammary development after weaning in calves younger than 3 mo of age. Using calves fed for 2 rates of gain after weaning, Petitclerc et al. (1999) showed that ad libitum feeding increased extraparenchymal tissue volume but reduced the amount of parenchymal tissue when data were adjusted for differences in BW at slaughter, indicating the potential for nutritional impairment of mammary development after the time of weaning around 2 mo of age.

The objective of this experiment was to determine if increasing energy and protein intake using commercial milk replacers and calf starters would affect mammary growth and development in heifer calves younger than 4 mo of age. Results of treatment effects on body growth rate and carcass composition are reported in a companion article (Brown et al., 2005).

MATERIALS AND METHODS

Management of Calves

All procedures were approved by the Animal Use and Care Committee of Michigan State University. Female Holstein calves (44.1 ± 0.9 kg BW) were blocked by date of purchase, as described earlier by Brown et al. (2005). Following the 1-wk adaptation period, calves were randomly assigned to 1 of 4 treatments in a 2 × 2 factorial arrangement so that mean BW was not different among treatments. Withers height was not different among treatments at the start of the experiment.

In period 1, the moderate (**M**) diet consisted of milk replacer (Calvita Supreme, Milk Specialties Co., Dundee, IL; 21.3% CP, 21.3% fat, ~4.7 kcal of ME/g of DM guaranteed analysis) fed on a DM basis at 1.1% of BW (reconstituted to 11.8% DM), and starter grain (20.5% CP guaranteed analysis; Gold Flake Calf Starter, Nutrena Feeds–Cargill, Inc., Minneapolis, MN) fed at restricted intake to achieve 0.40 kg of average daily gain from 2 to 8 wk of age. The high (**H**) diet consisted of a high-protein milk replacer (Excelerate, Milk Specialties Co.; 30.3% CP, 15.9% fat, approximately 4.4 kcal of ME/g of DM guaranteed analysis) fed on a DM basis at 2.0% of BW (reconstituted to 14.1% DM) and high-protein starter grain (25.0% CP guaranteed analysis; Herd Builder Calf Starter, Nutrena Feeds–Cargill, Inc.) fed ad libitum. Calves were gradually weaned from milk replacer by 7 wk of age.

From 8 to 14 wk of age, calves on the low (**L**) diet were fed grain at restricted intake to achieve 0.4 kg of average daily gain, whereas calves on the H diet were fed high-protein grain for ad libitum intake. From 8 to 9 wk of age, calves were fed only the respective calf starters, but beginning at 9 wk of age rolled corn was added to both diets. The new diets contained 70% of the respective calf starters and 30% rolled corn. According to laboratory analyses, nutrient values for the new diets were 16.5% CP and 3.8% crude fat for the L diet, and 21.3% CP and 3.7% crude fat for the H diet. Calves had fresh water available at all times.

Tissue Collection

One subset of calves (1 or 2 calves from each block; n = 5 calves on M diet; n = 6 calves on H diet) was randomly selected and slaughtered at 8 wk of age to assess mammary development at the end of period 1. The remaining 41 calves were slaughtered at 14 wk of age.

Calves were weighed on the afternoon of the day before slaughter. Calves were then fed and allowed 1 h to eat and drink water before being shipped to the Michigan State University Meats Laboratory at 1630 h. Calves were slaughtered approximately 14 to 16 h after last feeding using captive bolt stunning followed by exsanguination. Within 15 min of slaughter, the mammary glands were collected. Mammary glands were bisected into right and left hemiglands and weighed. The left half was frozen flat in liquid nitrogen and stored at –20°C for later analysis. Using the right half, samples of parenchymal tissue from the distal (**D**), midgland (**MID**), or proximal (**P**) region relative to the teat were excised from each calf, fixed for 24 h in buffered formalin, and then transferred to 70% ethanol for later analysis.

The reproductive tracts were examined to confirm that animals were not freemartins and had not reached puberty. One heifer was a freemartin and her data were eliminated from the results.

Mammary Tissue Analysis

The frozen left half of the udder was cut transversely using a band saw into slices 5 to 10 mm thick. Slices from both the anterior and posterior ends that did not contain parenchymal tissue were discarded. Skin, teats, and supramammary lymph nodes were removed. Extraparenchymal fat located outside the border of the parenchyma was weighed. The remaining parenchymal tissue