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Effects of milk replacer formulation on measures of mammary growth and composition in Holstein heifers

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ABSTRACT

Overfeeding prepubertal heifers may impair mammary parenchymal growth and reduce milk production, but evidence suggests that increased intake of a high-protein milk replacer before weaning may be beneficial. This study was designed to evaluate effects of milk replacer (MR) composition on mass and composition of mammary parenchyma and fat pad, growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis gene expression, and putative mammary epithelial stem cells. Specifically, we hypothesized that positive effects of faster rates of gain during the preweaning period alter the development, persistence, or activity of populations of putative mammary epithelial stem cells, possibly through involvement of GH/IGF-I axis molecules. Twenty-four newborn heifers were fed 1 of 4 MR diets (n = 6/diet): control [20% crude protein (CP), 21% fat MR fed at 441 g of dry matter (DM)/d], high protein, low fat (28% CP, 20% fat MR fed at 951 g of DM/d), high protein, high fat (27% CP, 28% fat MR fed at 951 g of DM/d), and high protein, high fat+ (27% CP, 28% fat MR fed at 1,431 g of DM/d). Water and starter (20% CP, 1.43% fat) were offered ad libitum. Animals were killed on d 65 and mammary tissue was subjected to biochemical, molecular, and histological examination. No differences in mammary parenchymal mass or composition, with or without adjusting for empty body weight, were detected. Mass was increased and composition of the mammary fat pad was altered by nutrient intake. No diet differences in putative mammary epithelial stem cell abundance or abundance of transcripts for genes of the GH/IGF-I axis were detected. In this study, growth of the mammary epithelium, size of the mammary epithelial stem cell population, and components of the GH/IGF-I axis did not depend on diet. However, an underlying positive correlation between telomerase, a marker of mammary stem cells, and growth of the mammary parenchyma was detected. Implications of diet-induced effects on mammary fat pad and possible effects on subsequent development and function remain to be determined.
Key words: stem cell, mammary gland, milk replacer, prepubertal heifer

INTRODUCTION

The effect of nutrition on prepubertal development of the bovine mammary gland has yet to be fully elucidated. Accelerated rearing of heifers reduces the age and mass of mammary parenchyma (**PAR**) at puberty (Sejrsen et al., 1982; Capuco et al., 1995). This apparent reduction in PAR mass was universally interpreted to represent an inhibition of mammary epithelial proliferation induced by overnutrition during this allometric phase of mammary growth, which in turn supported the concept of a critical period during which the mammary gland was sensitive to nutrient intake. Recent data indicated that the rate of mammary growth through the majority of the prepubertal period was not affected by feed intake (Meyer et al., 2006a,b); thus, an effect of diet may be caused by truncation of the allometric phase of mammary growth or an artifact of comparing heifers at different ages or developmental stages. The authors pointed out that when mammary development of heifers reared at different rates of gain is assessed at the same or similar BW, dietary effects are confounded by heifer age. Nonetheless, recent studies focusing on the period from birth to weaning suggest a beneficial role of elevated nutrient intake on PAR growth (Brown et al., 2005; Meyer et al., 2006a,b).

Growth hormone (**GH**), IGF-I, their receptors, and IGF binding proteins (**IGFBP**) are components of the GH/IGF-I axis. Some components of this axis are influenced by nutrition and have been implicated as mediators of inhibited mammary growth in heifers fed elevated levels of nutrients (Sejrsen et al., 1983; Berry et al., 2003b; Weber et al., 1999, 2000). In other cases, specific components of the GH/IGF-I axis were not affected by level of nutrient intake (Meyer et al., 2007). These conflicting results demonstrate the need for fur-

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ther evaluation of the role of the GH/IGF-I axis in development of the bovine mammary gland (**MG**), with respect to both PAR and mammary fat pad (**MFP**) development.

Mammary epithelial stem cells provide for growth and development of the mammary epithelium (Capuco and Ellis, 2005). The response of these adult stem cells to nutrition may be important in determining the effect of nutrition on mammary growth during the prepubertal period (Meyer et al., 2006a). When mammary epithelial cell proliferation was assessed at a common BW of 100 kg, Meyer et al. (2006a) found that it was 44% higher in heifers fed at elevated levels of nutrient intake, compared with heifers fed for restricted intake; this effect was lost at 150 kg of BW. Meyer et al. (2006a) postulated that elevated nutrient intake increased epithelial cell proliferation, which may have resulted from increased proliferation of mammary stem cells or their daughters, or both. Although no mention of mammary stem cells appeared in the paper of Brown et al. (2005), evidence presented demonstrated that increased energy and protein intake associated with accelerated calf growth programs increased PAR growth in heifers from 2 to 8 wk of age. This growth may also have been influenced by stem cell activity. Further support for the idea that stem cells can be modulated by nutrition comes from Drummond-Barbosa and Spradling (2001), who noted that in some model organisms, stem cells and their more differentiated daughter cells change proliferation patterns according to nutritional status. When the data of Meyer et al. (2006a) and Brown et al. (2005) are considered together, the possibility of nutritional regulation of stem cell proliferation is plausible.

This study addresses the hypothesis that increased nutrient intake by preweaned heifers increases PAR mass by promoting proliferation of mammary epithelial stem cells, which is promoted by local changes in the GH/IGF-I axis. There were 3 objectives: The first was to confirm a positive effect of nutrient intake on growth of the mammary parenchyma in preweaned heifers and to expand upon previous studies by evaluating the effect of composition of milk replacer (**MR**) on growth of the PAR and MFP. The second objective was to characterize components of the GH/IGF-I axis in PAR and MFP of young heifers fed different diets. The third objective was to determine whether diet altered the number of putative mammary epithelial stem cells. To address these objectives, preweaned Holstein heifers of similar ages were fed different diets to achieve various rates of BW gain. Four MR regimens were used to make 3 diet comparisons at the end of the experiment. Heifers were harvested at a common age and mammary tissue was subjected to biochemical, molecular, and histological examination to assess effects of diet.

Results of diet effects on blood metabolite and hormone concentrations, body growth and carcass composition, and gastrointestinal tract development and selected gene expression are reported elsewhere (Daniels et al., 2008; Hill et al., 2008; Velayudhan et al., 2008; respectively).

MATERIALS AND METHODS

Animals and Treatments

The Virginia Tech Institutional Animal Care and Use Committee approved all animal procedures. This study was designed as a randomized complete block experiment. Heifers were managed and fed according to Daniels et al. (2008). Briefly, 24 Holstein heifers were acquired from a single commercial dairy farm within 3 d of birth (40.4 ± 2.2 kg of BW) and blocked into groups of 8 in the order acquired. Heifers were housed individually and fed 1 of 4 MR diets (n = 6/diet): control (**CON**; 20% CP, 21% fat MR fed at 441 g of DM/d), high protein, low fat (**HPLF**; 28% CP, 20% fat MR fed at 951 g of DM/d), high protein, high fat (**HPHF**; 27% CP, 28% fat MR fed at 951 g of DM/d), and high protein, high fat plus (**HPHF+**; 27% CP, 28% fat MR fed at 1,431 g of DM/d). Heifers were fed twice daily; water and starter (20% CP, 1.43% fat) were offered ad libitum. Diets are further characterized in Table 1. At 1 mo of age (32 d ± 0.4; LSM ± SEM) heifers were injected intravenously with 5 mg of 5-bromo-2'-deoxyuridine (**BrdU**) per kg of BW daily for 4 d. The BrdU (Sigma Chemical Co., St. Louis, MO, cat no. B5002-5G) solution was made in sterile 0.9% NaCl and contained 20 mg of BrdU/mL (pH 8.5). One heifer (group 1; HPLF) died unexpectedly at 6 wk of age from acute peritonitis and endotoxemia and was not replaced; all data from that animal were excluded.

Mammary Tissue Sampling

Thirty days after the last BrdU injection (65 d of age ± 0.4; LSM ± SEM) and an overnight fast, heifers were harvested to evaluate body composition (reported in Hill et al., 2008) and mammary development. Heifers were killed by phenobarbital injection (Euthasol, 10 mg/kg BW; Virbac AH Inc., Fort Worth, TX) and immediately exsanguinated. Udders were removed. The whole udder was weighed and bisected along the median suspensory ligament. The right hemi-udder was reweighed, wrapped in foil, submerged in liquid nitrogen, and stored at −80°C until later compositional analysis. The left rear quarter was sampled for later RNA analysis and the left front quarter was sampled for histology. At the time of slaughter, it was noted that