J. Dairy Sci. 100:1–9
https://doi.org/10.3168/jds.2016-11826

Effects of preweaning nutrient intake in the developing mammary parenchymal tissue

F. Soberon* and M. E. Van Amburgh†[1]
*Nutreco USA, 115 Executive Drive, Highland, IL 62249
†Department of Animal Science, Cornell University, Ithaca, NY 14850

ABSTRACT

Historically, mammary gland growth has been considered isometric the first 2 mo of life and then allometric until peripuberty. However, recent work indicated that the mammary gland might be responsive to nutrient intake preweaning. The objectives of this study were to describe the effects of nutrient intake preweaning on mammary gland development and to investigate cell specific proliferation during this phase of development. Twelve dairy heifer calves were fed either a fixed amount of milk replacer (MR; control, n = 6) or an amount of MR adjusted for BW (enhanced, n = 6). Control calves received a constant amount of a 28% crude protein, 15% fat milk MR per day that was equivalent to 2.8 Mcal of metabolizable energy intake per day; enhanced calves received 0.3 Mcal of metabolizable energy intake per kilogram of metabolic body weight (from 4.2 to 8.4 Mcal of metabolizable energy intake per day). All calves had constant access to water and a 22% crude protein commercial calf starter. Calves were killed at 54 ± 2 d. Control calves consumed 32.6 ± 2.4 kg of MR and 6.7 ± 0.5 kg of calf starter per calf, whereas the enhanced calves consumed 69.5 ± 2.4 kg of MR and 1.9 ± 0.5 kg of calf starter per calf over the 54-d period. Further, to evaluate putative stem cell proliferation, BrdU (5-bromo-2′-deoxyuridine; 5 mg/kg) was injected intramuscularly once per day between 12 to 15 d and again once per day between 24 to 27 d of life. Initial and final body weight for the control and enhanced treatments were 39.2, 61.0, 39.7, and 83.2 kg, respectively. At euthanasia, weights of liver, kidneys, pancreas, whole skinned mammary gland, and mammary parenchyma were measured. The growth rate of each organ was calculated using the concept of allometry as the difference in the change in organ weight as a percentage of body weight. The mammary glands of calves fed the enhanced diet were significantly heavier at euthanasia; when mammary parenchymal weight was analyzed, enhanced calves had 5.9 times greater mammary parenchymal mass, indicating the mammary gland was responsive to nutrient intake before weaning. Allometric growth of the mammary gland was initiated preweaning in the calves fed the enhanced treatment. Further characterization of mammary cells that retained BrdU label revealed no significant differences among the tissue slices analyzed between treatments; however, as calves fed the enhanced diet had more mammary parenchymal mass, if the number of label-retaining cells per counted slide were similar between treatments then the enhanced calves had a larger total population of putative mammary stem cells present in the mammary gland.

Key words: mammary gland, preweaning nutrition, growth rate

INTRODUCTION

Historically, prepubertal mammary development has been described as having 2 distinct phases: isometric and allometric growth (Sinha and Tucker, 1969). Allometry was a term coined by Huxley and Tessier (1936) that describes the ratio between the growth of 2 body parts. When the ratio is equal to 1, then the growth of the 2 parts is considered isometric. In the case of positive allometric growth, the ratio is greater than 1, which implies a faster growth rate than the part of comparison (Huxley, 1950). Prior to approximately 3 mo of age, the bovine mammary gland has been shown to grow at a similar rate to the body (isometric growth), and then from approximately 3 mo of age to the peripubertal period, positive allometric growth has been observed, (Sinha and Tucker, 1969; Meyer et al. 2006b). In contrast, Brown et al. (2005) and Meyer et al. (2006b) reported that mammary epithelial cell proliferation could be influenced by diet during the preweaning period, but not postweaning. In the data of Meyer et al. (2006b), mammary epithelial cell proliferation assessed by BrdU (5-bromo-2′-deoxyuridine) labeling as a marker for DNA proliferation was allo-

Received August 4, 2016.
Accepted February 15, 2017.
[1]Corresponding author: mev1@cornell.edu

metric from birth in calves fed higher levels of nutrient intake, suggesting that the previously observed phases of growth in the data of Sinha and Tucker (1969) might have been due to differences in nutrient intake relative to maintenance, at least before weaning. Furthermore, Meyer et al. (2006b) suggested that cells within the mammary gland are nutritionally responsive in the early neonatal period. Given the emerging data describing the effects of early nutrition on long-term productivity (Moallem et al., 2010; Soberon et al., 2012; Soberon and Van Amburgh, 2013), there is a need to understand the factors responsible for this long-term enhancement of productivity.

Further, Ellis and Capuco (2002) and Capuco (2007), using the approach of BrdU label-retaining cells (**LRC**), described the identification of a primary proliferative cell population in the mammary parenchyma, considered a putative stem cell population. Capuco (2007) indicated this population was mixed, with some cells being estrogen-sensitive progenitors, and this might provide some insights into the mechanism behind the observation that increased nutrient intake and higher ADG in preweaned calves results in greater milk yield through the growth of more progenitor cells during the early postnatal development of the mammary gland. Increasing the number of putative stem cells, if they are progenitor cells, would provide one possible mechanism for greater milk yield through greater cell production of secretory cells in the mammary gland at the onset of lactation. For this experiment, our hypothesis was that mammary cells and the mammary gland in general are nutrient-responsive during the preweaning phase of development; thus, proliferation can be altered during this period as an outcome of specific cells types. Therefore, the objectives of our study were to determine the effects of the preweaning feeding program on organ size, mammary gland development, and putative stem cell proliferation in dairy calves using the LRC approach.

MATERIALS AND METHODS

All protocols involving the use of animals were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (Protocol number 2009–0120). Calves for this study were born between July 24, 2010, and August 1, 2010. Twelve Holstein heifer calves from the Cornell Research Farm (Harford, NY) were randomly assigned at birth to 1 of 2 treatments (**TRT**). All calves received 4 L of colostrum within 1 h of birth and 2 L after another 12 h. Calves assigned to the enhanced TRT were fed 0.3 Mcal of ME intake per kilogram of metabolic BW ($BW^{0.75}$) in 3 daily feedings; the amount of milk replacer (**MR**) was adjusted weekly according to changes in BW. Daily amounts of energy received by each calf increased from 4.2 to 8.4 Mcal of ME intake energy per day. Calves in the control group were fed 2.8 Mcal of ME per day in 2 daily feedings throughout the study; this TRT was designed to reflect the previous industry-standard preweaning MR feeding rates. All calves were fed a milk replacer containing 28% protein and 15% fat (Excelerate, Milk Specialties Inc., Carpentersville, IL; Table 1). Milk replacer refusals were recorded at each feeding. Starting on d 18, calves were offered a commercially available starter grain (22% CP, Cargill Inc., Minneapolis, MN; Table 1); starter grain consumption was recorded daily. Fresh water was available at all times.

Table 1. Milk replacer and starter grain chemical composition as reported by manufacturer

Analysis (DM basis)	Milk replacer[1]	Starter grain[2]
CP, %	28.5	22.0
Crude fat, %	15.0	4.3
NDF, %	0.2	33.1
Calcium, %	1.0	1.6
Phosphorus, %	>0.6	1.0
Vitamin A, IU/g	>16.5	43.1
Vitamin D_3, IU/g	>5.5	NA[3]
Vitamin E, IU/kg	>110.3	197.0
Gross energy, Mcal/kg	5.1	1.8

[1]Excelerate, Milk Specialties Inc., Carpentersville, IL.

[2]Cargill Inc., Minneapolis, MN.

[3]NA = not available.

All calves were housed in individual hutches bedded with sawdust throughout the study. Body weights and hip heights of each calf were measured weekly 1 h after the morning feeding. Calves received milk replacer until they were killed at 54 d. All calves received 8 daily intravenous injections of BrdU (5-bromo,2′-deoxyuridine; Sigma-Aldrich, Saint Louis, MO) at a concentration of 5 mg/kg of BW to label DNA in putative stem cells (Smith, 2005; Capuco, 2007; Huderson et al., 2011); injections were given from d 12 to 15 and 24 to 27 of study. Two injection periods were used to ensure the label was available through the early growth phase of the study. Daniels et al. (2009) injected BrdU for 1 period and Meyer et al. (2006b) used only 1 injection at the time of euthanasia; however, little information exists concerning mammary proliferation throughout the preweaning period, and we wanted to ensure we could capture any possible changes. Therefore, we chose to provide the label over 2 periods, as the putative stem cells should be the only cells that retain the label.

On the day of euthanasia, calves were fed at 0700 h, weighed, and loaded for transport to the Cornell University abattoir (Ithaca, NY). Calves were killed by stunning with a captive bolt, followed directly by