Maternal nutrient restriction followed by realimentation during early to midgestation on mammary gland development in beef cows

L.E. Camacho¹, C.O. Lemley², J.S. Haring¹, P.P. Borowicz¹, D.M. Hallford³, K.C. Swanson¹ and K.A. Vonnahme¹

The objective of this study was to examine the effects of early to midgestation maternal nutrient restriction followed by realimentation on mammary gland development in beef cows. The results indicate that nutrient restriction (40 percent less than National Research Council [NRC] recommendations) during early to midgestation does not appear to impact mammary gland weight; however, composition may be altered.

Introduction

Beef cows commonly are managed in grazing systems where forage quality varies according to regional conditions. Forage quality or availability often is poor, affecting the nutritional and physiological status of the animal (Funston et al., 2010). During this period of reduced nutrient availability, the dam will undergo a series of metabolic and physiologic adaptations to protect her body stores from depletion as the increase in nutrient demands by the conceptus occurs (Rosso and Streeter, 1979).

Maternal nutrition during pregnancy not only plays an important role in fetal and placental growth and development, but mammary development as well. To continue to nourish the offspring after birth, the mammary gland needs to be developed properly. Mammary gland milk production depends on several factors; one of them is the amount of secretory cells (alveoli) in the gland that secretes milk (Anderson et al., 1985). In addition, maternal nutrition also affects milk composition and production (Miranda et al., 1983).

Our laboratory (Swanson et al., 2008; Vonnahme et al., 2011) previously reported that nutritional plane during gestation decreased mammary gland size and proliferation, and altered mammary gland vascularity in sheep. Meyer et al. (2011) reported decreased colostrum and milk production in nutrient-restricted ewes; moreover, this decrease in milk production continued after ewes were realimented to control

Summary

On day 30 of pregnancy, multiparous, nonlactating cows (initial body weight [BW] = 620.5 ± 11.3 kilograms [kg], body condition score [BCS] = 5.1 ± 0.1) were assigned to three different dietary treatments: control (C; 100 percent NRC; n = 18) and restricted (R; 60 percent NRC; n = 28). On day 85, cows were slaughtered (C, n = 6; R, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12), or were realimented to control (RC; n = 11). On day 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On day 254, all remaining cows were slaughtered.

The diet consisted of grass hay to meet 100 or 60 percent net energy (NE) recommendations for maintenance and fetal growth and to meet or exceed metabolizable protein (MP) recommendations. At slaughter, mammary glands were removed and weighed immediately. Glands were analyzed for fat and cellular proliferation, and quantitative real time polymerase chain reaction (qPCR) was used to determine messenger ribonucleic acid (mRNA) expression of vascular endothelial growth factor (VEGF) and its receptors (fms-related tyrosine kinase 1 [FLT1] and kinase insert domain receptor [KDR]).

Mammary gland weight was not affected (P ≥ 0.15) by treatment. Fat (percent) did not differ (P ≥ 0.35) at days 85 and 140; however, at day 254, RRC and RCC cows had less (P = 0.02) fat vs. CCC. Maternal dietary treatment had no effect (P ≥ 0.45) on mammary alveolar cellular proliferation. We found no treatment effect (P ≥ 0.27) on mRNA expression of VEGF, FLT1, and KDR.

Nutrient restriction during early to midgestation does not appear to impact mammary gland weight; however, composition may be altered. Further information is needed to determine how nutritional interventions could improve lactation in beef cattle.

¹Animal Sciences Department, NDSU
²Animal and Dairy Sciences Department, Mississippi State University
³Animal and Range Sciences Department, New Mexico State University
diets during lactation (Meyer et al., 2011).

We hypothesize that longer nutrient restriction would impact maternal mammary gland development negatively compared with controls. Our objectives were to determine the effects of realimentation after maternal nutrient restriction during early to midgestation on mammary gland development.

**Experimental Procedures**

All procedures involving animals were approved by the NDSU Animal Care and Use Committee. A total of 54 nonlactating, multiparous crossbred beef cows of similar genetic background were synchronized using a Select Synch plus progesterone insert (CIDR; Pfizer Animal Health, New York, N.Y.) and fixed-time AI (TAI) protocol.

At the NDSU Beef Research and Teaching Unit (Fargo, N.D.), cows were assigned to one of six breeding groups, with breeding dates ranging from July 13 to Oct. 24, 2011. Cows received GnRH (100 µg as 2 mL of Factrel i.m.; Fort Dodge Animal Health, Fort Dodge, Iowa) and a CIDR on day 0. On day seven, CIDR devices were removed and cows were given an injection of PGF2α (25 mg as 5 mL of Lutalysie i.m.; Pharmacia & Upjohn Co., Kalamazoo, Mich.). Estroprotect Heat Detectors (Rockway Inc., Spring Valley, Wis.) were used to monitor estrous behavior for a minimum of 72 ours. Artificial insemination was performed utilizing the a.m./p.m. rule 12 hours after the first detected estrus. Cows not detected in estrus after 72 hours received a second GnRH injection and TAI was performed.

Inseminated cows were transported to the Animal Nutrition and Physiology Center (ANPC; Fargo, N.D.) within three days post-insemination. From arrival at the ANPC until confirmed pregnant, cows were grouped in pens (n = 4 to 5) and trained to use the Calan gate feeding system. At this time, all cows were fed chopped grass hay (8.02 percent crude protein [CP], 69.2 percent neutral detergent fiber [NDF], 41.5 percent acid detergent fiber [ADF] and 57.9 percent total digestible nutrients [TDN] [dry-matter, or DM, basis]), and a mineral and vitamin supplement to meet NE recommendations for maintenance and fetal growth and to meet or exceed recommendations for MP, minerals and vitamins (NRC, 2000) until pregnancy was confirmed. The hay net energy for maintenance (NE\textsubscript{m}) concentration was predicted using equations described by Weiss (1993) and NRC (2000).

On days 27 and 28 post-insemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 megahertz). Nonpregnant cows restarted the same breeding protocol. On day 30 of pregnancy, cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) were assigned randomly to dietary treatments: control (C; 100 percent NRC; n = 18) and nutrient restriction (R; 60 percent NRC; n = 28). On day 85, cows were slaughtered (C, n = 6 and R, n = 6), remained on control (CC, n = 12) and restricted (RR, n = 12) treatments, or were realimented to control (RC; n = 11). On day 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5) or were realimented to control (RRC, n = 6). On day 254, all remaining cows were slaughtered (CCC, n = 6; RCC, n = 5; RRC, n = 6).

The control diet consisted of grass hay (Table 1) to meet 100 percent NE recommendations for maintenance and fetal growth (NRC, 2000) and to meet or exceed MP recommendations. Nutrient-restricted cows received 60 percent of the same control hay diet. Cows were fed individually once daily in a Calan gate system at 10 a.m. and had free access to water. The mineral and vitamin supplement (Trouw dairy VTM with optimins; Trouw Nutrition International, Highland, Ill.) was top-dressed three times per week at a rate of 0.18 percent of hay dry-matter intake (DMI) to meet or exceed mineral and vitamin requirements relative to dietary NE intake (NRC 2000). Cows were weighed weekly at approximately 8 a.m. throughout the experiment, and dietary intake was adjusted relative to BW.

On days 85, 140 and 254, a randomly selected subset of cows from each treatment was slaughtered at the NDSU Meat Laboratory. The mammary gland was removed, weighed and processed. Glandular tissue from the mammary gland was snap-frozen in super-cooled isopentane (submerged in liquid nitrogen) and stored at minus 80°C until analysis for mRNA expression and fat content (Neville et al., 2010). Mammary gland mRNA was analyzed for relative expression of vascular endothelial growth factor (VEGF) and its receptors fms-related tyrosine kinase 1 (FLT1), and kinase insert domain receptor (KDR). Also, glandular tissue was fixed for proliferation analysis via histology using Ki-67 as the proliferation marker. Statistical analysis was performed to interpret our results.

**Results and Discussion**

Mammary gland weight was not affected (P ≥ 0.15) by dietary treatment. Average mammary gland

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<th>Ingredient</th>
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<tr>
<td>Ash</td>
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weights, regardless of dietary treatment, were 9.96 ± 1.30, 7.30 ± 1.01 and 13.67 ± 0.97 pound at days 85, 140 and 254 of gestation, respectively. Similarly, mammary gland weight did not differ (P ≥ 0.16) when expressed relative to eviscerated BW. Mammary gland fat (percent) did not differ (P ≥ 0.35) among groups at day 85 (35.1 ± 12.1 percent) and day 140 (31.6 ± 14.8 percent); however, at day 254, RRC and RCC cows had less (P = 0.02) fat vs. CCC (12.65 ± 2.2 and 13.88 ± 2.4 vs. 22.06 ± 2.2 percent, respectively). Maternal dietary treatment had no effect (P ≥ 0.45) on mammary alveolar cellular proliferation at day 85 (average = 0.74 ± 0.15 percent), day 140 (average = 0.82 ± 0.14 percent) and day 254 (average = 0.82 ± 0.12 percent) of gestation. We found no dietary treatment effect (P ≥ 0.27) on mRNA expression of VEGF, FLT1 and KDR at days 85, 140 and 254 of gestation.

The only mammary gland parameter measured in the current study that was influenced by nutrient restriction was fat content within the gland. Perhaps a depletion of this energy source would impact milk performance negatively. Our laboratory (Swanson et al., 2008; Vonnahme et al., 2011) previously reported that sheep fed 60 percent of nutrient recommendations during gestation had decreased mammary gland size and proliferation and altered mammary gland vascularity, compared with control ewes.

In the current study, maternal nutrient restriction in beef cows followed by realimentation did not affect maternal mammary gland weight or cellular proliferation. What is important to note is that the beef cow mammary gland might be less sensitive to nutrient restriction, compared with sheep, at the time points we have investigated. In addition, the restriction periods in Swanson et al. (2008) and ours are different and perhaps will have a different impact on mammary gland responses.

Nutrient restriction in crossbred dairy cows from two weeks before calving to 11 weeks postpartum resulted in decreased mammary gland weight and lower number of mammary cells, compared with control diets. However, mammary gland epithelial cell proliferation was not affected by nutrient restriction (Des sage et al., 2010). Because more mammary gland growth occurs after parturition, perhaps we did not investigate glandular growth long enough.

Previously, Swanson et al. (2008) showed that maternal nutrient restriction during late gestation decreased postpartum colostrum, which matches the decreased mammary gland weight of the underfed ewe. In addition, cellular proliferation in the alveoli of mammary glands from nutrient-restricted ewes was decreased, while the alveolar area was increased (Swanson et al., 2008).

In the current study, bovine mammary cellular proliferation was not altered by nutrient restriction followed by realimentation from early to midgestation. Neville et al. (2010) previously reported that mammary glands from ewes that were restricted to 60 percent of NRC recommendations had an increase in VEGF mRNA expression. In the present study, we did not alter mRNA expression of VEGF and its receptors due to nutrient restriction during early to midgestation.

In sheep, the mammary gland grows exponentially during pregnancy and continues to grow during early lactation until peak lactation, and it is controlled by hormones (Anderson et al., 1985). Mammary gland growth is slow during early pregnancy, but as pregnancy advances, the growth is accelerated (Anderson et al., 1985). Prolactin (PRL) plays an important role in the maintenance of mammary gland function (Flint and Knight, 1997), and synergistically with other mammotrophic factors, can control mammary gland development (Brisken et al., 1999). In our study, maternal nutrient restriction followed by realimentation did not affect PRL concentrations prior to slaughter or through gestation.

Maternal nutrient restriction during early to midgestation followed by realimentation does not appear to impact mammary gland weight; however, fat content was decreased. Our laboratory is analyzing mammary gland samples and serum samples for vascularity and other hormones involved in mammary gland development.

In conclusion, nutrient restriction during early gestation appears to alter mammary gland fat content without affecting weight and cellular proliferation. More research is necessary to further understand the effects of nutrient restriction followed by realimentation on mammary gland development and milk composition in beef cows.

Acknowledgments

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Literature Cited


