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Feeding a higher plane of nutrition and providing exogenous estrogen increases mammary gland development in Holstein heifer calves

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ABSTRACT

Feeding heifers a higher plane of nutrition postweaning but before puberty can negatively affect mammary gland development and future milk yield. However, enhanced nutrition preweaning may promote development and future production. Our objectives were to determine the effects of enhanced feeding preweaning and exogenous estrogen immediately postweaning on mammary gland development and the composition of the mammary parenchyma (PAR) and mammary fat pad (MFP). Thirty-six Holstein heifer calves (<1 wk old) were reared on 1 of 2 dietary treatments for 8 wk: (1) a restricted milk replacer fed at 0.45 kg/d (R; 20% crude protein, 20% fat), or (2) an enhanced milk replacer fed at 1.13 kg/d (EH; 28% crude protein, 25% fat). Upon weaning, calves from each diet (n = 6) were given either a placebo or estrogen implant for 2 wk, creating 4 treatments: R, R + estrogen (R-E2), EH, and EH + estrogen (EH-E_2). Calves were housed individually with ad libitum access to water. Starter feeding began at wk 5 and was balanced between treatments. Udders were evaluated by palpation and physical measurements weekly. Subsets of calves were killed at weaning (n = 6 per diet) and at the conclusion of the trial (n = 6 per treatment). Udders were removed, dissected, and weighed. At wk 8, EH calves had longer front and rear teats. Providing estrogen to EH calves increased the length of rear teats during wk 9 and 10. Enhanced-fed calves had 5.2-fold more trimmed mammary gland mass than R calves. Providing estrogen to EH calves further increased mammary gland weight. Masses of PAR and MFP were markedly greater for EH calves than for R calves (e.g., 7.3-fold greater PAR tissue). Estrogen increased the mass of both PAR and MFP in EH calves. Feeding a higher plane of nutrition increased total protein, DNA, and fat in the MFP and total protein and DNA in the PAR. Dual-energy x-ray absorptiometry estimates of mammary fat mass were highly correlated with biochemical analyses of fat content. From histological study, we observed that the degree of expansion of epithelium into the adjacent stromal tissue and the complexity of ductal development were minimal in R, increased in EH, and increased by estrogen in both dietary treatments. Results provide compelling evidence that preweaning nutrition and estrogen administration immediately postweaning markedly increase mammary gland development in dairy calves. Cellular and molecular mechanisms responsible for these differences are currently under study.
Key words: mammary gland, milk replacer, estrogen, calf

INTRODUCTION

The expense of raising heifers accounts for about 20% of annual dairy farm costs (Heinrichs, 1993). Consequently, producers seek to have heifers calve as early as practically possible to decrease their nonproductive period of life. To achieve earlier calving, heifers must reach puberty at an earlier age. Because puberty and BW are highly correlated, early puberty and calving can be achieved by increasing rates of gain (Sejrsen et al., 1982; USDA, 2007).

However, it is widely reported that an excessive rate of gain during the prepubertal period, which can sometimes result from trying to get calves to their pubertal BW earlier, can impair mammary growth and reduce future milk yield (Sejrsen et al., 1982; Lammers et al., 1999; Radcliff et al., 2000). Precise mechanisms responsible for these effects are not completely understood but are likely a combination of a shorter peripubertal allometric mammary growth phase and altered responses to mammogenic stimuli [e.g., estrogen (**E_2**); Meyer et al., 2006a,b], or both.

Before weaning, the dairy calf consumes a nutrient-dense, milk-based diet. During this time, feeding for an increased rate of gain does not impair mammary development (Daniels et al., 2009a). In fact, several studies (Brown et al., 2005; Meyer et al., 2006b) suggest that a higher plane of nutrition preweaning can stimulate

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mammary development. Soberon et al. (2012) reported that increasing ADG by 1 kg/d during the preweaning period was correlated with an increase in first-lactation milk yield of more than 1,000 kg. Additionally, as emphasized in the comprehensive review by Khan et al., (2011), improved preweaning nutrition benefits not only the growth but also the health and performance of the dairy calf.

Although the mechanisms responsible for the positive correlation between preweaning gain and future milk potential are unknown, it is clear that mammary gland development during early life is essential to future productivity. Consensus was that mammary parenchyma (**PAR**) growth was minimal during the preweaning period, until the onset of allometric mammary growth before puberty (Sinha and Tucker, 1969), but recent data have shown that allometric growth of the mammary PAR begins much earlier than once believed. Indeed, PAR mass can increase 60-fold from 30 d until approximately 90 d of life (Capuco and Akers, 2010; Esselburn et al., 2015).

Our hypothesis is that providing heifers with a higher plane of nutrition during the preweaning period creates a mammary gland and mammary cells that are primed to respond more readily to mammogenic stimuli. Estrogens are classic mammogenic hormones produced primarily by the ovaries (Yart et al., 2014). Moreover, mammary tissue in prepubertal ruminants is sensitive to E_2 (Woodward et al., 1993; Ellis et al., 1998; Capuco et al., 2002). Despite the fact that circulating concentrations of E_2 are very low in prepubertal calves (Purup et al., 1993; Velayudhan et al., 2012, 2015) when E_2 influence is removed from the calf via ovariectomy or the use of an E_2 receptor antagonist, mammary gland development is markedly reduced (Berry et al., 2003; Tucker et al., 2016). Perhaps enhanced early nutrition augments the action of mammogenic stimuli (such as E_2); for this reason, we selected E_2 to test our hypothesis.

Lammers et al. (1999) assessed the effects of exogenous E_2 and plane of nutrition on mammary gland development in older heifers beginning at 4.5 mo of age over a period of 20 wk. They noted that E_2 treatment increased teat lengths, but E_2 administration and feeding for an increased rate of gain actually decreased future milk yield by 5.2 and 7.1%, respectively. An increase in teat length as a result of E_2 treatment or exposure to E_2 in the diet has been used as a noninvasive bioindex for E_2 activity, and the effect has been noted in studies with beef heifers (Moran et al., 1991) and lambs (Ellis et al., 1998; Mahgoub et al., 2001).

To test our hypothesis, we fed calves 2 distinct preweaning diets. We have reported the effects of these diets on general body growth and the development of multiple organs (Geiger et al., 2016). In this report, we describe the effects of these dietary treatments on the udder, mammary PAR, and mammary fat pad (**MFP**). We also describe the effects of E_2 (Preston, 1999) administered during the 2 weeks postweaning on the same mammary parameters. We hypothesized that calves fed a higher plane of nutrition preweaning and given E_2 immediately postweaning would experience increased mammary gland development compared with controls.

MATERIALS AND METHODS

This experiment was conducted under the review and approval of the Virginia Polytechnic Institute and State University Institutional Animal Care and Use Committee (#14-045-DASC).

Animal Handling and Experiment Design

The experimental design and animal handling were as previously described (Geiger et al., 2016). Briefly, calves were assigned to 1 of 2 experimental milk replacers (n = 18 per group): (1) a restricted milk replacer (**R**; 20% CP, 20% fat; Southern States Cooperative Inc., Richmond, VA) fed at 0.45 kg/calf per day, or (2) an enhanced milk replacer (**EH**; 28% CP, 25% fat; Land O'Lakes Animal Milk Products Co., Shoreview, MN) fed at 1.13 kg/calf per day. Starter (22% CP, 3.5% fat, 8.0% crude fiber; Southern States Cooperative Inc.) was offered at the end of wk 4 of the trial. A subset of calves (n = 6 per diet) was killed upon weaning to assess the effects of diet on mammary gland development. The remaining calves (n = 24) were given an E_2 implant (Compudose, Elanco Animal Health, Greenfield, IN) or a placebo implant at weaning. This produced the following treatment groups (n = 6 per treatment): (1) calves fed a restricted diet and given a placebo implant (**R**), (2) calves fed a restricted diet and given an E_2 implant (**R-E2**), (3) calves fed an enhanced diet and given a placebo implant (**EH**), and (4) calves fed an enhanced diet and given an E_2 implant (**EH-E2**). After 2 wk of E_2 treatment, all remaining animals were killed to assess the effect of E_2 on calves fed the 2 different diets.

Sample Collection

Noninvasive mammary gland measures were collected once weekly and included front and rear teat length, distance from front to rear teats, distance between front teats, distance between rear teats, and gland length and width. All mammary measures (e.g., distance be-