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## The effects of increasing amounts of milk replacer powder added to whole milk on mammary gland measurements using ultrasound in dairy heifers

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### ABSTRACT

The aim of this study was to determine the effect of increasing total solids (TS) in the liquid diet during the preweaning phase on mammary gland development in dairy heifers. The increase in TS was obtained with the addition of milk replacer powder to whole milk. Cross-breed Holstein-Gyr heifers ( $n = 60$ ) were distributed in 4 treatments with different TS concentrations: 13.5% ( $n = 15$ ), 16.1% ( $n = 15$ ), 18.2% ( $n = 15$ ), and 20.4% ( $n = 15$ ). The liquid diets were provided from 5 to 55 d of age. From 56 to 59 d of age, the total amount of liquid feed was reduced by half. Heifers were weaned at 60 d and monitored until 90 d of age. Ultrasound mammary gland evaluations were performed weekly between 5 to 11 wk of age, using a B-mode ultrasound equipped with microconvex transducer at a frequency of 6 MHz. In those same weeks, the manual palpation of mammary parenchyma (PAR) was performed. Increased TS concentration of liquid diet during the preweaning period increased body weight of heifers, but did not alter PAR growth and the deposition of adipose tissue in the mammary gland evaluated by ultrasonography. The oval-shaped structure of PAR was altered after 2 mo of age. In the evaluated period, PAR growth was isometric with respect to the body growth rate. Palpation scoring of PAR had a strong correlation with the ultrasound evaluation of the PAR.

**Key words:** development, parenchymal area, palpation score

### INTRODUCTION

The conventional calf-feeding program adopted on dairy farms consists of feeding 10% of BW of liquid feed (Raeth-Knight et al., 2009), and this practice is also

known to stimulate starter intake and promote early rumen development (Azevedo et al., 2016a). However, the performance and feed efficiency of this system are often associated with unsatisfactory results (Khan et al., 2007). In intensive calf-feeding, higher amounts of nutrients are provided by increasing the total volume of liquid feed (Silper et al., 2014; Leão et al., 2016; Rosenberger et al., 2017), by increasing TS in milk replacer (MR; Terré et al., 2009; Chapman et al., 2016; Hill et al., 2016), or by the addition of milk balancer (Glosson et al., 2015) or milk replacer powder (MRP; Azevedo et al., 2016a,b).

According to Sejrsen and Purup (1997) and Radcliff et al. (2000), high ADG rates before puberty (90–320 kg of BW) in Holstein heifers may negatively affect the development of the mammary gland, with consequent lower milk yield in the first lactation. Weller et al. (2016) reported an alteration in mammary development with increased nutrient intake in prepubertal Holstein-Gyr heifers, with gains from 0.5 to 1.0 kg/d. The consequence is an increase in body fat content and fat accumulation in mammary gland, which may compromise PAR growth in heifers; however, during the preweaning phase (before 2 mo of age), this same effect has not been reported (Daniels et al., 2009). Meyer et al. (2006) and Geiger et al. (2016a,b) reported greater mammary development in heifers submitted to intensive feeding programs. According to Brown et al. (2005), the increased growth rate before weaning seems to have positive effects on mammary gland growth, as the increase in nutrient supply at that age almost doubled the PAR mass of heifers. This demonstrates that the rapid growth until weaning is not detrimental to mammary gland development. In this way, the effects of the nutrition plan on mammary gland development seem to differ between preweaning and prepubertal period, which may be due to the low fat content of the young calf at birth.

Evaluation of mammary gland development (PAR and mammary fat pad) in heifers is usually done after slaughter (Brown et al., 2005; Geiger et al., 2016a,b;

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Weller et al., 2016). This technique does not allow for the monitoring the animal during its development and presents high costs, which limits the research. In this context, alternative and noninvasive methods to reduce time and cost of such analyzes become important. Ultrasonography is a widely used technique for assessing fat deposition in animal carcasses (Arnold et al., 1991), and it has been used to evaluate developmental characteristics and lesions of the mammary gland in dairy calves and heifers (Nishimura et al., 2011; Esselburn et al., 2015, Albino et al., 2017). Another alternative used to monitor the development of the mammary gland in dairy heifers is palpation scoring, which is easy, does not present a cost in its execution, but is a subjective technique (Esselburn et al., 2015).

The objective of our study was (1) to determine the effect of increasing TS of whole milk (WM) by the addition of MRP in the mammary gland development of dairy heifers from wk 5 to 11 of age, using ultrasonography; and (2) to evaluate the relationship between the weekly measurements of PAR growth using ultrasound with the manual palpation scoring. Our hypotheses were (1) heifers fed higher TS contents in a liquid diet would have greater PAR growth in relation to heifers fed lower TS concentrations; and (2) the palpation scoring may be an option to monitor PAR development in dairy heifers.

## MATERIALS AND METHODS

The Ethics Committee of Embrapa Dairy Cattle, Brazil (protocol no. 06/2014), approved the study. The trial was conducted at the Embrapa Dairy Cattle Experimental Farm, located in Coronel Pacheco, Minas Gerais, Brazil.

### *Animals, Housing, Management, and Performance*

Holstein-Gyr crossbred heifers ( $n = 60$ ) were used; their genetic composition was 5/8 or more Holstein and 3/8 or less Gyr. These heifers were previously part of the study conducted by Azevedo et al. (2016a) to evaluate the effect of increasing solids concentration by the addition of MRP to WM on performance, intake, and health of heifers from 5 to 90 d of age. Detailed descriptions of the facilities, initial care, and dietary composition from preweaning (5–59 d of age) to postweaning (60–90 d of age) can be found in Azevedo et al. (2016a). One heifer (treatment 17.5% TS) was removed from the study in the last week of the postweaning period due to an accident that led to the fracture of its anterior right leg.

The animals were randomly distributed in 4 treatments after birth. Preweaning treatments consisted of

increasing amounts of MRP (Sprayfo Violet SSP, Sloten BV, Deventer, the Netherlands; 20% CP and 33% ether extract) added to WM ( $12.6 \pm 0.7\%$  TS, mean  $\pm$  SD; Table 1) to adjust the TS to expected concentrations of 12.5% (actual TS =  $13.5 \pm 0.53\%$ ;  $n = 15$ ), 15.0% (actual TS =  $16.1 \pm 0.03\%$ ;  $n = 15$ ), 17.5% (actual TS =  $18.2 \pm 0.14\%$ ;  $n = 13$ ), and 20.0% (actual TS =  $20.4 \pm 0.24\%$ ;  $n = 15$ ). The initial TS content in the WM was measured daily, immediately after milking and before each feeding, using a Brix refractometer (DD-3 Palm Abbe Digital, Misco, Solon, OH). Brix grade values were converted to TS content using the equation proposed by Moore et al. (2009) [ $TS = 0.9984 \times (\text{Brix refractometer reading}) + 2.077$ ] and the amount of MRP to be added to the WM was adjusted to achieve the desired TS content for each treatment. The MRP was added to the WM immediately before feeding the heifers. The total volume of treatment (6 L/d) was divided into 2 equal meals (0800 and 1600 h) and provided to heifers in buckets from 5 to 55 d of age. At 56 d of age, the total volume of liquid feed was reduced by half (3 L/d, provided at 0800 h only) and heifers were weaned at 60 d of age. Starter (Soylac Rumen 20% Flocculated, Total Alimentos, Três Corações, Brazil) and water were offered ad libitum during the preweaning period. At 70 d of age, corn silage was included ad libitum in a separate bucket.

### *Mammary Ultrasound*

Ultrasound images were obtained weekly between wk 5 to 11 of age, always by the same evaluator, using a B-mode ultrasound equipped with microconvex transducer at a frequency of 6 MHz (DP 2200, Mindray, Shenzhen, China). For the evaluation of the mammary glands (front and rear quarters), the animals were laid on lateral decubitus with the probe in a standardized position at a 45° inclination in relation to the teat position, always in caudal-cranial direction (Nishimura et al., 2011). To increase the contact of the probe with the skin and to improve the visualization of the images, the area to be evaluated was wetted with 70% alcohol and the ultrasound gel was placed (Carbogel ULT, Sao Paulo, Brazil).

The images obtained were saved in BMP format and then transferred to the ImageJ program (National Institutes of Health; <https://imagej.nih.gov/ij/docs/guide/user-guide.pdf>) for further analysis. In ImageJ, the scale of pixels per centimeter ( $1 \text{ cm}^2 = 48,148$  pixels) was calibrated using the straight tracer, and PAR areas were resultant by tracing the PAR area with the mouse and cursor using the freehand tracer.

Within in PAR area, we used number the pixels to calculate the average pixel brightness. In 8-bit images,

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