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Technical note: Mammary gland ultrasonography to evaluate mammary parenchymal composition in prepubertal heifers

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ABSTRACT

Bovine mammary gland development studies are often terminal or involve invasive biopsy procedures. Therefore, noninvasive means of assessing mammary development should be considered as alternative methods in live animals. The objective was to test if mammary ultrasonography can be used as a noninvasive way to estimate mammary parenchyma (PAR) composition in prepubertal dairy heifers with different average daily body weight gains. In the 84 d preceding, the ultrasound exam heifers were maintained in 1 of 3 treatment groups. Individual heifers were fed a high gain (1 kg/d; n = 6), low gain (0.5 kg/d, n = 6), or maintenance (n = 6) treatment diet. To achieve desired body weight gains, heifers were fed differing amounts of the same silage-based diet. Mammary glands of 18 crossbred heifers Holstein:Gyr underwent a single mammary ultrasound exam immediately before heifer slaughter, which took place when heifers weighed 142.0 ± 8.0 kg and were 200 d old. The 4 mammary glands of each heifer were evaluated using a real-time B-mode ultrasound machine equipped with a 6.5-MHz microconvex transducer. Digital images (8-bit) of glands were obtained and PAR was identified within gland. Average pixel values per unit of PAR area were determined for each gland and analyzed at the level of heifer. Pixel results were interpreted on the basis that lower average pixel values reflect PAR with relatively high amounts of protein as opposed to fat. To help validate that the pixel value within PAR is associated with composition of PAR, pixel findings were compared with histological [number of adipocytes in PAR (Nad) and epithelial area in PAR (Ep)] and biochemical [percent crude protein in PAR (%CP), percent ether extract in PAR (%EE), PAR weight (WPAR), and mammary fat pad weight (WFAT)] composition of PAR in these same heifers. Within PAR, %EE and WFAT were positively correlated with pixel values, whereas %CP, Ep, and Nad were negatively correlated. Parenchyma weight did not correlate with pixel values. Regression analyses (fixed effect log-pixel value; random effect treatment) were used to estimate Nad, Ep, %CP, %EE, WPAR, and WFAT. Sensitivity analysis of regression equations revealed that accuracy of tested equations ranged from 0.77 to 0.93 and precision ranged from 0.56 to 0.82. Concordance correlation coefficients of the equations ranged from 0.41 to 0.76. In conclusion, ultrasonography of PAR can accurately measure and predict PAR composition in prepubertal dairy heifers growing at various rates of gain.
Key words: dairy heifer, ultrasound, mammary gland

Technical Note

Mammary glands consist of 2 major types of tissues that serve different functions. As summarized by Esselburn et al. (2015), these are (1) epithelial tissue and surrounding stromal elements, collectively called mammary parenchyma (**PAR**), and (2) stromal tissue that lies adjacent to PAR and does not contain epithelial structures, and instead mainly consists of connective tissue structures such as adipose. For this reason, mammary stroma is often referred to as mammary fat pad.

Understanding of growth dynamics of these mammary tissues as well as the influence of nutrition on mammary gland development has been the subject of study over the years (Sejrsen et al., 1983; Brown et al., 2005; Meyer et al., 2006). Esselburn et al. (2015) recently showed that in heifers younger than 2 mo of age, obtaining weekly PAR measurements via ultrasound was an effective quantitative tool for measuring changes in PAR area in vivo. Although estimation of PAR composition via ultrasonography was not an experimental objective of Esselburn et al. (2015), those authors noted that such estimation is feasible. That was the motivation for our current work and is a distinguishing feature of this study.

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Ultrasound has been widely used in the meat industry to monitor both subcutaneous fat thickness and intramuscular fat accumulation (Williams, 2002). The measurement of intramuscular fat accumulation in carcasses is possible due to differences in the way that fat and protein reflect sound waves in body tissues (Brethour, 1990).

Our hypothesis was that mammary ultrasonography can be used as a noninvasive way to estimate PAR composition in prepubertal dairy heifers with different ADG.

The study was conducted in the Animal Science Department of the Universidade Federal de Viçosa, Viçosa-MG. The institutional ethics committee approved all procedures (protocol number, 20/2015). Eighteen crossbred heifers Holstein:Gyr were used in this experiment; they were part of a larger experiment that dealt with effects of nutrient intake on mammary growth and composition. Full experimental details are reported elsewhere (Weller et al., 2016). Briefly, heifers were allotted to 1 of 3 dietary treatments when they were 3 to 4 mo of age (BW, 102.2 ± 3.4 kg). Treatments were high gain [**HG** (n = 6); 1.0 kg/d of BW gain], low gain [**LG** (n = 6); 0.5 kg/d of BW gain], and maintenance [**MA** (n = 6); 0.0 to 0.1 kg/d of BW gain]. The MA treatment was kept slightly above 0.0 kg/d of ADG to avoid mobilization of body tissues. To achieve desired BW gains, heifers were fed differing amounts of the same silage-based diet. The amount of ration offered was adjusted every 2 wk based on heifer BW gains. Heifers were housed and fed individually; the experimental unit was heifer.

At the end of the 84-d experiment, heifers were slaughtered (140.0 ± 8.0 kg and 200 d old) to assess body and mammary composition (Weller et al., 2016). Relevant dependent variables reported by Weller et al. (2016) and also used here include number of adipocytes in PAR (**Nad**), epithelial area (**Ep**), percent crude protein of PAR (**%CP**), percent ether extract of PAR (**%EE**), PAR weight (**WPAR**), and mammary fat pad weight (**WFAT**) in these same heifers. For full description of methodology on these 6 dependent variables, the reader is directed to Weller et al. (2016).

The 4 mammary glands of each heifer were evaluated in a single ultrasound exam before slaughter using a real-time B-mode ultrasound machine equipped with a 6.5-MHz micro-convex transducer (DP2200, Mindray, Shenzhen, China). Heifers remained standing for the ultrasound exam; sedatives were not used. Commercial acoustic gel (Chattanooga Group Inc., Chattanooga, TN) was applied to each gland before ultrasounding. A single operator performed all ultrasound exams.

With the heifer restrained in a standing position, the lubricated micro-convex transducer was applied to the base of each teat at a 45° angle, in a caudal-to-cranial placement (Figure 1A). This procedure was obtained from Nishimura et al. (2011) and adapted (Albino et al., 2015).

Still image capture software available on the ultrasound machine was used to capture two 8-bit digital images for each gland (Figure 1B). These images were visually inspected and the image with the best PAR definition was chosen; subsequent evaluations were done based on 4 images per heifer.

The PAR within each digital image was evaluated using ImageJ software (National Institutes of Health, Bethesda, MD). The PAR is hypoechoic (black) on ultrasound when compared with the mammary fat pad (Esselburn et al., 2015), which is hyperechoic (white). Therefore, the most hypoechoic region of each image was identified as PAR (Figure 1B). Within the area identified as PAR (3 squares each measuring 0.4 cm^2) were randomly overlaid onto the image in ImageJ (Figure 2).

Finally, an average pixel value per unit area was determined for each square using a conversion factor

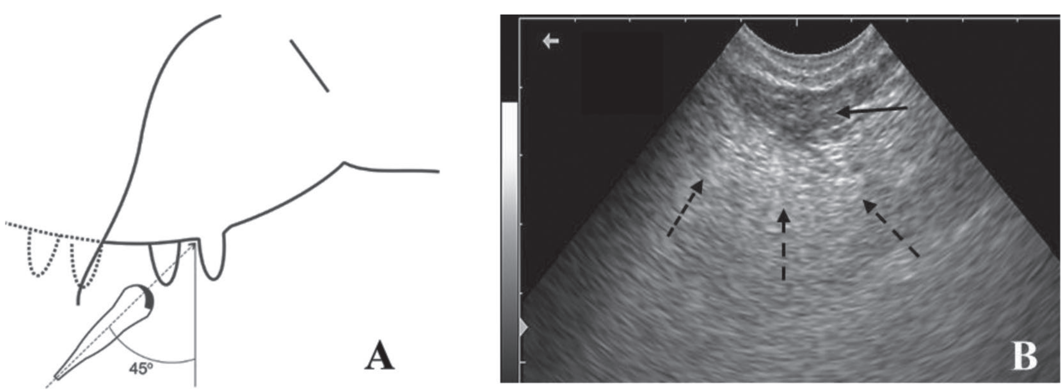

Figure 1. Drawing of the probe position at the heifer's mammary gland (Albino et al., 2015; A) and ultrasound image (B) with dashed arrows representing the mammary fat pad and solid arrows representing the parenchymal area.