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Examination of weekly mammary parenchymal area by ultrasound, mammary mass, and composition in Holstein heifers reared on 1 of 3 diets from birth to 2 months of age

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ABSTRACT

Monitoring in vivo growth of mammary parenchyma (PAR) has historically been difficult, necessitating slaughter studies to measure PAR quantity. Advances in ultrasound (US) technology warrant revisiting its use as a noninvasive tool to monitor PAR growth in vivo. The level of nutrient intake during the first 2 mo of life may affect measures of mammary growth and composition. Objectives were to examine the utility of US as an in vivo tool to quantify PAR cross-sectional area in Holstein heifers reared on 1 of 3 diets from birth to 2 mo of age, assessing potential dietary effects; assess the relationships between weekly US measurements, teat length, manual palpation of PAR scores, and PAR mass at 2 mo of age; and examine mammary composition in experimental animals. Holstein heifers (n = 24; 41 ± 1 kg initial body weight) from a single farm were randomly assigned to 1 of 3 milk replacers that differed in source and amount of fat. Milk replacer was fed at 660 g of dry matter/d until weaning at 42 d. Heifers had ad libitum access to a common calf starter (20% crude protein) and water for the duration of the 56-d trial. Teat length and palpation scores were obtained weekly. A real-time B-mode US with a 7.5-MHz convex probe was used to examine 2-dimensional PAR area in all 4 glands of heifers once weekly from 2 to 3 d of age to harvest at 56 d. The left front and left rear glands were also examined by US 24 h postharvest to validate final US measurements, and then bisected to produce a sagittal plane view of PAR for comparison with US images. Mass and composition of mammary gland tissue were determined at 8 wk using standard methodology. Over the course of this 8-wk trial, average teat length increased from 11 to 17 mm. The PAR area started small (6.6 ± 3.2 mm^2 per gland) and increased to 42.1 ± 2.5 mm^2 per gland by the end of the trial. As anticipated, based on measurements obtained at slaughter, US measurements were more related to amount of PAR (r = 0.74) than either teat length (r = 0.34) or palpation scoring (r = 0.63). Importantly, US is quantitative, whereas palpation scoring is subjective. Diet did not affect mass or composition of PAR in young heifers; total udder PAR mass averaged 1.40 ± 0.80 g. In conclusion, we showed that in heifers younger than 2 mo of age, obtaining weekly PAR measurements via ultrasound is an effective quantitative tool for measuring changes in PAR area in vivo. Future studies may incorporate and expand upon the methods developed here to determine what quantitative evaluation of PAR in young heifers can reveal about milk production capacity.
Key words: dairy heifer, mammary, ultrasound

INTRODUCTION

Initial growth and development of mammary glands begins in utero. When a heifer is born, a rudimentary mammary ductal system is present (reviewed in Rowson et al., 2012). The duct system extends from the teat cistern to the gland cistern and ends with the epithelial ducts. This epithelial tissue and its surrounding stromal elements (loose connective tissue, blood, and lymph vessels) are collectively known as mammary parenchyma (**PAR**). The mammary fat pad (**MFP**) lies adjacent to PAR and does not contain epithelial structures, only stromal elements. Mammary epithelial structures are derived from the embryonic ectoderm and stromal structures are derived from the embryonic mesoderm. Thus, any given mammary gland contains 2 major types of tissue that serve different functions. Tubulo-alveolar epithelial structures have the capacity to make and secrete milk, whereas stromal tissues primarily provide support and contribute to gland shape.

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Mammogenesis includes expansion of both PAR and MFP and occurs in embryonic, fetal, prepubertal, pubertal, and pregnant lifecycle stages, with most mammogenesis occurring during pregnancy.

Akers et al. (2005) and Meyer et al. (2006b) both note that in heifers younger than 30 d of age it is difficult to detect PAR and mass of PAR is typically around 150 mg/gland. By 90 d of age, mass of PAR can exceed 10 g/gland. This represents a near 60-fold increase in tissue mass and hints at a growth rate that far exceeds general body mass during this same period. This argues against isometric growth of PAR (with respect to body mass) and favors an allometric pattern of mammary growth in young heifers. To date, it is the general belief that an increased level of nutrient intake during the first 2 mo of age does not impair PAR growth in heifers, and may actually enhance growth (Brown et al., 2005a; Meyer et al., 2006a; Daniels et al., 2009). More research is needed on the effects of diet on PAR accretion in young heifers.

Advances in understanding potential effects of early life mammary growth (with and without nutrition program comparisons) and resultant long-term effects on PAR development and udder function have been hindered because of the need for serial slaughter data and the relatively large number of animals required to accurately evaluate mammary development over time (Brown et al., 2005a; Meyer et al., 2006a; Daniels et al., 2009). These studies have been informative; however, in each case, animals never realized lactation (Brown et al., 2005a; Meyer et al., 2006a; Daniels et al., 2009). Therefore, determination of PAR quantity and growth in young heifers in vivo would allow the same animals for longevity studies wherein milk yield is monitored in lactation.

Teat length measurement has been used in past studies as a noninvasive way to monitor mammary growth over time. Teat length increases when exogenous estrogen is administered (Moran et al., 1991; Lammers et al., 1999), and as a result, teat length has been used as a de facto bioassay to examine relative amount of circulating estrogen in prepubertal heifers. Estrogen stimulates PAR ductal elongation and branching, and is required for prepubertal mammary development (Purup et al., 1993). Work by Capuco et al. (2012) indicates that estrogen initiates paracrine signaling to promote mammary stem cell growth. Therefore, the effect that estrogen has on teat length may enable an indirect measurement of mammary epithelial cell development. However, at least one study showed no correlation between teat length and amount of secretory tissue present (Whitlock et al., 2002), potentially negating its usefulness as a noninvasive tool for measuring amount of PAR.

Manual palpation scoring of PAR has also been used as a noninvasive means of monitoring mammary growth over time. Documented ideas for how to do this were published as early as 1955, with experiments taking place in the preceding years. One example is from the doctoral studies of Donoho (1955); the use of calipers was employed as a means to measure size of PAR within each gland. It was hoped that this technique could be used to predict future milk production (Donoho, 1955). However, Donoho (1955) noted that the caliper method was difficult to perform due to amount and location of PAR within each animal. A second, perhaps more well known, example of a palpation technique was published in a Technical Bulletin by the USDA (Swett et al., 1955). The technique was established for 3- to 6-mo-old heifers; a relationship between palpation grade and milk yield was reported for the conditions of that experiment, but it was communicated that the procedure was not likely to be widely implemented because of the subjective nature of the evaluation, the time that it would take for the technician to be trained, and the high possibility of error (Swett et al., 1955). To illustrate the potential pitfalls of that technique in practice, a researcher in the United Kingdom attempted to use the exact technique developed by Swett et al. (1955) in a field trial that collected measurements on 727 four-month-old heifers (Elliot, 1957). First-lactation records were obtained for 244 of the heifers. Palpation grade at 4 mo was not correlated with first lactation milk yield (Elliot, 1957). Although the study was conclusive, the author commented that the experiment had notable limitations: the effect of nutrition was not considered and palpation scores were only obtained once before calving (Elliot, 1957). In the decades since these initial reports were published, it seems the pursuit of minimally invasive ways to monitor PAR growth fell out of favor, perhaps due in large part to technological limitations and conflicting results.

Advances in ultrasound (**US**) technology have enabled researchers to obtain in vivo quantitative information that has not been formerly accessible. Examination of mammary tissue by US has been previously reported in goats to examine gland cistern size (Nudda et al., 2000); dairy goats, dairy ewes, and dairy cattle to examine teat characteristics (Díaz et al., 2013, Alejandro et al., 2014, and Vetter et al., 2014, respectively); and goats to assess pathological changes of gland tissue (Fasulkov et al., 2014). Fasulkov (2012) provides a review of mammary US in ruminants. Nishimura et al. (2011) performed a single-event, nonquantitative US on Holstein heifers aged 2 to 25 mo. To our knowledge, no US experiment has been performed over time on heifers younger than 2 mo of age. The hypothesis was that 2-dimensional (**2D**) area of PAR, as measured by