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Computed tomography study of the fetal development of the dairy cow stomach complex

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ABSTRACT

In the fetal development of animals, critical physiological and anatomical events influence the long-term health and performance of the offspring. To identify the critical growth phases of the fetal bovine stomach, we used computed tomography imaging on 30 German Holstein fetuses to examine the fetal bovine stomach in situ. Computed tomography allows the study of diverse parameters such as the volume of the stomach chambers in situ without the need for sophisticated filling preparation techniques. The absolute volume, relative volume, and monthly volume increase of each stomach chamber were determined. Computed tomography was a reliable method for in situ examination of the fetal bovine stomach complex from the third month of gestation onward. It was able to detect an abnormal position of the abomasum in 2 fetuses. The crown-rump length of the fetuses studied ranged from 9.5 to 89 cm (from 2.2 to 8.3 mo of gestation). Over this timeline, the changes in the relative volumes of the ruminoreticulum and abomasum were inversely related. Until mo 5 of gestation, the relative volume of the ruminoreticulum increased steadily, whereas that of the abomasum decreased. Thereafter, the relative volume of the ruminoreticulum became gradually smaller, and that of the abomasum became larger; by mo 8, the abomasum was larger than the ruminoreticulum. All stomach chambers had large increases in volume over the gestation period and we observed differences in development patterns and volume changes of the individual stomach chambers over this period. The largest monthly volume increase of the stomach complex was between mo 4 and 5 of gestation. In this period, the volume of the ruminoreticulum increased 43.8 times, that of the omasum 38.9 times, and that of the abomasum 30.03 times. Between mo 5 and 6 of gestation, the abomasum had another growth spurt, with a monthly volume increase of 10.4 times. These 2 time points in the gestation period may be critical phases of fetal development that should be considered in the management of pregnant cattle.
Key words: cow stomach, intrauterine programming, fetal development, abomasum

INTRODUCTION

The evolution of the ruminant stomach complex occurred about 50 million years ago and was the result of adaptive processes for the efficient use of cellulose-based diets (Langer, 1988; Hofmann, 1989). This has resulted in modern ruminants having a suite of complex morphological changes that occur both prenatally and postnatally to allow the neonate to effectively and efficiently digest milk as its primary diet and then to transition to a plant-based diet.

During fetal development of the ruminant stomach, complex growth and differentiation processes result in a stomach having 4 functional compartments: rumen, reticulum, omasum, and abomasum. After birth, the initial dependence on milk and subsequent transition to a plant-based diet is critical. During the transition period of 2 to 3 mo, the forestomachs (rumen, reticulum, omasum) must develop both morphologically and physiologically to undertake their roles in the microbial fermentation of plant material after weaning (Silper et al., 2014).

Recent studies have shown that metabolic programming of calves postpartum plays a major role in their lifetime performance and productivity (Moallem et al., 2010; Soberon et al., 2012; Soberon and Van Amburgh, 2013). A meta-analysis of 12 neonatal studies in cattle showed that long-term milk productivity was promoted by increased nutrient intake from milk or milk replacer during the preweaning period (Soberon and Van Amburgh, 2013). Intrauterine development is at least as important as extrauterine development in the subsequent health and performance in later life (Bell, 2006; Funston et al., 2010). Intrauterine programming de-

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scribes the process by which stimuli at critical phases of fetal development during gestation create long-lasting changes in tissue structure and function (Fowden et al., 2006; Opsomer et al., 2016). The timing, duration, and exact nature of the stimuli are crucial determinants of the pattern and extent of intrauterine growth and of specific physiological outcomes (Fowden et al., 2006).

By the end of the bovine embryonic period on d 44 of gestation, the different stomach compartments—ruminoreticulum, omasum, and abomasum—can be distinguished and have an independent shape (Vivo et al., 1990). In a 110-mm-long (mo 3) bovine fetus, the shape and position of the stomach is very close to its final appearance (Schummer, 1932). In the subsequent fetal period, the stomach chambers grow in size and their proportions change. The development of the fetal bovine stomach has been described in several previous studies (Becker et al., 1951; Wardrop and Coombe, 1961; Kano et al., 1981; Schnorr and Kressin, 2011). However, those studies were mainly observations of formalin-fixed stomachs or used filling preparation techniques (Kano et al., 1981). Recently, computed tomography (**CT**) has been used to demonstrate the in situ development of the bovine stomach (Flor et al., 2012). Computed tomography scanning generates sets of data that can be operated to demonstrate various organs and tissues based on their ability to absorb the x-ray beam, and allows these data to be reformatted in various planes or as volumetric (3-dimensional, **3D**) visualizations of structures. The post-processing methods facilitate qualitative and quantitative evaluation of structures, including determinations of volume, area, or distance. Finally, CT allows the study of diverse parameters such as the volume of the stomach chambers without the need for complex filling preparation techniques.

We hypothesized that the stomach compartments of fetal cattle develop at different rates during gestation and that critical development phases exist that should be considered. The aim of the present study was to investigate the pattern of development of the bovine stomach of German Holstein fetuses using CT, focusing on volumetric and topographic changes of the stomach complex from mo 3 to 9 of gestation.

MATERIALS AND METHODS

Animals

Thirty bovine fetuses (16 female and 14 male; Table 1) obtained from a slaughterhouse (Teterower Fleisch GmbH, Teterow, Germany) were used in this study. The fetuses were taken from German Holstein cows slaughtered between October 2011 and June 2013. The history of the slaughtered cows was unknown and there were no multiple fetuses. Approximately 30 min after each cow had been killed, its fetus was collected and sex was determined immediately.

The crown-rump length (**CRL**) of each fetal calf was measured with the fetus in lateral recumbency. Using a measuring tape, the CRL was measured as a straight line from the foramen occipital magnum (opisthion) to the first caudal vertebra. The age of the fetuses (mo) was estimated according to Michel (1995) where gestational month $= \sqrt{(\mathrm{CRL} + 1)} - 1$. The BW of fetuses less than 2,000 g was measured using an electronic scale (Univers; Petermann, Dombühl, Germany). Heavier animals were weighed using a separate electronic scale (PJ 3600 DeltaRange; Mettler, Gießen, Germany). Then, fetuses were transported immediately within a cool box to the CT room.

CT

Computed tomography of the abdomen was performed to evaluate the morphology and morphometry of the stomach complex. The CT scan was done using a third-generation 16-slice scanner (Phillips Brilliance, Phillips Healthcare, Hamburg, Germany). To prevent deformation of stomach compartments by gravitational effects, the fetuses were positioned on the table in a supine position and held in place using foam wedges and hook-and-loop fastening straps. They were scanned using standardized scan parameters (voltage 120 kV, 200 mA; collimated slice thickness: 16 × 0.75 mm; increment: 1.5 mm; pitch: 0.938).

For volumetric determinations, 2-mm images were reconstructed without overlap. Because the junction between the rumen and reticulum could not be identified reliably on the CT images, the 2 compartments were considered a single functional unit, the ruminoreticulum. Basic image analysis was performed and the volume of each stomach chamber (ruminoreticulum, omasum, and abomasum) was calculated using Amira 5.4 software (FEI Visualization Sciences Group, Mérignac, France). A manual segmentation technique was used to separate each stomach chamber from the surrounding context. The image segmentation of the stomach chamber was carried out by hand in the axial plane, along their inner contour at the junction of the soft tissue and the fluid filled lumen of each stomach compartment (Figure 1, panels A, B, and C). The segmentation process was controlled along all 3 view axes. To validate the method, the segmentation process was performed twice at different times by the same person for all 30 fetuses and the mean value taken.

The position of the stomach chamber was defined in all 3 axes and the craniocaudal extent of the ruminoreticulum, omasum, and abomasum was determined