Technical note: Comparison between two tracing methods with ultrasonography to determine lumen area of the caudal artery in beef cattle

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ABSTRACT: Two experiments were conducted to compare variation between 2 tracing methods in measuring cross-sectional lumen area of the caudal artery in 5 beef heifers on 3 different dates (Exp. 1) and to compare tracing methods in detecting changes in artery lumen area after 5 heifers were switched from a diet containing nonendophyte-infected tall fescue [Schedonorus arundinaceus (Schreb.) Dumort] seed to one containing endophyte-infected tall fescue seed (Exp. 2). Lumen area determined by tracing the Doppler flow signal was 25% less than that determined by tracing the intima of the connective tissue, but there was no difference (P = 0.90) in the variation of measures between the 2 methods in Exp. 1. Declines in lumen area were detected at the same level of significance (P < 0.01) for both tracing methods after cattle in Exp. 2 were switched from noninfected to infected tall fescue diets. Variation in lumen areas was different between noninfected and infected diets with tracing the Doppler flow signal (P < 0.05) or the intima of the connective tissue (P < 0.01). Results indicated that lumen area of the caudal artery can be measured with similar precision by tracing the intima of the connective tissue in the artery wall or the outer boundary of the Doppler flow signal.

Key words: artery area, caudal artery, Doppler ultrasonography, ergot alkaloid

INTRODUCTION

Doppler ultrasonography has been used to evaluate blood flow characteristics in dogs (Brown et al., 1991; Lee et al., 2004) and horses (Cipone et al., 1997; Raisis et al., 2000). The technology also has potential use as a noninvasive diagnostic or research tool in evaluating hemodynamics of cattle that are exposed to plants composed of toxic secondary metabolites.

Measurements of cross-sectional areas of vessels are needed to quantify blood flow rate and determine if vessels are in a constricted or relaxed state (Aiken et al., 2007). Ultrasound units and transducers used in human medicine and research have the resolution that is necessary to measure blood vessel diameter at the interface between the lumen and the endothelial intima (Poulin and Robbins, 1996; Shaw et al., 2007). Resolution of ultrasound units and transducers that are often used by veterinary practitioners and researchers is generally insufficient to discern the endothelial intima in the blood vessel (Figure 1A, B). Therefore, lumen areas often are traced using either inner aspects of the elastin tissue layer at the interface between the adventitia-media layers in the artery wall (Cipone et al., 1997; Meinders and Hoeks, 2004) or the outer boundary of the flow signal obtained with Doppler color ultrasonography (Aiken et al., 2007). However, differences in precision between the 2 tracing methods are not known. A study was conducted with cattle to determine if there are differences in the variation of lumen area measurements of the caudal artery between tracing the interface between the adventitia-media layers or the outer boundary of the color Doppler flow signal methods, and if the 2 tracing methods have the precision needed to detect a vasoconstriction response to ergot alkaloids.

MATERIALS AND METHODS

Two experiments were conducted with crossbred (Angus × Brangus) heifers (12 to 14 mo) that were on diets devoid of ergot alkaloids from birth until the beginning
of the experiment. The experimental protocol was reviewed and accepted by the Institutional Animal Care and Use Committee at the University of Kentucky.

**Experiment 1**

Five crossbred Angus × Brangus heifers [mean initial BW = 293 ± 35 (SD) kg] were maintained on free-choice corn silage-concentrate rations and ultrasonically scanned on 3 dates (Feb. 20, Feb. 22, and Mar. 2, 2007). Cross-sectional ultrasound scans of the caudal artery at the 4th coccygeal vertebrae were taken using an Aloka 3500 Ultrasound Unit (Aloka Inc., Wallingford, CT) with a UST-5542 linear array (13 MHz) transducer set to a 2-cm depth. Ten B-mode images (Figure 1C) and 10 Doppler flow images (Figure 1D) were taken to determine cross-sectional artery lumen area. Overall gain was set at 90 and the flow gain was set at 14; the maximum gain before noise became apparent. A pulse frequency of 6 MHz was used for B-mode and Doppler flow images. Sample volume was set at 0.5 mm, which sets the size of the pulsed-wave Doppler region to be examined. Following the freezing of an individual flow image, frames stored in the cine memory of the unit (42 frames with a 15-Hz frame rate) were searched to store the image exhibiting the maximum flow signal and assumed to be at peak systolic phase. For B-mode scans, the inner aspect of the acoustic reflection from the elastin connective tissue in the artery wall was used to trace for estimation of lumen area. For color Doppler images, the outer aspect of the flow signal in the artery was traced for estimation of lumen area.

For each tracing method, individual measurements of lumen area from 10 images were taken for 5 heifers on 3 dates. Comparisons between tracing method variances and means were made. For comparing tracing method means, the experimental design was a split plot where the main unit had a randomized complete block design with 2 tracing method treatments and 5 heifers as a block effect; the subunit was a repeated measure over 3 dates, and multiple images on each heifer × tracing method × date were subsamples. Tracing method

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![Figure 1](image-url)
Experiment 2

Variance of lumen area measurements for each tracing method, without assuming common variance between tracing methods, consisted of variance between images plus variance between dates and heifers partitioned into 3 components for date, heifer, and date × heifer. This variance was expressed in the following equation:

\[ V(Y_{ijkl}) = \sigma^2_{ij} + \sigma^2_{ik} + \sigma^2_{ijk} + \sigma^2_{i(jk)l}, \quad \text{for each } i, \]

where \(i\) = tracing method, \(j\) = heifer, \(k\) = date, \(l\) = image.

The model used to compare variances was different from the model used to compare means because 1) heifer as a block effect can only be removed from error for mean comparison purposes given an assumption of common variance between treatment effects being compared and 2) the effect of date was considered a random source of error. Variance components, defined above, were analyzed using mixed models (SAS Inst. Inc., Cary, NC). A model allowing different variances for each tracing method was compared with a model assuming equal variances between tracing method with a likelihood ratio test (Littell et al., 2006). Statistical differences were determined using the \(\alpha = 0.10\) level of significance.

**Experiment 2**

Five crossbred Angus × Brangus heifers [mean initial BW = 346 ± 20 (SD) kg] were placed in individual pens and fed a mixture of chopped alfalfa hay (54% DM) and a concentrate (46% DM) that contained ground soybean hulls (47.5% DM), mineral premix (1% DM), molasses (4% DM), and ground nonendophyte-infected tall fescue seed (47.5% DM) for 7 d. Following this 7-d period, the heifers were fed the same chopped alfalfa-concentrate mixture with the exception that the concentrate contained endophyte-infected seed instead of nonendophyte-infected seed. Before feeding, 12 samples of infected seed were assayed for ergovaline (mean = 3.67 μg/g of DM; CV = 2.1%) by HPLC florescence (Yates and Powell, 1988). Sensitivity of detection was 0.1 μg/g of DM. Quantity of endophyte-infected seed was formulated to provide 0.85 μg of ergovaline/g of DM in the total diet.

Cross-sectional ultrasound scans of the caudal artery at the 4th coccygeal vertebrae were taken on d 5 during the period when the diet containing nonendophyte-infected seed was fed to all heifers, and on d 8 during the period when the ration containing endophyte-infected seed was fed. Scanning and measurements followed the same procedures as Exp. 1, with the exception that 3 B-mode and 3 color Doppler flow scans were collected from each heifer during each of the 2 scanning sessions. Differences in cross-sectional lumen area and variation between nonendophyte-infected and endophyte-infected rations were determined for each tracing method using the \(t\)-test procedure of SAS. Measurements were averaged within each heifer × diet combination resulting in heifers being the experimental units. Statistical differences were determined using the \(\alpha = 0.10\) level of significance.

**RESULTS**

**Experiment 1**

Mean cross-sectional lumen area was greater \((P = 0.009)\) with tracing the intima of the connective tissue \((9.6 \text{ mm}^2)\) than with the Doppler flow signal \((7.2 \text{ mm}^2)\). Total variance was 2.41 for connective tissue and 3.65 for Doppler flow signal based on a likelihood ratio test comparing a model with separate variances components for each tracing method to a model with equal variances components for both tracing methods. Variation in measurements did not differ \(\left(\chi^2 = 4.0, \text{ df} = 4, \ P = 0.406\right)\) between the 2 tracing methods. There was a correlation \((r = 0.44, \ P < 0.09)\) between the tracing methods.

**Experiment 2**

Variation in lumen area was different for the noninfected and infected diets by tracing both the Doppler flow signal \((P < 0.01)\) and the intima of the connective tissue \((P = 0.07)\). Mean cross-sectional artery lumen areas were different between nonendophyte-infected and endophyte-infected diets when tracing connective tissue \((P = 0.066)\) or the Doppler flow signal \((P = 0.052)\). Switching from endophyte-free to endophyte-infected diets caused lumen area to decline from 6.3 ± 1.4 to 2.9 ± 0.2 mm² when tracing the Doppler flow signal and from 8.9 ± 1.3 to 5.4 ± 0.5 mm² when tracing the connective tissue.

**DISCUSSION**

Greater lumen area with tracing the connective tissue was expected because layers of smooth muscle and endothelial cells could cause slight overestimation of artery area. Conversely, tracing the Doppler flow signal could underestimate lumen area due to lack of detection of reduced velocity blood flow in the outer aspects of the lumen.

Variation in measurements for Exp. 1 was similar between the 2 tracing methods even though the sources of error between the methods are different. The observer is likely the major source of error in measuring lumen area by tracing the connective tissue. Although observer is a source of error in tracing the flow signal, as previously discussed, there likely are some inaccuracies in detection of blood flow in the outer aspects of the lumen where flow velocities are reduced.

The correlation coefficient was less than 0.50; however, ranges in areas for both methods were low and
could have reduced an ability to detect a close association between the 2 measurements (Cornell and Berger, 1987). Range in lumen areas using connective tissue to trace was 7.2 to 11.5 mm², and the range using the Doppler flow signal to trace was 4.1 to 9.4 mm².

Caudal arteries in Exp. 2 were determined to constrict following the switch from nonendophyte to endophyte-infected diets for both tracing methods. A vasoconstriction response to ergot alkaloids has been shown with the bovine caudal artery (Aiken et al., 2007) and the bovine saphenous vein (Klotz et al., 2007). However, artery lumen areas in the present experiment were more variable with the nonendophyte diet. Aiken et al. (2007) reported greater variation in caudal artery lumen areas and blood flows in heifers on a nonendophyte-infected diet than those on an endophyte-infected diet and concluded that caudal arteries in cattle on nonendophyte-infected diets are more responsive to changes in ambient temperature than those on the endophyte-infected diet. The precision in detecting differences in lumen area between the 2 diets could have been increased if more than 3 ultrasound scans had been collected; however, a previous study showed that 3 ultrasound measures per animal were sufficient to detect changes in blood flow for cattle that were switched from nonendophyte-infected to endophyte-infected tall fescue diets (Aiken et al., 2007).

Conclusions

Results indicated that changes in caudal artery lumen area can be detected with B-mode or color Doppler ultrasound tracing methods after the cattle undergo a vasoconstriction response. Either method can be used to measure lumen area with approximately the same precision.

LITERATURE CITED

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