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# The use of ultrasound to measure muscle depth and area in postmortem Holstein dairy calves

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**ABSTRACT:** Monitoring growth of neonatal dairy calves is a useful management tool to assist producers in achieving goals for reproduction and performance. The goal of this study was to examine ultrasound as an *in vivo* tool to quantify longissimus dorsi muscle (ribeye) linear depth and *extensor carpi radialis* (ECR) and *semitendinosus* (ST) muscle cross-sectional areas in postmortem preweaned Holstein calves. Postmortem preweaned calves ( $n = 137$ , age  $13.1 \text{ d} \pm 15.5 \text{ SD}$ , body weight  $36.5 \text{ kg} \pm 7.2 \text{ SD}$ ) were obtained from two California calf ranches between April and July 2013. Two operators collected ultrasound images of the ribeye, ECR, and ST muscles using an Aloka 500V equipped with a 5-cm 7.5-MHz linear transducer. Ultrasound ribeye linear depth and ECR and ST cross-sectional areas were calculated using the Ultrasound Image Capture System. Ultrasound measurements were compared to dissected (carcass) measures. Carcass ribeye linear depth was estimated using a ruler. Dissected ECR and ST muscle cross-sectional areas were estimated by tracing muscle cross sections onto transparency paper and then photocopying, cutting out, and weighing individual paper muscle tracings. Weights of the tracings were then converted to areas using

the known area of a standard  $8.5 \times 11$  inch paper. Data were analyzed by regressing carcass estimates on observed ultrasound measurements. The coefficient of determination ( $R^2$ ) indicated that ultrasound measurements were most closely associated with carcass measurements for the ST muscle ( $R^2 = 0.60, 0.62$  for operator 1 and 2, respectively) when compared to the ribeye and ECR muscles ( $R^2 = 0.27, 0.41$  for ribeye and  $0.43, 0.32$  for ECR for operator 1 and 2, respectively). The mean bias showed consistent underestimation by the ultrasound measurements when predicting carcass measurements for all three muscles and for both operators (ribeye bias =  $0.15, 0.40$ ; ECR bias =  $0.95, 1.15$ ; and ST bias =  $0.73, 0.27$  for operator 1 and 2, respectively). Operator contributed significantly in explaining a proportion of the variation in the regression equation for the ST muscle only, whereas calf body weight contributed significantly in explaining a proportion of the variation in the regression equation for all three muscles. The results of this study demonstrated that ultrasound measurements of the ST were the most accurate for quantifying the cross-sectional area when compared to both the ECR and ribeye in postmortem Holstein calves.

**Key words:** ultrasound body composition, ultrasound muscle area, ultrasound muscle depth

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## INTRODUCTION

Monitoring the growth of neonatal dairy calves is a useful management tool to assist

producers in improving growth to meet reproduction and performance goals. A variety of methods exist for estimating growth in calves, with the most accurate involving routine weighing of individuals on an electronic scale (Hoffman, 1997). However, surveys have found that dairy producers see this as being too time consuming and labor intensive (Heinrichs et al., 1992). The most common and least expensive method of estimating growth in calves was a heart girth tape with the heart girth circumference corresponding to an estimated body weight (Heinrichs and Hargrove, 1987). Similar measurements include hip height, hip width, and body length measurements to assess skeletal size in neonatal calves. These methods provide body weight and skeletal size information, but fail to define muscle growth, which may be more useful when evaluating nutritional management programs and assessing feed efficiency (Hoffman, 1997).

One tool that could be used to evaluate muscle growth in calves is ultrasonography. Ultrasonography has been used to quantify muscle area and depth in both cattle and small ruminants (Perkins et al., 1992; Hassen et al., 1998; Greiner et al., 2003). In beef cattle, carcass ultrasound data were used to calculate expected progeny differences and improve overall genetic selection of breeding stock (Lambe et al., 2010). Ultrasound was also used to monitor body fat changes in periparturient dairy cows (Ivings et al., 1993; Domecq et al., 1995; Schröder and Staufenbiel, 2006). Small ruminant producers also used ultrasound measurements in their national genetic programs for carcass quality improvement (Edwards et al., 1989; Teixeira et al., 2008; Ripoll et al., 2010). Despite an extensive use of this technology in both beef and dairy cattle and small ruminants, ultrasound has yet to be used to measure muscle growth in neonatal dairy calves.

The purpose of this study was to evaluate the use of ultrasonography for measurement of both muscle depth and area in postmortem neonatal Holstein calves as compared with the actual carcass muscle depth and area, as measured by dissection and tracing. Differences between operator results were evaluated to determine the level of training needed to employ the ultrasound method. Although this research was performed on postmortem preweaned Holstein male calves, relationships between muscle measurements and calf body weight were investigated to determine if ultrasound measurements could be used as a proxy for body weight increases associated with calf growth.

## MATERIALS AND METHODS

Because this experiment used postmortem prewean male Holstein dairy calves that had died on two commercial calf ranches, approval of procedures by an animal care and use committee was not needed.

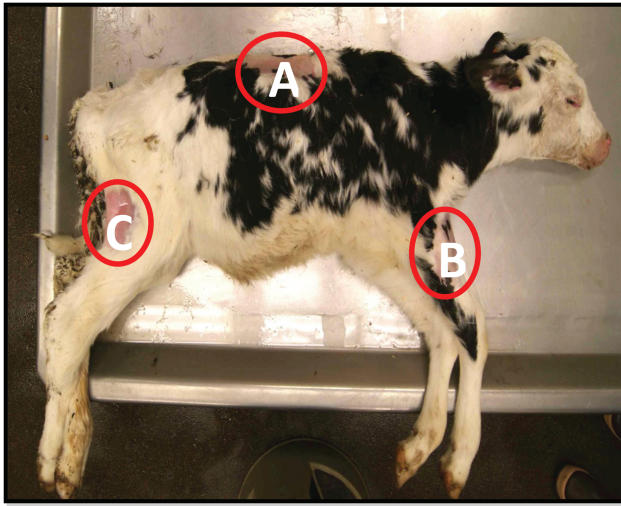
### *Animals and Experimental Procedure*

This prospective cross-sectional study was conducted between April and July 2013 at the Veterinary Medicine Teaching and Research Center (VMTRC) in Tulare, California. Holstein calves were collected from two calf ranches that primarily raise male calves in the San Joaquin Valley of California. Calves that were bloated or emphysematous were excluded from the study. All calves collected were deceased for less than 24 h and the cause of death was unknown. Calves were transported to the VMTRC and weighed using a portable floor scale (Model MTI-500 WB Class III Weigh Systems). Weight, date of birth, and calf ranch were recorded for each calf.

### *Ultrasound Measurements*

Ultrasound measurements of the longissimus dorsi (ribeye), extensor carpi radialis (ECR), and semitendinosus (ST) muscles were performed by two operators. Scanning was performed using an Aloka SSD-500V ultrasound machine with a 5-cm, 7.5-MHz linear transducer. All calves were placed in left lateral recumbency on a metal table in order to minimize errors that could be associated with positioning. Before each ultrasound session, the scanning sites were shaved with a surgical size 40 blade and conductive solution was applied (vegetable oil).

Ultrasound measurements were taken on the right side at three different sites on the calf labeled A, B, and C (Figure 1). The ribeye linear depth was measured between the 12th and 13th ribs, 3 cm lateral and parallel to the spine (Figure 2A). This probe placement enabled capture of an image of the ribeye over its length and muscle depth corresponding to the maximal height between the vertebral transverse processes (Thériault et al., 2009). Ribeye depth was defined between the 12th and 13th ribs delineated as a hyperechoic upside-down “u” on the left and right side of the screen (Figure 2B). The ECR muscle area was measured at proximal end of the ulna, or olecranon, perpendicular to the muscle (Figure 3A) with a



**Figure 1.** Three anatomic sites for ultrasound scanning on post-mortem preweaned Holstein male calves: (A) longissimus dorsi (ribeye), (B) ECR, and (C) ST muscles.

small circle of moderate echogenicity surrounded by hyperechoic lines delineating fascia that surrounds the ECR muscle (Figure 3B). The ST muscle area was measured at the point of the right femoropatellar joint perpendicular to the muscle (Figure 4A) with a cone to heart-shaped area of dense echogenicity surrounded by hyperechoic lines delineating the fascia that surrounds the ST muscle (Figure 4B).

### *Carcass Measurements*

To estimate ribeye depth, a longitudinal cut was made through the ribeye at the level of the 12th and 13th ribs and a plastic ruler was inserted at the approximate midpoint of the muscle to measure depth (Figure 2C). To estimate ECR and ST muscle cross-sectional areas, skin segments covering the ECR and ST muscles were removed and each muscle was dissected (Figures 3C and 4C). Cross-sectional cuts were made in each muscle and transparency paper was superimposed onto the cross-sectional cuts while muscles were stabilized using the left thumb and index finger to trace the cross-sectional area of the muscle (Figures 3D and 4D).

After ultrasound scanning, carcass measurements were made via dissection by both operator 1 and 2. Both operators also traced each muscle. The transparency paper with the muscle tracings was photocopied and the muscle tracings were cut out and weighed in grams (Sartorius LA-120S scale). Grams were converted to area using the following equation (Cruz et al., 2013):

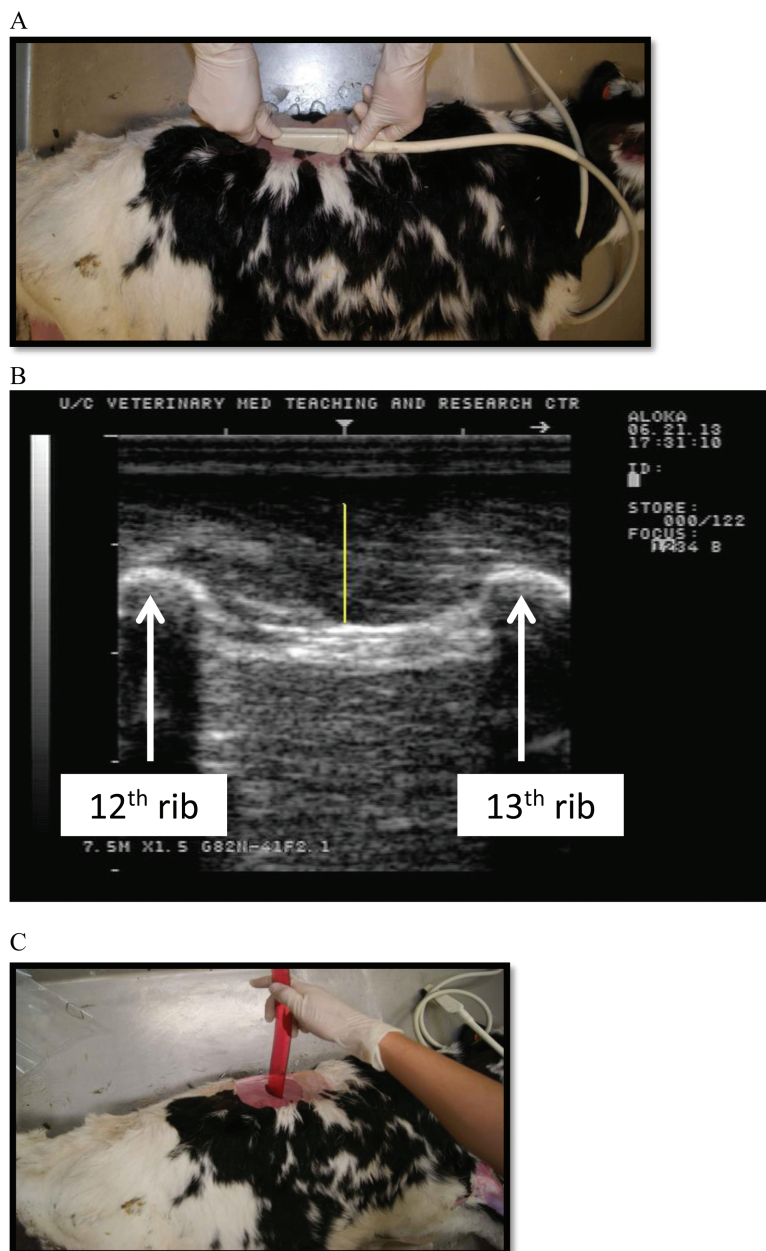
$$\frac{\text{Area of muscle (cm}^2\text{)}}{\text{Area 8.5} \times \text{1 lin paper (603.5 cm}^2\text{)}} = \frac{\text{Weight muscle tracing (g)}}{\text{Weight 8.5} \times \text{1 lin paper (4.672 g)}}$$

### *Image Analysis*

Images were captured and processed using the Ultrasound Image Capture System (UICS) within the Centralized Ultrasound Processing (CUP) Software (UICS, v. 2.0, Walter and Assoc, 2007) at a magnification of 1.5. Estimates of muscle depth using the CUP software were recorded in centimeters and muscle cross-sectional area in square centimeters.

### *Statistical Analysis*

Descriptive statistics of the calf population carcass and ultrasound means, medians, SD, and minimum and maximum values for ultrasound and carcass values for the ribeye, ECR, and ST muscles were obtained using Univariate Procedure of Statistical Analysis System (SAS; v9.2, 2008, SAS Inst. Inc., Cary, NC). The mean bias was defined as the absolute value of the mean of individual ultrasound-carcass measurements and was calculated for each of the three muscles. Pearson correlation coefficients ( $r$ ) were calculated to measure the strength of the relationship between the carcass and ultrasound measurements using the CORR Procedure in SAS. The reported  $P$ -value indicated the probability of observing a correlation at least as extreme as the one observed under the null hypothesis that the correlation equals zero. A  $P$ -value < 0.01 was considered significant. Using linear regression analysis by the GLM and REG Procedures of SAS, carcass muscle measurements were regressed on ultrasound measurements. Coefficient of determination ( $R^2$ ) and the root mean square error (RMSE) were estimated to further examine the relationship between carcass and ultrasound measurements (Herring et al., 1994; Thériault et al., 2009). The  $R^2$  indicated the proportionate amount of variation in the predicted variable  $y$  (carcass) explained by variability in the observed, independent variable  $x$  (ultrasound) in the linear regression model. The RMSE was the SD of the error and estimated the deviation of the predicted carcass values from the regression line. The effects of weight and age were included in the statistical model only if significant.



**Figure 2.** Ultrasound image capture and analysis of longissimus dorsi (ribeye). (A) An ultrasound image of the ribeye muscle depth is captured by aligning the probe 3 cm lateral and parallel to the spine using the 12th and 13th ribs as landmarks. (B) The ribeye depth is outlined in yellow. (C) The hide of the calf was removed and a longitudinal cut was made midway between the 12th and 13th ribs in order to measure the linear depth of the ribeye.

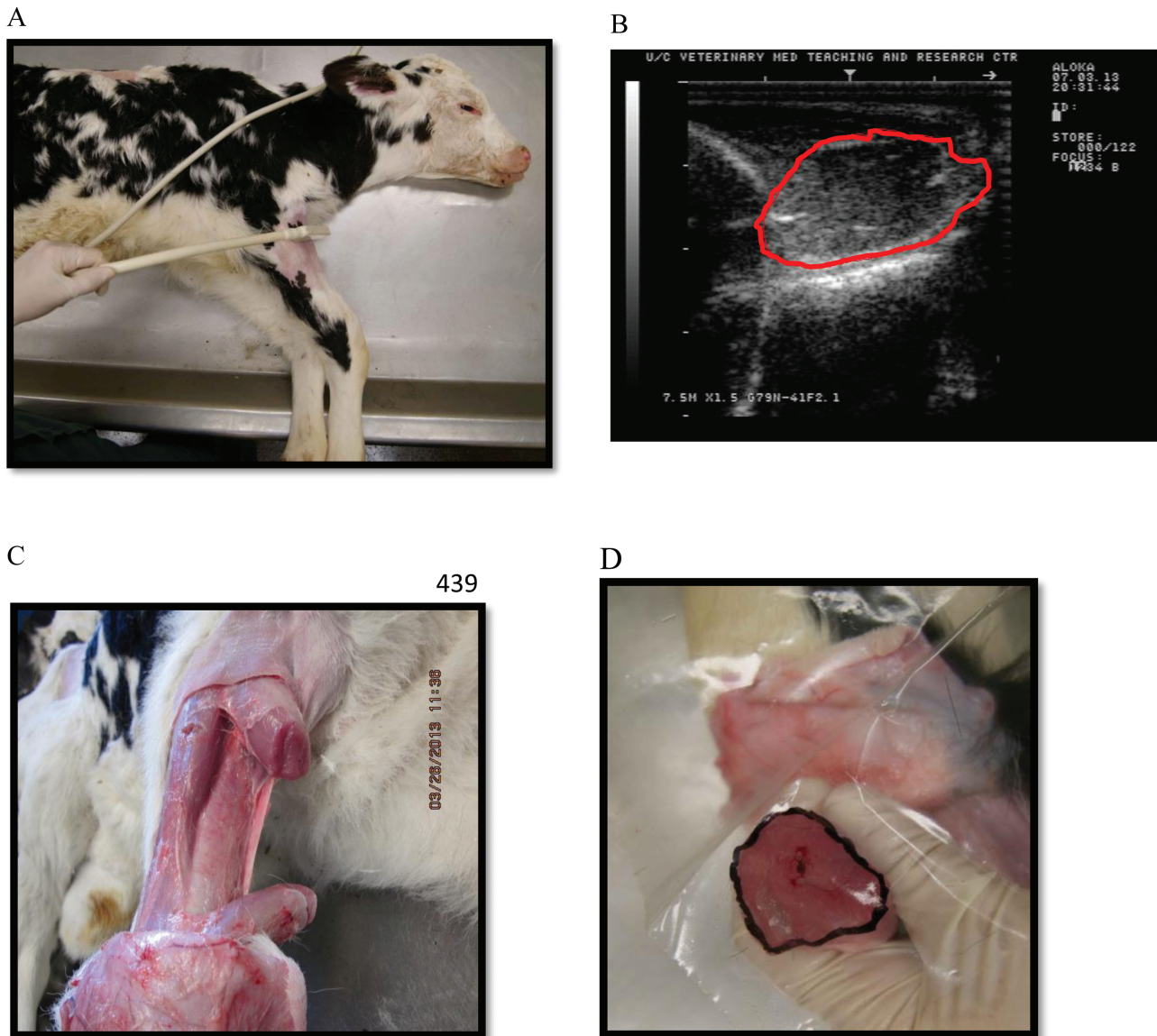
Residuals were regressed on ultrasound values to test the assumptions of linearity and equal variances and  $R^2$  was reported to test for bias of residuals using GLM procedure of SAS. The plots for the ribeye, ECR, and ST muscles all showed a relatively random scatter of points above and below the horizontal line at  $(y_i - \bar{y})$  equal to 0 indicating no violations of the assumptions listed.

## RESULTS AND DISCUSSION

Data from 137 postmortem preweaned Holstein male calves were used in this study. Five calves did

not have a recorded weight due to weigh scale malfunction. Age and weight distributions of the calf population are presented in [Table 1](#). Age of the calves displayed a right-skewed distribution, with 60% of the calf population less than 10 days of age. This distribution was not unexpected given the high risk of mortality in dairy calves in the first week of life ([Waltner-Toews et al., 1986](#); [Svensson et al., 2006](#)).

Means, SD, and minimum and maximum values for the carcass and ultrasound measurements of the ribeye, front, and hind muscles for both operators were presented in [Table 2](#). Discrepancies



**Figure 3.** Ultrasound image capture and analysis of ECR. (A) An ultrasound image of the ECR area is captured by aligning the probe with the right elbow perpendicular to the long axis of the muscle. (B) The ECR cross-sectional area ( $\text{cm}^2$ ) is outlined in red. The red line was drawn around the oval area of moderate echogenicity between the two hyperechoic lines to the left and bottom. (C) The hide of the calf was removed and the ECR was dissected. A cross-sectional cut was made through the ECR. (D) Transparency paper was placed over the ECR and traced with a fine-tipped marker.

in the number of observations for the ribeye muscle were due to poor image quality that interfered with image interpretation. Mean biases for the three muscles showed ultrasound underestimated the carcass linear depth and areal measurements for both operators with the mean bias for the ribeye muscle for operator 1 being greatest (bias = 0.20 and 0.15, respectively).

Similar to this study, [Smith et al. \(1992\)](#), [Perkins et al. \(1992\)](#), and [Ripoll et al. \(2010\)](#) working with feedlot steers and heifers, yearling steers, and commercial male lambs, respectively, also found that ultrasound underestimated carcass muscle area. [Thériault et al. \(2009\)](#) reported similar results when

using ultrasound to predict ribeye muscle depth at the 12th and 13th ribs in Suffolk and Dorset lambs. The underestimation could be caused by difficulty in differentiating muscle from subcutaneous fat and skin on the ultrasound image and not correctly tracing the muscle depth. The pressure applied to the ultrasound probe may have distorted the images of the three muscles, making them appear smaller. [Purchas and Beach \(1981\)](#) reported similar observations, in which certain tissues were more compressible than others leading to distortion of images and subsequent underestimation or overestimation depending on amount of fat within the muscle.

A



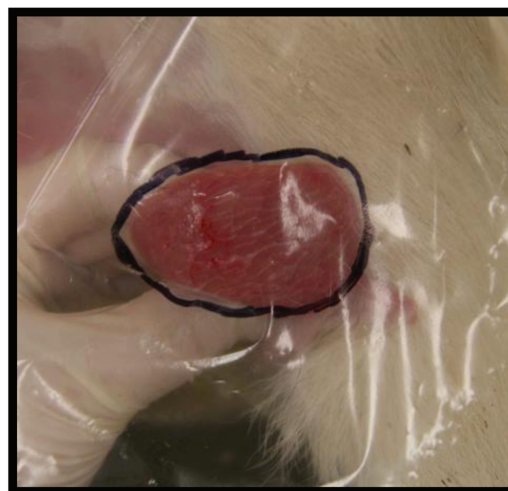
B



C



D



**Figure 4.** Ultrasound image capture and analysis of ST. (A) An ultrasound image of the ST muscle area is captured by aligning the probe with the right femoro patellar joint perpendicular to the long axis of the muscle. (B) The ST cross-sectional area (cm<sup>2</sup>) is outlined in red. The red line was drawn around the cone to heart-shaped oval area of moderate echogenicity. (C) The hide of the calf was removed and the ST was dissected. A cross-sectional cut was made through the ST muscle. (D) Transparency paper was placed over the ST and a tracing with a fine-tip Sharpie marker was performed.

**Table 1.** Mean, median, SD, and minimum and maximum values for age and weight of postmortem preweaned male Holstein calves

Variable	N	Mean	Median	SD	Min	Max
Age, d	137	13.1	6.0	15.5	1.0	85.0
Weight, kg	132	36.5	36.6	7.2	16.9	69.5

**Relationship between Ultrasound and Carcass Measurements**

All Pearson correlations between carcass and ultrasound measurements were significant ( $P < 0.01$ ; Table 3). Carcass and ultrasound ST

muscle were most closely correlated for both operators ( $r = 0.79, 0.77$ , respectively) followed by ECR muscle area and ribeye muscle depth ( $r = 0.66, 0.56$ , and  $0.52, 0.64$ , respectively). On the basis of results from Houghton and Turlington (1992),  $r = 0.65$  was chosen as a Pearson correlation cut-off value to determine predictability of carcass estimates by ultrasound. There were no cattle or small ruminant studies that examined correlations between carcass and ultrasound measurements for the ST or ECR muscle area, and only a few studies that looked at the correlations between carcass and ultrasound measurements for ribeye muscle depth. Therefore,

**Table 2.** Means, SD, minimum and maximum values, and mean bias for longissimus dorsi (ribeye), ECR, and ST muscles carcass and ultrasound measurements, by operator

Muscle	Operator	N	Carcass			Ultrasound			Bias <sup>  </sup>
			Mean	SD	Range	Mean	SD	Range	
Ribeye <sup>†</sup>	1	137	1.59	0.44	0.70–2.60	1.39	0.32	0.35–2.24	0.20
Ribeye <sup>†</sup>	2	131	1.69	0.43	0.60–2.60	1.54	0.39	0.49–2.70	0.15
ECR <sup>‡</sup>	1	137	6.38	1.79	2.84–11.76	5.43	1.32	1.77–8.83	0.95
ECR <sup>‡</sup>	2	137	6.65	2.05	2.62–12.18	5.50	1.45	1.26–9.96	1.15
ST <sup>‡</sup>	1	137	9.37	2.41	4.26–17.05	8.64	2.32	2.79–15.71	0.73
ST <sup>‡</sup>	2	137	9.11	2.31	3.81–16.29	8.84	2.29	2.39–13.68	0.27

<sup>†</sup>Ribeye measurements in centimeters.

<sup>‡</sup>ECR and ST measurements in square centimeters.

<sup>||</sup>Mean bias = absolute value of the mean of individual ultrasound-carcass measurements.

**Table 3.** Pearson correlation coefficient (*r*) as a measure of the relationship between the ultrasound and carcass measurements for the longissimus dorsi (ribeye), ECR, and ST, by operator

Operator	Muscle	N	Pearson ( <i>r</i> )	P-value
1	Ribeye	137	0.52	<0.01
2	Ribeye	131	0.64	<0.01
1	ECR	137	0.66	<0.01
2	ECR	137	0.56	<0.01
1	ST	137	0.79	<0.01
2	ST	137	0.77	<0.01

identification of adequate Pearson correlation values for ribeye depth was based on results from previous studies on ribeye area. Correlation values for ST muscle for both operators exceeded 0.65 and were greater in this study than in Hassen et al. (1998), who evaluated the accuracy of ultrasound to predict carcass ribeye area at the 12th–13th rib in crossbred steers ( $r = 0.48$ , RMSE = 9.73).

Pearson correlation values were not as high for the ribeye muscle area as they are for back fat in steers because of the difficulties in obtaining traceable ribeye images. Pearson correlation values for ribeye depth and area in this study were lower when compared to Silva et al. (2006) who evaluated the accuracy of ultrasound to predict ribeye area at the 13th thoracic vertebrae in sheep ( $r = 0.95$ , calculated from given  $R^2 = 0.895$ , residual SD = 1.18). The differences in the results of this study when compared to the results of the Silva et al. (2006) study may be explained by the different species scanned. Smaller than expected Pearson correlations could also be attributed to the fact that muscle depth and cross-sectional carcass cuts were not made at the exact location as where the probe was placed and ultrasound images taken. Different operators also had greater bias in their estimates of muscle area and depth (Table 2) emphasizing the importance

of operator training in placing the probe for ultrasound images, tracing of ultrasound images and incising the muscles for carcass measurements.

Initially, ribeye area was intended to be one of the measurements (Houghton and Turlington, 1992; Perkins et al., 1992; Smith et al., 1992). However, the probe was not long or wide enough to capture the entire ribeye area. Ribeye depth was more commonly measured in both goats and sheep to assess carcass composition and was chosen because of the similarity in size between small ruminants and calves (Teixeira et al., 2008; Thériault et al., 2009; Ripoll et al., 2010). Pearson correlation values for ribeye for both operators ( $r = 0.52$  and  $0.64$ , respectively) were below 0.65, which indicates a weak association between the carcass and ultrasound. However, the correlation value for ribeye depth was greater than those in Thériault et al. (2009) who evaluated the accuracy of ultrasound to predict carcass ribeye depth at both the 12th rib and 3rd lumbar vertebrae in weaned Suffolk and Dorset lambs ( $r = 0.34$  and  $0.42$ , respectively). The discrepancy between the values in Thériault et al. (2009) and this study's values were probably due to differences in the anatomy of species, differences in anatomical placement of the probe, and differences in equipment used. Correlation values for ribeye depth were similar to what Silva et al. (2006) reported at both the 13th thoracic vertebrae and between the 3rd and 4th lumbar vertebrae in sheep (calculated from given  $R^2 = 0.749$ , and  $0.763$ ,  $r = 0.56$ , and  $0.58$ , respectively,  $P < 0.01$ ). Because correlation values were also affected by the variation of the sample population, some of the differences in correlations among studies could be due to uniformity in ribeye muscle depth in the sample population.

Small differences in Pearson correlation values for the ribeye, ECR, and ST muscles between operator 1 and 2 can be attributed to differences



in operator experience with ultrasound (Table 3). Operator 1 was a veterinarian who had experience interpreting ultrasound images of bovine uteri and diagnosing pregnancies. Operator 2 was an undergraduate student with very little experience with ultrasound and image interpretation. Neither operator 1 or 2 had had any experience interpreting muscle depth or area images prior to this study. However, the high correlation values for both operator 1 and 2 for ECR and ST muscles implies individuals with limited knowledge of ultrasound for carcass evaluation can learn how to take usable ultrasound images of muscles (ECR  $r = 0.66$  and  $0.56$ , ST  $r = 0.79$  and  $0.77$ , respectively; Table 3). The small differences between correlation values between operators 1 and 2, who differed in previous experience, suggested that previous experience with ultrasound did not affect accuracy, although testing with more operators would be necessary to confirm this hypothesis.

**Linear Regression for Examining Predicted vs. Observed Measurements**

Estimations of carcass measurements for the ribeye, ECR, and ST muscles were achieved by regressing carcass measurements on ultrasound measurements (Tables 4 and 5). Predictions of carcass measurements by ultrasound were best for ST muscle. Predictions of ST carcass estimates accounted for 62% and 60% of the variation in ultrasound measurements for operator 1 and 2, respectively (Table 4). Predicted carcass vs. observed ultrasound measurements were compared using the regression line  $y = x$ . Intercepts and slopes were significantly different from 0 and the RMSEs were small (<20%) compared to observed means for both operators (RMSE = 1.44 and 1.46 compared to ultrasound mean = 8.64 and 8.84, respectively; Tables 2 and 4). However, estimates of ST carcass measurements are biased for both operators, with

ultrasound underestimating carcass measurements by 8% and 3%, respectively.

Further investigation of this underestimation for the ST muscle was accomplished by regressing the difference between ultrasound and carcass values on the difference between carcass and the mean carcass value. This allowed for a more robust interpretation of the intercept and gave information about how much underestimation occurred with regard to smaller or larger muscles sizes (that could be potentially extrapolated to calf sizes in a healthy population of calves). The intercept and slope for the ST muscle were both negative (intercept =  $-0.50$ , slope =  $-0.24$ ,  $P < 0.01$ ; data not shown). The negative intercept indicated that ultrasound did underestimate carcass by  $-0.50$  cm<sup>2</sup> for an average muscle size (or area). The negative slope indicated that the difference between ultrasound and carcass was greater for larger muscles and smaller for smaller muscles. This may be important for future ultrasound operators to know because ultrasound was more accurate for smaller muscles. This may be due to probe size because the software utilized was programmed for a larger probe for larger muscles (and larger cattle). A computer software program more suited for smaller muscles (or smaller animals like calves) may be warranted if this technology is deemed appropriate for estimating body composition.

Estimates of the ECR muscle were not as accurate when compared to ST. Predictions of the carcass measurements for ECR muscle accounted for 43% and 32% of the variation in ultrasound measurements for operator 1 and 2, respectively (Table 4). Predicted carcass vs. observed ultrasound measurements were compared using the regression line  $y = x$ . Intercepts and slopes were significantly different from 0 and the RMSEs were small compared to observed means for both operators (RMSE = 1.00 and 1.20 compared to ultrasound

**Table 4.** Simple linear regression equations for assessing the relationship between carcass measurements ( $y$ ) and ultrasound measurements ( $x$ ) of longissimus dorsi (ribeye), ECR, and ST, by operator

Operator	Muscle	<i>N</i>	a	SE	CI	b	SE	CI	<i>R</i> <sup>2</sup>	RMSE
1	Ribeye <sup>†</sup>	137	0.78	0.05	0.60, 0.95	0.38	0.09	0.28, 0.49	0.27	0.28
2	Ribeye <sup>†</sup>	129	0.67	0.10	0.48, 0.86	0.53	0.06	0.42, 0.64	0.41	0.27
1	ECR <sup>‡</sup>	137	2.33	0.32	1.70, 2.96	0.49	0.05	0.39, 0.58	0.43	1.00
2	ECR <sup>‡</sup>	137	2.86	0.35	2.17, 3.55	0.40	0.05	0.30, 0.50	0.32	1.20
1	ST <sup>‡</sup>	137	1.53	0.49	0.55, 2.51	0.76	0.05	0.66, 0.86	0.62	1.44
2	ST <sup>‡</sup>	137	1.89	0.51	0.88, 2.89	0.76	0.05	0.66, 0.87	0.60	1.46

All coefficients were significantly different from zero ( $P < 0.01$ ). a = intercept, b = coefficient.

<sup>†</sup>Ribeye measurements in centimeters.

<sup>‡</sup>ECR and ST measurements in square centimeters.

**Table 5.** Multiple linear regression equations to predict carcass measurements looking about both body weight and the interaction of ultrasound and body weight, by operator

Operator	Dependent	Independent		a	SE	b	SE	R <sup>2</sup>	RMSE	P-value
	variable	variable								
1	C_Rib <sup>†</sup>	US_Rib		0.61	0.14	0.71	0.10	0.27	0.38	<0.01
		Weight				0.02	0.005	0.35	0.36	<0.01
		US_Rib*Wt				0.01	0.01	0.33	0.37	0.7
2	C_Rib <sup>†</sup>	US_Rib		0.47	0.13	0.79	0.08	0.41	0.33	<0.01
		Weight				0.01	0.005	0.42	0.33	0.2
		US_Rib*Wt				0.01	0.01	0.41	0.34	0.5
1	C_ECR <sup>‡</sup>	US_ECR		1.53	0.49	0.89	0.09	0.43	1.4	<0.01
		Weight				0.05	0.02	0.48	1.3	0.02
		US_ECR*Wt				0.01	0.01	0.48	1.3	0.3
2	C_ECR <sup>‡</sup>	US_ECR		2.28	0.57	0.79	0.1	0.32	1.7	<0.01
		Weight				0.02	0.02	0.35	1.7	0.3
		US_ECR*Wt				0.002	0.01	0.35	1.7	0.8
1	C_ST <sup>‡</sup>	US_ST		2.31	0.49	0.82	0.06	0.62	1.5	<0.01
		Weight				0.07	0.03	0.65	1.4	0.01
		US_ST*Wt				0.0008	0.004	0.65	1.5	0.9
2	C_ST <sup>‡</sup>	US_ST		2.21	0.50	0.78	0.06	0.60	1.5	<0.01
		Weight				0.05	0.02	0.61	1.5	0.05
		US_ST*Wt				-0.003	0.005	0.61	1.5	0.6

C\_Rib = ribeye carcass, C\_ECR = extensor carpi radialis carcass, C\_ST = semitendinosus carcass, Wt = weight, a = intercept, b = coefficient, R<sup>2</sup> = adjusted coefficient of determination for partial model.

<sup>†</sup>Ribeye measurements in centimeters.

<sup>‡</sup>ECR and ST measurements in square centimeters.

mean = 5.43 and 5.50, respectively; [Tables 2 and 4](#)). However, estimates of ECR carcass measurements were biased for both operators, with ultrasound underestimating carcass measurements by %15 and 17%, respectively. Similar to the ST muscle, further investigation of this underestimation for the ECR muscle was accomplished by regressing the difference between ultrasound and carcass values on the difference between carcass and the mean carcass value. The intercept and slope for the ST muscle were both negative (intercept = -1.04, slope = -0.57,  $P < 0.01$ ; data not shown). The negative intercept indicated that ultrasound did underestimate carcass by -0.57 cm<sup>2</sup> for an average muscle size (or area). The negative slope indicated that the difference between ultrasound and carcass was greater for larger muscles and smaller for smaller muscles.

Finally, estimates of accuracy of ribeye depth were similar to those of ECR muscle. Model predictions of the carcass measurements for the ribeye muscle accounted for 27% and 41% of the variation in ultrasound measurements for operator 1 and 2, respectively ([Table 4](#)). Predicted carcass vs. observed ultrasound measurements were compared using the regression line  $y = x$ . Intercepts and slopes were again significantly different from 0 and the RMSEs were small compared to the observed means for

both operators (RMSE = 0.28 and 0.27 compared to ultrasound mean = 1.39 and 1.54, respectively; [Tables 2 and 4](#)). However, estimates of ribeye carcass measurements were biased for both operators, with ultrasound underestimating carcass measurements by 13% and 9%, respectively. Predictions of ribeye carcass estimates accounted for 62% and 60% of the variation in ultrasound measurements for operator 1 and 2, respectively ([Table 4](#)). Just like the ST and ECR muscles, further investigation of this underestimation for the ribeye was accomplished by regressing the difference between ultrasound and carcass values on the difference between carcass and the mean carcass value. The intercept and slope for the ST muscle were both negative (intercept = -0.17, slope = -0.60,  $P < 0.01$ ; data not shown). The negative intercept indicated that ultrasound did underestimate carcass by -0.17 cm<sup>2</sup> for an average muscle depth. The negative slope indicated that the difference between ultrasound and carcass was greater for larger muscles and smaller for smaller muscles.

Model predictions of carcass measurements improved with the addition of calf body weight to the regression equation ([Table 5](#)). Many authors have found that body weight was a useful variable to predict carcass measurements in livestock. [Teixeira et al. \(2008\)](#) identified body weight as a significant

predictor variable in adult goats, accounting for up to 82% of the variation in carcass measurements. Silva et al. (2006) described similar results when looking at female sheep to study carcass composition using ultrasound. Finally, Ripoll et al. (2010) identified 95% of the variation in carcass measurements to be a result of body weight in male lambs. In this study, body weight was statistically significant when added to the regression model for all three muscles (Table 5). All reported  $R^2$  and RMSE in Table 5 were from a full model with either weight or the interaction of weight and ultrasound as the extra variable (not both together). The largest improvement in  $R^2$  and RMSE was seen for the ribeye for operator 1 (Table 5). All other improvements in  $R^2$  and RMSE were less than 10% (Table 5). This was most likely due to the fact that the calves were dead and may have been unhealthy prior to death with less than ideal body condition, muscle mass, and fat deposition. No statistically significant interactions between ultrasound and calf body weight were observed with any of the muscles, indicating there was no difference in the results for light weight calves when compared to heavier calves.

Ultrasound measurements taken of the ST muscle area were the most appropriate predictor of the ST carcass muscle area, demonstrating an acceptable correlation coefficient and a high coefficient of determination for both operators followed by ECR and ribeye muscles. Similar results obtained from both operators suggest previous experience with ultrasound for other diagnostics other than carcass composition is not necessary. Ultrasound as a tool to evaluate muscle growth in preweaned calves is a promising technology because it is relatively easy to use and noninvasive. But, further research needs to be carried out using live calves that are measured from birth to weaning to show that this method could be used to assess muscle growth in the prewean period.

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