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Accuracy of real-time ultrasound measurements of total tissue, fat, and muscle depths at different measuring sites in lamb[1]

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ABSTRACT: Accuracy of live ultrasound measurements to evaluate the total tissue depth (GR), as well as fat and LM depths at different scanning sites, was studied in 96 purebred Suffolk and Dorset lambs of both sexes slaughtered between 36 and 54 kg of BW. Before slaughter, 7 real-time ultrasound measurements were taken on lambs: fat and LM depths between the 12th and 13th ribs (transverse) and between the 3rd and 4th lumbar vertebrae (transverse and longitudinal), and GR. After slaughter, the measurements equivalent to ultrasound measurements were taken on digitized images of the cuts on the left half carcass of each lamb. Ultrasound GR and fat depth measurements were closely correlated with the corresponding carcass measurements ($0.76 \leq r \leq 0.81$). Ultrasound GR measurement exhibited a large error of central tendency, but the level of error due to the disturbance (ED) was comparable with fat depth measurements (ED = 8.5%; residual SD = 2.24 mm; CV of residuals = 9.5%). Ultrasound fat depth measurements were more accurate between the 12th and 13th ribs (error due to regression = 1.20; ED = 0.82) than between the 3rd and 4th lumbar vertebrae (error due to regression = 5.58 and 5.4; ED = 1.10 and 0.93, transverse and longitudinal, respectively), mainly due to image interpretation errors in the lumbar region. Measurements of LM depth demonstrated low variability in the population under study (SD = 2.6 mm), and these ultrasound measurements showed low correlation with the corresponding carcass measurements ($0.34 \leq r \leq 0.43$). The results of this study demonstrated that ultrasound measurements were more accurate for evaluating fat depth and the GR measurements than for estimating LM depths. Ultrasound GR measurement is a promising measurement, especially where carcass grading systems are based on this carcass measurement.

Key words: fat depth, lamb, live measurement, loin muscle depth, total tissue depth measurement, ultrasound

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INTRODUCTION

In hogs and cattle, ultrasound fat and muscle depths have been used for several years in genetic selection programs for improving carcass quality, and much research has been published on this topic (Moeller, 2002; Williams, 2002). In sheep, few scientific data are available to evaluate and compare the accuracy of the different scanning sites, and conclusions on their usefulness were often conflicting (Houghton and Turlington, 1992). Besides, it is difficult to compare results because of the various statistical methods used.

The most common site to evaluate transverse fat and LM depths in lamb is at the 12th-13th ribs (Wilson, 1992). Some researchers obtained greater correlation between ultrasound and carcass measurements at the 3rd-4th lumbar vertebrae (Fernández et al., 1998; Silva et al., 2006). In hogs, longitudinal measurements, parallel to the backbone, are commonly used in Canada, but this method is rarely reported in lambs (Berg et al.,

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1996) and has never been compared with the transverse measurement. Total tissue depth over the 12th rib at 11 cm from the midline of the carcass (**GR**) is a measurement included in carcass grading systems to predict lean and fat yield in Canada (Jones et al., 1996), Australia (Hopkins, 1994), and New Zealand (Kirton and Johnson, 1979). Despite the proven usefulness of carcass GR measurement, few researchers demonstrated interest in this ultrasound scanning site in live lambs (McEwan et al., 1989; Ramsey et al., 1991; Hopkins et al., 1993).

This investigation aimed to determine the better scanning sites, in regard to accuracy and feasibility, to validate and refine the evaluation of carcass quality traits in genetic selection programs for lamb. Specifically, the objectives of this study were to assess the accuracy of ultrasound fat and LM depths at 2 scanning sites and the accuracy of ultrasound GR, as well as to compare transverse and longitudinal ultrasound measurements.

MATERIALS AND METHODS

Care and handling of the lambs used in this study were conformed to the guidelines established by the Canadian Council on Animal Care (1993).

Animal Sampling and Husbandry Conditions

A total of 144 purebred Suffolk (**SU**; n = 72) and Dorset (**DP**; n = 72) lambs were selected at weaning, at around 55 d of age, from 9 Quebec sheep producers. Lambs representative to their respective breed in terms of weaning weight were retained. The Dorset breed is a maternal type and is characterized by moderate growth and a greater fat content. Conversely, the Suffolk is a leaner, fast-growing terminal breed.

Lambs entered the test station at around 65 d of age and were assigned according to sex (male and female), breed (SU and DP), and slaughter weight classes (36 to 39 kg, 41 to 44 kg, 46 to 49 kg, and 51 to 54 kg) to a 2 × 2 × 4 factorial design arranged in 8 randomized complete blocks, each block consisting of 1 pen of each sex. Treatments were used for studying growth and tissue deposition of heavy lambs as part of another research project (F. W. Castonguay, unpublished data). Lambs were fed ad libitum a pelleted, complete grower diet (18% CP; 2.76 Mcal/kg of ME) to approximately 35 kg of BW and then a finisher diet (15% CP; 2.79 Mcal/kg of ME) until slaughter. High quality hay also was available for ad libitum intake during the entire experiment.

Live Measurements

Body weight and the ultrasound measurements were recorded less than 48 h before slaughter. Ultrasound measurements were taken by an experienced operator using a real-time ultrasound device (Ultrascan50, Alliance Médicale Inc., Montreal, Canada) with a 120-mm, 3.5-MHz linear probe. Lambs were restrained and measured in a standing position on a preparation table to minimize errors related to movement and tissue compression. Before each ultrasound session, the different scanning sites were sheared with a surgical blade (0.1 mm) and a conductive solution (mineral oil or P-net, DGF, Pintendre, Canada) was applied. Depending on the scanning site, a flat or curved gel pad (Superflab, Mick Radio Nuclear Instruments, Bronx, NY) was placed under the probe coated with ultrasound gel (Ecogel200, Eco-Med Pharmaceutical Inc., Mississauga, Canada). The flat gel pad was fitted with guides (at 4 cm and 11 cm) to assist in the longitudinal measurements.

Ultrasound measurements were taken on the left side at 4 sites on the live animal: total tissue depth ($\mathbf{GR}_{\mathbf{us}}$) between the 11th and 12th ribs, 11 cm lateral to the spine and parallel to it (longitudinal measurement, flat gel pad; Figure 1); fat depth ($\mathbf{FD12}_{\mathbf{us}}$) and LM depth ($\mathbf{LD12}_{\mathbf{us}}$) between the 12th and 13th ribs perpendicular to the body midline (transverse measurement, curved gel pad); fat depth ($\mathbf{FD3T}_{\mathbf{us}}$) and LM depth ($\mathbf{LD3T}_{\mathbf{us}}$) between the 3rd and 4th lumbar vertebrae, taken perpendicular to the spine (transverse measurement, curved gel pad); fat depth ($\mathbf{FD3L}_{\mathbf{us}}$) and LM depth ($\mathbf{LD3L}_{\mathbf{us}}$) between the 3rd and 4th lumbar vertebrae, taken parallel to the body midline (longitudinal measurement, flat gel pad). The same operator performed the ultrasound measurements throughout the experiment. The images were captured at each site, and measurements were taken immediately using the cursor of the device. For the transverse measurements, the probe was placed perpendicular to the backbone capturing the entire lamb chop from which the maximal height of the LM, perpendicular to the surface, and the fat depth over this height was assigned respectively to muscle and fat depths (Pálsson, 1939). Longitudinal measurements, parallel to the backbone, captured an image of the LM over its length. In this case, the muscle depth corresponded to maximal height between transverse processes and fat depth was the fat cover over this muscle depth (Figure 2). Skin depth was included in all the ultrasound fat measurements because this tissue is not easily distinguishable from the fat layer. Skin layer was thin (2.5 to 3.0 mm) and showed little between-animal variation (Gooden et al., 1980; Cameron and Bracken, 1992). Our measurements and analyses of skin thickness performed in this experiment at around 110 d of age corroborated these observations (3.5 ± 0.4 mm; data not shown).

Slaughter and Grading

Lambs were slaughtered weekly on a fixed day for the entire duration of the experiment. After feed withdrawal for at least 12 h, the BW was recorded before slaughter. Lambs were slaughtered in a commercial abattoir. Particular attention was given to the pelt re-

moval to keep the subcutaneous fat intact. Hanging carcasses were weighed before chilling at 4°C. After 24 h of chilling, the carcasses were graded according to the method of Agriculture Canada (1992). Using a metal ruler, total tissue depth was measured over the 12th rib, 11 cm from the midline (GR measurement). Finally, the carcasses were split longitudinally, and the left half-carcasses were sent to Agriculture and Agri-Food Canada's Dairy and Swine Research and Development Centre.

Cuts and Carcass Measurements

Five days after slaughter, the half-carcasses were cut into primary cuts (shoulder, loin, leg, and flank). Two cuts parallel to the ribs were made in the loin region, one between the 12th and the 13th ribs and one behind the 13th rib, to extract the last chop. This chop was digitized using an image digitizer (Scanmaker 2, Microtek, Taiwan) at a resolution of 100 pixels per inch. Similarly, a cut was made between the 3rd and 4th lumbar vertebrae, and an image of this surface (posterior region of the loin) was digitized. Measurements corresponding to the live ultrasound measurements, fat depth (**FD12**: between the 12th and 13th ribs; **FD3**: between the 3rd and 4th lumbar vertebrae), LM depth (**LD12**: between the 12th and 13th ribs; **LD3**: between the 3rd and 4th lumbar vertebrae), and LM area (**LMA12**: between the 12th and 13th ribs; **LMA3**: between the 3rd and 4th lumbar vertebrae), were evaluated using image analysis software (Pomar et al., 2001).

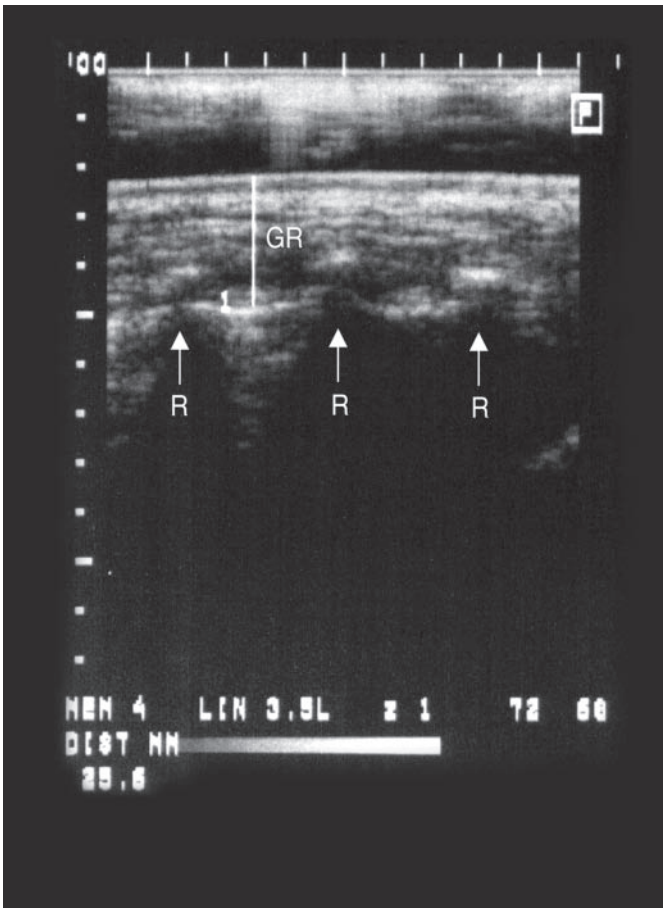

Figure 1. Longitudinal ultrasound image of total tissue depth between 11th and 12th ribs at 11 cm from the midline (GR) in lamb. Ribs are designated by letters R.

Statistical Analyses

Analyses were performed on animals having valid data for all the studied variables. Pearson's correlation coefficients between the ultrasound measurements and the corresponding carcass measurements were calculated using the CORR procedure (SAS Inst. Inc., Cary, NC), indicating the intensity of the relationship between these 2 sets of variables. Coefficients of correlation (r) and determination (r^2) are, however, strongly influenced by the population distribution (Houghton and Turlington, 1992). For the purpose of comparing studies, it is therefore preferable to refer to the residual SD (**RSD**) of the relationship between the carcass and ultrasound measurements. The relationship between ultrasound measurements (dependent variable) and measurements taken on the digitized images (independent variable) was studied using the SAS REG procedure. The inverse relationship was also determined, with the same SAS procedure, to compare our results with those of other studies. Outliers and data having undue influence were identified using influence diagnostics and graphic analysis.

Additionally, error decomposition was used to determine the accuracy of the ultrasound measurements, in terms of trueness and precision (ISO, 1993). According to the method described by Theil (1966), the total measurement error is equal to the mean square prediction error (**MSPE**). In our case, the error is the difference between measurements taken on the carcass and the value obtained using ultrasound imaging; that is,

$$MSPE = \frac{\sum (carcass_i - ultrasound_i)^2}{n}.$$

The MSPE also is equal to the square of the root mean square error (**RMSE**) as described by Herring et al. (1994). Graphically, the error represents the difference between each point (ultrasound measurement) and the line of identity (perfect match between carcass and ultrasound measurements). The MSPE can be broken down into 3 components: error of central tendency (**ECT**), error due to regression (**ER**) and error due to disturbance (**ED**), as proposed by Benchaar et al. (1998) and Pomar and Marcoux (2005). The ECT evaluates the closeness of the agreement between the mean value obtained using an instrument and the accepted reference value. The ECT is equal to the square of the bias of the ultrasound measurements (bias = mean difference between the ultrasound and carcass measurements), as used in studies of measurement precision (Moeller and Christian, 1998; Greiner et al., 2003).

$$ECT = bias^2 = (mean_{carcass} - mean_{ultrasound})^2 = \left(\frac{\sum(carcass_i - ultrasound_i)}{n}\right)^2.$$

The ER refers to the difference between the slope of the regression line between the ultrasound and carcass measurements and the slope (b = 1) of the identity line. The ED is the component of the error that cannot be explained by the regression. It represents the dispersion of the points around the regression line, the random error. The ED is the square of the RSD.

In the present study, the trueness of the measurements is evaluated as the sum of ECT and ER, whereas the precision is evaluated by the ED. Presence of bias does not mean that the measurement is not useful (ECT > 0). The ECT can easily be corrected by adding the value of the bias to the ultrasound measurement. Although it also can be corrected by regression, the ER implies that the bias is not constant and varies depending on the magnitude of the measurements. The ED, on the other hand, cannot be corrected and needs to be minimized.

Few authors use the SE of prediction (**SEP**) for evaluating the precision of ultrasound measurements (Herring et al., 1994; Moeller and Christian, 1998). The SEP is similar to the RMSE of Herring et al. (1994):

$$SEP = \sqrt{\frac{\sum(carcass_i - ultrasound_i - bias)^2}{n-1}}.$$

Thus, an SEP of 1.5 mm for fat depth indicates that, in 68% of cases, an ultrasound measurement will be within 1.5 mm of the carcass measurement (Moeller and Christian, 1998). Within the SEP, the ER and the random error are merged when n is very large, because

$$\sqrt{ER + ED} = \sqrt{\frac{\sum(carcass_i - ultrasound_i - bias)^2}{n}}.$$

The error decomposition method proposed here gives additional information about the type of errors made with ultrasound imaging. The SEP was presented for the purposes of comparison with previous published results. All error calculations were performed using SAS software.

RESULTS AND DISCUSSION

Data from 96 lambs (44 SU and 52 DP) were used in this study. Information from other animals was removed from the data set because of mortality of the animal, disease, or because carcasses were not properly split. Average daily gain of the lambs was 417 g/d and ranged between 272 and 620 g/d (data not shown). Lambs were slaughtered at an average age of 130.9 d with an average fasted BW of 47.0 kg and HCW of 24.4 kg (Table 1). The high variability showed in Table 1 for carcass fat depth reflects the discrepancy in the pattern of fattening of the 2 breeds of lamb used in this study (maternal and terminal types).

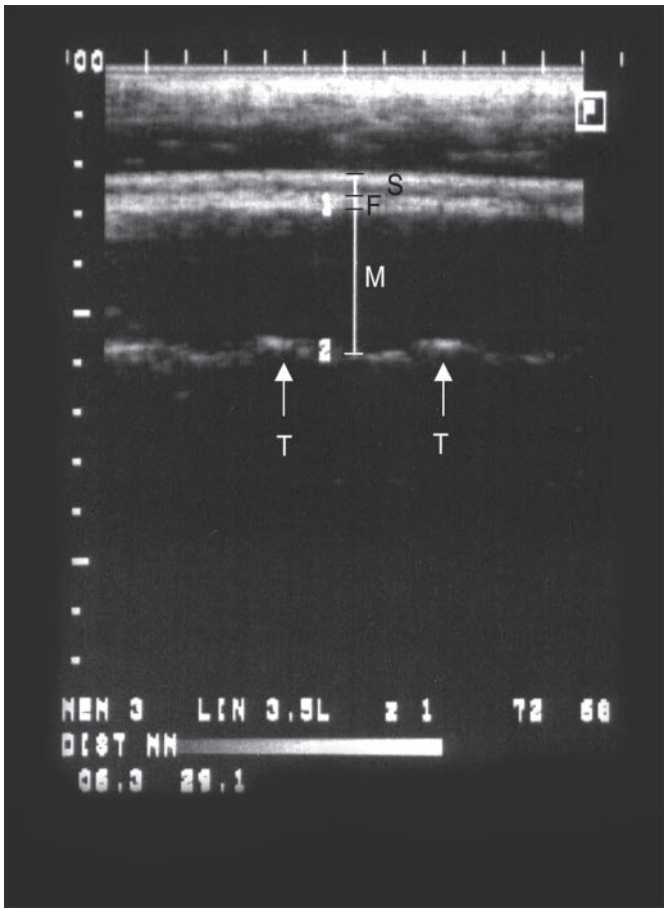

Figure 2. Longitudinal ultrasound image between the 3rd and 4th lumbar vertebrae at 4 cm to the midline in lamb. Letters S, F, and M represent skin, fat, and muscle depths, respectively. Transverse processes are identified by letter T.

Relationship Between Live Ultrasound and Carcass Measurements

Total Tissue Depth. Ultrasound GR measurements (GR_{us}) and those measured on the carcass with a ruler (GR) were correlated (Table 2; r = 0.83; $P < 0.001$) in agreement with other studies (McEwan et al., 1989; Ramsey et al., 1991; Hopkins et al., 1993). The GR also was correlated with ultrasound fat depth measurements ($FD3T_{us}$, $FD3L_{us}$, $FD12_{us}$; $0.76 \leq r \leq 0.81$; $P < 0.001$).

Fat Depth. Correlations between the various ultrasound and corresponding carcass fat depth measurements on digitized images were high (Table 2; r = 0.82, 0.78, and 0.82 for $FD12_{us}$ vs. FD12, $FD3T_{us}$ vs. FD3, and $FD3L_{us}$ vs. FD3, respectively; $P < 0.001$) and were similar to the coefficients of correlation reported elsewhere (Thompson et al., 1977; Delfa et al., 1991; Fernandes, 2000) but greater than those below 0.6 obtained by Turlington (1990) and Hopkins et al. (1996).

Table 1. Means, SD, CV, and minimum and maximum values for live lambs and carcass traits (n = 96)

Trait	Mean	SD	CV, %	Minimum	Maximum
Live, before slaughter					
Age, d	130.9	19.5	14.9	96.0	177.0
Empty BW, kg	47.0	5.4	11.5	36.0	55.8
Total tissue depth, mm	23.6	4.0	16.9	14.5	31.6
Ultrasound fat depth, mm					
12th-13th rib	8.5	1.6	18.8	5.9	12.0
3rd-4th lumbar vertebrae transverse	8.8	1.7	19.3	5.2	13.1
3rd-4th lumbar vertebrae longitudinal	8.9	1.7	19.1	5.9	13.8
Ultrasound LM depth, mm					
12th-13th rib	31.3	2.1	6.7	27.4	35.9
3rd-4th lumbar vertebrae transverse	31.5	2.0	6.3	26.6	36.1
3rd-4th lumbar vertebrae longitudinal	30.8	2.3	7.5	24.5	36.3
Ultrasound sum of depths, mm					
12th-13th rib	39.8	3.0	7.5	34.0	46.5
3rd-4th lumbar vertebrae transverse	40.2	2.9	7.2	33.7	46.9
3rd-4th lumbar vertebrae longitudinal	39.8	3.2	8.0	31.1	47.9
Carcass					
HCW, kg	24.4	3.0	12.3	18.1	29.5
Total tissue depth, mm	16.4	4.4	26.8	8.0	25.0
Fat depth, mm					
12th-13th rib	6.2	2.4	38.7	2.1	12.7
3rd-4th lumbar vertebrae	7.9	3.7	46.8	1.8	17.9
LM depth, mm					
12th-13th rib	33.7	2.6	7.7	27.4	40.1
3rd-4th lumbar vertebrae	34.0	2.6	7.6	28.6	41.9
Sum of depths, mm					
12th-13th rib	39.8	3.3	8.3	31.8	47.3
3rd-4th lumbar vertebrae	41.9	3.7	8.8	33.2	49.5
LM area, mm^2					
12th-13th rib	1,628.0	169.4	10.4	1,133.0	1,929.0
3rd-4th lumbar vertebrae	1,682.0	207.1	12.3	1,172.0	2,213.0

The RSD obtained in our study were between 1.39 and 2.31 mm (data not shown; ultrasound = independent variable). Silva et al. (2006) reported better precision than our ($0.77 \leq$ RSD ≤ 0.95 mm) with greater probe resolution (5 and 7.5 MHz). In ultrasound imaging, it is recommended to use probes with focal depths close to the tissue of interest (Ginther, 1986). For lambs with BW of 35 to 55 kg, the depth of fat measurement, including the thickness of the gel pad (20 mm), ranged between 25 and 42 mm. For a probe of 3.5 MHz, the focal depth is around 80 mm. A probe of 5.0 MHz and having a focal distance about 40 mm improves fat layer image definition.

LM Depth. For LM depths measured between the 12th and 13th ribs, the coefficients of correlation between ultrasound and carcass measurements reported in the literature were generally less than those calculated for the fat depth measurements ($0.4 \leq r \leq 0.7$; Fortin and Shrestha, 1986; McEwan et al., 1989; Hopkins et al., 1996). In our study, a correlation of 0.34 (Table 2; $P < 0.001$) was found between $LD12_{us}$ and the corresponding carcass measurement on digitized images. In the lumbar region, the coefficients of correlation between the $LD3T_{us}$ and $LD3L_{us}$ with the LD3 measurements were 0.43 and 0.42, respectively. Values reported by Fernández et al. (1998) and Fortin and Shrestha (1986) for measurement at this site ranged from 0.49 to 0.76. Coefficient of correlation between measured and reference values for a given measuring device are dependent on both the precision of the device (RSD) and the SD of the population under study. In fact, for a given device and measured variable, the coefficient of correlation increases with the increase of the population variation. Therefore, the low muscle depth variability observed in the studied population (Table 1; SD = 2.6 mm) could explain the reduced correlations observed in our study compared with those greater than 0.85 observed by Silva et al. (2006) and Binnie et al. (1995). In both studies, SD of muscle depth was greater than 5 mm. Even with the high correlation, RSD values observed by Silva et al. (2006) were greater ($2.27 \leq$ RSD ≤ 4.09 mm) than the 1.74 mm obtained by Binnie et al. (1995) and ours ($2.38 \leq$ RSD ≤ 2.42 mm; data not shown). Despite our low r-value, the RSD obtained for ultrasound and carcass muscle depth regressions are comparable with or less than RSD between 2.4 and 2.8 mm observed by McEwan et al. (1989) and Hopkins et al. (1996).

Errors of Live Ultrasound Measurements

A measurement taken using a given device corresponds to the sum of the true value and the measurement error. Magnitude of the error will vary depending on the accuracy of the device; it is this error that is presented in Table 3. However, it is important to note that in

Table 2. Simple coefficient correlation between live ultrasound measurements and corresponding carcass measurements in lamb (n = 96)

Variable[1]	GR_{us}	$FD12_{us}$	$FD3T_{us}$	$FD3L_{us}$	$LD12_{us}$	$LD3T_{us}$	$LD3L_{us}$	GR	FD12	FD3	LD12	LD3	LMA12	LMA3
GR_{us}	1.00	0.74***	0.76***	0.78***	0.52***	0.49***	0.57***	0.83***	0.72***	0.71***	0.00	0.03	0.13	0.08
$FD12_{us}$		1.00	0.89***	0.91***	0.35***	0.26**	0.30**	0.78***	0.82***	0.82***	−0.08	−0.16	−0.06	−0.18
$FD3T_{us}$			1.00	0.97***	0.33***	0.25*	0.32***	0.76***	0.76***	0.78***	−0.10	−0.14	−0.01	−0.12
$FD3L_{us}$				1.00	0.34***	0.25*	0.32***	0.81***	0.80***	0.82***	−0.09	−0.15	0.01	−0.15
$LD12_{us}$					1.00	0.72***	0.69***	0.43***	0.43***	0.37***	0.34***	0.38***	0.46***	0.43***
$LD3T_{us}$						1.00	0.90***	0.42***	0.30**	0.34***	0.30**	0.43***	0.51***	0.53***
$LD3L_{us}$							1.00	0.49***	0.34***	0.36***	0.30**	0.42***	0.48***	0.52***
GR								1.00	0.78***	0.78***	−0.02	−0.09	0.07	−0.03
FD12									1.00	0.82***	−0.13	−0.15	−0.01	−0.09
FD3										1.00	−0.08	−0.35***	−0.06	−0.22*
LD12											1.00	0.34***	0.72***	0.48***
LD3												1.00	0.55***	0.79***
LMA12													1.00	0.73***
LMA3														1.00

[1]GR_{us} = ultrasound GR—total tissue depth between the 11th and 12th ribs at 11 cm from the midline, longitudinal measure; $FD12_{us}$ = ultrasound fat depth between the 12th and 13th ribs, transverse measure; $FD3T_{us}$ = ultrasound fat depth between the 3rd and 4th lumbar vertebrae, transverse measure; $FD3L_{us}$ = ultrasound fat depth between the 3rd and 4th lumbar vertebrae, longitudinal measure; $LD12_{us}$ = ultrasound LM depth between the 12th and 13th ribs, transverse measure; $LD3T_{us}$ = ultrasound LM depth between the 3rd and 4th lumbar vertebrae, transverse measure; $LD3L_{us}$ = ultrasound LM depth between the 3rd and 4th lumbar vertebrae, longitudinal measure; GR = total tissue depth over the 12th rib at 11 cm from the midline, Canadian carcass grading site; FD12 = fat depth between the 12th and 13th ribs on digitized image; FD3 = fat depth between the 3rd and 4th lumbar vertebrae on digitized image; LD12 = LM depth between the 12th and 13th ribs on digitized image; LD3 = LM depth between the 3rd and 4th lumbar vertebrae on digitized image; LMA12 = LM area between the 12th and 13th ribs on digitized image; LMA3 = LM area between the 3rd and 4th lumbar vertebrae on digitized image.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

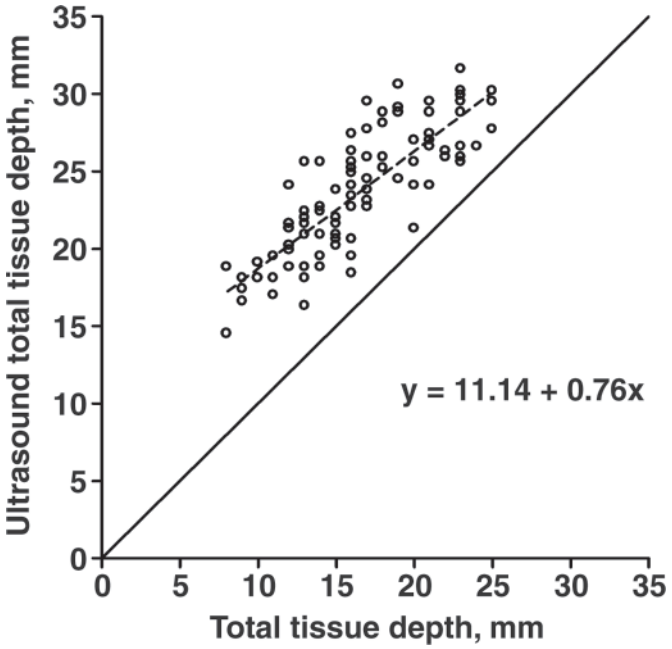

Figure 3. Relationship between live ultrasound total tissue depth and carcass total tissue depth measurements in lamb (n = 96). Regression line (– – –). Solid line represents perfect relationship between ultrasound and carcass measurement, y = x.

our study, measurements taken on the digitized images were used as a reference and considered as true values. Although they represent an acceptable compromise in terms of technical feasibility, carcass measurements are not a perfect reference and also entail their own degree of error that is included in the total measurement error. We used the error decomposition method to study the accuracy of ultrasound measurements in terms of precision and trueness and to understand the nature of the disagreements between the measurements taken on the live animal and the reference measurements (Table 3).

Total Tissue Depth. The GR_{us} measurement exhibited a significant bias as indicated by the ECT error, which represents 87% of the MSPE error (Table 3). The ER of the GR_{us} measurement was low and represented only 2% of the total error. However, carcasses with a smaller GR were more overestimated by ultrasonography than those with a larger GR, which is indicated by a regression slope less than unity ($P < 0.001$; Figure 3). Finally, the ED was 4.91 and represents 8.5% of the total error.

Because GR ultrasound measurement included the skin, the GR_{us} should be approximately 3.5 mm greater than the carcass measurement. In addition, the GR_{us} was evaluated between the ribs, whereas the GR was obtained with a ruler knife directly on the rib. In our preliminary trials, repeatable assessment of total tissue depth was obtained only between the 12th and 13th ribs due to the presence of connective tissues, giving a clear boundary with ultrasound device. These 2 variants could partly explicate the difference of 7.19 mm observed between GR_{us} and GR (ECT). Additionally, the greater propensity of thicker tissue to be compressed by the pressure on the ultrasound probe compared with thinner tissue (Purchas and Beach, 1981) could explain the error due to regression for GR measurement. Finally, our observations showed that the movements of the animal during the scanning process (movement of the head, breathing, etc.) influenced the total tissue depth, as it is generally admitted for all ultrasound measurements (Stouffer, 2004). Animal movement, combined with the differences specific to the measurements themselves (ultrasound vs. carcass), could explain the ED of the measure.

Fat Depth. For ultrasound fat depths, the MSPE and the trueness (ECT + ER) were similar between the different scanning sites (Table 3). It was mainly in the partitioning of the systematic errors (ECT and ER) that differences between scanning sites were noted. In the thoracic region ($FD12_{us}$), most of the error was due to central tendency (ECT/MSPE = 74%). If the skin was the only source of discrepancy between the ultrasound and carcass fat depth measurements, it would be plausible to obtain a difference of approximately 3.5 mm, or an ECT of 12.3 (3.5^2). However, the ER

Table 3. Accuracy of live ultrasound measurements (dependent variable) relative to carcass measurements (independent variable) in lamb[1] (n = 96)

Dependent variable	r^2	RSD, mm	CVe, %	MSPE	ECT	ER	ED	SEP, mm
Total tissue depth	0.689	2.24	9.51	57.70	51.69	1.10	4.91	2.47
Fat depth								
12th-13th rib	0.670	0.92	10.74	7.70	5.68	1.20	0.82	1.43
3rd-4th lumbar vertebrae trans.	0.615	1.06	12.08	7.53	0.85	5.58	1.10	2.60
3rd-4th lumbar vertebrae long.	0.667	0.97	10.87	7.52	1.18	5.41	0.93	2.53
LM depth								
12th-13th rib	0.113	1.99	6.35	12.82	5.56	3.40	3.86	2.71
3rd-4th lumbar vertebrae trans.	0.185	1.78	5.65	12.66	6.43	3.14	3.10	2.51
3rd-4th lumbar vertebrae long.	0.179	2.06	6.70	16.94	10.02	2.74	4.17	2.64
Sum of depths								
12th-13th rib	0.457	2.26	5.66	6.44	0.00	1.46	4.98	2.55
3rd-4th lumbar vertebrae trans.	0.694	1.61	4.01	6.82	2.60	1.67	2.54	2.06
3rd-4th lumbar vertebrae long.	0.697	1.78	4.47	8.47	4.32	1.05	3.10	2.05

[1]RSD = residual SD; CVe = CV of the residuals; MSPE = mean square prediction error, total measurement error; ECT = error of central tendency; ER = error due to regression; ED = error due to disturbance; SEP = SE of prediction; trans. = transverse; and long. = longitudinal.

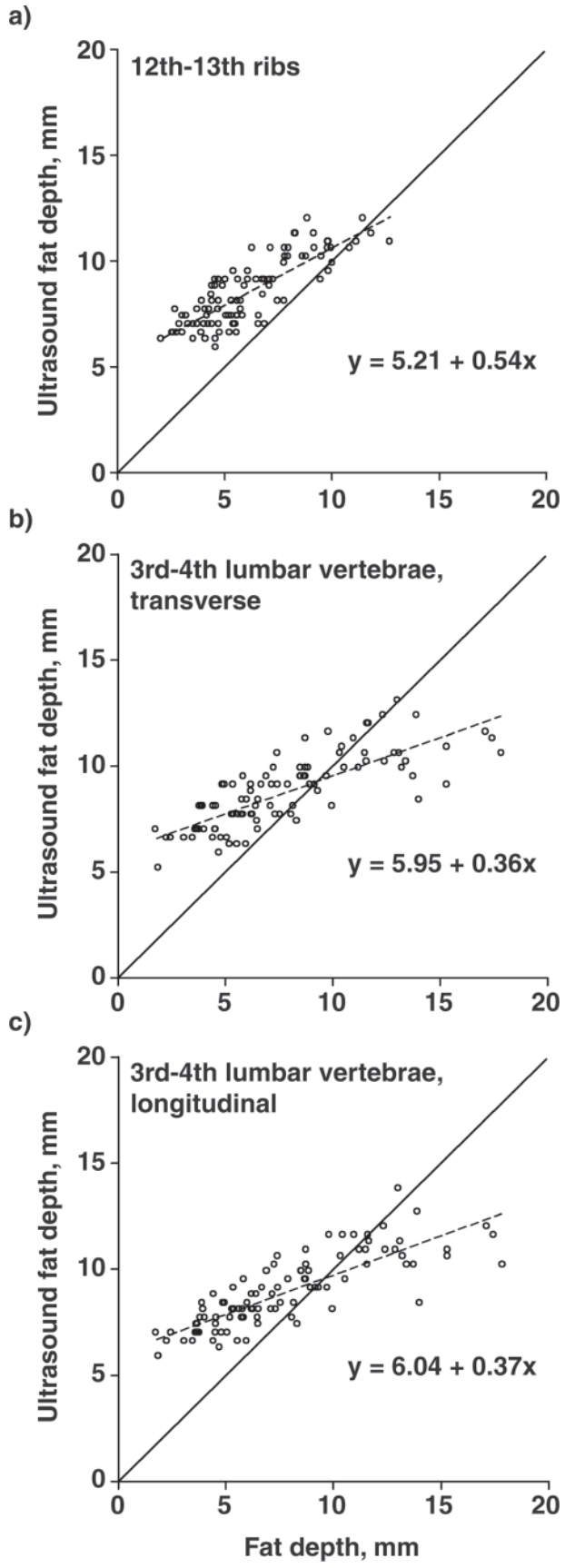

Figure 4. Relationship between live ultrasound fat depths and corresponding carcass measurements on digitized image in lamb (n = 96) a) between the 12th and 13th ribs; b) between the 3rd and 4th lumbar vertebrae, transverse; and c) between 3rd and 4th lumbar vertebrae, longitudinal. Regression line (– – –). Solid line represents perfect relationship between ultrasound and carcass measurement, y = x.

also must be taken into account, which was low for the $FD12_{us}$ (1.20), but high for the $FD3T_{us}$ and $FD3L_{us}$ measurements (5.58 and 5.41, respectively). These values indicate that the slope of the regression line was different from unity for the 3 fat depths (Figure 4) and that ultrasound measurements tend to overestimate the fat thickness in lean animals and to underestimate these measurements in the fat ones. This observation is reported by several authors for lambs (Fernandes, 2000), cattle (Greiner et al., 2003), and pigs (Moeller and Christian, 1998). However, after correcting our fat depth measurements to subtract the skin thickness, it appears that the ultrasound fat depths were truer in leaner lambs but underestimated carcass fat depth in fatter lambs (data not shown). These results were in agreement with those of Robinson et al. (1992) in cattle and those of Purchas and Beach (1981) and Fernandes (2000) in lambs. In the lumbar region, the underestimation of fat depth by the ultrasound measurements was even greater in fatter lambs. In fact, for these measurements, the MSPE was mainly due to ER. For all fat measurements, precision was good as indicated by their low ED and RSD of around 1.0 mm (Table 3).

For fat depth measurements, the root squares of the MSPE are comparable with the RMSE of 2.7 to 3.3 mm obtained between the 12th and 13th ribs by Herring et al. (1994) in cattle. In addition, the SEP of the ultrasound fat measurements calculated here (Table 3) was equal to the 1.4 obtained by Leeds et al. (2008) and compared favorably with those ranging from 1.8 to 3.2 mm reported by various authors in pigs (Moeller and Christian, 1998; Schwab et al., 2003) and cattle (Herring et al., 1994; Greiner et al., 2003). According to Tait et al. (2005), an SEP less than 2.54 mm would be an acceptable standard for fat measurements between the 12th and 13th ribs in lambs.

Fat depths were greater in the carcass than in the equivalent ultrasound measurements. This ECT appears to be mainly related to differences between ultrasound and reference measurements: inclusion of the skin in the ultrasound measurements but not in the carcass measurements, ultrasound measurements on hot living tissue vs. reference measurements on chilled dead tissue, etc. Pelt removal and carcass hanging can generate ECT, causing, respectively, expansion of fat layers (Robinson et al., 1992) and sliding of fat from the posterior (fatter) region toward the anterior, both modifying the fat depth measurement of hanged carcasses compared with live animals in a standing position (Mersmann, 1982; Turlington, 1990; Robinson et al., 1992). Moreover, because they have a greater influence in fatter than in leaner animals or tissues, these 2 phenomena together with pressure on the scanning probe can explain the observed ER values at given measurement sites and the difference of ER values between sites (Purchas and Beach, 1981; Robinson et al., 1992).

The ER in the lumbar region can be explained by the difference in tissue depth between sites (Table 1; 7.9 vs.

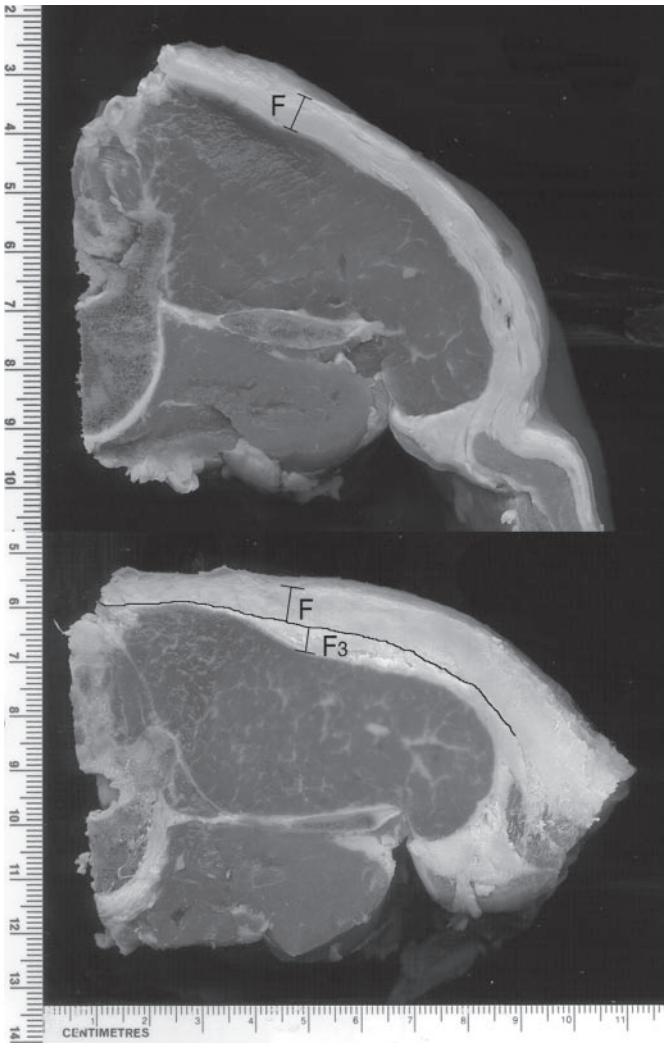

Figure 5. Digitized images between the 3rd and 4th lumbar vertebrae in lamb carcass. Fat depth is designated by letter F. Third fat layer (F_3) is apparent only on the second image.

6.2 mm; $P < 0.001$, for FD3 vs. FD12). The presence of a third fat layer between the 3rd and 4th lumbar vertebrae was observed in some digitized images (Figure 5), but never between the 12th and 13th ribs. Appearance of this additional fat layer has been documented in hogs (Fortin, 1986) but not in lambs. Lambs showing a third fat layer were fatter than those without it (31.8 vs. 23.1% dissected fat, respectively; data not shown). In addition, this fat layer seemed to increase from the ventral end of the loin eye muscle toward the backbone. We presumed that the third fat layer was an image artifact because its appearance in the images was inconsistent and because its definition at the loin extremity was usually poor. Ultrasound fat depth in fatter lambs having a third fat layer was underestimated by the exclusion of this unclear layer. Leaner lambs had better agreement between carcass and ultrasound measurements, probably as a result of the absence of this third fat layer.

As previously explained, the low probe resolution adds imprecision, increasing ED, in fat depth measure-

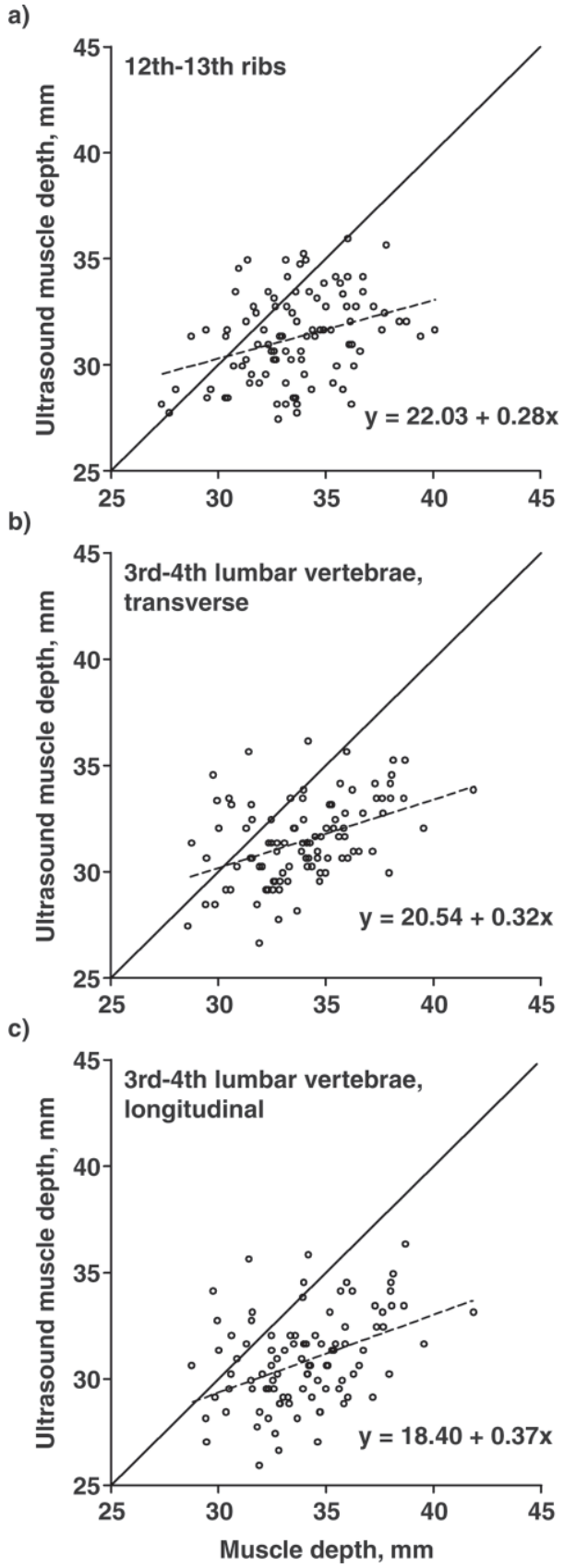

Figure 6. Relationship between live ultrasound LM depths and corresponding carcass measurements on digitized image in lamb (n = 96) a) between the 12th and the 13th ribs; b) between 3rd and 4th lumbar vertebrae, transverse; and c) between 3rd and 4th lumbar vertebrae, longitudinal. Regression line (– – –). Solid line represents perfect relationship between ultrasound and carcass measurement, y = x.

ments. Even if it was easier to distinguish the probe/skin interface than the skin/fat interface (Alliston, 1983), variation in skin thicknesses, as included in fat measurements, also increases ED in ultrasound fat depths. Pelt removal could also generate random errors of variable magnitude because some fat may be torn off the carcass (McLaren et al., 1991; Young and Deaker, 1994). Carcass fabrication and the handling of the pieces during image digitization could have caused some deformation in fat layers and thus increased ED. According to Pomar et al. (2001), it would be preferable to freeze the cuts before digitizing them to allow the fat to solidify and minimize fat layer deformation.

LM Depth. The magnitude of ultrasound LM measurement errors was greater than those observed for fat depths. Within scanning sites, the MSPE was greater for $LD3L_{us}$ than for $LD12_{us}$ and $LD3T_{us}$ measurements (Table 3). This difference was mainly caused by a larger ECT of the $LD3L_{us}$ measurement (Table 3; 10.02 vs. 5.56 and 6.43, for $LD3L_{us}$ vs. $LD12_{us}$ and $LD3T_{us}$, respectively). The ER was less for the $LD3L_{us}$ measurement, but the slopes of the lines between ultrasound and carcass LM depths were significantly different from unity in all cases ($P < 0.001$; Figure 6). Several authors reported such slopes meaning that ultrasound measurements overestimate the smaller LM and underestimate the larger ones in hogs (Moeller and Christian, 1998), lambs (Fernandes, 2000), and cattle (Greiner et al., 2003). Precision of ultrasound LM depths ($3.10 \leq ED \leq 4.17$) and their relationship with carcass measurements ($0.11 \leq r^2 \leq 0.19$) were low, even if the RSD and coefficient of variation of residuals (**CVe**) values appeared to be acceptable (Table 3). Despite the fact that the studied population was composed of lambs of 2 breeds with very different growth characteristics, the SD for carcass muscle depth was 2.6 mm, and the majority of this variation, nearly 2.0 mm (RSD), remained unexplained by ultrasound measurements. Leeds et al. (2008) also concluded that the low variability of the LM depth, in comparison with LM area, reduced its usefulness in prediction of carcass yield and value in lamb. With such muscle depth variability and such random error, it appears that ultrasound measurement could not be used to distinguish between the heavier- and lighter-muscled lambs in the current study. Nevertheless, the SEP of the ultrasound LM measurements (Table 3) is comparable with the 2.6 mm reported by Leeds et al. (2008). Comparison between species is limited because most of the authors measured loin area rather than depth.

Difficulty of identifying the deepest part of the LM due to its proximity to the ribs could explain the high ED of the $LD12_{us}$ measurements. Young et al. (1992) mentioned that the pressure on the probe deforms the fat layer uniformly, but not the muscle, due to the presence of the ribs in the thoracic region. On the other hand, the $LD3T_{us}$ and $LD3L_{us}$ random errors could be attributed to the incorrect interpretation of the fat-muscle boundaries. Underestimation of the fat depth resulting from the unintentional exclusion of the third

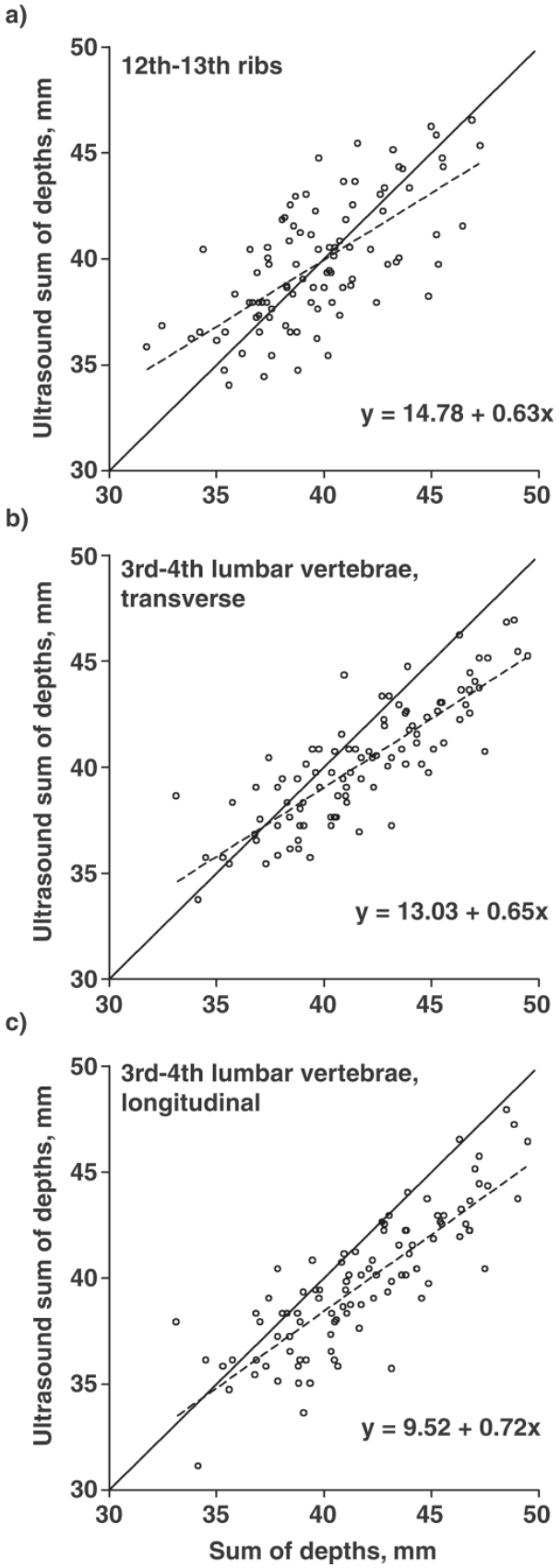

Figure 7. Relationship between sum of fat + muscle depths taken by ultrasound and sum of corresponding fat + muscle depths on carcass digitized image in lamb (n = 96) a) between the 12th and 13th ribs; b) between the 3rd and 4th lumbar vertebrae, transverse; and c) between 3rd and 4th lumbar vertebrae, longitudinal. Regression line (– – –). Solid line represents perfect relationship between ultrasound and carcass measurement, y = x.

fat layer could cause overestimation of muscle depths. To confirm these hypotheses, the sum of the ultrasound fat and muscle depths was compared with the sum of the same values assessed on the digitized images (Table 3 and Figure 7). Because the skin boundary (gel pad/skin interface) was easily identifiable, the agreement of these ultrasound values with the carcass measurements means that the lower muscle boundary were clearly distinguished on the ultrasound images. As expected, the sum of depth in the thoracic region contained a high proportion of random error (Table 3; ED/MSPE = 77.35%) compared with the other total measurements. Difficulty of distinguishing the LM end appears to explain the majority of the error for ultrasound total depths at this site, and then, muscle depth as speculated previously. Conversely, ultrasound and carcass sums of depths between the 3rd and 4th lumbar vertebrae were strongly related (R^2 of 0.69 and 0.70 and RSD of 1.61 and 1.78 mm, for transverse and longitudinal measures, respectively; Table 3 and Figure 7). Errors of these measures were, in fact, smaller than those of the fat and muscle depths at this site. In the lumbar region, the inner muscle boundary seems to be clearly discerned on the ultrasound images. These results suggest that the difficulty in distinguishing the fat/muscle boundary can reduce accuracy of muscle and fat measurements in the lumbar region and that image interpretation errors related to the presence of the third fat layer were responsible for the inaccuracy of these measurements between the 3rd and 4th lumbar vertebrae.

In regard to carcass measurements, changes on LM shape occurring during postslaughter chilling, hanging and handling of the carcass could be implicated in the lack of agreement between live ultrasound and carcass measurements (Fortin and Shrestha, 1986; Turlington, 1990; Hopkins et al., 1993). However, it is difficult to quantify how these phenomena affect muscle shape and the type of errors that will occur. Freezing the meat pieces before digitization could, as for fat, minimize muscle deformation (Pomar et al., 2001). Binnie et al. (1995) has performed measurements on frozen carcasses and obtained better precision (RSD = 1.74 mm).

Finally, the ED and ECT for longitudinal measurements could partly be explained by the variation in the angle of wave penetration and, hence, by the angle of measurement of LM depth. Depths will be larger when the probe is perpendicular to the skin and smaller when it is directed toward the backbone or toward the side of the animal (Youssao et al., 2002).

Comparing Measurements and Measuring Sites

As the accuracy of ultrasound measurement was established, comparison of scanning sites can be realized in regard to both precision (minimizing ED) and technical considerations.

Total Tissue Depth. Showing r-values comparable with those observed for fat depths, the GR ultrasound measurement appears to offer advantages in terms of accuracy, despite the greater absolute value of its random error (Table 3). In term of relative error variation (Table 3; CVe), GR seems to be slightly more precise than fat depths. Difficulty of distinguishing small differences in depth together with image interpretation errors would proportionally be less important when tissue depth or variability increase (Thompson et al., 1977; Simm, 1992; Young and Deaker, 1994), giving advantage to ultrasound GR over fat depth measurements.

Fat Depth. In regard to precision, the ED was smaller for fat depth between the 12th and 13th ribs than between the 3rd and 4th lumbar vertebrae. These findings agree with those of Fernández et al. (1998) based on coefficient of correlation at both scanning sites. However, Silva et al. (2006) obtained similar precisions for these 2 measurements sites (RSD ~0.8 vs. 0.9 mm).

In our study, the growth of a third layer of fat, between the 3rd and 4th lumbar vertebrae in the fattest lambs, makes this scanning site less useful in terms of precision of the fat measurements as well as in terms of practical application. Image interpretation problems encountered at this site demonstrate, as reported by Starck et al. (2001), the importance of having a good knowledge of the morphology of the studied tissues. Recognition of the existence of this additional fat layer will improve the accuracy of fat and muscle measurements at the lumbar region.

LM Depth. From a practical standpoint, measurements near the last ribs are the most used mainly due to the presence of ribs, which is an easily identifiable anatomical reference (Alliston, 1983; McLaren et al., 1991). However, our results, like those of Young et al. (1992), demonstrate that the proximity between the ribs reduces the accuracy of the LM measurements. Given the greater distance between the 3rd and 4th transverse processes than between the ribs, the exact site—maximum depth between the transverse processes—is easier to locate. Despite the low precision of ultrasound muscle depths in the present study, slight advantage goes to transverse measurements in the lumbar region, as confirmed by the greater r-values reported by Jensen (1977) and Silva et al. (2006) in the lumbar compared with the thoracic region. Results of Miles et al. (1972) in cattle also indicated that the boundary of the LM is more clearly defined in the lumbar than in the thoracic region.

Transverse vs. Longitudinal Measurements. This study is the first to compare the accuracy of transverse and longitudinal ultrasound measurements in lambs. Longitudinal measurements are very popular in hogs, and the work of Cisneros et al. (1996) demonstrated that there was no difference in the precision of the 2 types of measurement in this species. However, our results showed that transverse measurements were more precise than longitudinal ones for lumbar muscle depth

(ED = 4.17 vs. 3.10; Table 3) and sum of depths (ED = 2.54 vs. 3.10), most likely because of the greater stability in the angle of wave penetration. For ultrasound fat depth, the precision of longitudinal measurements compared favorably with transverse measurements (ED = 0.93 vs. 1.10; r^2 = 0.67 vs. 0.62). Longitudinal measurements were not performed between the 12th and 13th ribs because of the irregularity of the vertical line due to the presence of the ribs.

Conclusions

The primary focus of this study was to understand the discrepancies between ultrasound measurements taken on the live animal and carcass measurements. In vivo fat and GR depths in lambs were successfully measured using real-time ultrasound. However, in the population studied, variability in muscle depths was minimal, and ultrasound muscle depth measurements were neither precise nor correlated with carcass measurements. Therefore, we were unable to use real-time ultrasound measurements of muscle depth to rank the lambs according to their degree of muscle development. When only fat depth was assessed, the site between the 12th and 13th ribs seemed to be the most appropriate for measurement, but the accuracy of muscle depth measurement at this site was low. Transverse measurements of fat and muscle depths between the 3rd and 4th lumbar vertebrae were of an acceptable accuracy and were more accurate than longitudinal measurements. Based on the results of this study, we suggest that particular attention should be given to image interpretation to correctly identify and measure all fat layers. Moreover, because the depth of tissue studied in lamb was small, unlike in swine, ultrasound fat thickness in lambs might be better evaluated with probe resolution greater than 3.5 MHz. Ultrasound GR showed potential to be included in genetic selection programs, especially in countries where the GR measurement was used to estimate carcass quality. Ultimately, further studies are needed to establish the relationships between these different ultrasound measurements and carcass composition (fat, muscle, and bone) with the intention of predicting carcass quality.

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