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## **Editors:**

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*P u b l i s h e r s*

## ***Longissimus thoracis et lumborum* muscle volume calculation using *in vivo* real time ultrasonography**

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### **Abstract**

The *Longissimus thoracis et lumborum* muscle (LM) volume was measured *in vivo* by real-time ultrasonography (RTU) in 13 female sheep. Animals were scanned over 6 identified sites (7th, 9th, 11th and 13th thoracic vertebrae and 2nd and 4th lumbar vertebrae). After RTU measurements, the animals were weighed and slaughtered. RTU volume measurements were calculated by multiplying the LM area obtained at each site by the slice lengths. Equivalent measurements to those taken *in vivo* were obtained on the carcass using a digital camera and image analysis software. Correlation analysis was carried out to evaluate relationships between LM volume measured *in vivo* by RTU and in carcass. The LM volume measured in carcass and *in vivo* by RTU was highly correlated ( $r=0.96$ ,  $0.96$  and  $0.98$  for lumbar, thoracic and all vertebrae, respectively). These results strongly support that LM volume can be predicted *in vivo* by RTU.

**Keywords:** *longissimus* muscle, sheep, volume, ultrasound

### **Introduction**

The determination of three-dimensional structure from serial sections is a common problem in animal corporal composition studies. Computer tomography (CT) and magnetic resonance image (MRI) analysis are frequently used for *in vivo* three-dimensional structure determination by using consecutive images that can be reconstructed to render a volume measurement of a region of interest (Szabo *et al.*, 1999; Mitchell *et al.*, 2001). Studies conducted with pigs (Baulain, 1997; Mitchell *et al.*, 2001) and with lambs (Jopson *et al.*, 1995; Kvame and Vangen, 2006) showed that volume measured *in vivo* by CT and MRI was highly correlated ( $r > 0.90$ ) with the weights of the dissected tissues. Thus, these techniques have been pointed out as very accurate but its high cost limits the routinely application in animal science (Fuller *et al.*, 1994). The real time ultrasonography (RTU) can also be used for volume determinations.

Since almost five decades, ultrasounds have been used for evaluating animal composition based on tissue depth and area measurements (Stouffer *et al.*, 1961; McEwan *et al.*, 1989; Hopkins *et al.*, 1993; Silva *et al.*, 2005; Teixeira *et al.*, 2006). Compared to tissue area and depth, much less information is available about volume measurements. Results obtained with lamb (Maghoub, 1998) and with broilers (Silva *et al.*, 2006a) showed that muscle volume measurements obtained *in vivo* by RTU were able to explain carcass composition. Maghoub (1998) found that *longissimus thoracis et lumborum* muscle (LM) volume measured *in vivo* by RTU was correlated ( $r = 0.59$ ;  $n = 18$ ) with LM volume determined on carcass.

The study herein reported was undertaken to evaluate the capacity of RTU to measure *in vivo* LM volume in female sheep.



## Materials and methods

The experimental group consisted of 13 female sheep from the Île-de-France breed ( $59.3 \pm 8.9$  kg) selected from the research herd of the University of Trás-os-Montes and Alto Douro (Vila Real, Portugal). All animals were kept under the same management conditions and were fed according to the AFRC (1993) recommendations. Animal handling followed the EU directive number 86/609/EEC concerning animal care. Prior to ultrasound measurements and subsequent slaughter, animals were shorn and deprived of food for 12 h.

### Ultrasound measurements

Just before slaughter, animals were scanned with an Aloka SSD 500V real time scanner using a linear probe of 5.0 MHz (UST-588U-5, 64 mm, Tokyo, Japan). The wool at each measurement point was clipped close to the skin and shaved and a medical ultrasound gel was used as a coupling medium. The probe was placed perpendicular to the backbone over the following sites: 7th, 9th, 11th and 13th thoracic vertebrae and 2nd and 4th lumbar vertebrae, where RTU images were taken (Figure 1a). During ultrasound scanning, sheep were individually restrained. Once a satisfactory image had been obtained at each site, it was captured on a video printer for image analysis.

### Slaughter procedure and carcass measurements

After RTU measurements, the live weight of all animals was recorded. All the animals were stunned with a captive bolt gun and slaughtered by exsanguination. The fore and the hind limbs (feet) were then separated at the radio-carpal and tarso-metatarsal articulations, respectively and the pelt, head and all internal organs were removed. After being stored at 4 °C for 24 h, carcasses were weighed and split down by the vertebral column with a band saw. A segment of the thoracic/lumbar region (6th thoracic vertebra to the 5th lumbar vertebra) was removed from the right half to take carcass measurements equivalent to those taken *in vivo* by RTU. This segment was frozen over a horizontal surface in order to minimize muscle shape deformation. Then this segment was split by each vertebra with an electric saw and the planes where RTU measurements were taken were exposed (Figure 1b). After that, it was used a digital camera to capture images of the planes where the carcass measurements were taken and performed image analysis.

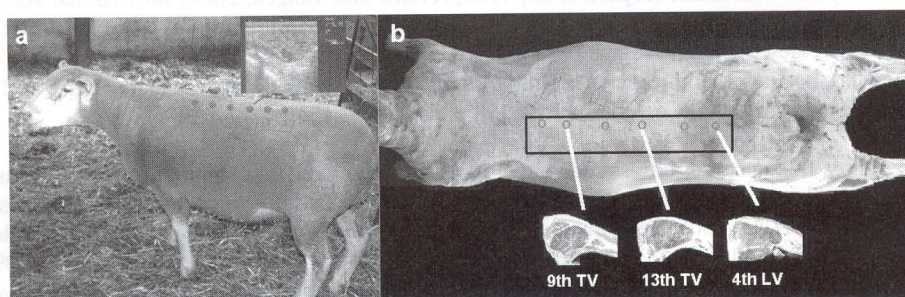


Figure 1. (a) Measurements points for RTU. Shown example for RTU image over the 13th thoracic vertebra. (b) carcass measurements points and joint examples for 9th, 13th thoracic vertebrae (TV) and 4th lumbar vertebra (LV) showing the planes where the LM area was taken.

## Image analysis and volume calculation

The printed images were digitized and RTU measurements taken. The area of LM for both RTU and carcass images, at each site, were determined after image analysis using the Image J software (<http://rsb.info.nih.gov/ij/>). The area was obtained by tracing the contour of LM and by counting the number of pixels on each of the 6 sites. The number of pixels was then converted into area measurements corresponding to the image plane of the 6 mentioned sites. Six areas one for each site was obtained.

The LM volume (LMV) was calculated by multiplying the areas obtained by *in vivo* RTU and carcass measurements by the slice lengths. The slice lengths were obtained after physical measurement *in vivo* or in carcass, using a ruler, of the length between the 6th thoracic vertebra and the 5th lumbar vertebra and divided by 6 to obtain the length of each slice. The following equation was used for LM volume calculation (cm<sup>3</sup>):

$$\text{Volume} = \sum_{i=1}^6 A_i d_i$$

where: d - is the slice length (cm); A - is the site area (cm<sup>2</sup>); i - is the number of slices.

The LM total volume (LMV<sub>tot</sub>) was calculated as the sum of the volumes of 6 slices: LMV7, LMV9, LMV11, LMV13, LMV2 and LMV4. Volumes of the LM from the 7th to the 13th thoracic vertebrae (LMV7–13) and from the 2nd to the 4th lumbar vertebrae (LMV2–4) were also calculated.

## Statistical analysis

Data were subjected to correlation analysis to study relationships between carcass and RTU measurements. All analyses were performed with SAS software (v. 8.2; SAS Institute Inc. Cary, NC).

## Results and discussion

### Live and carcass weight

Mean values, range of the values, standard error (SE) and coefficient of variation (CV) for live weight (LW) and carcass weight (CW) are presented in Table 1. These data show that the sheep presented a large range of variation in LW and CW.

Table 1. Mean values, range of values, standard error (SE) and coefficient of variation (CV, %) for live weight (LW) and carcass weight (CW).

	Mean	Range	SE	CV
Live weight (kg)	59.3	47.1-74.5	2.46	29.3
Carcass weight (kg)	31.2	26.1-40.6	1.31	15.1



## Carcass and ultrasound measurements

Mean values, SE and CV for carcass and RTU measurements and the simple correlation coefficients ( $r$ ) between RTU and carcass LM volume are presented in Table 2. The variation observed for all measurements is large. The LM volume increased from the 7th thoracic vertebra to the 4th lumbar vertebra for both carcass and RTU measurements. This finding was expected and it is, obviously, related to the LM shape (Korn *et al.*, 2005).

The LM volume measured *in vivo* by RTU and in carcass were different; the RTU measurements were lower than the carcass measurements. Similar trends were also reported in sheep for LM volume by Mahgoub (1998) and for LM area by Hamby *et al.* (1986), Edwards *et al.* (1989), Ward *et al.* (1992) and Fernández *et al.* (1998). This is also in agreement with results for LM area found in other animal species (cattle, Greiner *et al.*, 2003; poultry, Silva *et al.*, 2006a). The reasons generally accepted for this underestimation of carcass measurements by RTU are associated with difficulties on image analysis, differences between operators, and differences in muscle shapes due to slaughter procedure. In the present study all the measurements were done by the same operator and all the images were analysed using a computer program. So, it is reasonable to accept that differences in muscle shapes due to slaughter procedures were responsible for the underestimation of carcass measurements.

The ability of RTU for predicting carcass LM volume was high ( $r$  varied between 0.703 and 0.981;  $P < 0.01$ ). The lower correlation coefficients were found when muscle measurements were taken over the 7th and the 9th thoracic vertebrae. In these sites the LM is inside a more complex tissue distribution compared with the other sites along the thoracic-lumbar axis (Simm, 1983; Russel, 1995). This anatomical difference and the low absolute values of muscle measurements taken over the 7th and the 9th thoracic vertebrae are the possible reasons that explain these results. The image analysis

Table 2. Mean values, standard error (SE) and coefficient of variation (CV, %) for *Longissimus thoracis et lumborum* muscle volume (LMV) measured *in vivo* by real-time ultrasonography (RTU) and in carcass of sheep ( $n = 13$ ) and the simple correlation coefficients ( $r$ ) between *in vivo* real-time ultrasonography (RTU) and carcass measurements.

LMV (cm <sup>3</sup> )	Carcass			In vivo RTU			$r$
	Mean	SE	CV	Mean	SE	CV	
LMV7 <sup>a</sup>	63.7	4.5	25.6	61.1	4.1	24.1	0.813
LMV9 <sup>a</sup>	86.7	3.4	14.1	82.6	3.8	16.6	0.703
LMV11 <sup>a</sup>	101.9	5.0	17.7	100.9	5.1	18.1	0.942
LMV13 <sup>a</sup>	130.5	7.2	19.8	127.9	6.6	18.5	0.935
LMV7-13 <sup>b</sup>	382.8	18.3	17.2	372.4	18.6	18.0	0.958
LMV2 <sup>a</sup>	138.4	6.1	16.0	127.0	5.6	15.8	0.921
LMV4 <sup>a</sup>	119.5	12.5	37.8	112.4	9.0	29.0	0.951
LMV2-4 <sup>c</sup>	257.9	17.9	25.1	239.3	13.9	21.1	0.957
LMVtot <sup>d</sup>	640.7	35.6	20.0	611.8	32.0	18.9	0.981

<sup>a</sup> LMV7, LMV9, LMV11, LMV13, LMV2, LMV4: *Longissimus thoracis et lumborum* muscle volume above the 7th, 9th, 11th and 13th thoracic vertebrae and the 2nd and 4th lumbar vertebrae, respectively.

<sup>b</sup> LMV7-13: *Longissimus thoracis et lumborum* muscle volume between the 7th and the 13th thoracic vertebrae.

<sup>c</sup> LMV2-4: *Longissimus thoracis et lumborum* muscle volume between the 2nd and the 4th lumbar vertebrae.

<sup>d</sup> LMVtot: *Longissimus thoracis et lumborum* muscle total volume.

All correlation coefficients ( $r$ ) are significant \*\*\*  $P < 0.01$ .

using a computer program combined with the ultrasound equipment used in this study, namely the probe length (64 mm), were able to accurately measure the LM volume, allowing the assessment of differences between animals. Improvement in the predicting ability of RTU can be achieved by using large probes and image analysis (McLaren *et al.*, 1991; Young and Deaker, 1994; Williams, 2002; Silva *et al.*, 2006b). The length of the probe may have contributed to the good prediction ability of LM volume in our study because it allows the identification of the lateral boundaries of the LM on RTU images, as observed by Stouffer (2004) in cattle and Silva *et al.* (2006b) in sheep.

Few studies (Mahgoub, 1998; Silva *et al.*, 2006a) have been published on LM volume determinations *in vivo* by RTU. Mahgoub (1998) reported a correlation coefficient of 0.59 ( $P < 0.01$ ) between LM volume measured *in vivo* by RTU and in carcass, in sheep ( $n = 18$ ), and Silva *et al.* (2006a) obtained a correlation coefficient of 0.866 ( $P < 0.01$ ) between breast volume measured *in vivo* by RTU and in carcass, in broilers ( $n = 103$ ). In the present study, correlation coefficients obtained between LM volume determined *in vivo* by RTU and in carcass were higher ( $r = 0.968$ ;  $P < 0.01$  for LMVtot) than those obtained by these authors. Several authors determined LM volume using CT (Jopson *et al.*, 1995; Kvame and Vangen, 2006) and found high correlations between *in vivo* and carcass measurements in sheep ( $r > 0.90$ ). Findings of the current study show that LM volume measured *in vivo* by RTU is able to accurately predict carcass LM volume in sheep.

## Conclusions

This study showed that LM volume measurements taken *in vivo* by RTU are highly correlated to corresponding carcass measurements. Results issued from this study encourage the use of LM volume measured by *in vivo* RTU. This approach is non-invasive, accurate, reliable, and easy to use and require an inexpensive common RTU machine. Moreover, such measurements in everyday practice are easy to obtain due to the equipment mobility.

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Livestock production systems will only be sustained in the long term if their products continue to meet the demand of consumers. The quality of ruminant carcasses, meat and meat products is of predominant importance in a competitive market where consumers tend to have a preconceived idea about the criteria that define meat quality such as flavour, tenderness, juiciness, smell, colour and texture. The carcass evaluation could be interesting as a precocious classification of the final quality of meat coming from each carcass. Today the quality characteristics of the meat must be different according to its utilisation (supermarket, butcher, catering, refectory, etc.) and so it is very important to choose very early the final destination of the carcass. Obviously, the carcass classification must correlate with meat quality characteristics required by final consumer. Other important factors that have to be taken into account in order to maintain a demand for ruminant meat are safety and traceability.

This book reviews the historical and recent developments for carcass evaluation and grading for meat quality assessment in beef and sheep. It places special emphasis on new concepts and approaches to define carcass and meat quality and on the use of modern technologies for composition and quality evaluation. A range of technologies are presented such as ultrasounds and colour reflectance, X-ray computerised tomography, spectral and thermal imaging, image analysis and NIRS. The use of phenotypic markers such as the plasma hormones and genetic markers to predict carcass composition and meat quality are also presented.

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