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Similarity of *longissimus thoracis* parameters and fat thickness measured by ultrasound and digital image analysis

Similitud de los parámetros de *longissimus thoracis* y el espesor de la grasa medidos mediante ecografía y análisis digital de imágenes

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ABSTRACT. The aim of the present study was to evaluate the similarity of area ($LTMA_{US}$, cm^2), depth ($LTMD_{US}$, cm) and width ($LTMW_{US}$, cm) of the longissimus thoracis muscle and fat thickness (SFT_{US}) measured by ultrasound and ImageJ software. Ultrasound measurements were performed on 36 male sheep 24 h prior to slaughter and immediately measured using electronic callipers with a resolution of 0.1 cm. Images were stored on a flash drive, opened on a computer and measured using ImageJ ($LTMD_{DIA}$, $LTMW_{DIA}$, $LTMA_{DIA}$ and SFT_{DIA}). There were no differences between ultrasound and ImageJ measurements (P = 0.3610) for Hotelling's T^2 test. Our results show that ImageJ software is a potential tool for in vivo measurement of longissimus thoracis parameters and fat thickness in hair sheep.
Keywords: Carcass characteristics, ImageJ software, Meat Sheep, Tropical sheep.

RESUMEN. El objetivo del presente estudio fue evaluar la similitud del área ($AMLT_{US}$, cm^2), la profundidad (PLT_{US}, cm) y la amplitud (ALT_{US}, cm) del músculo *longissimus thoracis* y el espesor de la grasa (EGS_{US}) medidos mediante ecografía y el programa informático ImageJ. Las mediciones ecográficas se realizaron en 36 ovinos, 24 h antes del sacrificio y se midieron inmediatamente utilizando calibradores electrónicos con una resolución de 0.1 cm. Las imágenes se almacenaron en una unidad flash, se abrieron en un ordenador y se midieron con ImageJ (PLT_{DIA}, ALT_{DIA}, $AMLT_{DIA}$ y EGS_{DIA}). No hubo diferencias entre las mediciones ecográficas e ImageJ (P = 0.3610) para la prueba T^2 de Hotelling. Nuestros resultados demuestran que el software ImageJ es una herramienta potencial para la medición in vivo de los parámetros del *longissimus thoracis* y el grosor de la grasa en ovejas de pelo.
Palabras clave: Características de la canal, software ImageJ, ovino de carne, ovino tropical.

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INTRODUCTION

The use of ultrasound measurements can improve the accuracy and precision of predicting carcass traits in sheep. It can also help in selection programmes and assist decision support systems in determining the optimum carcass endpoint, thus improving the productivity of sheep production systems (Camacho-Pérez *et al.* 2023, Muñoz-Osorio *et al.* 2024). In addition, it is a non-invasive technique to know the state of different body tissues of farm animals. This could be developed as a practical tool to predict the optimal time to slaughter animals with care and comfort to ensure animal welfare (Gastelum-Delgado *et al*. 2024). The good correlation between ultrasound measurements and carcass measurements of back fat thickness and longissimus dorsi muscle area has been reported by some authors (Sahin *et al*. 2008, Grill *et al*. 2015, Aguilar-Hernandez *et al*. 2016). However, when using this technique, hair sheep are usually handled to shave the area to be measured first to promote the best coupling of the transducer to the skin and thus obtain quality images (Gastelum-Delgado *et al*. 2024). The animal must then be restrained while the parameters of the longissimus thoracis muscle are measured using the instrument's digital calliper (Gastelum-Delgado *et al*. 2024). All of this takes approximately 3-5 minutes, which can be stressful for the animal. It is therefore necessary to evaluate tools or applications capable of reducing the time required for the measurement and handling of animals. In this sense, Pimentel *et al*. (2023) reported that it is possible to subsequently analyse the images obtained from ultrasound measurements of the parameters of the longissimus thoracis muscle using digital image analysis technology with ImageJ software, which reduces the time spent handling the animals and avoids stressing them.

However, there are still some questions regarding the similarity of longissimus thoracis muscle parameters and fat thickness measured by ultrasound and Image J software in hair sheep. For more than 25 years, the NIH Image and ImageJ software has been a pioneering open tool in image processing for the analysis of scientific images (Schneider *et al*. 2012). One of the main advantages of this software is its open-source code, which is available under a BSD-2 licence. As a result, many plug-ins have been developed by its users, greatly enhancing the capabilities of digital image processing and analysis (ImageJ 2024). Over the past 25 years, applications of ImageJ have included materials science, soil science, astronomical and climatological data, medical imaging, and crystallographic analysis (Schneider *et al*. 2012). Recently, the usefulness of ImageJ software for the evaluation of the size and shape of starch granules has been investigated. The results showed that ImageJ can be used successfully for this purpose (Boruczkowski *et al*. 2022). Similarly, ImageJ provided pollen counts in 60 seconds or less per image (Costa and Yang, 2009). This is very similar to human eye counting (5-68 minutes). However, it provides a fast, cheap and reliable solution for counting pollen from digital images because it is much faster and reduces the amount of labour required. In addition, Martin *et al*. (2020) used the ImageJ software instead of a leaf area meter in both white oat and black oat crops. The accuracy of the results was not affected. ImageJ software is a useful tool for agricultural science. In animal science, recent applications have been in the comparison of ultrasound images of reproductive and productive structures (ovaries, muscle depth and width) in livestock (Brito *et al*. 2022, Pimentel *et al*. 2023). We therefore hypothesised that the use of ImageJ software would be effective in facilitating the evaluation of ultrasound measurements and would also be a potentially useful tool for field measurements and subsequent computer measurements. The aim of the present study was to evaluate the similarity of area, depth, and width of the *longissimus thoracis* muscle and fat thickness measured by ultrasound and ImageJ software in hair sheep.

MATERIALS AND METHODS

The animals used in this study were treated in accordance with the guidelines and regulations for ethical animal experimentation of the Department of Agricultural Sciences of the Universidad Juárez Autónoma de Tabasco (CIEI: Folio 1173-2022). The experiment was conducted at the Southeast Sheep Integration Centre (Centro de Integración Ovina del Sureste [CIOS]; 17° 78'' LN, 92° 96'' LW; 10 masl), located 25 km from the Villahermosa-, Tabasco state, Mexico. A total of 18 male Katahdin lambs, 6-9 months old, with a mean BW of 36.64 ± 11.42 kg and 18 male Black Belly lambs, 6 months old, with a mean BW of 32.07 ± 2.33 kg were used. Lambs were weaned at 60 days of age and fattened in a cage system with raised slatted floors and group feeding (ten animals per cage) for 60 to 100 days in a feedlot system. Animals were selected from the fattening groups when they reached the target BW for slaughter. The diet consisted of 80% concentrate and 20% forage with an estimated 15% crude protein (CP) and 12 MJ of metabolizable energy (AFRC 1993) and was offered ad libitum as described previously Gastelum-Delgado *et al.* (2024).

Ultrasound measurements (US) were performed 24 h before slaughter. A Mindray DP Vet 50® B-mode real-time ultrasound device (Mindray Ltd. and National Ultrasound Inc.; Wuxi, Jiangsu, China) with a 7.5 MHz linear probe was used to determine subcutaneous fat thickness (SFT_{US}) and *Longissimus thoracis* muscle depth ($LTMD_{US}$, cm), width ($LTMW_{US}$, cm) and area ($LTMA_{US}$, cm^2) as previously described by Sahin *et al.* (2028). The probe was positioned perpendicular to the spine above the 13th thoracic vertebra (Morales-Martinez *et al.* 2020, Muñoz-Osorio *et al.* 2024). Previously, sheep were shaved between the 12th and 13th thoracic vertebrae. The acoustic gel was used to improve contact between the animal's skin and the device after the sheep had been manually immobilised and passed through the probe. The pressure on the head of the transducer was kept to a minimum to avoid compression of the subcutaneous fat layer (Chay-Canul *et al*. 2019, Muñoz-Osorio *et al.* 2023). Furthermore, the same trained operator performed all measurements from the right side of the sheep. Images, $LTMD_{US}$, $LTMW_{US}$, $LTMA_{US}$ and fat thickness (SFT_{US}) were frozen and measured immediately using the electronic callipers of the devices (Morales-Martinez *et al.* 2020, Muñoz-Osorio *et al*. 2024).

In addition, images were stored on a USB stick, opened on a computer, and manually measured using ImageJ ver. 1.54g. LTMD, LTMW, LTMA and SFT were measured using the DIA software tools (LTMDDIA, LTMWDIA, LTMADIA and SFTDIA) as described by Pimentel *et al*. (2023). Image processing was performed by the same operator. The operator placed a mobile cursor at both ends of each measurement to optimise the orientation of the image. Several steps were involved in the digital measurements. First, using the depth scale on the ultrasound monitor, the pixel/cm scale was set by converting pixels to cm units (Figure 1). The straight-line tool was then used to measure the fat thickness, width and depth of the *longisimus thoracis* (Figure 1). The polygon selection tool was then used for the total muscle area (Figure 1).

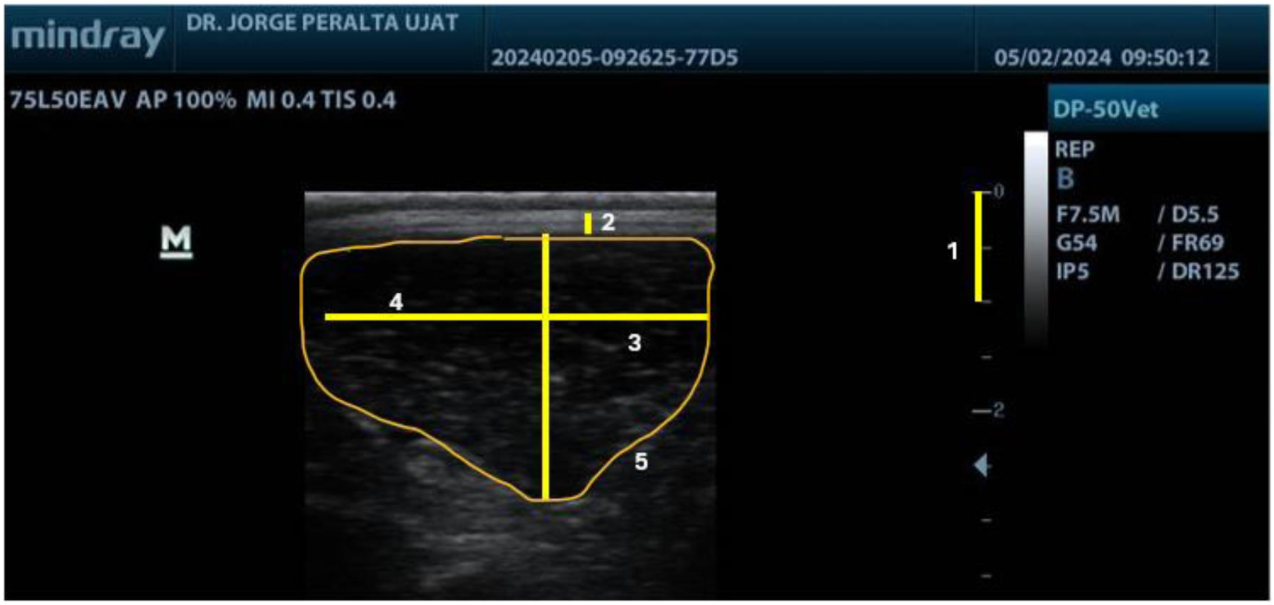

Figure 1. Measurements recorded on the digital images of set the scale (1), fat thickness (2), depth (3), width (4) and area of the *longisimus thoracis*).

Due to the presence of one factor (treatment: ultrasound versus ImageJ) and four dependent variables (LTMD, LTMW, LTMA and SFT) measured on the same experimental units (animals), a multivariate analysis approach was chosen. Firstly, to explore differences between measurement methods, variable profiles (Manly and Navarro 2016) were constructed by treatment, using box plots for each variable analysed. Second, the null hypothesis of no significant differences between treatments was tested with a Hotelling's T^2 test for paired samples, because, as mentioned above, each animal is considered a sample measured twice (for two treatments, ultrasound versus ImageJ) and four dependent variables, so the samples are considered matched or paired and not independent (Johnson and Wichern 2002). This test is based on the assumptions of multivariate normality and equality of the variance-covariance matrices (Rencher 2002), which in this case were tested with the Doornik-Hansen and Box's M tests, respectively. All calculations were performed with the PAST program (Hammer *et al*. 2001), version 4.15.

RESULTS AND DISCUSSION

According to the profiles of variables plot, there are no significant differences between the two treatments in all variables considered in the present studies (Figure 2). In addition, the results of the paired Hotelling’s T^2 test confirmed the pattern described above, as there were no significant differences between the area, depth and width of the *Longissimus thoracis* muscle and fat thickness measured by ultrasound and image analysis (ImageJ) in hair sheep (F = 1.1273, P = 0.36108 with 4, 32 d.f.).

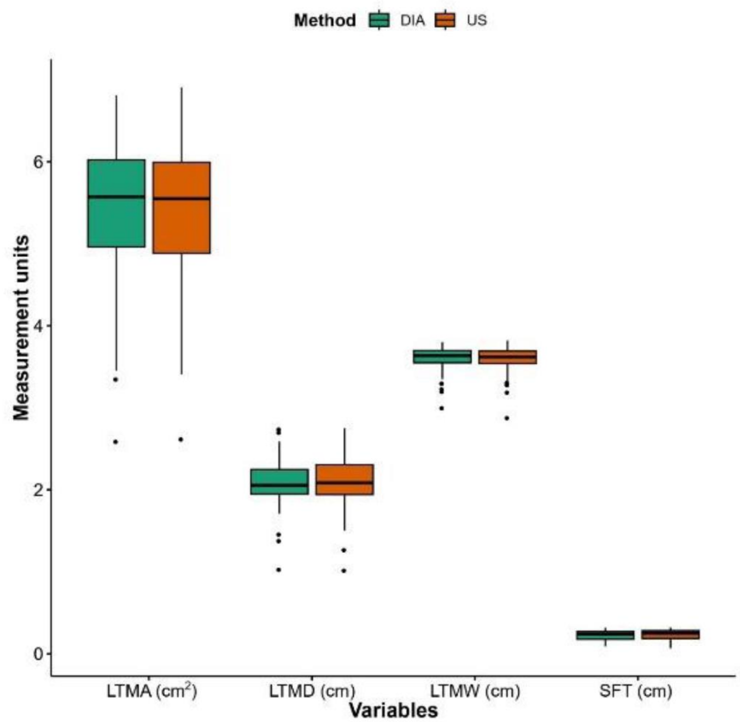

Figure 2. Profiles of variables measured by Ultrasound (US) and ImageJ software (DIA) methods. LTMW = *Longissimus thoracis* muscle width, LTMD= *Longissimus thoracis* muscle depth, LTMA= *Longissimus thoracis* muscle area and SFT= subcutaneous fat thickness

This study investigated the similarity of area, depth and width of the *Longissimus thoracis* muscle and fat thickness measured by ultrasound and ImageJ software in hair sheep as an alternative to reducing the time spent on ultrasound assessment of animals. To provide animals with care and comfort to ensure animal welfare (Gastelum-Delgado *et al*. 2024). Ultrasound (US) techniques have been used to predict carcass composition and meat quality in vivo for more than 50 years. Since its inception, and especially in the last two decades, US has proven to be a valuable tool for estimating carcass composition and meat traits in live animals (Silva *et al*. 2006). This technique has undergone tremendous development in the last decade and is currently presented as indispensable in meat production as a non-invasive, versatile, easy-to-use, and low-cost tool for the assessment of meat traits (Afonso *et al*. 2022). However, it is still little used in sheep, when compared to beef cattle.

In the present work, the results confirmed that there were no differences between the techniques of measuring the area, depth and width of the *Longissimus thoracis* muscle and fat thickness in hair sheep (F= 1.1273, P= 0.36108 with 4, 32 d.f.). Similarly, Brito *et al.* (2022) compared the effectiveness of two methods of evaluating B-mode and Doppler ultrasound images: analysis using ultrasound software and using a computer with ImageJ software to evaluate the morphological and vascular characteristics of follicular dynamics and luteal function in crossbred cattle. They concluded that the use of ImageJ software is an effective biomedical technique for analysing ultrasound images of morphological and vascular characteristics before and after ovulation in cattle. Pimentel *et al*. (2023) also concluded that it is possible to analyse images obtained from ultrasound measurements of *L.*

thoracis muscle parameters using the ImageJ programme. In addition, ImageJ can be used for post-analysis of the images, reducing the time spent handling the animals and avoiding stress to them. Silva *et al.* (2006) noted that efforts have focused on automated image analysis systems that improve image analysis and reduce operator and image acquisition effects.

There were no significant differences between the area, depth, and width of the longissimus thoracis muscle and fat thickness measured by ultrasound and image analysis (imageJ) in hair sheep (F = 1.1273, P = 0.36108 with 4.32 d.f.). Our results showed that ImageJ software is a potential tool for use in the in vivo measurement of longissimus thoracis muscle parameters and fat thickness in hair sheep.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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