



Use of ultrasonographic examination for *in vivo* evaluation of body composition and for prediction of carcass quality of sheep



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ABSTRACT

Since the first use in examination of soft tissue in 1950, the ultrasound technique has had an enormous success as an excellent diagnostic tool for the medical field. As a consequence of this relation with the medical field, a huge technological evolution of equipment and image analytic methods have been observed. Alongside with medical applications, ultrasound techniques have been used for evaluation of body composition of farm species since the late 1950s. The technique has been used to predict *in vivo* traits related to carcass composition in various animal species. This review summarises developments in the application of ultrasound technique in sheep. Experimental results are analysed and aspects that influence accuracy and precision of the technique are reviewed. In general, the ultrasound technique shows to be able to evaluate *in vivo* traits related to carcass composition and quality in sheep. In the future, further developments expected for the technique together with other imaging techniques will allow deeper understanding of the body composition of that species.

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1. Introduction

Development of accurate methods for predicting carcass composition in animal species used for meat production is of great significance for performance testing, grading to establish a value-based market system and to implement breeding selection schemes (Bünger et al., 2011; Notter et al., 2014; Scholz et al., 2015). In recent years, there have been important developments in non-invasive and non-destructive image techniques to obtain objective data on carcass and body composition of meat-producing animal species (Silva and Cadavez, 2012; Scholz et al., 2015). Among the various image techniques available, real-time ultrasonographic examination has become the most common technique for carcass and body composition assessment of meat species (Stouffer, 2004; Scholz et al., 2015). Although remarkable improvements of other image technologies, e.g., magnetic resonance imaging (MRI – Scholz et al., 2015) or computed tomography (CT – Bünger et al., 2014) ultrasonographic examination still maintains its utility in predicting carcass traits in live sheep. In recent years, several reports have shown the merit of a dual approach using both ultrasonographic examination and computed tomography for selective breeding to improve lamb carcass quality (Bünger et al., 2014). Ultrasono-

graphic examination can be used as an initial screening tool, with subsequent use of computed tomography on a subset (~10%) of potentially elite breeding males (Macfarlane et al., 2006; Bünger et al., 2014). For over 50 years, extensive experimental work has been carried out to collect information about the relationship between measurements obtained with ultrasonographic examination and body composition and carcass traits of sheep (Teixeira et al., 2006; Silva et al., 2006; Hopkins et al., 2007; Leeds et al., 2008). The aim of much of this work has been to describe the ability of ultrasonographic examination to predict carcass and body composition of sheep (Thwaites, 1984; Silva and Cadavez, 2012). Objective of the present review is the presentation of a broad overview of the implementation of ultrasonographic examination for assessing body and carcass composition in sheep.

2. Milestones and developments of ultrasonographic examination to assess body composition in sheep

The discovery of the piezoelectric properties of certain crystals in the late 19th century by the Curie brothers (Woo, 2006) and, later, the military applications of pulse-echo techniques during the two world wars (Van Tiggelen and Pouders, 2003) are the roots of the use of ultrasonographic techniques for medical and animal science purposes. In fact, throughout that period, and in parallel with the military development, it had been comprehended that pulse-echo techniques had a potential for employment for medical

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purposes; the first diagnostic ultrasonographic examination study was published during the 2nd World War (Dussik, 1942). Later, in the early 1950s, the first work for measurement of biological tissues and detection of tissue density with ultrasounds has been published (Wild, 1950). With that work, a step was done to make possible the identification, non-invasively and non-destructively, of the organs and tissues of animals. These attributes are at the base of the huge success that ultrasonographic examination has reached in the medical field and in animal science to predict *in vivo* carcass traits.

In fact, the first studies to predict carcass composition of meat-producing animal species were published in the late 1950s (cattle: Temple et al., 1956; pigs: Claus, 1957; Dumont, 1957). The first relevant study in sheep has been published a couple of years later (Campbell et al., 1959). At that time, simple devices designated A-mode (A from 'amplitude') were used. In the A-mode equipment, echoes formed from boundaries of target tissues appear as peaks on an oscilloscope screen. The height (amplitude) of peaks is related to the acoustic properties of the target tissues. In the oscilloscope display, the horizontal axis expresses the distance and the vertical axis the height (amplitude) of peaks (Thwaites, 1984; Wells, 1991; Whittaker et al., 1992). The distances between successive peaks represent different tissues and in devices utilised in animal science, the horizontal axis of the oscilloscope is usually calibrated in millimetres so that tissue thickness can be read directly (Simm, 1983). The A-mode device could measure tissue thickness, but was not capable of measuring areas (Thwaites, 1984). Further, the equipment lacks anatomical information and, often, thickness measurements of subcutaneous fat (SF) show erroneous results as a consequence of the formation, in some animals, of fat layers echoes that are confounded with the subcutaneous fat:muscle interface (Nicol and Parratt, 1984; Hopkins, 1990; Russel, 1995).

To overcome those limitations, the B-mode presentation (B from 'brightness') was introduced. In the B-mode display, an image of the object was built by integrating multiple A-mode signals (Amin, 1995). A popular B-mode device was the Scanogram, which had been introduced in 1969 and was the main system used in the following decade for most live animal evaluations (Stouffer, 2004). In that instrument, the transducer is motor-driven along a track, shaped to fit the back of different animal species; movement of the probe was captured by a camera photographing successive echoes that are displayed on an oscilloscope. A two-dimensional scan is built up by holding the shutter of the camera open as the probe travels the length of the track (Andersen et al., 1983). The Scanogram was utilised in several works with sheep from 1977 (Thompson et al., 1977; Shelton et al., 1977) until the mid-1980's (Leymaster et al., 1985; Fortin and Shrestha, 1986). From images obtained in those works, it has become possible, beyond thickness measurements, to determine the area of the muscles *longissimus thoracis* and *longissimus lumborum*. Launch of real-time ultrasonographic examination systems led to the end of the B-mode scanners, which had completely disappeared by late 1980s (Szabo, 2004).

Real-time ultrasonographic examination is a technique which uses repeated scans to form an almost instantaneous (real-time) image (Simm, 1983). The entire image frame must be displayed in 33 ms or less to be able to update the information at real-time frame rates (Insana, 2006). The transducer emits a wider ultrasound beam producing images of a section of an anatomical region under study. Its potential is increased by the ability of a single scan providing a complex image. The technique was developed for human medicine with the aim of viewing fast-moving organs, e.g., heart and its valves (Szabo, 2004). Horst (1971) was the first to use this technique in animal science. The first equipment employed in sheep was the Danscanner (Busk, 1984), which had a probe with 80 piezoelectric elements arranged in a line in a fluid-filled head, with the dorso-lumbar form of the species in which it is used (Thwaites, 1984).

In the 1980s and 1990s, the devices developed for human medicine began to be the most used to predict sheep carcass traits. Various devices have been utilised in that work, although the most utilised equipment was the Aloka 500, which is small, portable and sufficiently robust for use in field situations. Additionally, transducers specifically developed for use in animals have become available for this equipment. Frequency of transducers used in work with sheep varies between 3.5 and 7.5 MHz. Their length also shows variation, but some models have sufficient length to allow all the area of the muscles *longissimus thoracis* and *longissimus lumborum* to be viewed in one image (Tait, 2016).

Currently, there is a large supply of ultrasound equipment for animal science that can be used in work with sheep. Also, the suppliers can always provide transducers suitable to the objective of the work and often transducers with multiple frequencies features (Silva and Cadavez, 2012). Aspects associated with portability, robustness and ease of use in the field make the actual equipment appealing for the current use with sheep (Tait, 2016). Nevertheless, when a system would be selected for evaluation of body composition traits of animals, its capacity to provide accurate measures is the central and foremost issue to be taken into account (Tait, 2016).

Table 1 reviews the major milestones and developments of ultrasonographic equipment for *in vivo* assessment of body composition of sheep.

3. Causes of inaccuracy related to the application of ultrasonographic examination in sheep

Several factors can contribute to the inaccuracy of the measurements obtained during the ultrasonographic examination and, consequently, to the estimates of body and carcass composition of sheep. The most significant factors are the preparation and localisation of the reference points, the animal restraint, the coupling of the probe with the animal skin, the pressure applied to the probe, the operator, the level fat of animals and the interpretation and analysis of the images. Despite the fact that some factors are evident, attention should be given to them all, to minimise inaccuracy of the measurements obtained during the examination.

3.1. Preparation of reference points

In sheep, preparation of reference points is a critical step to capture ultrasonographic images. In fact, as sheep have wool, it is necessary to prepare reference points to ensure a good probe-skin coupling. Usually, two approaches have been used. One by shearing and the other by parting the wool at the reference points. Both methods have the objective to guarantee a skin field, where the probe would be placed. Some authors have mentioned the need for clipping and trimming the wool close to the skin before carrying out the measurements (Kempster et al., 1982; Leymaster et al., 1985; Fortin and Shrestha, 1986; Stouffer, 1991; Silva et al., 2016), whereas others (Hopkins et al., 1996; Teixeira et al., 2006; Hopkins et al., 2007; Esquivelzeta et al., 2012) have indicated that this operation would not be necessary.

The former have supported the opinion that it would be required to shear the wool to avoid strange echoes from trapped air bubbles between coupling agent and wool. Little work has been conducted to compare both approaches, but it seems that larger linear probes are more susceptible to have coupling problems (Bass et al., 1982; McLaren et al., 1991). However, with A-mode equipment equipped with a 5.0 MHz transducer of 5 mm diameter, Gooden et al. (1980) have found that it could operate well only with parting of the wool fibres. Also, Bass et al. (1982), using the same type of device, have found no significant differences in estimation of subcutaneous fat thickness observed between sheared and non-sheared animals;

Table 1

Milestones and developments of use of ultrasound technology for *in vivo* evaluation of body composition and for prediction of carcass quality of sheep.

Milestone	Year	Scientists	Description	Reference
Piezoelectric effect	1881	P. Curie	Pierre Curie found a connection between electrical voltage and pressure on crystalline material	Szabo (2004)
First medical application	1942	K. Dussik	Ultrasound application to localise brain tumours	Dussik (1942)
First report of soft tissue examination	1950	Wild	The first scientific proof of sonic energy reflection from within soft tissue	Wild (1950)
First animal evaluation	1956	Temple et al.	First ultrasonographic study to evaluate <i>in vivo</i> carcass traits	Temple et al. (1956)
First B-mode study	1959	Stouffer	Cross-sectional image of beef rib eye produced by an early mechanical B-scan system	Stouffer (1959)
First sheep evaluation	1959	Campbell et al.	First study published with sheep	Campbell et al. (1959)
First B-mode application in sheep	1961	Stouffer et al.	Study showing the superior performance of mechanical B-mode over the A-mode	Stouffer et al. (1961)
First real time equipment	1965		Appearance of Vidoson from Siemens, the first commercial real-time scanner	Szabo (2004)
Scanogram	1969	Stouffer	Scanogram, commercial second generation of B-mode scanner	Stouffer (2004)
First real-time ultrasonographic examination in sheep	1976	Hans Busk	Use of the RTU Danscanner in pigs, cattle and sheep for carcass traits	Busk (1984)
First use of Aloka 500 in sheep	1991	Stouffer	First application of the Aloka 500 in sheep for predict carcass traits	Stouffer (1991)

however, when they used equipment with a larger probe, special care in shearing animals was necessary, in order to obtain a good acoustic contact. The need for shearing animals was also verified by McLaren et al. (1991), who, after discarding from analysis results related to 7 animals that had not been sheared, have ultimately found that the significance of the correlation between the subcutaneous fat thickness measured with ultrasound and in carcass increased from $P>0.05$ to $P<0.01$.

With regard to advocates of not shearing the reference points, their views are related mainly with the speed in obtaining results. Shearing the reference points of animals under examination can be of considerable economic importance when the examination is performed in a large number of animals, which may occur in commercial flocks and takes a considerable time. This might have been a prime reason that led Hopkins et al. (1996), who used ultrasonographic examination to describing changes in growing lambs using a transducer with frequency of 3.5 MHz and scanning depth of 9.6 cm, over the skin after parting the wool and applying vegetable oil. The procedure was also suitable to avoid damages in the skin and to reduce the commercial value of the animal (Teixeira et al., 2006). When shearing would not be performed, one must use a great amount of coupling agent in the reference point to ensure good contact between the skin and the probe.

3.2. Transducer-skin coupling

Application of a coupling agent that allows acoustic contact between transducer and animal skin is essential to ensure transmission of the ultrasound beam. It can be a cause of poor-quality images, because the ultrasonic waves are rapidly dissipated into the air. In initial relevant works, engine oil (Leymaster et al., 1985) or derivatives such as petroleum gel (Gooden et al., 1980) had been used as coupling agents. Use of liquid paraffin (Kempster et al., 1982) also allowed good results. More recently, other agents have been used, e.g., vegetable oil (Hopkins et al., 2007; Emenheiser et al., 2010a; Notter et al., 2014) or products specific for ultrasonographic equipment (Thériault et al., 2009; Notter et al., 2014; Silva et al., 2016). Vegetable oil has proved as very effective in establishing acoustic contact between the transducer and the skin and has the advantage of being inexpensive and widely available.

The amount of the agent to be used depends on the breed, type of transducer and potential shearing of the animal. Ambient temperature is also important; if it is below 7 °C during the examination, supplementary heat should be applied to the acoustical couplant oil

(Tait, 2016). Warming the vegetable oil has also been performed in studies with sheep (Leeds et al., 2008; Notter et al., 2014).

3.3. Animal restraint

During the examination, it is necessary to restrain movements of the animals, as the operator's attention is divided by observing the image on the screen and for placing the transducer on the reference point(s). The degree of restraint depends on various factors, e.g., time of reading, number of readings, temperament of an animal, experience of the operator and availability of assistance. In studies performed in cattle, adverse effects in consistency and repeatability of ultrasonographic measurements non-restrained animals have been reported (Tait, 2016). Restraint of sheep might be necessary, especially if a systematic ultrasonographic measurement would be implemented for carcass trait evaluation (Thériault et al., 2009). Restraint equipment must allow full freedom of operator movements around the animal, whilst it is important that animals are in comfort and a natural stance during the time necessary to perform all procedure to image capture. For lambs, a moderate restraint would be sufficient, by applying gentle pressure under the jaw to prevent forward movement, and by placing around the back an arm to stop backward movements (Ripoll et al., 2009; Esquivelzeta et al., 2012).

3.4. Pressure applied on the probe

Pressure necessary to ensure good acoustic contact between the transducer and the skin has been reported as a cause of tissues deformation, which creates underestimation of ultrasonographic measurements in sheep (Gooden et al., 1980; Hamby et al., 1986; McEwan et al., 1989; McLaren et al., 1991). The problem is particularly noticeable in sheep, because skin is flexible and thickness of subcutaneous fat is reduced (Thwaites, 1984). Pressure on the transducer largely depends on coupling quality between transducer and skin and probe length. Sheared sheep and sufficient amount of coupling agent would lead to requiring smaller pressure on the transducer for the same acoustic contact. Also, when using small and light probes (Gooden et al., 1980), only a slight contact with the skin would be required. In contrast, larger transducers require increased pressure for good acoustic contact (Gooden et al., 1980; McLaren et al., 1991). Currently, to overcome this problem, the transducers have a coated head of a soft membrane that ensures proper skin contact with slight pressure. Also, possible

tissue deformations may be followed through the screen of real-time ultrasonographic equipment, with immediate correction in transducer pressure being possible (Ramsey et al., 1991; Young and Deaker, 1994). Since tissue deformation can impact in accuracy of measurement, various accessories have been developed to minimise the problem. Examples are water bag between the skin and the probe (McEwan et al., 1989) or a standoff pad made in flexible material, with a curvature to adjust the probe to the natural rounded shape of animals (Emenheiser et al., 2010a; Notter et al., 2014; Tait, 2016).

3.5. Reference point selection

Work in sheep to assess body composition traits use several anatomical regions as reference points for image capture and tissue measurements. Most frequently, reference points have been reported along the thoracic and lumbar regions, using the *longissimus thoracis* and *longissimus lumborum* muscles and vertebral bones as major anatomical structures for localisation of the reference point and as a guide for ultrasonographic measurements. These regions are easy to access in a standing animal and the reference points can be located rigorously by detectable anatomical features like spinous and the ribs, which allow an accessible identification both in live animals as well as carcasses. Correct location of the reference points and thus transducer position can be accurately reproduced for each animal. Several reports (Ramsey et al., 1991; Young and Deaker, 1994; Fernández et al., 1998; Silva et al., 2006; Teixeira et al., 2006; Grill et al., 2015; Tait, 2016) have shown the importance of exactness in the reference point identification for accuracy and repeatability of *in vivo* measurements. Actually, correlation between *in vivo* ultrasonographic and corresponding carcass measurements can be affected by misidentification of anatomical bases of the reference points (McLaren et al., 1991).

In a typical lamb evaluation, weights and ages acquiring the cross-sectional ultrasonographic image between the 12th and 13th thoracic vertebrae is a challenge, because the vertebrae are closer to each other (Tait, 2016). Furthermore, subcutaneous fat thickness and the *longissimus thoracis* and *longissimus lumborum* muscles vary over small distances: cranial-caudal (Silva et al., 2007a; Grill et al., 2015) or medial-lateral (Simm, 1983). It has also been found that the area of *longissimus thoracis* and *longissimus lumborum* muscles varies significantly over short sections of a vertebra to the next. For example, Grill et al. (2015) have observed a cranial-caudal reduction of the *longissimus thoracis* and *longissimus lumborum* muscles, from 20.5 mm to 19.5 mm at the 10th to 11th thoracic vertebra to the 3rd to 4th lumbar vertebra, respectively. Inversely, the subcutaneous fat got thicker: 5.5–6.9 mm at the above regions. Other authors (Fernández et al., 1998; Ripoll et al., 2009, 2010) have also observed a similar craniocaudal increase trend for the combined width of *longissimus thoracis* and *longissimus lumborum* muscles in lambs.

3.6. Operator

Several causes of potential problems related to the operator have been identified. These include: anatomy knowledge, prior involvement in carcass work (especially dissection), familiarity with equipment, image acquisition and interpretation.

Awareness of the anatomy of the reference points and its variations is of great importance, in order to take full advantage of the technique (Ramsey et al., 1991; Silva et al., 2007a). In this regard, Andersen et al. (1983) have suggested that operators should carry out a prior anatomy training of the reference points. It should be noted that in an ultrasonographic image it is necessary to categorise tissues and boundaries. Some obvious anatomical structures will serve orientatively for the operator. A good example has been

presented by Hopkins et al. (2008) with measurement of combined depth of *longissimus thoracis* and *longissimus lumborum* in live animals, because the anatomical presence of the 12th rib enhances the muscle boundary definition. In cattle, Stouffer et al. (1961) with the small *iliocostalis* muscle located ventral and lateral to the edge of the *longissimus thoracis* and *longissimus lumborum* muscles, giving a consistent idea of *longissimus thoracis* and *longissimus lumborum* muscles location in the images.

Prior experience of the operator would also influence the ability to interpret ultrasonographic images; for example, it is hard for an operator with experience in visual assessment of an animal, to avoid pre-defined impressions when interpreting ultrasonographic images (Thwaites, 1984). Skills in operating ultrasound equipment and in interpreting images is one of the causes considered for improvement of the accuracy of ultrasonographic measurements (Ramsey et al., 1991; Hopkins et al., 1996; Silva et al., 2005; Hopkins et al., 2007). Considering the nature of images obtained by ultrasound devices, which are generated from tissue interfaces with different acoustic impedance, some interfaces visible in the carcass may not appear in images obtained and, conversely, acoustic images may not match with visible structures of the carcass. Thus, the operator, sometimes, has to decide between two ambiguous interfaces, which have repercussion on the accuracy and consistency of measurements. An excellent example has been reported by Thériault et al. (2009), who observed that ultrasonographically-measured fat depth in fatter lambs with a third fat layer, had been underestimated by exclusion of this unclear layer from the measurements, whereas leaner lambs had better agreement between carcass and ultrasonographic measurements, what had been pointed as a result of the absence of a third fat layer.

3.7. Thickness of the tissues and probe frequency

It has been noted by several authors (Simm, 1987; McLaren et al., 1991; Stouffer, 2004) that, in sheep, less accurate estimates of carcass composition had been observed than that in cattle or pigs, as a result of the smaller thickness of tissues. The same problem has further been identified between various types of sheep (Young and Deaker, 1994); these authors have found better results in estimation of carcass fat with thickness measurement of soft tissue over the 12th rib, 11 cm from dorsal midline (GR) than with measurement of thickness of subcutaneous fat, which had been attributed to lower thickness of the latter measurement which implied a higher measurement error. This finding has been corroborated by others (Hopkins et al., 1993; Silva et al., 2006). For example, Hopkins et al. (1993) have reported that in 3- to 4-month-old lambs there were small differences between *in vivo* and on carcass findings for GR, fat depth and area of the *longissimus thoracis* and *longissimus lumborum* muscles, although accuracy observed for fat depth was smaller compared with GR measurement; this had been attributed to greater relative error in measurement determination. Subsequently, Hopkins et al. (1996), in heavier lambs, obtained more accurate ultrasonographic measurements of subcutaneous fat.

Significant interpretation should be performed in the first 6 mm of thickness, if it would be required to analyse thickness of subcutaneous fat (Gooden et al., 1980). Despite reduced skin thickness (e.g., 1.9 mm (Brown et al., 2000) or 3 mm (Tait et al., 2015)), the skin:fat interface must be precisely located when the aim is to measure the thickness of subcutaneous fat, as this variable can measure only few millimetres (Gooden et al., 1980; Silva et al., 2006). The difficulty of distinguishing the skin:fat interface, led some authors to include the skin in the thickness measurements (Fortin, 1980; Kempster et al., 1982; Silva et al., 2005; Teixeira et al., 2006; Thériault et al., 2009). This approach is considered feasible, because the skin shows little variation between animals (Gooden et al., 1980; Cameron and Bracken, 1992). Also it has been

shown that a small amount of fat can influence muscle measurements (Stanford et al., 1995). These authors have pointed out that in lean lambs, who had reduced intermuscular fat between *longissimus thoracis* and *longissimus lumborum* muscles and the smaller neighbouring muscles (*spinalis* and *iliocostalis*), the combined area of *longissimus thoracis* and *longissimus lumborum* appeared overvalued as a result of the inability to distinguish *in vivo* all these muscles.

Problems in interpretation of images in fatter animals have also been reported, because confusion that two or three layers of subcutaneous fat might have been introduced in tissue boundary identification (Thwaites, 1984; Thériault et al., 2009). Misinterpretation of fat layers in ultrasonographic images can have serious implications when it aims to select animals for leaner carcasses (Simm, 1987). The problem with boundaries identification has also been observed by Hopkins et al. (2007) in sheep of either sexes and of one of five genotypes; they have indicated problems with tissue boundary demarcation, due to high-fat levels of animals. The issue has been attributed to high levels of intramuscular fat, which might have influenced clarity of images or potentially separation of fat layers.

Others researchers (Nicol and Parratt, 1984) working with an A-mode 5 MHz equipment to predict fat-free carcass weight in rams have also found problems with fat layers, which had lead to erroneous low readings of fat thickness. Although it is understandable that confounding readings can be observed with A-mode equipment, those problems can also be seen with real-time ultrasonographic equipment (Thériault et al., 2009) and may further be amplified if associated with use of a transducer with frequency <3.5 MHz. A low-frequency probe has a small resolution of surface tissue layers, e.g., subcutaneous fat. Thériault et al. (2009) have reported that a low-resolution transducer adds imprecision, increasing error in fat depth measurements. This constraint has also been pointed by Hajji et al. (2015), who used a 3.5 MHz frequency to study effects of sheep breed as a cause of reduced accuracy of muscle and fat carcass predictions. Therefore, when the aim is to identify subcutaneous fat layers in heavier animals and/or reduced subcutaneous magnitude of lambs, use of a high-frequency probe (e.g., 7.5 MHz) with higher resolution at the surface and lower penetration capacity would be preferable (Silva et al., 2006). Currently, ultrasonographic equipment operate with multi-frequency probes, which can be very convenient to adjust with scanning depths close to the tissue of interest. Ripoll et al. (2010) have adjusted the frequency to achieve a clear image for each tissue, using high frequencies (8.0–10.0 MHz) for fat measurements and a 7.0 MHz frequency for muscle depth.

3.8. Analysis of images

Images obtained during ultrasonographic evaluation require analysis. Typically, this analysis has been performed to obtain thickness, area or volume measurements, which require identification and mapping of tissues and their interfaces (Glasbey et al., 1996). The concern in development and application of image analysis in images obtained during ultrasonographic evaluation is primarily based on evidence that it is possible to have more accurate *in vivo* measurements and thus predict more accurately carcass compositions (Young and Deaker, 1994; Silva et al., 2006; Tait, 2016).

Previously, it has been discussed that an important cause of inaccuracy in *in vivo* measurements is the reduced thickness of subcutaneous fat in sheep, especially in lambs or adults in low body condition. It is particularly apt for such animals that image analysis would be a necessary tool. Most ultrasonographic equipment carry with an internal calliper with a 1 mm resolution (McEwan et al., 1989; Fernández et al., 1997). This might not be adequate for animals with subcutaneous fat thickness of a magnitude value

close to 1 mm. Low resolution undermines accuracy of the technique when applied in lambs, particularly those for light carcass production (Ripoll et al., 2009), or for analysis of tissue thickness change in growing animals (Hamby et al., 1986; Silva et al., 2005). The problem has been recognised by Young et al. (1992), who carried out thickness measurements of fat and muscle in a group of sheep using ultrasonographic equipment with a system for image analysis. These authors have concluded that measurement repeatability could be superior with an approach including image analysis software other than one in which measurements were performed directly on the monitor. Repeatability differences between the two methods were associated with the resolution. In the case of the monitor, that was 1 mm, whilst with the image analysis system it was 0.1 mm. This is undoubtedly a strong argument for saving images at the time of the examination for later analysis.

Along the years, it has been possible to obtain images with increasing quality and in different supports suited for image analysis. Examples include photographs obtained directly from the monitor (Glasbey et al., 1996) or on thermal paper from a video printer (Silva et al., 2005; Teixeira et al., 2006) and subsequently digitised in a scanner with 256 grey levels. In both examples, the images were analysed with an image analysis software showing that it is possible a resolution measurement near to the 0.2 mm (0.157 (Glasbey et al., 1996) or 0.2 mm (Silva et al., 2005)). Regarding this approach, Hopkins et al. (2007) have stated that this technique is worthy of consideration as a method to identify potential causes of variation in ultrasonographic image measurement. In recent years, the process of acquiring and analysing ultrasonographic images has suffered significant progress, as the result of advances in computer and ultrasound processing capability, improvements in image quality and development of image analysis software (Szabo, 2004; Whitsett, 2009). Also, capacity for online analysis and image storage (Whitsett, 2009; Li, 2010) and reduction of equipment and operational costs (Szabo, 2004) are the features that will have vast impact on evaluation of body composition and meat traits of sheep breeds. In fact, image quality information and data obtained from an image processing laboratory (Tait, 2016) and a procedure that ultrasonographic images would be taken by accredited scanners (Australia LAMBPLAN (Huisman et al., 2016), USA (Tait, 2016)) can have impact in consistency and accuracy of carcass traits used in selection programs to improve lamb body composition (Emenheiser et al., 2010a; Tait, 2016).

4. Applications of ultrasonographic examination for evaluation of body composition and for prediction of carcass quality in live sheep

There are many examples of use of the ultrasonographic examination for evaluation of body composition and for prediction of carcass quality in live sheep. For example, in genetic improvement programmes, improvements in carcass traits have been achieved by using selection of animals based on results of ultrasonographic examination (Macfarlane and Simm, 2008; Márquez et al., 2013; Huisman et al., 2016). Also, with regard to work where it is necessary to know sequential body composition, ultrasonographic examination has been used to describe changes in body carcass composition of growing animals (Hamby et al., 1986; Hopkins et al., 1993, 1996; Silva et al., 2005; Emenheiser et al., 2010b). Prediction of carcass composition and meat quality traits by non-invasive and non-destructive means has also been the aim of studies using ultrasonographic evaluation (Silva and Cadavez, 2012). Finally, ultrasonographic examination has been used as an objective approach for predicting body fat reserves of sheep (Russel, 1995).

In these fields, it is necessary to obtain accurate and precise ultrasonographic measurements. Usually, relevant studies have been orientated to evaluate relationships between ultrasonographic findings and corresponding carcass measurements or to predict carcass composition (weight or proportion of tissues and cuts) by means of *in vivo* ultrasonographic and bodyweight variables.

4.1. Relationship between *in vivo* ultrasonographic measurements and corresponding carcass measurements

Research into ultrasonography application in live sheep has examined relationships between ultrasonographic findings and corresponding carcass measurements. The correlation between those has been typically used as indicator of accuracy (Notter et al., 2014). Nevertheless, caution should be necessary, because correlation coefficient, although useful as an accuracy indicator, does not reflect population variation or bias (Houghton and Burlington, 1992). There are obvious difficulties in comparing different trials directly due to differences in methodology. As equipment, settings used, image analysis approach, animal factors, operator experience etc. vary widely from trial to trial, additional information about the studies are provided to help in the evaluation of correlation values (Tables 2 and 3).

Choice of reference points is important step in the application of the technique to obtain carcass measures of fat, muscle and tissues. The reports in Tables 2 and 3 indicate that the thoracic and lumbar regions have been mostly used for ultrasonographic measurements in sheep. In these regions, the reference points on the 12 to 13th thoracic vertebrae and between the 3rd and 4th lumbar vertebrae have been mostly utilised. Choice for these reference points is due to the widespread use of measures of the muscles *longissimus thoracis* and *longissimus lumborum* during *in vivo* and carcass studies. These reference points have several attributes that make them the most widely used in this work. First, measurements of subcutaneous fat and *longissimus thoracis* and *longissimus lumborum* muscles measurements taken in these reference points show consistent and significant correlation with carcass traits; second, these reference points are easy to locate and assess by palpation of ribs and vertebral apophyses in a standing animal; third, there are few muscles at those locations, with *longissimus thoracis* and *longissimus lumborum* muscles assuming a major contribution, and their anatomy is fairly straightforward which aids interpretation of ultrasonographic findings (Simm, 1987; Glasbey et al., 1996).

Despite the merit of these reference points, other ones have also been investigated. For example, the body wall thickness between the last two ribs 12 cm from the dorsal midline (Ramsey et al., 1991) or over the 12th rib 11 cm from the dorsal midline where total depth of soft tissues was measured (GR) (Ramsey et al., 1991). Correlations of results of measurements of the latter parametre with respective results in subcutaneous fat are presented in Table 2. Most studies describe that subcutaneous fat in the thoracic and the lumbar regions have been measured by ultrasonographic examination. In 21 of the 32 studies, a correlation coefficient $r > 0.5$ ($P < 0.01$) has been found for the subcutaneous fat measurements. A similar trend has been observed for the GR or related tissue wall thickness measurement. Although this measure is used less extensively compared with the subcutaneous fat, it shows a high accuracy ($0.54 < r < 0.87$; $P < 0.01$) and, in some countries, is included in the carcass grading systems (Australia: Hopkins, 1994; Canada: Jones et al., 1996; New Zealand: Kirton and Johnson, 1979). The GR can also be considered for *in vivo* studies, especially for selection decisions made in young lambs, as it shows a greater magnitude than measurement of subcutaneous fat (Ramsey et al., 1991). Reduced dimension and variation has been reported

by Hopkins et al. (1993) as an explanation of low accuracy observed with measurement of subcutaneous fat ($r = 0.17$; $P > 0.05$), whereas measurement of GR shows a higher correlation value ($r = 0.60$; $P < 0.01$).

Other studies have nevertheless shown reduced correlation; in those cases, explanations vary and relate to factors discussed previously. For example, Fortin and Shrestha (1986) have shown measurement errors and problems with movement of the tissue when hanging the carcass for cooling; McLaren et al. (1991) have indicated that results found were associated with the different position from that measured *in vivo* (13TV) and the corresponding carcass (12TV).

Table 3 presents correlations between *longissimus thoracis* and *longissimus lumborum* muscles ultrasonographic measurements and corresponding measurement in carcass. Correlations observed with *longissimus thoracis* and *longissimus lumborum* muscles measurements indicate a high variation between studies (r from 0.02; $P > 0.05$ –0.89; $P < 0.01$), but, in general, the combined area and depth of *longissimus thoracis* and *longissimus lumborum* are more accurate than respective width. Also, with few exceptions, the combined area and depth of *longissimus thoracis* and *longissimus lumborum* show similar accuracy.

Correlation variation observed for *longissimus thoracis* and *longissimus lumborum* muscles measurements may be of varying origin. Difficulties in identification of *longissimus thoracis* and *longissimus lumborum* muscles borders in ultrasonographic images can impact accuracy of measurements (Silva et al., 2006). The difficulty in identifying vertical interfaces, e.g., side edges of the *longissimus thoracis* and *longissimus lumborum* muscles, has been indicated as a cause for the reduced correlation found with the combined width of *longissimus thoracis* and *longissimus lumborum* (McEwan et al., 1989). Further, Hopkins et al. (1993) have found a smaller correlation with combined width of *longissimus thoracis* and *longissimus lumborum* than with combined area or depth of these muscles ($r = -0.15$, $P > 0.05$ versus $0.36 < r < 0.42$, $P < 0.01$), which made them conclude that combined width of *longissimus thoracis* and *longissimus lumborum* was of little value in sheep breeding programmes.

4.2. Prediction of carcass composition from ultrasonographic measurements taken *in vivo*

The number of papers regarding use of ultrasonographic evaluation for predicting carcass composition in sheep has increased considerably after 1985. Although prediction of carcass composition has been the subject of previous reviews (Allen, 1990; Simm, 1987; Houghton and Burlington, 1992; De Campeneere et al., 2000; Scholz et al., 2015) little reference has been made to sheep in those papers. Typically, prediction models have been established by use of multiple linear regression; coefficient of determination (R^2), which indicates accuracy, and residual standard deviation (rsd), which indicates precision, have been employed to assess the model fit (Hopkins et al., 2008).

4.2.1. Prediction of carcass or body fat

Table 4 presents the R^2 and rsd values for prediction of carcass fat components from linear models with bodyweight and ultrasonographic measurements.

In most studies, the best prediction models include animal bodyweight and ultrasonographic measurements. Bodyweight has been introduced in carcass prediction models, being the most efficient predictor, as it could provide a very precise prediction of carcass composition when used alone; it could also provide the highest precision when ultrasonographic measurements were used with it in multiple regression (Kempster et al., 1982). Carcass fat components can be explained by bodyweight and/or ultrasonographic mea-

Table 2

Correlations (r) between *in vivo* ultrasonographic measurements of subcutaneous fat/thickness of soft tissues over the 12th rib (GR) and corresponding measurements in carcass in sheep.

Reference	n	Breed	BW (kg)	Device	Mode	Frequency (MHz)	Reference point(s)	Subcutaneous fat	GR
Moody et al. (1965)	69, 73, 93, 235	Mixed	40–44	Branson 5 A	A	2.25	13 TV	0.27–0.34	
Shelton et al. (1977)	102	Rambouillet		Scanogram 722	B		12–13 TV	0.77	
Gooden et al. (1980)	30, 32, 97, 106	3 genotypes		HP 7215A, AIDD1, AIDD2, AIDD3	A	5.0	12–13 TV	0.72–0.91	
Purchas and Beach (1981)	65	Mixed		AIDD3	A	5.0	12–13 TV	0.54–0.91	
Bass et al. (1982)	20	Southdown × Romney	34	BCM, AIDD3	A	5.0	12–13 TV	0.87–0.95	
Fortin and Shrestha (1986)	273	3 genotypes	37	Scanogram 722	A		13 TV, 3 LV	0.05–0.27	
Nicol et al. (1988)	28, 32	Border Leicester	60–66	AIDD3	A	5.0	12 TV	0.81–0.85	
Edwards et al. (1989)	30	Finewool × Blackface		Aloka 210	RT	5.0	12–13 TV, 1–2 sacral vertebrae	0.36–0.59	
Turlington (1989)	162			Technicare 210	RT		13 TV	0.42–0.63	
McEwan et al. (1989)	15, 30	Romney		AIDD3, Aloka210, Toshiba SAL22A	A, RT	5.0	12 TV, GR	0.71–0.78	0.54–0.81
Hopkins (1990)	123, 51	Mixed		Delphi 1017A	A		12–13 TV	0.93–0.95	
McLaren et al. (1991)	32		49	Ithaco 731, J&J210	A, RT	2.0, 3.0	13 TV versus 12 TV	0.15–0.42	
Delfa et al. (1991)	14	Aragonesa		Toshiba SAL-32B	RT	5.0	3–4 LV	0.73–0.87	
Stouffer (1991)	15			Toshiba SAL-22A	RT		12–13 TV	0.93	
Ramsey et al. (1991)	99, 147			Toshiba SAL-22A	RT	5.0	GR, BWT		0.79–0.87
Hopkins et al. (1993)	58			Aloka 500	RT	3.5	12 TV, GR	0.17	0.60
Fernández et al. (1997)	60	3 genotypes		Toshiba SAL-32B	RT	5.0	12–13 TV	0.74	
Fernández et al. (1998)	10	Manchego	25, 35	Toshiba SAL-32B	RT	5.0	12–13 TV, 3–4 LV	(−0.06)–0.92	
Teixeira et al. (2006)	67	Churra Bragançana	36	Aloka 500	RT	5.0, 7.5	12–13 TV, 3–4 LV	0.31–0.42	
Hopkins et al. (2007)	147	5 genotypes		Honda HS-1201	RT	5.0	12–13 TV	0.67	
Orman et al. (2008)	13	Awassi	40	Dynamic	RT	7.5	12–13 TV	0.79–0.82	
Sahin et al. (2008)	40	Akkaraman	42	PieMedical100	RT	8.0	12–13 TV	0.77	
Leeds et al. (2008)	168	Mixed	63	Aloka 500	RT	3.5	12–13 TV	0.81	
Thériault et al. (2009)	96	2 genotypes	47	Ultrscan 50	RT	3.5	12–13 TV, GR, 3–4 LV	0.78–0.82	0.83
Ripoll et al. (2010)	114	Tensina	22	Aloka 900	RT	8.0–10.0	10–11 TV, 12–13 TV, 1–2 LV, 3–4 LV	0.70–0.74	
Emenheiser et al. (2010a)	163	Suffolk		Aloka 500	RT	3.5	12–13 TV, WT	0.78	0.73
Orman et al. (2010)	30	Awassi	39	Dynamic	RT	7.5	12–13 TV	0.93	
Esquivelzeta et al. (2012)	124	Mixed	27	Vet 180 Plus	RT	5.0	12–13 TV, 1–2 LV	0.32–0.60	
Notter et al. (2014)	512	Mixed		Aloka 500	RT	3.5	12–13 TV	0.69	
Vardanjani et al. (2014)	99	Torki	65	PieMedical100	RT	8.0	12–13 TV	0.70	
Agamy et al. (2015)	15	Barki	34	PieMedical100	RT	8.0	12–13 TV	0.34–0.62	
Grill et al. (2015)	36	6 genotypes	39	Mindray DP-6900	RT	5.0	12–13 TV	0.82	

Abbreviations. BW: bodyweight, RT: real-time, TV: thoracic vertebra, LV: lumbar vertebra, GR: total depth of soft tissues over the 12th rib, 11 cm from dorsal midline, BWT: body wall thickness between 12th and 13th ribs, 12 cm from the dorsal midline, WT: body wall thickness between the 12th and 13th ribs, including the lateral edge of the *longissimus thoracis* and *longissimus lumborum* muscles, but not the spine.

Table 3Correlations (r) between *in vivo* ultrasonographic measurements of *longissimus thoracis* and *longissimus lumborum* muscles and corresponding measurements in carcass in sheep.

Reference	n	Breed	BW (kg)	Device	Mode	Frequency (MHz)	Reference point(s)	LMA	LMD	LMW
Campbell et al. (1959)	33, 32			Somascope	A		12–13 TV	0.44–0.62	0.49–0.68	
Moody et al. (1965)	69, 73, 93, 235	Mixed breed	40–44	Branson 5 A	A	2.25	13 TV	0.52–0.66	0.31	0.36
Shelton et al. (1977)	102	Rambouillet		Scanogram 722	B	2.0	12–13 TV	0.69		
Fortin and Shrestha (1986)	273	3 genotypes	37	Scanogram 722, Krautkramer	A	2.0	13 TV, 3 LV	0.61–0.63	0.52–0.69	
Hamby et al. (1986)	30	Rambouillet × Finn		Technicare 210	RT		12–13 TV	0.80		
Edwards et al. (1989)	30	Finewool × Blackface		Aloka 210	RT	5.0	12–13 TV; 2 sacral vertebra	0.36		
Turlington (1989)	162			Technicare 210	RT		13 TV	0.58		
McEwan et al. (1989)	15, 30	Romney		AIID3, Aloka210, Toshiba SAL22A	A, RT	5.0	12 TV		0.04–0.38	0.41–0.72
McLaren et al. (1991)	32		49	Ithaco 731, J&J210	A, RT	2.0, 3.0	13 TV versus 12 TV	0.28		
Delfa et al. (1991)	14	Aragonesa		Toshiba SAL-32B	RT	5.0	3–4 LV			0.22
Stouffer (1991)	15			Aloka 500	RT		12–13 TV	0.89		
Ward et al. (1992)	89			Dynamic	RT	3.5	12 TV	0.76	0.79	
Hopkins et al. (1993)	58			Aloka 500	RT	3.5	12 TV	0.42	0.36	(−0.15)
Fernández et al. (1997)	60	3 genotypes		Toshiba SAL-32B	RT	5.0	12–13 TV	0.88	0.56	
Mahgoub (1998)	19	Omani	26	Microimager	RT	7.5	6 TV, 12 TV, LV		0.27–0.48	
Fernández et al. (1998)	10	Manchego	25, 35	Toshiba SAL-32B	RT	5.0	12–13 TV, 3–4 LV	0.13–0.76	0.40–0.83	
Hopkins et al. (2007)	147	5 genotypes		Honda HS-1201	RT	5.0	12–13 TV		0.55	
Orman et al. (2008)	13	Awassi	40	Dynamic	RT	7.5	12–13 TV	0.87–0.89	0.58–0.60	(−0.17)–0.48
Sahin et al. (2008)	40	Akkaraman	42	PieMedical100	RT	8.0	12–13 TV	0.82	0.60	
Leeds et al. (2008)	168	F1 wether lambs	63	Aloka 500	RT	3.5	12–13 TV	0.75	0.71	
Thériault et al. (2009)	96	2 genotypes	47	Ultrasound 50	RT	3.5	12–13 TV, 3–4 LV	0.34–0.42		
Ripoll et al. (2010)	114	Tensina	22	Aloka 900	RT	7.0	10–11 TV, 12–13 TV, 1–2 LV, 3–4 LV	0.42–0.59	0.16–0.37	
Emenheiser et al. (2010a)	163	Suffolk	66	Aloka 500	RT	3.5	12–13 TV	0.66		
Orman et al. (2010)	30	Awassi	39	Dynamic	RT	7.5	12–13 TV	0.88	0.77	0.58
Esquivelzeta et al. (2012)	124	Mixed	27	Vet 180 Plus	RT	5.0	12–13 TV, 1–2 LV	0.72–0.78	0.61–0.88	0.69–0.70
Notter et al. (2014)	512	Mixed		Aloka 500	RT	3.5	12–13 TV	0.65		
Vardanjani et al. (2014)	99	Torki	65	PieMedical100	RT	8.0	12–13 TV	0.80	0.77	0.54
Agamy et al. (2015)	15	Barki	34	PieMedical100	RT	8.0	12–13 TV	0.06–0.83	(−0.18)–0.67	0.21–0.42
Grill et al. (2015)	36	6 genotypes	39	Mindray DP-6900	RT	5.0	12–13 TV	0.76		

Abbreviations. BW: bodyweight, RT: real-time, TV: thoracic vertebra, LV: lumbar vertebra, LMA: combined area of *longissimus thoracis* and *longissimus lumborum* muscles, LMD: combined depth of *longissimus thoracis* and *longissimus lumborum* muscles, LMW: combined width of *longissimus thoracis* and *longissimus lumborum* muscles.

Table 4

Prediction of carcass and body fat components from bodyweight and ultrasonographic measurements in sheep.

Reference	n	Breed	BW (kg)	Device	Mode	Frequency (MHz)	Dependent variable	Independent variables						R ²	RSD		
								BW	SFTV	LMDTV	LMATV	SFLV	GR	Other			
Hopkins (1990)	123	Mixed		Delphi 1017A	A		CF (%)	●	●						0.62	2.83	
Ramsey et al. (1991)	147			Toshiba Sal-22A	RT	5.0	CF (%)	●						●	0.66	2.40	
Hopkins et al. (2007)	147	5 genotypes		Honda HS-1201	RT	5.0	CF (%)	●	●					●	0.48	2.85	
Young and Deaker (1994)	76	Coopworth		Aloka SSD-210	RT	5.0	CF (kg)							●	0.67	0.36	
Sahin et al. (2008)	40	Akkaraman	42	Pie Medical Falco 100	RT	8.0	CF (kg)	●	●						0.84	0.24	
Agamy et al. (2015)	45	3 genotypes		Pie Medical Falco 100	RT	8.0	CF (kg)	●							0.39		
Hajji et al. (2015)	14	Noire de Thibar		Falco Vet	RT	3.5	CF (kg)	●	●	●					LMP	0.90	0.15
Teixeira et al. (2006)	67	Churra Bragançana	36	Aloka 500	RT	5.0 and 7.5	logCF	●	●						0.88	0.07	
Ripoll et al. (2009)				Aloka 900	RT	7.0 to 10.0	logCF	●	●						0.51	0.06	
Silva et al. (2006)	46			Aloka 500	RT		CIF (g kg^{-1})	●	●					●	0.68	8.30	
Silva et al. (2006)	46			Aloka 500	RT		CIF (kg)	●	●					●	0.92	0.30	
Teixeira et al. (2006)	67	Churra Bragançana	36	Aloka 500	RT	5.0 and 7.5	logCIF	●	●						0.84	0.07	
Ripoll et al. (2010)	114	Tensina		Aloka 900	RT	7.0 to 10	logCIF	●	●					●	0.84	187.2	
Teixeira et al. (2006)	67	Churra Bragançana	36	Aloka 500	RT	5.0 and 7.5	logCSF	●	●						0.85	0.09	
Kempster et al. (1982)	273	Several breeds		Scanogram 722	B	2.0	CSF (g kg^{-1})	●	●							23.4	
Kempster et al. (1982)	254	Several breeds	39–47	Danscanner	RT	2.2	CSF (g kg^{-1})	●	●							28.6	
Silva et al. (2006)	46			Aloka 500	RT		CSF (g kg^{-1})	●						●	SFA	0.89	8.00
Ripoll et al. (2010)	114	Tensina	22	Aloka 900	RT	7.0 to 10.0	CSF (g)	●	●					●	SFst	0.75	214.9
McEwan et al. (1989)	15	Romney		AIDD	A		ChemCF (%)	●	●							0.67	2.40
McEwan et al. (1989)	15	Romney		Aloka	RT	3.0	ChemCF (%)	●								0.52	2.90
Ramsey et al. (1991)				Toshiba SAL-22A	RT	5.0	ChemCF (%)	●						●		0.66	2.44
Silva et al. (2005)	31	2 genotypes		Aloka 500	RT	7.5	ChemBF (g kg^{-1})	●	●						TD11	0.83	19.40
Leymaster et al. (1985)	37	Suffolk	73	Scanogram	B	2.0	ChemCF (kg)	●	●						SF3CV	0.31	1.30
Silva et al. (2005)	31	2 genotypes		Aloka 500	RT	7.5	ChemBF (kg)	●	●						TD11	0.95	0.60
Teixeira et al. (2006)	67	Churra Bragançana	36	Aloka 500	RT	5.0 and 7.5	logKPF	●	●							0.66	0.16
Fernández et al. (1998)	10	Manchego		Toshiba SAL-32B	RT	5.0	KPF (g)	●	●					●		0.46	112.0
Vardanjani et al. (2014)				Pie Medical Falco 100	RT	8.0	TailF (kg)	●	●						LMW	0.18	1.34

Abbreviations. BW: bodyweight, RT: real-time, CF: carcass fat, CIF: carcass intermuscular fat, CSF: carcass subcutaneous fat, ChemCF: chemical carcass fat, ChemBF: chemical body fat, KPF: kidney and pelvic fat, TailF: tail fat, ●: variables included in model, SFTV: subcutaneous fat in thoracic vertebrae area, LMDTV: combined depth of *longissimus thoracis* and *longissimus lumborum* muscles in thoracic vertebrae area, LMATV: combined area of *longissimus thoracis* and *longissimus lumborum* muscles in thoracic vertebrae area, SFLV: subcutaneous fat in lumbar vertebrae area, GR: total depth of soft tissues over the 12th rib, 11 cm from dorsal midline, LMP: combined perimetre of *longissimus thoracis* and *longissimus lumborum* muscles, SFA: subcutaneous fat area, SFst: subcutaneous fat over 3rd sternum, TD11: total tissue depth over 11th rib, 16 cm from dorsal midline, SF3CV: subcutaneous fat at 3rd coccygeal vertebra, LMW: combined width of *longissimus thoracis* and *longissimus lumborum* muscles, R²: coefficient of determination, rsd: residual standard deviation.

surements ($0.18 < R^2 < 0.95$). Subcutaneous fat ultrasonographic measurements obtained at thoracic level are used most often, whereas subcutaneous fat present in the lumbar area was used more rarely (Fernández et al., 1998). Although this difference in frequency of using subcutaneous fat measurements is not surprising, given that region had been chosen for inclusion in ultrasonographic measurement protocols, there are further references that support superiority of thoracic region measures (Teixeira et al., 2006; Ripoll et al., 2010).

In addition to carcass, fat depots were also estimated the kidney and pelvic fat depots, which in some countries belong to the carcass (Fernández et al., 1998; Teixeira et al., 2006). In adult animals, these deposits have also been given attention during ultrasonographic examination, e.g., Silva et al. (2007b) whose findings have explained up to 70% of variation of omental, mesenteric and perirenal fat. Although the results do not allow definite conclusions regarding ability of ultrasonographic examination for *in vivo* measurements to predict this type of fat depots, they still provide opportunities for development in future studies. In fact, tomography studies have shown the need to estimate these deposits to monitoring the seasonal depletion and repletion of fat and muscle in breeding ewes (Lambe et al., 2003).

4.2.2. Muscle and protein prediction

The example of what has been described for fat prediction, has also been observed for muscle and protein prediction. Several authors have calculated prediction equations for muscle and protein, including bodyweight, and either muscle or fat during *in vivo* ultrasonographic measurements (Table 5). In general, it has been found that bodyweight and results of ultrasonographic measurements could explain well muscle and protein variation ($0.26 < R^2 < 0.99$). In most prediction equations, bodyweight could explain the largest part of the variation; in some cases, it is the only variable in the equation. For example, Turlington (1989) and Teixeira et al. (2006) have reported that bodyweight would account for 89 and 96%, respectively, of variation in muscle weight, whereas bodyweight for the amount of protein would explain 97% (Silva et al., 2005). An aspect to note is that use of fat ultrasonographic measurements included in the equations is in larger number than for muscle measurements. Often, equations include both fat and muscle measurements (Puntilla et al., 2002; Silva et al., 2005, 2006; Hajji et al., 2015). However, this approach has limitations, as, sometimes, inclusion of a variable does not represent an increase in accuracy; this would justify work necessary to get this variable, especially if it would be obtained in a different reference point (Kempster et al., 1982). Nevertheless, if additional accuracy is necessary to estimate composition, a combination of measures would justify the extra cost (Hopkins et al., 2008).

4.2.3. Prediction of carcass and cut yields

Prediction of carcass and cuts yields from *in vivo* measurements is a major concern, not only for research, but above all for the industry, as it is in this form (carcass or cuts) that the final product is presented to consumers. Results of studies that have provided predictions of yield of carcass and cuts from bodyweight and ultrasonographic measurements are summarised in Table 6. Generally, bodyweight and ultrasonographic measurements show a high coefficient of determination with the weight of carcass or cuts (e.g., Notter et al., 2014). However, when data were presented as a percentage basis the relationships become weaker. For example, Shelton et al. (1977) worked to predict relationship between ultrasonographic *in vivo* measurements and carcass cutability and found that inclusion of bodyweight, average daily gain, subcutaneous fat and combined area of *longissimus thoracis* and *longissimus lumborum* in multiple regression equations could allow significant

predictions of weight of total cuts ($R^2 = 0.85$), weight of primal cuts ($R^2 = 0.88$) and weight of dissected leg ($R^2 = 0.81$). However, when cuts were expressed as a proportion of cold carcass weight, accuracy was smaller for total cuts ($R^2 = 0.51$), primal cuts ($R^2 = 0.53$) and dissected leg ($R^2 = 0.42$). These results had been confirmed by Fortin (1980), who used subcutaneous fat obtained at 12th to 13th thoracic vertebrae to predict trimmed or boneless cuts (shoulder, loin, rack, leg). In that case, best equation to predict trimmed or boneless weight explained >94% of the variation, whereas for proportions trimmed ($R^2 = 0.08$, $P > 0.05$) or boneless ($R^2 = 0.35$; $P < 0.05$) equations showed a smaller prediction ability.

Inability to estimate proportion of cuts lied in the little variation, exemplified by the coefficient of variation being 5–8 times greater for cuts weight than for proportion. Later, Fortin and Shrestha (1986) found that both bodyweight and ultrasonographically measured subcutaneous fat and *longissimus thoracis* and *longissimus lumborum* muscles were of no value for prediction of proportion of trimmed boneless meat; it was further concluded that ultrasonographic evaluation lacked the level of precision necessary for practical application. Other studies (Stanford et al., 1995; Hopkins et al., 1996), though with varying results, found that was possible to establish a meaningful relationship between cuts or saleable-meat yields from bodyweight and carcass ultrasonographic measurements (subcutaneous fat and/or *longissimus thoracis* and *longissimus lumborum* muscles), while Fernández et al. (1998) found impossible to use *in vivo* subcutaneous fat measurements to predict carcass yield. For carcass and cuts yield, a major constraint when comparing studies is the difference with regard to the cutting technique applied, which vary widely from country to country. Therefore, some harmonisation would be necessary to compare data and to have a clear view of the usefulness of the different measurement reference points and ultrasonographic procedures.

4.2.4. Prediction of intramuscular fat

It is well documented that intramuscular fat ('marbling') has an important role in eating quality of meat (Wood et al., 2008). Intramuscular fat represents the chemically extractable fat, whereas the term 'marbling' derives from the appearance of the white flecks of fatty tissue between bundles of muscular fibres (Silva et al., 2015). For sheep meat, the minimum amount of intramuscular fat to be acceptable for consumer satisfaction is about 5% (Hopkins et al., 2006) and the lamb loin usually contains 4–5% intramuscular fat (Pethick et al., 2005). This amount can be achieved in growing lambs, as some data indicate that intramuscular fat shows an early relative growth (Pethick et al., 2007; Mcphee et al., 2008). In fact, the proportion of intramuscular fat in the loin about total carcass fat decreases as animals mature, that way indicating that a prolonged feeding period of lambs to reach a target of intramuscular fat is not required (Mcphee et al., 2008). Therefore, it can be possible to monitor intramuscular fat variation during growth or to improve its content genetically without compromising carcass quality by means of *in vivo* imaging techniques like ultrasonographic examination or tomography (Williams, 2002; Clelland et al., 2014). Intramuscular fat and 'marbling' have been extensively examined by such methods (Bünger et al., 2011; Silva and Cadavez, 2012).

Most relevant publications refer to carcasses of cattle or pigs. Some research related to sheep has been published, e.g. for ultrasonographic examination: Stouffer (1991), Slóśarz et al. (2001), and for tomography: Clelland et al. (2014), Pannier et al. (2014). The work of Slóśarz et al. (2011) has indicated that it could be possible to predict intramuscular fat with ultrasonographic examination ($r = 0.858$; $P < 0.01$). In the future, the ability to predict intramuscular fat could help to maintain level of this fat depot around 5%, as suggested by Pethick et al. (2006).

Table 5

Prediction of carcass and body fat components from bodyweight and ultrasonographic measurements in sheep.

Reference	n	Breed	BW (kg)	Device	Mode	Frequency (MHz)	Dependent variable	Independent variables							R ²	RSD		
								BW	SFTV	LMDTV	LMATV	SFLV	LMDLV	GR	Other			
Puntila et al. (2002)	83	4 genotypes	46, 77	Dynamic Imaging	RT	7.5	Lean (%)	●	●				●			0.67		
Hopkins et al. (2007)	147	5 genotypes		Honda HS-1201	RT	5.0	Lean (%)	●	●							0.46	2.71	
Puntila et al. (2002)	83	4 genotypes	46, 77	Dynamic Imaging	RT	7.5	Lean (kg)	●		●						0.68		
Hopkins et al. (2007)	147	5 genotypes		Honda HS-1201	RT	5.0	Lean (kg)	●		●						0.86	1.62	
Agamy et al. (2015)	45	3 genotypes		100 LC, Pie Medical	RT	8.0	Lean (kg)	●				●				0.82		
Ripoll et al. (2010)	114	Tensina	22	Aloka 900	RT	7.0 to 10.0	logLean	●					●			0.96	154.2	
Fortin and Shrestha (1986)	273	3 genotypes		Krautkramer	A		Muscle (kg)	●			●					0.79	0.27	
Fortin and Shrestha (1986)	273	3 genotypes		Scanogram 722	B	2.0	Muscle (kg)	●			●					0.78	0.27	
Turlington (1989)	162			Technicare 210	RT		Muscle (kg)	●								0.89		
Young and Deaker (1994)	76	Coopworth		Aloka 210	RT	5.0	Muscle (kg)	●		●						0.32	0.72	
Silva et al. (2006)	46			Aloka 500	RT	7.5	Muscle (kg)	●	●	●						0.99	0.48	
Sahin et al. (2008)	40	Akkaraman	42	Pie Medical Falco 100	RT	8.0	Muscle (kg)	●								0.80	0.63	
Hajji et al. (2015)	14	Noire de Thibar		Falco Vet	RT	3.5	Muscle (kg)	●	●	●	●	●				0.88	0.24	
Teixeira et al. (2006)	67	Churra Bragançana	36	Aloka 500	RT	5.0 and 7.5	Muscle (g)	●								0.96	214.6	
Ripoll et al. (2009)				Aloka 900	RT	7.0 to 10.0	Muscle (g)	●	●	●						0.59	144.46.00	
Kempster et al. (1982)	273	Several breeds	39–47	Scanogram 722	B	2.0	Muscle (g kg ⁻¹)	●	●								32.90	
Silva et al. (2006)	46			Aloka 500	RT	7.5	Muscle (g kg ⁻¹)	●					●	●		0.87	12.70	
Ramsey et al. (1991)	99			Toshiba Sal-22A	RT	5.0	Protein (%)	●						●		0.51	0.87	
Silva et al. (2005)	31	2 genotypes		Aloka 500	RT	7.5	Protein (g kg ⁻¹)	●	●							0.54	5.35	
Leymaster et al. (1985)	37	Suffolk	73	Scanogram	B	2.0	Protein (kg)	●	●							SF3CV	0.26	0.25
Silva et al. (2005)	31	2 genotypes		Aloka 500	RT	7.5	Protein (kg)	●								0.97	0.18	

Abbreviations. BW: bodyweight, RT: real-time, ●: variables included in model, SFTV: subcutaneous fat in thoracic vertebrae area, LMDTV: combined depth of *longissimus thoracis* and *longissimus lumborum* muscles in thoracic vertebrae area, LMATV: combined area of *longissimus thoracis* and *longissimus lumborum* muscles in thoracic vertebrae area, SFLV: subcutaneous fat in lumbar vertebrae area, LMDLV: combined depth of *longissimus thoracis* and *longissimus lumborum* muscles in lumbar vertebrae area, GR: total depth of soft tissues over the 12th rib, 11 cm from dorsal midline, LMP: combined perimetre of *longissimus thoracis* and *longissimus lumborum* muscles, SF3CV: subcutaneous fat at 3rd coccygeal vertebra, R²: coefficient of determination, rsd: residual standard deviation.

Table 6

Prediction of carcass and cut yields from bodyweight and ultrasonographic measurements in sheep.

Reference	n	Breed	BW (kg)	Device	Mode	Frequency (MHz)	Dependent variable	Independent variables				R ²	RSD
								BW	SFTV	LMATV	SFLV		
Fortin (1980)	33	Mixed breed	29	Scanoprobe 731	A		Trim4cuts (kg)	●	●			0.96	0.02
Fortin (1980)	33	Mixed breed	29	Scanoprobe 731	A		Trim4cuts (%)	●	●			0.08	3.84
Fortin (1980)	33	Mixed breed	29	Scanogram 722	B	2.0	B4cuts (kg)	●	●			0.95	0.02
Fortin (1980)	33	Mixed breed	29	Scanogram 722	B	2.0	B4cuts (%)	●	●			0.35	4.62
Hopkins et al. (1996)	86	Ewes	43	Aloka 500	RT	3.5	TLC yield (%)	●	●			0.43	2.21
Hopkins et al. (1996)	84	Cryptorchid lambs	51	Aloka 500	RT	3.5	TLC yield (%)	●	●			0.39	1.8
Fernández et al. (1998)	10	Manchego	25	Toshiba SAL-32B	RT	5.0	Carcass yield (%)	●	●		●	0.20	2.05
Orman et al. (2010)	20	Awassi	40	Dynamic	RT	7.5	Carcass yield (%)	●	●			0.31	1.23
Notter et al. (2014)	512	Mixed breeds		Aloka 500	RT	3.5	TrHVW (kg)	●	●	●	●	0.98	0.38

Abbreviations. BW: bodyweight, RT: real-time, Trim4cuts: trimmed shoulder, loin, rack and leg, B4cuts: boneless shoulder, loin, rack and leg, TLC: trimmed lamb cuts (topside, silverside and boneless loin), TrHVW: weight of trimmed high-value cuts (trimmed rack and loin and trimmed boneless leg and sirloin). ●: variables included in model, SFTV: subcutaneous fat in thoracic vertebrae area, LMATV: combined area of *longissimus thoracis* and *longissimus lumborum* muscles in thoracic vertebrae area, SFLV: subcutaneous fat in lumbar vertebrae area, R²: coefficient of determination, rsd: residual standard deviation.

5. Concluding remarks

Real-time ultrasonographic examination is a versatile and useful technology applied in various areas of animal science with proven results in the ability to accurately evaluate *in vivo* body composition and predict carcass composition of sheep. Accurate information achieved with the technology can play an important role in the sheep industry, providing accurate and objective information in live animals related with body and carcass composition, which is necessary for performance testing, carcass classification, breeding programmes or for a precise feed of animals. Accuracy of measurement by means of existing equipment can be optimised by rigorously following the scanning protocols. Nevertheless, it is expected that the technology will benefit from developments in image quality, portability, image analysis capabilities, which will enhance accuracy and precision. These aspects will be useful in rigorous marketing systems and in precision sheep meat production. Both aspects will contribute to more efficient production systems. Developments in other fields, e.g., genomics, which need very accurate phenotypic information will largely benefit from the evolution of the technology. It will also be expected a new look for new measurement points, besides the thickness and area measurements, the volume measurements will also be pursued; this will be an attractive approach for predicting meat carcass composition traits. From current knowledge, it will also be expected a strengthening of the ultrasonographic examination association with computerised tomography in animal study of body composition in longitudinal experiments, as well as in two-stage selection programmes.

Conflict of interest statement

The author certifies that has no conflicts of interest to declare.

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