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Factors affecting repeatability of tissue depth determination by real-time ultrasound in sheep

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ABSTRACT

Measurement accuracy for ultrasonic tissue depths was examined in order to determine the relative importance of factors affecting it. Repeatabilities for ultrasonic fat depth (UFD) and ultrasonic muscle depth (UMD) were assessed from repeated runs (2) in three groups of animals; Dorset Down ewes (LW=45.4kg, 7.5mo, n=50), Coopworth rams (LW=51.5kg, 11mo, n=50) and Dorset Down rams (LW=38kg, 4.5mo, n=50). Repeatability values were higher for UMD (0.77, 0.95 & 0.87 for the 3 groups, respectively) than for UFD (0.63, 0.84 & 0.82, respectively). Lower repeatabilities were due to high within-animal variance rather than low between-animal variance. Measurements made on videotaped images demonstrated that image registration had a great effect on repeatability, with image interpretation having a negligible effect. A comparison of two operators differing in experience showed that fat depth was measured equally accurately by both operators but that the experienced operator measured muscle depth more accurately. It was concluded that tissue depths can be accurately assessed from one measurement.

Keywords Repeatability, backfat, fat depth, muscle depth, ultrasound.

INTRODUCTION

Repeatabilities for ultrasonic fat depth (UFD) measurements for sheep by A-mode machines have been reported (*eg.* Purchas & Beach, 1981), but equivalent data for measurements obtained with real-time B-mode ultrasound machines are less well known, particularly for ultrasonic muscle depth (UMD). Repeatabilities are important to commercial scanning services where there is a trade-off between accuracy of measurement and speed of scanning. This paper reports repeatabilities for UMD and UFD, and assesses the relative importance of some factors influencing repeatability.

MATERIALS & METHODS

Data were available for repeated scans of UMD and UFD on three groups of animals; (i) 50 Dorset Down (DD) ewe hoggets, (ii) 50 Coopworth (CPW) ram hoggets, and (iii) 50 DD ram lambs.

An experienced operator scanned the sheep which were restrained in a head stall, in a standing position. There were 24h between the two scanning runs for DD ewes and CPW rams, and 1.5h between runs for DD rams.

Field measurements of UMD and UFD were made directly on the monitor of the scanner. Images were obtained from scans made over the last rib lateral to the spinous process and over the dorsal surface of *M. longissimus dorsi* on the left side only. UMD was measured as the greatest depth of muscle at right angles to the skin surface, while fat depth was measured at a similar angle but slightly more lateral than UMD. These measurements correspond to B and C of Palsson (1939).

Additional measurements were available from the DD ewe group which was part of a larger study examining breed differences in subcutaneous fat distribution (Deaker & Young, 1992). For these sheep, as well as scanning at one site for measurements B & C, four other anatomical locations were scanned twice and the following measurements made.

- R = rib fat depth, from a scan of the lateral surface of the 5th rib, caudal to and at the level of the major tuberosity of the humerus and dorsal to the olecranon of the ulna.
- GR = the fat grading criteria for New Zealand sheep carcasses, at a site over the last rib as described by Kirton *et al.*, (1984).
- L = loin fat depth, over *M. longissimus dorsi*, equivalent to C but lateral to the penultimate lumbar vertebra.
- T = tail fat depth, the maximum depth of subcutaneous fat lateral to the 8th (approximately) coccygeal vertebra and medial to the ischiatic tuber of the pelvis.

Measurements C, L and T are fat depths. R and GR are predominantly fat but include some traces of muscle. B is a muscle depth. Scanning sites were selected to cover important areas of the carcass and for ease of location in the study of fat distribution by Deaker & Young (1992).

For all three groups, repeated measurements (2) of B and C were made in the field using a real-time B-mode ultrasound scanner (Aloka SSD-210 DXII, Aloka Co. Ltd., Japan) fitted with a UST-58101-5 probe operating at 5MHz with an image refreshing rate of 15 frames per second. For the DD ewes only, images obtained for all five anatomical locations were recorded onto videotape. Subsequently, measurements of tissue depth were made on these images using computer image analysis software (MIA -Morphological Image Analysis (1988), written by Dr W.R. Fright, Department of Medical Physics, Christchurch Hospital) run on an IBM compatible AT computer linked to a video recorder. Computer measurements were obtained for the two sets of field measurements and were repeated on one set.

Field measurements for B & C were repeated for the DD ewes for two further runs (runs 3 & 4) by the same experienced operator (>10 000 previous scans) and a trained but inexperienced operator (<5 previous scans).

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Repeatabilities were calculated as the intra-class correlation, t (Falconer, 1989).

RESULTS & DISCUSSION

Comparison of means for the three groups showed that the older animal groups were larger and had greater tissue depths for all measurements (Table 1). For the DD ewes, field measurements of muscle depth, B, closely matched the computer measurement, but for fat depth, C, the computer measurement was 22% greater. Two factors may account for this bias. Firstly, the operators excluded tissue interfaces from depth measurements when using ultrasound but measured depth from interface to interface using computer image analysis. However, this does not explain why one measurement was biased upward (C) while the other (B) was not. Secondly, it may have been that C was measured more laterally for the computer measurement since fat depth increases with movement in that direction. Generally smaller tissue depths were relatively more variable which is expected given similar absolute measurement errors.

TABLE 1 Means (± sem) within animal groups for field and computer measurements.

	DD ewes		CPW rams	DD rams
n	50		50	50
age (mo)	7.5		11	4.5
live weight (kg)	45.4		51.5	38[1]
	(0.7)		(0.7)	
tissue depths (mm)	field	computer	field	field
B	24.9	24.7	26.5	23.3
	(0.29)	(0.22)	(0.17)	(0.28)
C	3.50	4.27	3.80	2.69
	(0.14)	(0.11)	(0.08)	(0.12)
R	-	18.9	-	-
	(0.35)			
GR	-	14.3	-	-
	(0.21)			
L	-	5.77	-	-
	(0.17)			
T	-	14.6	-	-
	(0.24)			

[1] approximate because data obtained several weeks before scanning

Differences in repeatability occurred between the groups studied (Table 2). DD ewes had the lowest and CPW rams the highest values. Interestingly, these differences were not due to differences in between-animal variability, with the highest repeatability being associated with the lowest between-animal variance (CPW rams). Instead differences in repeatability were due to differences in within-animal variance, suggesting different conditions at scanning for the three groups. During scanning, animals in each group were handled and restrained in a similar manner, and scanned by the same experienced operator. However, the rate of scanning was different. CPW rams were scanned at a normal commercial rate of c.30/h. DD ewes were scanned at c.10/h since more sites were being scanned. It was observed that these animals grew progressively more restless while restrained for long periods, which may have had a negative effect on image quality. Also, compared with the CPW rams, the DD ewes had been handled less previously. Those involved in the scanning operation also observed that the DD groups were less tractable than other groups (CPW, this study, and Border Leicester (Deaker & Young, 1992)) suggesting that genetic differences in temperament occur. As well, much effort was put into the acquisition of good images at other sites for animals in this group, for which the operator had little experience (R, L and T), which may have led to less emphasis on obtaining the image at the B/C site. Together these factors combined to increase within-animal variance in the DD ewe group. DD rams were scanned at almost double the normal commercial rate because of time constraints, which appeared to compromise repeatability. Repeatabilities obtained under commercial scanning conditions (CPW rams) were very good, and probably serve to highlight the importance of a smoothly run scanning operation where animal stress is minimised and image quality maximized.

TABLE 2 Repeatabilities, t (± s_t), and variances (between-animal and within-animal) for field measurements. Runs 1 & 2.

	DD ewes	CPW rams	DD rams
Muscle depth, B			
t	0.77	0.95	0.87
	(0.13)	(0.03)	(0.08)
between-animal variance	3.25	2.85	3.34
within-animal variance	0.96	0.14	0.51
Fat depth, C			
t	0.63	0.84	0.82
	(0.19)	(0.10)	(0.11)
between-animal variance	0.70	0.51	0.60
within-animal variance	0.42	0.10	0.13

Repeatability estimates obtained in this study were lower than those of Purchas & Beach (1981) but higher than those of McLaren *et al.*, (1991). Data presented by Purchas & Beach (1981) indicated that their sheep had lower mean values for C but no estimates of variance were presented to allow comparison with data of the present study. McLaren *et al.*, (1991) used the same model of scanner as that used in this study but obtained very low estimates of repeatability due to very high within-animal variances. This may indicate that experience of the operators was very low compared to that of the experienced operator in the present study.

Errors can occur through obtaining a poor image (registration) or inconsistent measurement of images (interpretation). Results from the present study showed that image registration had a greater effect on repeatability than image interpretation (Table 3). Repeated measurements on the same run of images had a very high repeatability (method (a)) while repeated measurements on different images (method (b)) were less repeatable. The latter computer measurements were more repeatable for fat depth, C, than were field measurements (method (c)) on the same images, due to greater within-animal variance for field depth

TABLE 3 Repeatabilities, t (± s_t), for measurements B & C by each of three methods: (a) repeated (2) computer measurement of the same set of (video) images, (b) computer measurement of repeated (video) images, and (c) field measurement of repeated images. Data from DD ewes only, runs 1 & 2.

	B	C
(a) Repeated computer measurement	0.98	0.95
	(0.01)	(0.03)
(b) Repeated scans (computer)	0.74	0.80
	(0.15)	(0.12)
(c) Repeated scans (field)	0.77	0.63
	(0.13)	(0.19)

measurements indicating greater measurement errors. These could be due to the small size of the ultrasound machine monitor and the low resolution for measurement (± 1.0mm) compared with that of the computer analysis system (± 0.1mm). In contrast to these findings, McLaren *et al*., (1991) concluded that image interpretation was more important than image registration since repeatability increased markedly when their most experienced operator made measurements on images obtained by other operators. High within-animal variance in their study indicated that the operators were not as experienced at scanning sheep as those in the present study which may account for the different conclusion.

Consideration of analyses for repeated measurements made at all sites supports the finding that image interpretation was only a minor source of error (Table 4). However, registration was more important at some sites than others. Those sites at which the operator had experience (B/C and GR) had high repeatability (c.0.75) while R and T, for which the operator had no experience, had low values (c. 0.43).

TABLE 4 Repeatabilities, t (± s_t), for six computer measurements at five sites by each of two methods; (a) repeated (2) computer measurement of the same set of (video) images, and (b) computer measurement of repeated (video) images. Data from DD ewes only, runs 1 & 2.

	two measurements repeated on one image		two measurements from repeated images	
B	0.98	(0.01)	0.74	(0.15)
C	0.95	(0.03)	0.80	(0.12)
R	0.98	(0.01)	0.41	(0.22)
GR	0.97	(0.02)	0.73	(0.15)
L	0.98	(0.01)	0.81	(0.12)
T	0.99	(0.01)	0.45	(0.22)

Both the experienced and inexperienced operators had similar repeatabilities for fat depth, C, but the experienced operator had a higher repeatability for muscle depth, B (Table 5). Such a difference is supported by the observation of the experienced operator that it is easy to get an image that is adequate for measuring fat depth but it takes considerable experience to get good images of muscle. This is not surprising given that fat depth is relatively uniform around the measurement site and pressure will uniformly compress the fat layer creating a biased, but repeatable, measurement. In contrast, muscle depth is greater between the ribs than it is over the ribs and pressure variably distorts muscle shape. Furthermore, the angle of the ultrasound head would bias muscle depth relatively more than fat depth due to the more complex shape of muscle. Hence positioning of the ultrasound head and pressure applied have a great effect on repeatability for muscle measurements.

TABLE 5 Repeatabilities, t (± s_t), for field measurements of ultrasonic muscle depth, B, and ultrasonic fat depth, C, made by two operators on DD ewes, runs 3 & 4.

Operator	B		C	
experienced	**0.83**	(0.11)	**0.68**	(0.17)
inexperienced	**0.50**	(0.21)	**0.69**	(0.17)

Low repeatabilities can be countered by taking more than one measurement to estimate more accurately ultrasonic tissue depths. This study has shown that image registration has a large effect on accuracy of measurement of ultrasonic tissue depths. Consequently, extra measurements should be made on rescanned images, not on the same image. Additionally, it is not desirable to rescan an animal immediately, as obtaining the second image may be biased through knowledge of the first image. Rescanning animals in random order will overcome this problem but virtually doubles scanning time, the major determinant of scanning costs per animal. Taking more than one measurement is only valuable when repeatability is low (Falconer, 1989). Such was not the case with the traits studied here. Consequently the benefit of extra measurements is small, while costly to obtain.

To maximise repeatability, efforts should be made to maximise between-animal variation and minimize within-animal variation. Increasing between-animal variation can be achieved through nutrition, to maximise growth of muscle and fat, or by delaying measurement to a later stage of growth. Decreasing within-animal variation (measurement error) can be minimised through use of an experienced operator who has been trained in image registration and interpretation (particularly for measurements on muscle) and through use of a "smooth" scanning operation that minimizes the time animals are restrained and any stress associated with handling, provided sufficient time is given to allow accurate image registration and interpretation.

CONCLUSIONS

Given the high repeatabilities obtained under commercial scanning conditions in this study (CPW rams), single measurements of muscle and fat depth would be sufficiently accurate for most purposes. Best results will occur when using a trained and experienced operator, particularly for muscle measurements. Measurement errors can also be controlled by running a "smooth" scanning operation that minimises animal behaviour problems and maximizes image quality.

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