The measurement of skin thickness in Merino sheep using real time ultrasound

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Summary

The measurement of skin thickness in sheep may offer a number of potential benefits for sheep producers in terms of wool growth and monitoring body condition. The relationship between skin thickness measurement made using skin fold callipers to those made using real time ultrasound equipment was compared in 16 grazing Merino wethers. The relationship between the measurements made using the two techniques varied throughout the year. The measurements were not significantly different ($P<0.05$) at 7 of the 12 measurement times throughout the year. The measurement techniques were also moderately to highly correlated at 6 of the 12 measurement times however these were not exactly the same times as those with means not significantly different. With modified equipment, accurate estimation of average skin thickness could be measured using ultrasound.

Keywords: Skin fold callipers, real time ultrasound, seasonal variation

Introduction

It has been suggested that differences between sheep in skin thickness may be associated with differences in wool production. Utilising skin thickness may improve genetic selection of wool growth rate and quality. Skin thickness is positively related to clean fleece weight (0.39 ± 0.13), mean fibre diameter (0.20 ± 0.11) and staple length (0.35 ± 0.12) (Gregory 1982b). These relationships have been reviewed in more detail by Hynd *et al.* (1997). Sheep selected for high fleece weight also have a thicker skin than those selected for reduced fleece weight (Williams and Thornberry 1992). Skin thickness was not related to the differences in liveweight between the two groups. When individual animals from these groups where pen fed, these differences were not observed (Williams and Thornberry 1992) suggesting that diet and or climatic conditions may influence these relationships. Significant differences in skin thickness have also been observed between bloodlines of Merino sheep (Murray 1996). In contrast to these results, Masters *et al.* (2000) found no significant differences between sheep of similar mean fibre diameter but selected for and against fleece weight. As skin thickness is a heritable trait (0.25 to 0.60) (Gregory 1982a; Slee *et al.* 1991) it is anticipated that this trait would respond to selection.

It has been shown that the weight of the skin per unit area shows similar seasonal trends to that of body weight (Hutchinson 1957; Wodzicka 1958b; Lyne 1964; Williams and Morley 1994; Murray 1996; Schlink *et al.* 1996; Briegel *et al.* 2000). It is possible that differences in average skin thickness and seasonal variation in skin thickness between animals is associated with differences in the environmental responsiveness of wool growth. Average skin thickness and seasonal variation in skin thickness are significantly correlated with characteristics of the fibre diameter profile, mean fibre diameter, variation in fibre diameter, staple length and staple strength (Brown and Crook 2000). Sheep that are able to maintain skin thickness in spite of decreases in body condition may be able to maintain superior follicle nutrition and wool growth.

While the most widely used and accepted technique for measuring skin thickness is to measure the thickness of a fold of skin with skin-fold callipers, variation in the levels of subcutaneous fat and pinching force can contribute to measurement error (Alexander and Miller 1979). Ultrasound scanning is an objective method for estimating subcutaneous fat thickness and is frequently used in both sheep and cattle (Dicker *et al.* 1988). Ultrasound scanning provides an objective, non-invasive and simple way to fat and muscle depths and potentially skin thickness. Ultrasound also provides a real-time image of the skin, its structure and characteristics. The high frequency, high-resolution ultrasonic echo technique is capable of determining human skin thickness quickly and accurately (Alexander and Miller 1979).

Butler (1991) noted that variation may exist between sheep in the depth and relative proportions of epidermis, reticular layer and papillary layer of the dermis, sebaceous gland, sudoriferous gland and echo-pattern. These differences may influence wool production and the environmental responsiveness and fibre diameter profiles of sheep. By using high frequency and high-resolution ultrasound scanning differences between sheep in these characteristics may be identified.

This study was designed to examine the relationship between skin thickness measurements made using skin fold callipers and those using real time ultrasound equipment throughout the year.

Materials and Methods

A mob of 16 2-year-old fine wool Merino wethers were maintained for 12 months on improved pastures at Armidale NSW. Skin thickness was measured using both skin fold callipers and ultrasound at 12 times throughout the experiment, approximately 4 weeks apart.

The calliper technique was a modification of the technique used by Williams and Thornberry (1992). Skin thickness was measured at two points randomly selected on the right mid-side after close clipping (Oster small animal clippers size 30 blade). These two points were marked with a permanent-marking pen and used for the skin measurements at each sampling. A double fold of skin was measured using dial-gauge callipers that exerted a constant pressure of $1250 g/cm^2$ (Lyne 1964). A measurement was made at each site with the callipers facing in anterior / posterior direction relative to the body of the sheep and a second measurement was made in a dorsal / ventral direction. Average skin thickness at each sampling time was calculated by dividing the sum of these measurements by 8 (2 measurements x 2 locations x 2 layers).

The thickness of skin was also measured with a real time ultrasound scanner at the C site (over the eye muscle at the 12th rib) at the same time as the calliper measurements. These measurements were made using an Aloka 500V real time ultrasound scanner at a frequency of 3.5 Mega Hertz. The probe was 17.5cm long and designed for use on cattle. The existing wool staples at the C site were separated to gain access to the skin and vegetable oil was applied to the skin to enable clear image transmission. The site was not clipped as clipping has been shown to influence skin thickness (Wodzicka 1958c; Lyne 1964).

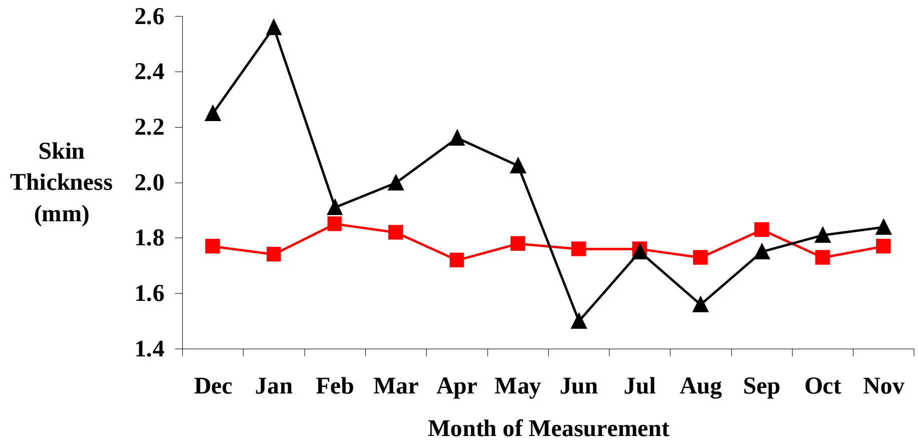
For each animal mean skin thickness and variation (standard deviation and coefficient of variation) in skin thickness over the experimental period were calculated for both techniques.

To compare measurements, least squares multivariate analysis of variance of the skin thickness measurements was conducted using the General Linear Model procedure in SAS (1990). Residual partial correlation coefficients were calculated to compare the skin thickness measurements from the calliper and ultrasound techniques.

Results

The means, standard errors and correlation coefficients for each skin thickness measurement technique at each sampling time are shown in Table 1. The relationship between the calliper and ultrasound techniques for estimating skin thickness varied throughout the experiment (Figure 1).

Figure 1 The mean values for the ultrasound (—▲—) and skin fold calliper (—■—) techniques throughout the experiment



The ultrasound measurements were significantly (P<0.05) higher than the calliper measurements in the months of December, January, April and May while significantly (P<0.05) lower in June. The measurements made in the remaining months were not significantly (P>0.05) different from the measurements made using the calliper technique. The two techniques were highly correlated in July (r=0.84) and moderately correlated in February, April, June, September, October and November (r=0.41 to 0.66). The mean, standard deviation and coefficient of variation of the measurements throughout the experiment were significantly different (P<0.05) between the calliper and ultrasound techniques but moderately and significantly correlated (r=0.66, 0.51 to 0.56 respectively). Both Figure 1 and Table 1 suggest that the accuracy of the ultrasound technique in measurement skin thickness improved throughout the experiment.

Table 1 The mean values (mm), standard errors of the means (s.e.) and correlation coefficients for the relationship between the skin thickness measurement made using the calliper and those made using real time ultrasound technique throughout the experiment

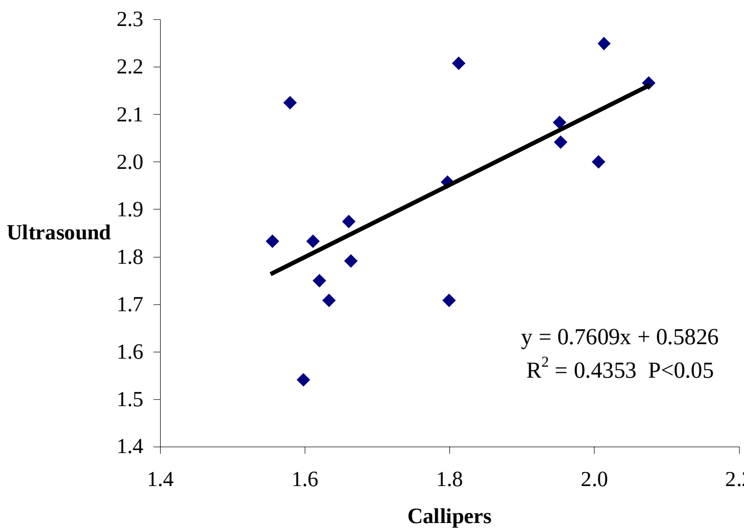
Month	Callipers	Ultrasound	s.e.	Correlation	Month	Callipers	Ultrasound	s.e.	Correlation
Dec	1.77^{a}	2.25^{b}	0.11	0.05	**Aug**	1.73^{a}	1.56^{a}	0.08	0.19
Jan	1.74^{a}	2.56^{b}	0.13	0.09	**Sep**	1.83^{a}	1.75^{a}	0.07	0.41
Feb	1.85^{a}	1.91^{a}	0.06	0.46*	**Oct**	1.73^{a}	1.81^{a}	0.06	0.55*
Mar	1.82^{a}	2.00^{a}	0.10	0.07	**Nov**	1.77^{a}	1.84^{a}	0.07	0.50*
Apr	1.72^{a}	2.16^{b}	0.06	0.18	**Mean**	1.77^{a}	1.93^{b}	0.03	0.66*
May	1.78^{a}	2.06^{b}	0.06	0.24	**SD**	0.11^{a}	0.47^{b}	0.03	0.51*
Jun	1.76^{a}	1.50^{b}	0.04	0.66*	**CV**	6.15^{a}	24.22^{b}	1.14	0.56*
Jul	1.76^{a}	1.75^{a}	0.03	0.84*					

Means within each column with different superscripts are significantly different ($P<0.05$)

* Correlation significant ($P<0.05$)

Figure 2 illustrates the relationship between the mean skin thickness measurements from the ultrasound technique and those from the calliper technique. This graph suggests that the positive bias of the ultrasound technique is more prominent in sheep with thinner skins.

Figure 2 The relationship between mean skin thickness measured using callipers and mean skin thickness measured using real time ultrasound



Discussion

The relationship between calliper and ultrasound measurements of skin thickness varied throughout the year and were only highly correlated at the eighth sampling time. However five of the monthly measurements were significantly correlated (r=0.46 to 0.84) suggesting that the ultrasound measurements were similar to those made by the callipers. A number of factors may have influenced these results. The ultrasound equipment used in this experiment was designed for the measurement of fat and eye muscle depth in cattle. The machine operated at a relatively low frequency and is designed to measure larger ranges. As the skin of sheep is very thin, generally less than 2mm, the image on this machine was very small. This made identification of the various tissue layers difficult. Furthermore the ultrasound equipment only measured to the nearest 0.5mm which equates to approximately 25% of the overall thickness of the skin. The skin fold callipers measured to the nearest 0.1mm, which resulted in a greater ability to distinguish differences between sheep. Subcutaneous fat measurements can be made using conventional scanners operating at 5 to 7 MHz (Butler 1991). Ultrasound equipment operating at higher frequency and resolution are required to produce adequate images to identify features of the skin (Alexander and Miller 1979; Dines *et al.* 1984). As the equipment used in this study operated at 3.5 MHz it is anticipated that these differences influenced the relationship between the ultrasound and calliper based measurements.

Results suggest that the accuracy of the ultrasound method improved as the experiment progressed. This may have been a direct result of the experience gained from the measurements at the previous sampling times. As the experiment progressed the operator found it easier to identify the layers of the dermis, subcutaneous fat and muscle and therefore to judge the top and bottom surfaces of the skin. Further experimentation may benefit from the incorporation of a leading in period to adapt operators to measurement and perhaps calibration against calliper measurements.

The two techniques used a different site to measure skin thickness. It is well known that wool traits vary over the body of the sheep (Sumner and Craven 2000). Variations in skin thickness of the body of sheep are not well documented, however, significant differences in skin thickness have been observed between sites in Merino sheep (Wodzicka 1958a; Williams and Thornberry 1992). Associated with this, to enable accurate measurements the site at which the calliper measurements were made had to be clipped prior to measurement whereas the ultrasound measurement did not always require the site to be clipped, except of rare occasions when the oil reacted unfavorably with the wool. As clipping has been shown to influence skin thickness (Wodzicka 1958c; Lyne 1964) this may have influenced the relationship between the measurement from the two techniques.

The fact that the wool generally does not have to be clipped is another benefit of the ultrasound technique. Clear images could be obtained simply by parting the wool and applying a small amount of vegetable oil. In a small percentage of animals (12.5%) however, the oil reacted unfavorably with the wool, discoloring it and creating a potential fly-strike problem. The above average rainfall that was received throughout the experiment may have influenced these reactions. It may be that the use of water based scanning gels, available for medical applications, would provide a more appropriate couplant for use in sheep.

Conclusion

This study found that real time ultrasound scanning could measure skin thickness and offered many potential advantages over the calliper technique. Further research is required to identify the most appropriate real time ultrasound equipment for this application, and it is likely to produce a viable alternative to the current skin fold calliper technique.

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