Wool Technology and Sheep Breeding

Volume 44, *Issue* 4 1996 *Article* 7

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ISSN 0043 - 7875/96 Wool Tech. Sheep Breed., 1996, 44 (4), 295 - 302

Precision of OFDA Fibre Diameter Measurements of Midside Wool Samples

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Summary

This paper gives details of a round trial designed to provide preliminary estimates of the precision of mean fibre diameter (MFD) test results on midside samples when measured using the OFDA. Estimates of between and within laboratory variance are needed in the draft Australian/New Zealand Standard for Wool Fleece Testing and Measurement (DR 96157-96163). The estimate of the 95% confidence limits of MFD measurements from this trial (±0.60μm) was smaller than the reported confidence limits of airflow and Laserscan measurements on midside samples. The 95% confidence limits of standard deviation (SD) measurements were ± 0.41μm and the 95% confidence limits of coefficient of variation (CV) measurements were ± 1.73%. Until a larger trial is conducted it is suggested that the Laserscan precision estimates for midsides are used. By estimating the components of variance, recommendations can be made on the most cost effective methods of improving precision. Future trials should therefore include any components of variance affecting between and within laboratory variance, eg. operators, software, number of slides, and number of fibres measured.

Keywords: Wool, fibre diameter, OFDA, midside

Introduction

Test methods for midside samples of wool for sheep breeding or clip preparation purposes are not yet covered by any standard test methods. There has been some concern in both the Australian and New Zealand industry that some laboratories may be producing inaccurate and imprecise results. A draft Australian/New Zealand Standard for Wool Fleece Testing and Measurement (DR 96157-96163) is in preparation. It requires precision estimates for the measurement of fibre diameter by airflow and sonic (DR 96160), Sirolan-Laserscan (DR 96161), OFDA (DR96162) and FDA 200 (DR 96163).

The precision estimates for airflow were obtained from two round trials in 1988 and 1989 reported by Morgan (1990). These trials involved each of 10 laboratories providing a single measurement of 3 replicate samples of 10 midsides. The between replicates

Reprinted from IWTO Technical Paper No. 8, Nice December 1996.

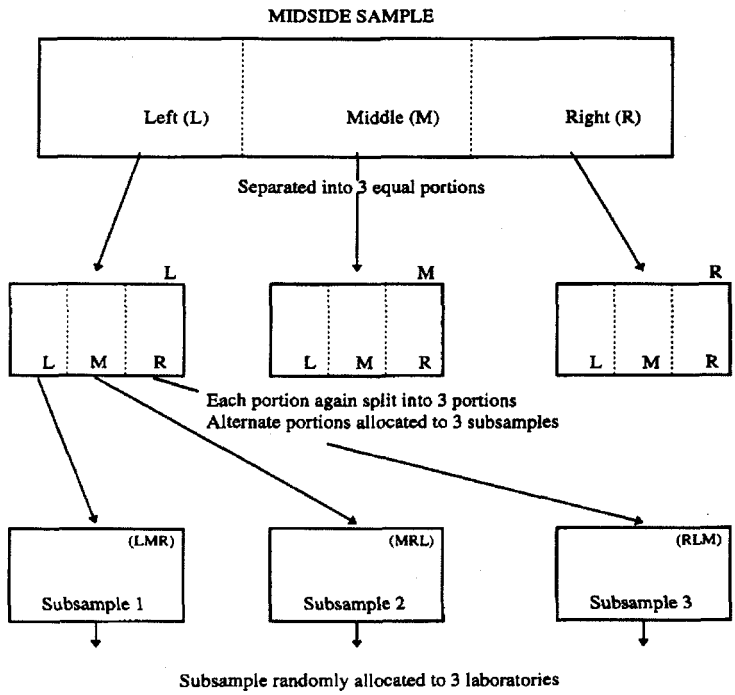
(residual) variance was termed within laboratory variance and was used to calculate the 95% confidence limits. Thus between measurement variance and between replicate variance were not partitioned. The precision estimates for Laserscan and FDA 200 were obtained from 8 round trials involving up to 27 Woolplan accredited laboratories using the same expermental design of 3 replicates from 10 midsides (Morgan, unpubl. data). Laserscan and FDA 200 results do not appear to have been treated separately from OFDA results in the round trials. The draft standard has no published estimates available for the precision of OFDA measurements and the trial reported here was conducted to provide some preliminary precision estimates for the standard.

Materials and Methods

Three fleece testing laboratories in 2 countries participated in the round trial. Fifty Merino midside fleece subsamples from each of two flocks were sent to each participant. Each set was selected to provide a wide range of mean fibre diameter.

A subsample from each midside was prepared according to the procedures in Fig. 1. Each midside was divided into three equal portions. Each of these portions was again divided into three portions. These were alternately allocated to make up three subsamples, which were then randomly allocated to the three laboratories.

Fig. 1 Midside subsampling procedure



Measurements were carried out according to the Draft Australian/New Zealand Standard for Wool - Fleece Testing and Measurement (DR 96157-96163). In laboratory 1 and 3 each greasy midside was weighed, then scoured, dried and oven dry weight determined. The scoured subsample was split into two halves. Each half was minicored, re-packed and minicored again to provide a total of four snippet samples. One slide was prepared from each snippet sample, giving four measurements per midside subsample from each laboratory. Laboratory 2, instead of adopting the suggested experimental design, followed their normal commercial procedures, ie. minicored the greasy subsample and solvent scoured the snippets. The midside subsample was not split prior to minicoring. Consequently, results from laboratory 2 were from two slides, where each was prepared from snippets from separate minicores from the entire midside subsample provided. The results from laboratory 2 were therefore of limited use in the analyses.

Each OFDA was calibrated according to IWTO-47 and set to 'wholeslide x 1', ensuring that at least 2000 counts were made per slide. The following information was provided: greasy subsample mass prior to scouring, scoured oven dry weight, washing yield at 16% regain, MFD, SD and CV, percentage of fibres >30µm and number of snippets measured. Only MFD, SD and CV results are presented here.

Statistical analysis

The 95% confidence limits (CL) for OFDA MFD measurements in any laboratory are calculated from 1.96* $\sqrt{}$(total variance). The components of variance making up the total variance are estimated from an analysis of variance of the data. The data were analysed in total and split into the finest 35 midsides (fine dataset; MFD 18.7µm±0.80, SD 3.8µm±0.52, CV 20.3%±2.61), 27 coarsest midsides (strong dataset; MFD 22.6µm±1.00, SD 4.5µm±0.51, CV 20.1%±2.39) and 38 intermediate midsides (medium dataset; MFD 20.5µm±0.61, SD 4.1µm±0.38, CV 19.9%±1.81). One model for the total variance is as follows:

$$\text{Total variance} = \sigma^2_{lab}/n + \sigma^2_{subs}/np + \sigma^2_{minicores}/npm + \sigma^2_{slides}/npms + \sigma^2_{fibres}/npmsf \qquad (1)$$

where the components of variance are:

σ^2_{lab} = between laboratory, σ^2_{subs} = between subsamples, $\sigma^2_{minicores}$ = between minicores, σ^2_{slides} = between slides, and σ^2_{fibres} = between fibres, and
n = number of laboratories, p = subsamples/lab, m = minicores/subsample,
s = slides/minicore, and f = fibres/slide.

If samples are always measured in the one laboratory, then $\sigma^2_{lab} = 0$, and the confidence limits of measurements are improved.

In this trial, s = 1, f = 2000-6000. Between slide and between fibre variances were not separated from between minicore variance. The residual variances in the analyses of variance have been termed *measure* variance and include between minicore, slide and fibre variances.

Data from laboratories 1 and 3 were analysed by analysis of variance using the model:

MFD, SD, CV = flock + midside(flock) + lab + flock*lab + midside* lab(flock) + subsample(flock*midside*lab) + residual, (2)

where:

flock = the source of the midsides (2 flocks), midside= midside identity, lab= testing laboratory (2 laboratories)and subsample (2 subsamples).

The components of variance were estimated as follows:

$MS_{lab} = \sigma^2 + 2^* \sigma^2_{subs(flock*midside*lab)} + 4^* \sigma^2_{midside*lab(flock)} + 400^* \sigma^2_{lab}$

$MS_{subs(flock*midside*lab)} = \sigma^2 + 2^* \sigma^2_{subs(flock*midside*lab)}$

$MS_{midside*lab(flock)} = \sigma^2 + 2^*\sigma^2_{subs(flock*midside*lab)} + 4^* \sigma^2_{midside*lab(flock)}$

Thus between laboratory variance, $\sigma^2_{lab} = (MS_{lab} - MS_{midside*lab(flock)})/400$ and within laboratory variance = $\sigma^2_{subs} + \sigma^2$, where $\sigma^2_{subs} = (MS_{subs} - \sigma^2)/2$ and $\sigma^2 = \sigma^2_{measure}$.

Data from all 3 laboratories were analysed to calculate the least squares means (LSM) of all laboratories as follows:

MFD, SD, CV = flock + midside(flock) + lab + flock*lab + residual (3)

The data from each individual laboratory (laboratory 1 and 3) were analysed as follows:

MFD, SD, CV = flock + midside(flock) + subsample(flock*midside) + residual, (4)

As stated above, residual variance ($\sigma^2_{measure}$) included between minicore, slide and fibres variance.

Data from Laboratory 2 were analysed as follows:

MFD, SD, CV = flock + midside(flock) + residual, (5)

For laboratory 1 and 3 results, $MS_{subs(midside+flock)} = \sigma^2 + 2^* \sigma^2_{subs}$.

Thus $\sigma^2_{subs} = (MS_{subs(midside+flock)} - \sigma^2)/2$ and $\sigma^2 = \sigma^2_{measure}$. For laboratory 2, only $\sigma^2_{measure}$ could be determined and this also included an unknown fraction of σ^2_{subs}.

Results

The least squares means of MFD, SD and CV values in the two flocks are given in Table 1.

Table 1 Least squares means of MFD, SD and CV

Lab	MFD			SD			CV		
	Flock 1	Flock 2	All	Flock 1	Flock 2	All	Flock 1	Flock 2	All
1	19.37	21.60	20.48	3.84	4.46	4.15	19.85	20.73	20.29
2	19.01	21.28[a]	20.15	4.19	4.84	4.51	22.05	22.81	22.43
3	19.28	21.36[a]	20.32	3.78	4.33	4.06	19.62	20.34	19.98

[a] Means are all significantly different (P<0.01), except for superscripted means.

MFD measurements were highest in laboratory 1, while MFD variability measurements were highest from laboratory 2. The laboratories provided significantly different values. The analysis of variance results for the balanced data from laboratories 1 and 3 are given in Table 2.

Table 2 Means squares of selected sources of variation in fine, medium, strong and all midside samples measured by laboratories 1 and 3

Source	df	Fine	df	Medium	df	Strong	df	All
Fibre diameter (MFD)								
Laboratory	1	.0773	1	2.0428*	1	7.2270*	1	5.3073*
Midside*lab(flock)	33	.2596*	36	.1109*	26	.1809*	98	.2074*
Subsample(flock*midside*lab)	70	.0653	74	.1009*	54	.1600*	200	.1042*
Residual	140	.0655	148	.0422	108	.0719	400	.0592
Standard deviation (SD)								
Laboratory	1	.1807*	1	.5819*	1	1.2135*	1	1.8442*
Midside*lab(flock)	33	.0670*	36	.0377*	26	.0786*	98	.0581*
Subsample(flock*midside*lab)	70	.0458*	74	.0315*	54	.0907*	200	.0524*
Residual	140	.0308	148	.0197	108	.0317	400	.0268
Coefficient of variation (CV)								
Laboratory	1	6.5516*	1	5.4152*	1	6.5452*	1	19.5625*
Midside*lab(flock)	33	1.0627*	36	.6598*	26	1.1145*	98	.9187*
Subsample(flock*midside*lab)	70	1.1365*	74	.5163	54	1.3038*	200	.9462*
Residual	140	.6773	148	.4139	108	0.4566	400	.5162

* Means squares effects are significant (P<0.01).

For MFD using the pooled results, σ^2_{lab} =(5.3073 - 0.2074)/400=0.013. σ^2_{subs}= (0.1042-0.0592)/2=0.023 and $\sigma^2_{measure}$= 0.059. Therefore within laboratory variance = 0.082 μm^2. The 95% confidence interval (for 1 laboratory, 1 subsample, 1 minicore measurement) was therefore $\pm 1.96 * \sqrt{(0.082+0.013)} = \pm 0.60$ µm.

When calculated separately for medium and strong samples between and within laboratory variances were 0.0141 and 0.0716 μm² for medium wools (95% CL = 0.57 μm), and 0.0652 and 0.1160 μm² for strong wools (95% CL = 0.83 μm) respectively. The variances for fine samples were poorly estimated due to sampling error or selection of data.

For SD the pooled varinaces were: σ^2_{lab} =0.0045. σ^2_{subs}= 0.0128 and $\sigma^2_{measure}$= 0.0268. Therefore total pooled within laboratory variance = 0.0396 μm². The 95% confidence interval (for 1 laboratory, 1 subsample, 1 minicore measurement) was therefore ±1.96 * √(0.0045+0.0396) = ±0.41 μm. When calculated separately for fine, medium and strong samples between and within laboratory variances were 0.001 and 0.038 μm² for fines, 0.004 and 0.026 μm² for mediums and, 0.011 and 0.061 μm² for strongs respectively, giving 95% CL estimates of 0.39, 0.34 and 0.53 mm respectively.

For CV the pooled variances were: σ^2_{lab} =0.0466. σ^2_{subs}= 0.215 and $\sigma^2_{measure}$= 0.5168. Therefore total pooled within laboratory variance = 0.7318 %². The 95% confidence interval (for 1 laboratory, 1 subsample, 1 minicore measurement) was therefore ±1.96 * √(0.0466+0.7318) = ±1.73 %. When calculated separately for fine, medium and strong samples between and within laboratory variances were 0.039 and 0.907%² for fines, 0.032 and 0.465%² for mediums and, 0.050 and 0.880%² for strongs respectively, giving 95% CL estimates of 1.91, 1.38 and 1.89% respectively.

The analyses of results from the individual laboratories are shown in Table 3.

Table 3 Mean squares of subsamples nested in midsides and residual

Source	df	Lab 1		Lab 2		Lab 3
MFD						
subsample(midside)	100	0.1770*			100	0.0314*
Error	200	0.1015	100	0.2406	200	0.0168
SD						
subsample(midside)	100	0.0920*			100	0.0128*
Error	200	0.0424	100	0.1595	200	0.0113
CV						
subsample(midside)	100	1.6474*			100	0.2450*
Error	200	0.7964	100	0.3830	200	0.2360

* Significant effect (P<0.01).

For laboratory 1, MFD σ^2_{subs} = 0.0378 and $\sigma^2_{measure}$= 0.1015, therefore within laboratory MFD variance was 0.1392 μm². SD σ^2_{subs}= 0.0248 and $\sigma^2_{measure}$= 0.0424, therefore within laboratory SD variance was 0.0672 μm². CV σ^2_{subs}= 0.4255 and $\sigma^2_{measure}$= 0.7964, therefore within laboratory CV variance was 1.2219%². 95% CL for testing within this laboratory were therefore 0.73μm, 0.51 μm and 2.17% for MFD, SD and CV respectively.

For laboratory 3, MFD σ^2_{subs} = 0.0073 and $\sigma^2_{measure}$ = 0.0168, therefore within laboratory MFD variance was 0.0241 μm². SD σ^2_{subs} = 0.0008 and $\sigma^2_{measure}$ = 0.0113, therefore within laboratory SD variance was 0.0121 μm². CV σ^2_{subs} = 0.0045 and $\sigma^2_{measure}$ = 0.2360, therefore within laboratory CV variance was 0.2405%². 95% CL for testing within this laboratory were therefore 0.30μm, 0.22 μm and 0.96% for MFD, SD and CV respectively.

The residual mean square from laboratory 2 was equal to $\sigma^2_{measure}$ plus an unknown fraction of σ^2_{subs} so it was not possible to carry out the above calculations to estimate the precision of measurements made in this laboratory.

A comparison of the mean squares by F test showed the laboratories were significantly different ($P<0.01$) in their variance between subsamples and measures for MFD, SD and CV.

Discussion

The levels of precision for MFD from this trial were equal or better than those reported in the draft test methods for airflow (± 1.2μm for 1 laboratory, ±1.6 μm over all laboratories) or for the Laserscan/FDA 200 (±1.0 μm for 1 laboratory, ±1.1 μm over all laboratories). However, due to the limited number of laboratories in the current trial, it is suggested that the values reported for Laserscan are used until a larger trial is conducted. This trial should also consider including operator, software, slide and fibre number sources of variation in the design.

Between and within laboratory MFD, SD and CV variance appeared higher for coarser wools, which is the case with airflow, Laserscan and OFDA measurements of core samples and sliver. The estimated 95% CL of MFD measured by airflow on greasy wool cores is ±0.45μm for 20 μm wool and ±0.57μm for 25 μm wool (IWTO-28-93).

The 95% CL of MFD measured by OFDA on greasy wool cores and sliver are ±0.36μm and ±0.30μm respectively for 20 μm wool and ±0.46 μm and ±0.42μm for 25 μm wool (IWTO -47-95, see Baxter and Marler, 1995; Marler and Baxter, 1995). The 95% CL of SD for greasy wool cores are ±0.27μm for wools with SD of 3μm and ±0.31μm for wools with SD of 5μm.

The 95% CL of MFD measured by Laserscan on greasy wool cores and sliver are ±0.32μm and ±0.25μm respectively for 20 μm wool and ±0.45μm and ±0.38μm for 25 μm wool (IWTO -12-95, see Baxter and Marler, 1995; Marler and Baxter, 1995). The 95% CL of SD for greasy wool cores and sliver are ±0.18μm for wools with SD of 3μm and ±0.23μm for wools with SD of 5μm.

In this trial the 95% CL for MFD was not much larger than that reported for core samples. Morgan (1990) noted that the 95% CL for midsides were larger than for core tests and suggested that midsides were more variable than blended core samples, but no published data were available to support this view. Core sample MFD variance includes between fleece, between region within fleece, between staple within region and within staple variance. Midside samples only contain the last two sources of variation, so it is possible that midside samples are not more variable than core samples.

Reference to equation 1 shows that, even though $\sigma^2_{measure} >> \sigma^2_{subs}$, it is preferable to take 2 subsamples and minicore each once, rather than taking 2 minicore samples from the 1 subsample and measuring them both. The former approach, using the trial results, has an estimated 95% confidence limit for mean MFD in 1 laboratory of $1.96 * \sqrt{(0.013 + 0.012 + 0.030)} = \pm 0.46\mu m$, the latter approach has a confidence limit of $1.96 * \sqrt{(0.013 + 0.023 + 0.030)} = \pm 0.50\mu m$. Following the same logic, improvements in precision are greatest in the order of increasing the number of laboratories, subsamples, minicores, slides and number of fibres/slide for each sample. The ease of logistics of increasing numbers is often in the reverse direction. Knowledge of all components of variation enables a proper cost-benefit analysis of improving precision to be undertaken.

Acknowledgment

Assocciate Professor John James, Department of Wool and Animal Science, University of New South Wales, provided valuable statistical advice.

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