

PRECISION OF MEASUREMENT OF DIAMETER, AND DIAMETER-LENGTH PROFILE, OF GREASY WOOL STAPLES ON-FARM, USING THE OFDA2000 INSTRUMENT

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The OFDA2000 is an instrument specifically developed to measure wool properties on farm without involved sample preparation. It allows an operator with minimal training to measure a greasy wool staple from an individual animal for mean fibre diameter, diameter distribution, and diameter profile along the staple length, all in 30 seconds or less. Issues related to sample selection and the measurement of greasy wool are examined, and precision data is given for two scenarios of use. This indicates that the single staple measurement regime is of similar precision to traditional midside sampling and testing in a typical fleece testing laboratory. Prediction of the mean fibre diameter of farm lots prepared using measurements on individual fleeces can be of adequate precision to allow financial benefits to accrue from such a selection process. Estimates are given for confidence limits for 10 key parameters from fibre diameter profile measurements.

The OFDA2000 instrument was developed as a fully-portable instrument, housed in a suitcase, specifically to allow diameter measurements to be made in real-time during animal selection or fleece classing (1). In the simplest method of use, a single staple is selected from a consistent location on each fleece or animal, and this staple is prepared and measured in its greasy form. The cycle of preparation and measurement takes 30 seconds or less, and throughputs of up to 1200 animals per day are commonly achieved in New Zealand by a single operator working alongside a selection team or shearing gang.

The diameter measurement algorithms used by the instrument are identical to those used in the OFDA100 (2). The OFDA2000 differs mainly in the way the sample is presented to the instrument, and the manner in which the sample is scanned. The operator teases or separates portions of one or more greasy staples so that they can be laid over a wire-framed slide. The tips of the staple portions are aligned at the top end of the slide. Once the slide is closed the fibres are held in position by the wire frame, and when placed on the instrument, the frame is traversed under a low-powered microscope objective, whilst being stroboscopically illuminated from below. The microscope and illuminant assembly moves across the slide at each increment along the staple, thereby building up a series of diameter distributions at discrete intervals along the length of each staple. The instrument can be set to measure at intervals of 3.5, 5, and 7 mm along the staple length, although normally the 5 mm setting is used for most average-length wools.

The fact that the instrument measures greasy fibres presents an interesting metrology scenario. Initially, experiments indicated that the grease factor was almost constant for many merino

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wools, and the grease factor was therefore defined simply as the mean difference between the mean fibre diameter for a greasy staple and the mean fibre diameter for that or an adjacent staple after cleaning in solvent. As the instrument was more widely used, it became apparent that not only did the grease factor vary between flocks, but also that it was diameter-dependent. The latter fact has a contingent effect on the distribution of diameters measured by the instrument, and whilst this has now been taken into account in the measurement software, at the time most of this work was carried out, this was not the case, and hence the paper focuses only on mean fibre diameter, and diameter profile along the staple length. It is therefore considered that the variance estimates given in this paper are likely to be conservative for operation of the instrument with the updated software (version 4.03 and above).

Variance models

In view of the two primary uses of the instrument - sheep selection and objective lot preparation, two models are necessary. Both must take account of the effect of the grease factor on individual measurements, and the objective preparation model additionally must allow for the effect of imprecision in the average grease factor on the precision of diameter of the prepared lots.

$$\text{Sale lots: } \beta(g, d, s) := \sqrt{\left[\frac{\left(\omega^2 + \Phi(d)^2 + \frac{\eta(d)^2 + \xi(d)^2}{s} \right)}{m \cdot f \cdot b} \right] + \sigma^2 + \frac{(\delta(d)^2 + \Phi(d)^2 + 2 \cdot \xi(d)^2)}{g}} \quad (1)$$

$$\text{Midsides: } \chi(g, d, s) := \sqrt{\left[\left(\frac{\eta(d)^2 + \xi(d)^2}{s} \right) + \Phi(d)^2 + \omega^2 + \sigma^2 + \frac{(\delta(d)^2 + \Phi(d)^2 + 2 \cdot \xi(d)^2)}{g} \right]} \quad (2)$$

Where:

d	mean fibre diameter of sample
f	number of fleeces in a bale
g	number of samples per mob used for determining grease factor
m	number of samples taken from a fleece
s	number of staples measured per sample
$\beta(g, d, s)^2$	total variance of diameter for an objectively-prepared sale lot
$\chi(g, d, s)^2$	total variance of diameter for a single animal or fleece
ω^2	component of variance for diameter between midside samples in a fleece
$\Phi(d)^2$	component of variance for grease factor variability between animals in a mob
$\eta(d)^2$	component of variance for diameter between staples in a midside sample
$\xi(d)^2$	component of variance for diameter between fibres in a sample
σ^2	component of variance for diameter between instruments
$\delta(d)^2$	component of variance for grease factor variability within samples

Experimental details

Midside samples were collected from at least 20 representative animals from each of 30 New Zealand flocks. Data was also made available from 26 Australian flocks, mainly, but not exclusively, from Western Australia. The overall dataset was used to examine the relationships between grease factor and mean fibre diameter, from which it was determined that for fine to medium flocks (below a flock average of approximately 30 μm), grease factor generally varied in a linear manner with diameter. (Coarser wools showed no correlation between these two parameters, but are not considered in the models since they do not represent the primary use of the instrument.)

Data involving replication was only available for the NZ flocks and for a limited number of Australian flocks. In the majority of cases, a single midside sample was used from each animal, and from this, two greasy staples were selected from separate parts of the sample. Each staple was split into two portions, one being measured greasy, and the other being measured clean, (after washing in dichloromethane, drying, and conditioning). All measurements were undertaken in the standard laboratory atmosphere of 20 °C and 65 % rh.

Hierarchical analyses of variance were undertaken on the clean mean fibre diameter results, and on the individual grease factor results with mean fibre diameter as covariate, for each set of samples from each flock. From these analyses, components of variance were determined for grease factor within samples (i.e. grease factor error), and between animals in a flock; and mean fibre diameter between staples in a sample. Additional data for the variance models was obtained from the literature, as outlined below.

Precision of mean fibre diameter measurements

Sources of data for each component of variance quoted in the models were as follows:

ω^2 Component of Variance for Diameter Between Midside Samples in a Fleece.
Most published data refers to variance determinations for between sites on an animal, rather than the circumstance where the aim is to sample from the same location. An attempt was made to determine this component by replicate sampling from thrown fleeces, but the resulting variance component was statistically non-significant. A conservative fixed value of 0.023 μm^2 has been used based on data quoted by Cottle et al. (3)

$\Phi(d)^2$ Component of Variance for Grease Factor Variability Between Animals in a Mob.
From an analysis of variance on 32 mobs, this component was determined to be heteroscedastic, but could be represented by a linear relationship:

$$\Phi(d)^2 = 0.0143 d - 0.1035 \quad (3)$$

$\eta(d)^2$ Component of Variance for Diameter Between Staples in a Midside Sample.
Analyses of variance on data from 25 mobs showed that this variance component could be modeled with a linear equation of the form:

$$\eta(d)^2 = 0.0130 d - 0.120 - \xi(d)^2 \quad (4)$$

$\xi(d)^2$ Component of Variance for Diameter Between Fibres in a Sample.

Continuous re-measurement of the same slides from a range of animals indicated a measurement error varying in a linear manner with diameter:

$$\text{Err}(d)^2 = 0.00169 d - 0.021 \quad (5)$$

However, a slightly more conservative estimate is available using the conventional model for this component, based on the measured diameter variance in the sample and the average number of fibres measured in each determination. This latter model was chosen since extensive relevant data was available for a very wide range of Australasian mobs (4).

$$\xi(d)^2 = (0.246 d - 1.07)^2 / 1000 \quad (6)$$

σ^2 Component of Variance for Diameter Between Instruments.

Instruments have an uncertainty associated with them in consequence of the calibration process, and whilst this manifests itself as a bias for the period of validity of the calibration, it nevertheless contributes to between-instrument variance and must be taken into consideration. Calibration and verification of these instruments follows similar procedures to those detailed for the OFDA100 in IWTO-47 (5). At the time this work was carried out, data was available from validations of 3 instruments, and this indicated that a uniform estimate of $0.0137 \mu\text{m}^2$ was appropriate.

$\delta(d)^2$ Component of Variance for Grease Factor Variability Within Samples.

This is the error variance associated with estimating the grease factor for an individual sample. It was determined by replicate measurements on 27 mobs. In the course of determining the grease factor, 2 measurements must be made of the mean fibre diameter (both greasy and clean), and hence the measurement error estimate appears in the equation:

$$\delta(d)^2 = 0.0087 d - 0.0436 - 2 \xi(d)^2 \quad (7)$$

Incorporation of these estimates into the two models shown in Equations 1 and 2 leads to estimates of total variance that are diameter-dependent, as shown in figure 1.

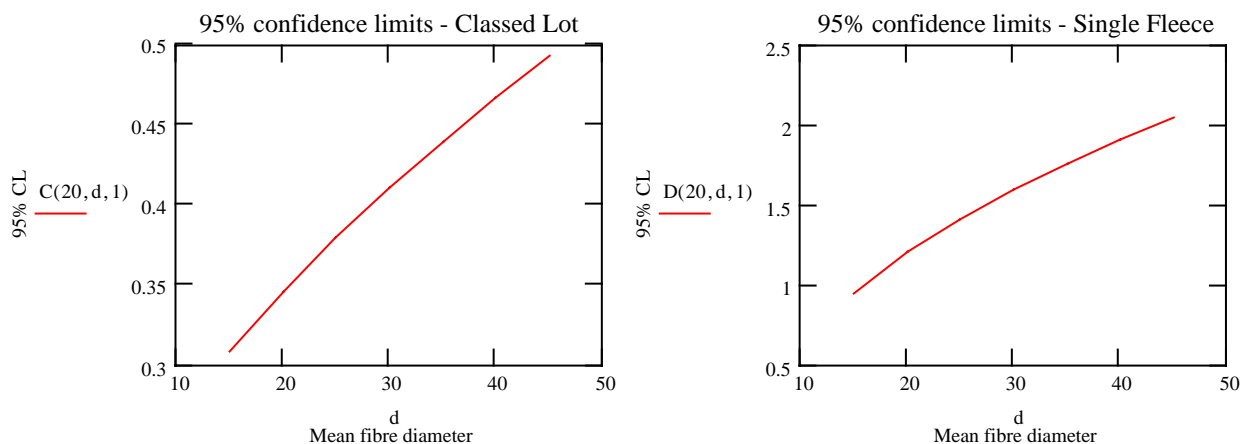


Figure 1: 95% confidence limits of MFD based on one staple per fleecce

Using the conventional 95% confidence limit expression of precision, the estimates for normal operation of the instrument at an MFD of 20 μm are $\pm 0.35 \mu\text{m}$ for a classed lot of 5 bales, and $\pm 1.2 \mu\text{m}$ for a single midside determination. These should be compared to equivalent estimates of, respectively, $\pm 0.33 \mu\text{m}$ deduced from NZPAC data (6), and $\pm 1.1 \mu\text{m}$ for the performance of an average fleece-testing laboratory (7).

These estimates incorporate both measured components of variance and estimates based on published data, and therefore must to be validated. Three validation schemes have been tested:

- Variances of differences between two independent sets of greasy measurements. The first set was carried out in the field on 19 mobs using one OFDA 2000 instrument and a single staple/animal, and the second was carried out in the laboratory using a separate instrument, with duplicate measurements on staples selected from a midside sample taken in the field. The variance of the differences is therefore expected to be 1.5 times the variance of a single measurement. This comparison is summarized in figure 2.
- Variances of differences between OFDA 2000 measurements on solvent-cleaned staples (undertaken in duplicate), and independent on-site measurements on 24 mobs using an OFDA 100 on solvent-cleaned snippets obtained by minicoring the greasy midside samples. The variance of the differences is expected to be equal to the sum of the variance of a conventional OFDA 100 solvent-based fleece test, plus half the variance of an OFDA 2000 measurement excluding the grease factor variance components – see figure 3.
- It is also possible to compare the duplicated greasy and cleaned measurements undertaken in the laboratory using the OFDA 2000 (for grease correction factor determinations – 26 mobs). In this case the expected variance of the differences is equal to the sum of half the variance for a greasy measurement plus half the variance for a clean measurement - see figure 4.

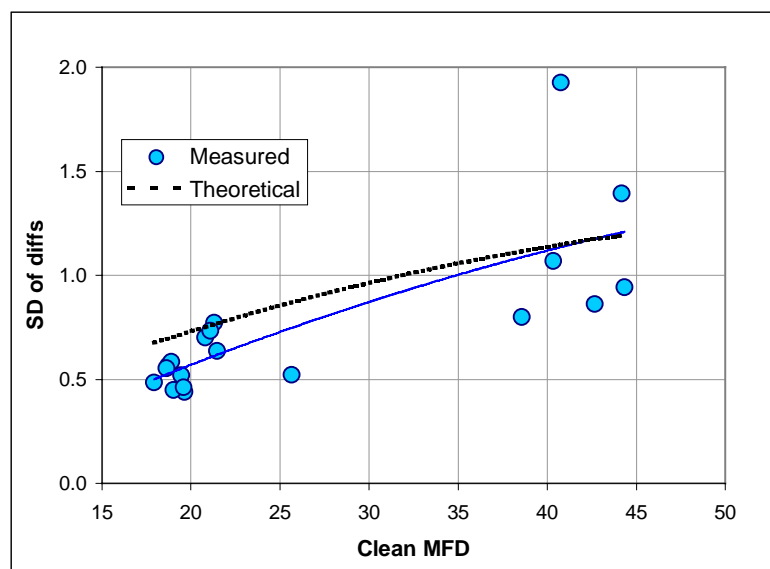


Figure 2: Comparison of SD of differences with expectation – greasy on-site v laboratory

Estimates of precision for OFDA100 measurements on solvent-cleaned greasy wool snippets were obtained from a round trial performed in 1992 (8), together with additional work carried out recently in SGS’s fleece testing laboratory, which indicated that the precision of this method is approximately 80% of the values estimated for the OFDA2000.

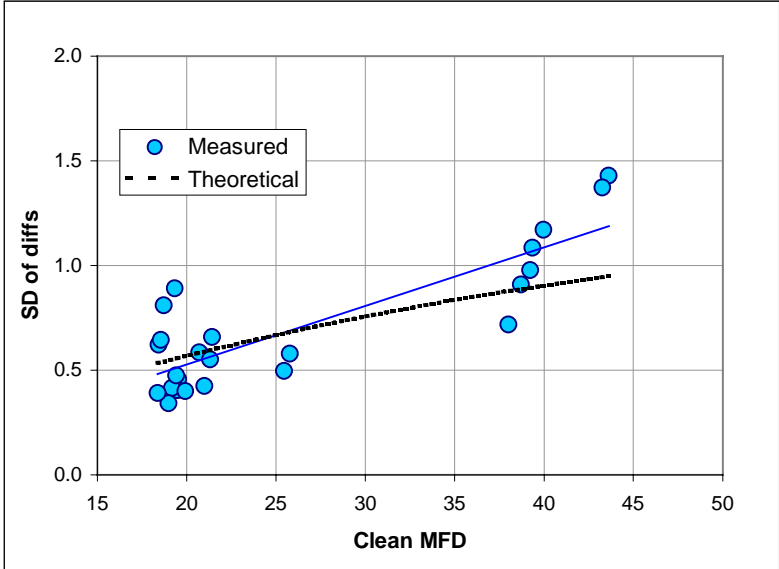


Figure 3: Comparison of SD of differences with expectation – OFDA2000 v OFDA100

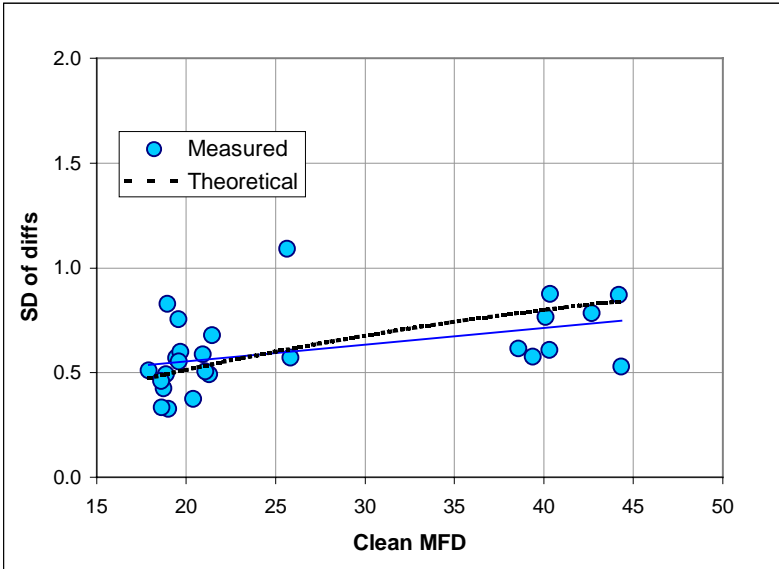


Figure 4: Comparison of SD of differences with expectation – greasy v clean staples

The apparent differences in the regression lines are of nil or marginal statistical significance. The three validations therefore confirm that the precision estimates for the midside sample model are realistic. The sale lots model uses the same variables, and recent work indicates that similar levels of agreement are being experienced for this model.

Precision of fibre diameter profile measurements

Over the last few years there has been considerable interest in fibre diameter profiles (FDP), particularly since they offer immediate benefits to growers using feed management to improve staple strength (9), and the possibility of significantly-improved prediction of processing length (10). These advantages provided much of the stimulus for development of the instrument (11).

During normal operation of the instrument staple profiles are built up in length increments of 5 mm. (Shorter increments may be used for short staples, and longer for longer staples.) Work carried out using conventional staple profiling techniques (12) emphasized the need to reduce the workload when 2 mm snippets are used, and it has been shown that when using this method, sampling at the equivalent of 8 mm increments is adequate for medium-length staples in order to obtain accurate FDP parameters (13).

The precision of the conventional staple profiling technique, using profiles generated at 2mm increments, has recently been examined by Brown et al. (14). The same FDP parameters and similar statistical techniques have been used in this work. Space restrictions prevent repetition of the method details outlined in the referenced paper, to which the reader is referred. In summary, Brown et al. focussed on the reproducibility under two conditions of sample preparation, of 9 key parameters that may be obtained from an FDP. In the work covered here, staple length measured by the OFDA2000 was examined in addition to the same 9 FDP parameters. For this work 20 samples were selected at random from a mixed-age flock of tender wools exhibiting mostly mid-point breaks. From each sample replicate staples were measured in both the greasy and cleaned condition. The staples used in this work were shorter than the examples diagrammatically illustrated in the figures in the referenced work, by 10 to 20 mm. The range of profiles examined is illustrated in figure 5, and a pair of replicated profiles for a typical sample are shown in figure 6.

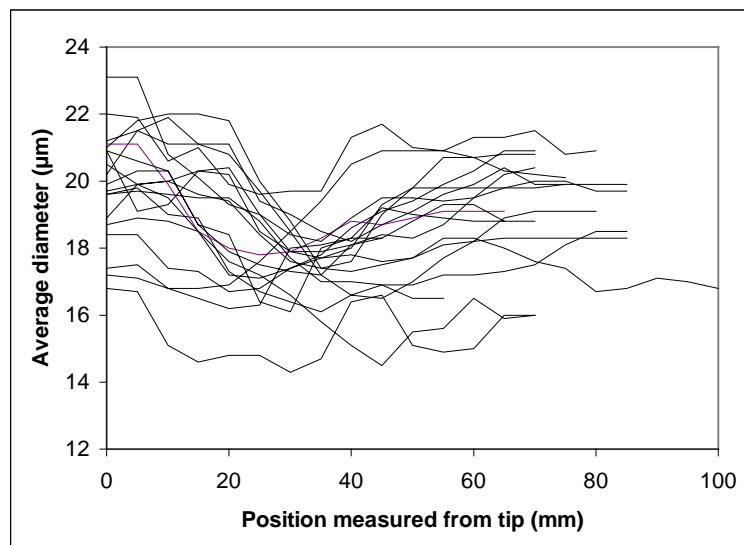


Figure 5: Range of individual FDPs examined for reproducibility

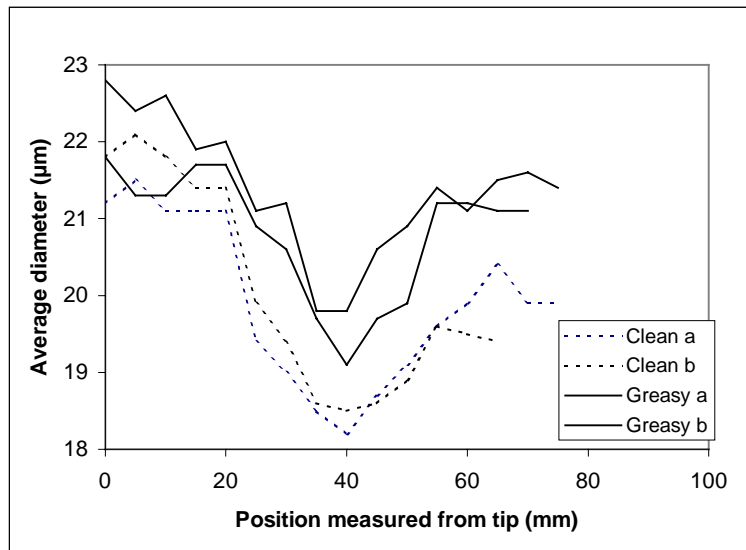


Figure 6: Paired FDPs on greasy and cleaned staples from a typical sample

The results of the statistical analyses for each of the 10 parameters are shown in an analogous form to Brown et al. in table I. For each wool state, the mean over 20 samples is shown for each replicate staple, together with coefficient of determination and standard error for the linear regression between the 2 sets of replicates, plus the 95% confidence limits associated with each single staple measurement. The paired differences of the 40 individual greasy and clean staples are also shown together with the regression statistics for the state comparison.

Table I: Statistical analysis results for FDP parameter reproducibility

State	Parameter	SL	Max	Min	Diff	AstVAR	Profmean	AstCV	Maxpos	Minpos	Roc
Greasy	Staple 1 mean	80.8	21.9	18.2	3.8	1.69	20.3	6.2	19.0	33.8	0.13
	Staple 2 mean	79.8	21.8	18.0	3.8	1.58	20.3	6.2	22.5	31.8	0.13
	Regression R ²	0.73 ***	0.70 ***	0.81 ***	0.46 ***	0.44 ***	0.82 ***	0.55 ***	0.18 *	0.83 ***	0.04 ns
	Regression se	5.6	0.70	0.61	0.97	0.78	0.54	1.4	22.3	6.5	0.04
	95% CL	8.3	1.2	1.0	1.2	1.0	0.8	1.9	43	15	0.09
Clean	Staple 1 mean	79.3	20.5	16.9	3.6	1.53	19.0	6.5	12	35	0.12
	Staple 2 mean	79.3	20.6	16.7	3.9	1.71	18.9	6.9	15	35	0.13
	Regression R ²	0.79 ***	0.58 ***	0.90 ***	0.23 *	0.44 ***	0.83 ***	0.55 ***	0.30 **	0.76 ***	0.25 *
	Regression se	4.8	0.86	0.39	0.71	0.54	0.44	1.0	18.5	7.1	0.04
	95% CL	7.2	1.2	0.7	1.2	0.9	0.6	1.7	26	10	0.06
Gsy-Clean diffs	Paired diffs	1.0 ns	0.0 ns	0.0 ns	0.05 ns	0.02 ns	0.06 ns	-0.51 *	7.3 ns	-2.1 ns	0.00 ns
	Regression R ²	0.73 ***	0.70 ***	0.77 ***	0.45 ***	0.43 ***	0.79 ***	0.44 ***	0.23 **	0.77 ***	0.10 *
	Regression se	5.9	0.74	0.57	0.63	0.55	0.50	1.1	17.7	6.9	0.04

Column headings are: SL - staple length (mm), Max - maximum fibre diameter along staple (μm), Min - minimum fibre diameter along staple (μm), Diff - Diameter range = Max minus

Min (μm), AstVAR - diameter variance along the staple (μm^2), Profmean - mean fibre diameter along the staple (μm), AstCV - coefficient of variation of diameter along staple (%), Maxpos - position of maximum diameter, measured from tip (mm), Minpos - position of minimum measured from tip (mm), Roc - rate of change of diameter between maximum and minimum ($\mu\text{m}/\text{mm}$). Statistically-significant differences and correlations are shown in the conventional manner (ns – not significant at 0.05 level, *, **, and *** - significant at the 0.05, 0.01, and 0.001 levels respectively). All the ‘Staple 1 mean’ to ‘Staple 2 mean’ differences were non-significant.

These statistics show that with the exception of Roc, good correlation was exhibited between clean and greasy results, and between replicates in each state. The Roc parameter is affected by the poor reproducibility of Maxpos in this dataset. This is partly because very similar values of maximum diameter were exhibited on both sides of the mid-season tenderness, and Maxpos was therefore sometimes closest to the tip, and sometimes closest to the base of the staple. Additionally, however, Roc was simply estimated in this work from the maximum and minimum diameters and their positions along the staple, whereas Brown et al used a linear regression technique on substantially more data points to improve their estimates of this parameter. Nevertheless, their paper also expressed concern about the need for a more robust method of estimating Roc. Approximately half the parameters show similar levels of precision in both the greasy and clean state. The other half show slightly better precision in the clean state, as might be expected.

In common with the referenced work, these results also show that variation in some FDP characteristics between staples prepared in the same way is small. SL, Max, Min, and Profmean gave 95% confidence limits of less than 10% of the mean values. The other parameters were less reproducible. The required number of staples to be measured for a specific FDP parameter will depend on the use to which the parameter will be put, and no judgement can be made here of the acceptability or otherwise of these precision estimates.

Conclusions

Two mean fibre diameter variance models are proposed for use of the OFDA2000 in objective preparation of sale lots and in sheep selection respectively. The variance components used in these models were obtained from both experimental data and published information. The 95% confidence limits arising from these models suggest that with appropriate quality controls over use of the instrument, it should be capable of producing similar precision to that already evidenced in NZPAC for objective lot preparation, and to that arising from normal implementation of fleece testing standards. Three sets of evidence have been shown to specifically validate the latter model. Since the same components of variance are used in the former model, albeit in a different manner, it is indicated that this model is similarly expected to validate in due course. It was noted that improvements made to the software subsequent to this work should improve the available precision.

Analyses of diameter profile data on animals from a mixed age flock indicate that key parameters may be determined reproducibly in both the greasy and clean staple condition, but that the latter state leads to slightly more precise values for some parameters. 95% confidence limits have been calculated for each of the key parameters, but until there is more information

available on the required precision for the two main uses of FDP data, recommendations cannot be made as to the number of staples required to be measured in each case.

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