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The detection of pigmented and medullated fibre in core samples of commercial sale lots from Merino ewes mated to Damara fat tail rams

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SUMMARY

A trial incorporating the testing of commercial core samples has provided further evidence of wool contamination arising from crossbreeding Merinos with Damara fat tail rams. The trial has also highlighted limitations of the CSIRO Dark Fibre Detector for this purpose in terms of both its subjectivity and the operator time required for a test.

Core samples from bales of fleece wool from Merino ewes known to have reared Merino lambs (5 lots), or to have reared Damara crossbred lambs (14 lots), or to have been mated to Damara rams but failed to rear a lamb (6 lots), were tested for pigmented and for medullated fibres by 3 laboratory methods. The tests were performed at AWTA Ltd and SARDI. The wool from the 3 different farm management practices had significantly different ($P<0.005$) concentrations of pigmented and medullated fibre. The ewes rearing the Merino lambs showed the lowest contamination, those rearing the Damara crossbred lambs showed the greatest contamination, and the remaining 6 lots showed intermediate concentrations of pigmented and medullated fibre.

The 2 laboratories were not significantly different ($P\geq0.05$) in their reported concentrations of medullated fibres and of the darkest (CSIRO darkness levels 6 to 8) pigmented fibres. However, for the pale-pigmented fibres (CSIRO darkness levels 4 and 5) and the total pigmented fibre concentration there were differences between the laboratories.

1 INTRODUCTION

During the 1990's a minority of Australian Merino wool producers diversified into fleece shedding breeds including the Damara fat tail breed. Some promoters of this breed had argued potential wool contamination was minimal or avoidable by farm management, but recent results/trials (Fleet et al. 2001) provide evidence to the contrary. In recognition of their concern about contamination from medullated and dark fibres, AWEX has introduced variations to clip preparation and marketing procedures, including the Woolclasser Development Program, that reinforce the risk of such wool contamination in certain crossbreeding situations. Indeed, they now require identification of affected lots in the marketplace (AWEX 2001).

There is no presale test or check currently available for dark or pigmented and medullated fibre. Diversification from entirely Merino production in Australia makes this deficiency more critical.

A trial was initiated by AWTA Ltd as a response to client inquiries to become involved in defining this contamination problem and in assessing the potential for presale testing using core samples of suspect wool lots. This report involves a limited survey of 25 sale lots to assess effects of crossbreeding Merino ewes to Damara fat tail rams as tested by 3 methods in 2 laboratories.

2 IDENTIFICATION OF CONTAMINANT SALE LOTS

AWTA Ltd and SARDI have investigated a number of possibilities for identifying wool contamination by dark and medullated fibres. The range of methods examined included:

- visual observation,
- image analysis,
- OFDA,
- near infrared reflectance,
- UV fluorescence,
- floatation, and
- measuring the Coefficient of Fibre Diameter.

Ideas were also explored for concentrating the proportion of contaminant fibres within a sample.

These methods had varying degrees of success. The simple visual examination of the cleaned cores by a trained wool operator produced a 50% success rate of identifying Merino ewe fleece wool that had been in contact with Damara rams and, possibly, Damara crossbred lambs. None of the other methods appears useful, at this stage, for the identification of greasy wool sale lots with potential contamination. However, a previous trial using CSIRO Dark Fibre Detection equipment (Fleet et al. 2001) had found contaminating fibres were present in each individual core sample tested in 3 consignments of fleece wool from Merinos that had reared Damara crossbred lambs. This finding lead to the use of the CSIRO Dark Fibre Detector in the current trial both to assess the levels of contamination in other commercial consignments and to determine the feasibility of using CSIRO Dark Fibre Detection equipment as a basis of a commercial presale test.

3 MATERIALS AND METHODS

3.1 Wool and farm management practices

The sale lots for the trial were selected by Wesfarmers-Landmark with the cooperating producers supplying the on-farm information. For the purpose of an experimental control group, 5 lots of Merino ewe fleece wool were selected from separate properties for which there were no known exposure to Damara or other “exotic” sheep breeds. A further 14 lots from 2 properties were chosen where Merino ewes were known to have reared Damara crossbred lambs for 4.5 months and to have been shorn 6 weeks after weaning of their crossbred lambs. The remaining 6 lots were from 3 properties (including the previous 2 properties). In this case, the ewes had been mated to Damara rams but failed to rear a crossbred lamb (classified as ‘dry’) and were drafted off and shorn separate from the main mob. The farm management practices assessed were: Damara lamb rearing; or, separation of ‘dry’ ewes from the mob in which crossbred lambs were reared.

3.2 Sample preparation, fibre detection and classification

The greasy core samples were firstly prepared for testing, then any dark or medullated fibres were detected, classified according to the intensity of their colour, extracted from the fibre mass and finally the extracted fibres were verified as being melanin pigmented or having pronounced medullation.

Specifically, blended greasy core samples (150g) from each of the 25 sale lots were scoured and dried at AWTA Ltd according to the procedures outlined in IWTO-19-98. The resulting clean wool was opened and blended either by a Shirley Analyser (at AWTA Ltd) as outlined in IWTO-28-00 or by a Shirley Fibreblender (at SARDI). Six (6) x 10g opened webs were produced from each of the 25 lots – 4 replicates using the Shirley Analyser and 2 replicates using the Fibreblender.

The samples thus had 2 possible preparation routes and were tested at 1 of 2 laboratories as follows:

2 x 10g replicates Analysed at AWTA Ltd, tested at AWTA Ltd;

2 x 10g replicates Analysed at AWTA Ltd, tested at SARDI; and,

2 x 10g replicates Fibreblended at SARDI, tested at SARDI.

Dark fibres in the webs were detected using a CSIRO Dark Fibre Detector according to the procedures for detecting dark fibres in top outlined in IWTO (E)-13-88(E); using hand drawn webs of up to 0.5g as opposed to the 0.2g stipulated in the Standard. Medullated fibres were detected after the inspection for dark fibres, by viewing with the top illumination only.

There were differences between the 2 laboratories in the manner in which the webs were tested. The 2 AWTA Ltd observers rotated in 30 minute shifts between detecting fibres using a prototype CSIRO Dark Fibre Detector and verifying and classifying fibres using a Leica microscope to examine the extracted fibres (Laboratory method 1). The 2 SARDI observers separately inspected the 2 AWTA Shirley Analysed replicates (Laboratory method 2) and the 2 Shirley Fibreblended replicates (Laboratory method 3), each of about 10g of the carded web, using separate CSIRO Dark Fibre Detectors model DFD-1 (CSIRO). The SARDI observer scored and extracted the fibres but the microscope classification was undertaken by M. Fleet using an Olympus BH-2 microscope. Both the identity of the samples and experimental design were unknown to the observers.

Each observer extracted suspected dark or heavily medullated fibres that were of at least 3 mm length and darkness was scored by comparison with the CSIRO Reference scale (Foulds et al. 1984; CSIRO). The extracted fibres were mounted on double-sided tape attached to formatted transparency sheets and when complete another strip of transparency was then placed over the fibres. These clear sheets then allow microscope examination (to 400x) for fibre classification without the need for mounting on glass slides (Fleet et al. 2001). Microscope examination confirmed melanin pigmentation or pronounced medullation (defined as medulla occupying over half of the fibre diameter for over 3mm of the fibre length). Inspected wool samples were conditioned and weighed and the rate of contamination per 10g was calculated for medullated fibres and for the total number of pigmented fibres, and the numbers with CSIRO darkness levels 4 and 5 or levels 6 to 8.

3.3 Statistical analysis

The counts of pigmented and medullated fibre were square root transformed prior to analysis to stabilise variance. The data were analysed with the procedure GLM of SAS (1996) using the following model:

$$Y_{ijk} = \mu + LM_i + R(LM)_j + FM_k + (LM*FM)_{ij} + e_{ijk}$$

Where: Y_{ijk} = the observed measurement on the individual sample;

μ = is the mean of the measurements;

LM_i the effect of the 3 types of laboratory method:

1 -AWTA Shirley Analysed and AWTA measured;

2 -AWTA Shirley Analysed and SARDI measured; and,

3 -SARDI Shirley Fibreblended and SARDI measured;

$R(LM)_j$ is the random effect of the 2 replicates nested within laboratory methods;

FM_k is the effect of the 3 types of farm management: Merino control; 'dry' Damara mated ewes; and, ewes that reared a Damara crossbred lamb;

$(LM*FM)_{ij}$ is the interaction between laboratory method and farm management; and

e_{ijk} is the random residual error.

The effect of LM_i was tested using the $R(LM)_j$ mean square, while other effects were tested against the residual error.

4. RESULTS AND DISCUSSION

4.1 Comparison of 3 types of farm management

The three types of farm management were significantly different (P<0.005) with the control ewes having low levels, the 'dry' ewes from matings to Damara rams having intermediate levels and ewes that reared Damara crossbred lambs having the highest numbers of pigmented and medullated fibres. These differences are shown pictorially in Figure 1 for samples measured by SARDI using AWTA Ltd preparation. The results in Figure 1 are similar to the average results of the 3 measurement systems. The very low contamination levels of some of the "Mild" set offer the possibility of some management practices, e.g. timing of separation of dry ewes that might further lower their risk of contamination.

Table 1 shows the least square means for the original and transformed data (√ with significant differences indicated) except for the concentration of pale-pigmented fibres alone (Darkness levels 4 and 5) that had a significant interaction ($P<0.001$).

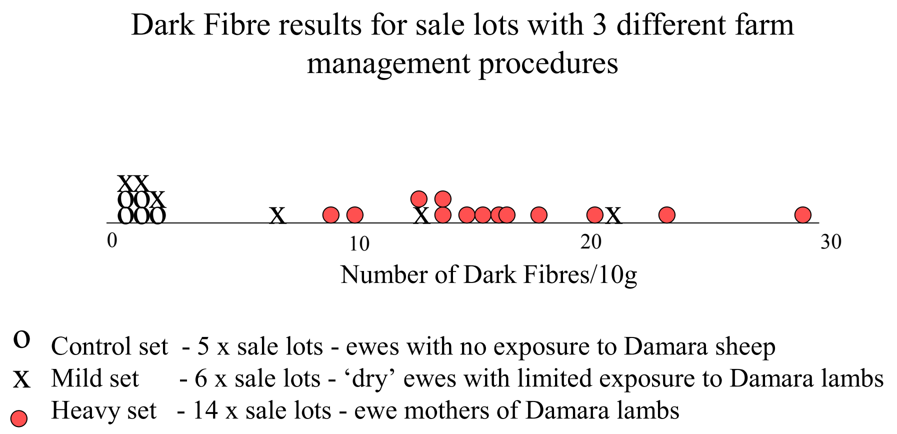

Figure 1. The overlap of Dark Fibre counts for sale lots with different exposure to Damara sheep, designated as 'Control', 'Mild contamination' and 'Heavy contamination'. SARDI(AWTA-preparation) testing of 2x10g samples/test.

Table 1: Least square means for effects of farm management and laboratory method

	Number of pigmented or medullated fibres per 10g					
	Farm management			**Laboratory method**		
Type of contaminated fibre	Control	'Dry' Damara mated	Damara mated & reared lamb	1	2	3
Total medullated (M)	0.6	1.7	4.8	3.4	1.9	1.9
(√ M)	*(0.4 **a**)	(1.0 **b**)	(2.0 **c**)	(1.4 a)	(1.0 a)	(0.9 a)
Pigmented Dark 6 to 8	1.5	8.7	9.2	12.0	8.6	8.9
(√ Dark 6 to 8)	(1.0 **a**)	(2.7 **b**)	(4.3 **c**)	(3.0 a)	(2.5 a)	(2.5 a)
Pigmented Dark 4 to 8	2.3	11.1	24.1	19.2	9.1	9.2
(√ Dark 4 to 8)	(1.2 **a**)	(3.0 **b**)	(4.7 **c**)	(4.0 a)	(2.5 b)	(2.5 b)

*Significance: Those means of each variable for Laboratory method or Farm management with a different superscript are significantly different ($P<0.05$) or if in **bold** ($P<0.01$).

The high numbers of pigmented and medullated fibres found in core samples from Merino ewes that reared Damara crossbred lambs are comparable with those reported by Fleet et al. (2001). The farm practice of separating 'dry' ewes from the crossbred mob apparently can reduce the level of exposure and wool contamination. However, these intermediate levels of contamination exceeded the control ewes by 3-fold for the medullated fibre and by 6-fold for the pigmented fibre, and may still be regarded as unacceptable for Merino wool destined for sensitive end-uses.

The concentration of medullated fibres apparently transferred during Damara crossbreeding was low relative to pigmented fibre. A reason for these differing proportions of contaminating fibres is the high degree of colouration of Damara crossbred lambs, but another possible explanation is that the CSIRO Dark Fibre Detector is less effective at detecting medullated than dark fibre contamination (Fleet et al. 2001).

4.2 Comparison of the 3 types of laboratory method

The 3 laboratory methods were not significantly different for the transformed concentration of medullated-pigmented fibres (P=0.117) or for the darker (levels 6 to 8) pigmented fibres (P=0.124); though means were highest in the AWTA Ltd laboratory (Table 1). However, for the total number of pigmented fibres (Table 1) and number of pale pigmented fibres (darkness levels 4 and 5) per 10g, laboratory method was significant (P<0.05 and P<0.01; respectively); the latter involving an interaction (Table 2).

Table 2: Least square means for the interaction between the effects of laboratory method and farm management for the number of pale-pigmented fibres (darkness 4 and 5) with the transformed variate (√ brackets).

	Farm Management		
Laboratory method	Control	'Dry' Damara mated	Damara mated & reared lamb
1. AWTA Shirley Analysed & AWTA measured	2.2	7.1	12.4
	*(1.3 **a**)	(2.5 **b**)	(3.4 **c**)
2. AWTA Shirley Analysed & SARDI measured.	0.0	0.1	1.4
	(0.0 a)	(0.1 a)	(0.9 **b**)
3. SARDI Shirley Fibreblended & SARDI measured	0.0	0.0	1.0
	(0.0 a)	(0.0 a)	(0.7 **b**)

*Significance: Those means within rows with a different superscript are significantly different (P<0.05) or if in **bold** (P<0.01)

The significant effects involving laboratory method, relate to the higher numbers of pale pigmented fibres detected within the AWTA Ltd laboratory (Table 2). The levels of darkness of the pale-pigmented fibres, darkness levels 4 and 5, are around the threshold of detection in a wool fabric. In fact, level 4 fibres are considered below the threshold of detection in a fabric and Foulds et al. (1991) noted that, "detection by (fabric) inspectors of level 5 fibres, even in white fabrics, is difficult". Also, the current method (IWTO-55-99) for determining the cleanliness of tops using automatic counting (the Optalyser) defines Dark and Very Dark fibres according to the levels "5.5 – 6.5" and "greater than 6.5", respectively. Fibres of levels less than 5.5 are, therefore, not reported under IWTO-55-99 (Optalyser testing).

Not only are these pale-pigmented fibres at the margin of being included as problem contamination, but also differences in their detection have been noted previously. Burbidge et al (1994), from a large inter-laboratory comparison using CSIRO Dark Fibre Detectors and wool tops, reported similar large differences between laboratories apparently relating to the identification and darkness classification of pale fibres. In that Inter-laboratory trial, higher-than-expected counts were sometimes associated with long inspection times and with removal of excess fibres that did not comply with the definition of a dark fibre. These factors reflect the subjectivity that is an inherent weakness in the method.

The analysis showed that the commercial Shirley Analyser preparation at AWTA Ltd was equivalent to the Shirley Fibreblender preparation performed at SARDI.

The observers in the AWTA Ltd laboratory took an average of 136 minutes per 10g sample though this included the microscope inspection of the fibres. The individual observers in the SARDI laboratory have taken less time 115 or 82 minutes per sample (excludes microscope checking of fibres) for the inspection of wool webs. This may have also contributed to fewer pale fibres being found.

The pigmented fibres transferred from Damara crossbred lambs also often have pronounced medullation (Fleet et al. 2001) which makes the assignment of darkness level more difficult since, in both reflected and transmitted light, they appear darker than is caused by the pigmentation alone. Both laboratories used different equipment and inspection routines (i.e. half-hourly rotations vs complete sample, microscope inspection of extracted fibres) and these factors may also have contributed to the laboratory differences.

5. CONCLUSION

Crossbreeding of Merino ewes with Damara rams exposes the resulting ewe fleece wool to the risk of contamination from dark and medullated fibres. Core samples from sale lots of Merino ewe wool after rearing Damara crossbred lambs had high levels of pigmented and medullated fibres relative those lots from self-replacing Merino ewe flocks. The practice of separating 'dry' ewes that had failed to rear a crossbred lamb produced levels of contamination intermediate between the other 2 groups.

Commercial core samples may provide useful information about the contaminant fibre burden arising in such crossbreeding situations. However, the CSIRO Dark Fibre Detector metrology applied is both labour intensive and subjective; leading to large differences between laboratories in the detection of pale-pigmented fibres.

It is clear that more information is required about other situations of Merino crossbreeding. This information could be used to reduce the uncertainty in the wool market about the level of contamination in sale lots. It is also clear that the metrology of dark fibre testing requires significant improvement and validation before a routine presale test for this type of contamination can be applied with confidence.

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7 REFERENCES

AWEX (2001) Why has the industry requested a Woolclasser Development Program. BOARDtalk the Australian Woolclassing Journal, March 2001 (Australian Wool Exchange; Sydney) p 3.

Burbidge A., McInnes C.B., Brown C.H., Foulds R.A and Stephens L. (1994). Inter-laboratory trial to evaluate IWTO(E)-13-88(E). IWTO T & S Committee, New Delhi, Report 22

CSIRO Dark fibre detector – Model DFD-1 - instructions for use, CSIRO; Ryde

Fleet M.R. (1999). Wool contamination – pigmented and heavily medullated fibres. PIRSA/SARDI Fact sheet Agdex 437/85. Available on Prime Notes CD-ROM (QDPI) and world wide web pages of SARDI (www.sardi.sa.gov.au), AWTA Ltd (www.awta.com.au) and AWEX (www.awex.com.au).

Fleet M.R., Turk J.A., Cottell M.A. and Keynes G.N. (2001). Merino wool contamination from rearing of Damara crossbred lambs. *Australian Journal of Experimental Agriculture* **41**(6): 725-731.

Foulds R.A., Wong P and Andrews MW (1984). Dark fibres and their economic importance. *Wool Technology and Sheep Breeding* **33**: 91-100.

Foulds, R.A., McInnes, C.B., Blenkenburg, G. and Philippen, H. (1991). Factors Influencing Final Inspections of White and Dyed Woven and Knitted Apparel Fabrics for Faults Due to Dark Fibre Contamination, IWTO T & S Committee, Lisbon, Report 11.

IWTO(E)-13-88 *Counting of Coloured Fibres in Tops by the Balanced Illumination Method*

IWTO-19-98 (1998) *Determination of Wool Base and Vegetable Matter Base of Core Samples of Raw Wool*

IWTO-55-99 *Method of Automatic Counting and Classifying Cleanliness Faults in Tops Using the Optalyser Instrument*

IWTO-28-00 *Determination by the Airflow Method of the Mean Fibre Diameter of Core Samples of Raw Wool*

Pattinson R. and Hanson P. (1993). An assessment of risk factors associated with dark fibre within the Australian wool clip, Australian Wool Corporation, Melbourne

SAS (1996), *SAS System for Windows version 6.12,* SAS Institute Inc., Carey, NC, USA