Unravelling the causes of wool yellowing Part II: Involvement of bacteria

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ABSTRACT

Wool was collected from five sheep with a propensity for wool yellowing. Sub-samples, from which the wool tip had been removed, were cut into 2cm lengths and incubated at 40°C for 6 days to provide an environment conducive to wool yellowing (YCT). Five treatments were applied to sub-samples of each fleece prior to YCT. Four sub-samples were gamma irradiated in an attempt to kill all bacteria present on the fleece (-B), the remaining subsample was not irradiated. Two -B sub-samples were re-inoculated with bacteria collected from the original fleece samples (+B). Each of the +B and -B samples was then either incubated at 0% humidity (-H) or 100% humidity (+H) for the YCT. Wool yellowness (Y-Z) was measured before and after YCT using standard methods.

No relationship was apparent between the number of bacterial colonies in the original fleece (plated onto nutrient agar), and the pre-YCT Y-Z (R^2=0.20; P=0.06), Y-Z after the YCT (R^2=0.19; P=0.06), or the change in wool Y-Z during the YCT (R^2=0.07; P=0.27). Gamma irradiation eliminated bacteria from the wool samples but increased their Y-Z pre-YCT (mean ± S.D, non-irradiated 2.46±0.55; irradiated 4.35±0.43; P<0.001).

Discolouration of wool (Y-Z) was increased by humidity during YCT (-H 1.06±0.45, +H 4.96±1.46; P<0.001). At 100% humidity, reinoculation with bacteria increased wool Y-Z during YCT (+B 6.13±0.87; -B 3.78±0.75; P<0.01). This effect of reinoculation was lost at 0% humidity. The interaction between humidity and reinoculation was significant (P<0.05). This study supports the hypothesis that bacteria are involved in the yellow discolouration of wool in the presence of high humidity.

Keywords: wool; yellowing; wool bacteria; wool pH.

INTRODUCTION

Wool varies in the degree to which it discolours and can be classed within the range from resistant to susceptible with respect to yellow discolouration (Aitken *et al*., 1994). There are both genetic and environmental components of this wool trait (Wilkinson, 1982). It has been shown in numerous reports that the incubation of wool samples at warm temperatures with high humidity provides a tool for distinguishing the two wool types (Wilkinson, 1981; Aliaga *et al*., 1996). The importance of heat and moisture in wool yellowing has led to the hypothesis that it is of bacterial origin.

Numerous bacteria have been identified in the sheep fleece. Bacterial proliferation following rain results in discolouration and may result in rotting of the fleece (Merritt *et al*., 1984). In particular, high colony numbers of *Pseudomonas aeruginosa* have been correlated with fleeces susceptible to discolouration and fleece rot (Merritt and Watts, 1978). Factors responsible for differences in bacterial populations between susceptible and resistant fleece types have not been defined, however moisture retention and wax composition have been suggested. Mulcock and Fraser (1958) found that fleeces susceptible to yellow discolouration had more bacteria than resistant fleeces during dry periods, and that fleeces susceptible to yellow discolouration retained more moisture, and for longer periods after rain, than fleeces resistant to yellow discolouration. It seems likely that moisture in the fleece provides an improved habitat for bacterial growth.

Merritt *et al*. (1984) showed a preference by *P. aeruginosa* for culture media made from waxes from susceptible, rather than resistant, sheep. The wax composition in susceptible sheep appears to make it more degradable by *P. aeruginosa* and thus supports bacterial proliferation. In 1992, Cottle *et al*. used a preliminary experiment involving wool sterilisation to demonstrate that bacteria play a role in colour change. This work is extended in the current contribution.

Aitken *et al*. (1994) and de Siqueira and Fernandes (1994) both described positive correlations between fleece yellowing and water extractable pH. However, Aitken *et al*. (1994) suggest that increased wool pH is an effect rather than a cause of colour development. De Siqueira and Fernandes (1994) observe that water extractable pH for a fleece needs to be below 7.8 for high quality wool, and that values above 9.3 would be likely to result from discoloured fibre. It is possible that this reflects specific pH requirements of fleece discolouring bacteria, a hypothesis examined here.

METHOD

Yellow challenge test (YCT)

The method used was a modification of that described by Aliaga *et al*. (1996) and is detailed in an accompanying publication (Winder *et al*., 1998). Briefly, greasy wool samples from which the tip had been removed were cut into

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2 cm lengths, sprayed with 6 ml $_dH_2O$ (thymol free) and mixed to ensure uniform wetting. Inclusion of thymol would have reduced establishment of bacterial populations during incubation (A P Maher, personal communication). In the current study it was deemed desirable not to disrupt what may be a natural fleece yellowing mechanism.

In contrast to the accompanying publication (Winder *et al*., 1998), the wet wool pieces were sealed into plastic bags during incubation, rather than the use of open containers. This was necessary to regulate humidity and prevent bacterial contamination of sterile samples. Samples were incubated at 40°C for 6 d. Mean measured base colour (Y-Z) of each wool sample was determined before and after the yellow challenge test (YCT) by measurement of tristimulus values to give △Y-Z data.

Gamma irradiation of bacteria

Greasy wool samples were sealed into plastic bags and gamma irradiated to kill fleece bacteria and endospores. Each sample received 25 kGy (2.5 Mrads) from a Cobalt-60 source (Mallinckrodt Veterinary Ltd., Upper Hutt). Following irradiation, and following YCT, samples were removed for measurement of colour and bacterial plate counts.

Bacterial cultures

Bacterial plate counts were determined for each sheep and experimental treatment following gamma irradiation and following YCT. In each case, bacteria from a 2 g sub-sample was extracted by thorough mixing with 8.9 ml sterile distilled water. Serial dilutions from 10^{-1} to 10^{-4} were made onto Nutrient agar. Duplicate plates were incubated at 40°C for 3 d.

Wool pH and non-treated samples

Wool samples were collected from the midside of 20 adult sheep. The pH of a water extract of the greasy wool samples was measured using a standard method (IWTO-2-86 (E)).

A 10 g sub-sample of the greasy wool collected from each animal was asceptically injected with 8.9 ml sterile distilled water and subjected to the YCT. Correlations between initial, final and change in Y-Z were undertaken, along with correlations between initial Y-Z and pH were evaluated.

Experimental treatments for yellow challenge test

Further wool samples were collected from the midside of five of the 20 sheep discussed above. These animals were selected on the basis of being most susceptible to wool yellow discolouration (L M Winder, unpublished data). Four duplicate 10 g sub-samples of greasy wet wool pieces from each animal were placed into sealed plastic bags and gamma irradiated (described above).

The sealed plastic bag for each irradiated sub-sample in the high humidity (+H-B) treatment was aseptically injected with 8.9 ml sterile distilled water. Irradiated sub-samples reinoculated with bacteria in the high humidity (+H+B) treatment were aseptically injected with 8.9 ml of a bacterial suspension produced by washing 10 g non-irradiated wool with 50 ml sterile distilled water. In each of the five fleeces examined, bacteria were collected and reapplied to wool from the same animal.

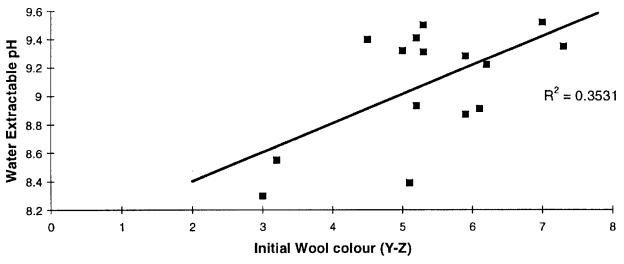
For irradiated sub-samples maintained at low humidity, the plastic bags were unsealed following injection of sterile water (-H-B) or bacterial suspension (-H+B) and samples placed on sterile metal racks inside a biohazard cabinet. The wool was dried using circulating air warmed by a Bunsen burner flame. Samples were dried to constant weight and resealed in plastic bags.

Additional wool sub-samples from each of these five sheep were not irradiated, and were aseptically injected with 8.9 ml sterile distilled water to ensure high humidity (control). Following each treatment, samples underwent a YCT incubation, with bacterial plate counts being determined before and after YCT.

RESULTS AND DISCUSSION

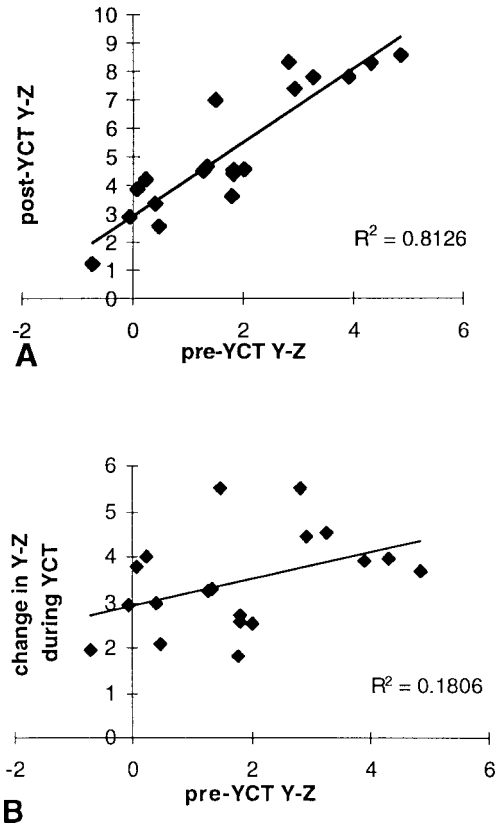
Data collected before the YCT suggests a relationship between pre-YCT wool pH and the susceptibility of wool to yellow under field conditions (Fig. 1; $p<0.05$). There was no relationship between pre-YCT pH and yellowness (Y-Z) post-YCT ($R^2 = 0.02$), or change in yellowness (△Y-Z) during YCT ($R^2 = 0.20$). That lack of correlation suggest that a high water extractable pH is an effect of wool yellowing rather than a cause, a finding which concurs with field observations by Aitken *et al*. (1994). Water extractable pH does not appear to be a useful predictor of yellowing susceptibility, although further studies to measure water extractable pH post-YCT would be of interest. Nonetheless, these findings do not support a hypothesis relating wool pH with environmental conditions preferential for specific wool discolouring bacteria.

FIGURE 1: Relationship between wool colour (Y-Z) prior to the yellow challenge test, and wool pH.



For wool samples collected from 20 sheep there was a highly significant relationship between pre-YCT wool yellowness and Y-Z following YCT ($R^2 = 0.81$; Fig. 2a) but not △Y-Z ($R^2 = 0.18$; Fig 2b). These data show that wool yellowness prior to the laboratory challenge indicates final colour but not change in colour. We would therefore recommend that wool susceptibility to yellowing be presented as △Y-Z, rather than post-YCT Y-Z as described by Aliaga *et al*. (1996) in their work optimising the YCT as a predictor for wool discolouration. However, post-YCT Y-Z data remain a valuable indicator of wool colour following the dyeing process.

FIGURE 2: Relationship between wool colour before the yellow challenge test (pre-YCT Y-Z) with A) wool colour after the YCT, and B) change in yellowness during the YCT.



For samples collected from five sheep the number of bacterial colonies on the pre-YCT wool sample and pre-YCT Y-Z, post-YCT Y-Z or △Y-Z were poorly correlated (R^2 = 0.20, 0.19 and 0.07 respectively). Number of bacterial colonies is therefore not an indicator of yellowing susceptibility. However no work was done in this study on the identity of bacteria in each fleece. It would be interesting to determine whether predominance of a particular bacterial species was associated with discolouration. This work is currently underway in our laboratory.

Gamma irradiation of the wool samples was found to eliminate all bacteria and is therefore an efficient technique for the removal of fleece bacteria. However, the irradiation treatment increased the pre-YCT Y-Z of all samples (Y-Z mean ± SD; non-irradiated 2.46 ± 0.55; irradiated 4.35 ± 0.43; $P < 0.001$).

FIGURE 3: Average change in wool colour (Y-Z) for sub-samples of wool subjected to one of four treatments prior to YCT (n = 5). Treatment codes describe high humidity, sterile (+H-B); high humidity, reinoculated with bacteria (+H+B); dry, sterile (-H-B); dry, reinoculated with bacteria (-H+B).

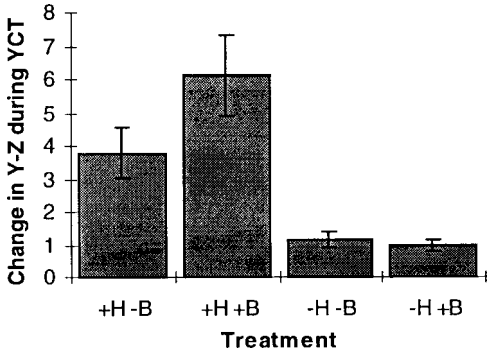


Figure 3 shows the average change in wool Y-Z following YCT for the four combinations of humidity and bacteria evaluated in this study. Incubation of samples under YCT conditions resulted in discolouration for all treatments examined. At high humidity (+H), reinoculation of samples with bacteria (+B) increased the change in wool Y-Z during YCT (△Y-Z mean ± SD; +H+B 6.13 ± 0.87; +H-B 3.78 ± 0.75; $P < 0.01$). This effect of reinoculation was not present at low humidity. In fact, in the absence of bacteria, the presence of moisture alone significantly increased yellowing during the YCT (△Y-Z mean ± SD; +H-B 3.78 ± 0.75; -H-B 1.17 ± 0.63; $P < 0.001$). The interaction between humidity and reinoculation was significant ($P = 0.002$). These data confirm that moisture is essential for significant yellow discolouration and that the presence of bacteria is a potent factor.

While it appears that bacteria do play a role in wool yellowing, the finding that moist wool yellowed significantly in the absence of bacteria suggests additional mechanisms are also involved. It is tempting to speculate on a role for suint, as noted in the accompanying paper (Winder *et al*., 1998). It seems likely that in the absence of moisture, any detergent action provided by suint is absent, thereby reducing it's role in yellow discolouration. Alternatively, bacterial enzymes may be involved. While this contribution has focused on effects of bacteria, other micro-organisms such as keratinolytic fungi have been shown to occur in the sheep fleece and may also be involved in the discolouration process by a moisture dependant mechanism (Al Musallam and Radwan, 1990).

CONCLUSIONS

Bacteria play a significant role in wool yellowing in the moist fleece. However, the observation of the change in yellowing in the absence of bacteria suggests other moisture dependant mechanisms are also involved. These may include a detergent or other action of suint, or the presence of bacterial enzymes. Changes in water extractable pH in the sheep fleece are an effect of yellow discolouration rather than contributing either directly or through the support of particular bacterial species.

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