THE EFFECTS OF SELECTIVE BREEDING FOR HIGH OR LOW CLEAN FLEECE WEIGHT ON THE CONCENTRATION OF CYSTINE IN PLASMA AND OF GLUTATHIONE IN ERYTHROCYTES OF MERINO SHEEP

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SUMMARY

Blood was sampled from sheep of 3 flocks, selectively bred for either high (Fl+) or low (Fl-) clean fleece weight or randomly bred (R). Under maintenance (M) and, subsequently, sub-maintenance (0.8xM) or supra-maintenance (1.6xM) feeding regimes, Fl+ sheep had significantly lower concentrations of cystine in plasma than R sheep, which in turn had significantly lower concentrations of cystine than Fl- sheep, indicating a probable negative genetic relationship between clean wool production and plasma cystine concentration.

The average concentration of glutathione in the red blood cells of GSHH sheep (i.e. sheep with higher concentrations of reduced GSH in their red blood cells, due to the action of a single dominant gene) was significantly greater in Fl- sheep than in Fl+ sheep, with R sheep having the lowest concentration. Within flocks, there was no significant correlation between cystine in plasma and glutathione in the erythrocytes.

The concentrations of both metabolites were greater in blood sampled from the sheep on 1.6xM than in those on 0.8xM (P < 0.01), but there was an interaction (P < 0.05) between flock and ration for GSH.

Keywords: clean fleece weight, plasma cystine, glutathione, wool production

INTRODUCTION

A long term selection experiment involving medium Peppin Merino sheep selectively bred for either high or low clean fleece weight per head has produced direct responses to selection, generally as predicted by the heritability estimated for clean fleece weight (Pattie and Barlow 1974). Correlated responses in other wool traits have also been described (Barlow 1974). Furthermore, a consistent difference has been observed in the concentration of cystine in plasma (Williams 1987). Sheep from the flock selectively bred for high clean fleece weight (Fl+) have exhibited lower concentrations of cystine in plasma than those from the flock selectively bred for low clean fleece weight (Fl-). As these comparisons have never included sheep from the randomly selected control (R) flock, the observed difference in cystine concentration may represent either a manifestation of an overall negative genetic relationship between cystine concentration and the rate of wool production (or, perhaps, another trait also genetically related to wool production), or an asymmetric or anomalous response to selection in one of the flocks. Lee and Williams (1994) provided evidence supporting the former interpretation. The wool production and cystine concentration in plasma were measured in sheep from 4 flocks, which varied in their average rate of wool production. The highest and lowest concentrations of cystine were found in the plasma of sheep from the flocks with the lowest and highest rates of wool production respectively; the sheep from the other 2 flocks having intermediate values for both traits. A direct comparison of the average concentration of cystine in the plasma of sheep from each of the three flocks in the selection experiment (Fl+, R and Fl-) would provide direct evidence to ascertain whether this correlated response was anomalous or symmetric. The results of this comparison are reported in this paper. While cyst(e)ine is the sulphur containing amino acid responsible for the high sulphur content of wool, and thus, for many of its physical and chemical properties, cyst(e)ine is maintained at relatively low concentrations in both extracellular and intracellular fluids, contrasting with the large intracellular concentrations of glutathione (GSH), a tripeptide containing cysteine, glycine and glutaminc acid. GSH is a reservoir for cystine (Higashi et al. 1977; Cho et al. 1981, 1984). The concentration of GSH in the red blood cells is also high, and the red cells of sheep from the selection flocks differ in GSH concentration (Hopkins et al. 1975), with Fl+ sheep having lower concentrations of GSH. In this experiment, we also investigated whether there is any relationship between these two metabolites among sheep. A close relationship would be convenient as red cell GSH is quickly and easily measured, relative to the assay of cystine in plasma.
MATERIALS AND METHODS

Thirty-three 20 month old wethers were individually housed indoors. There were 13 Fl+, 12 R and 8 Fl- sheep. The sheep were randomly selected from the three closed flocks of this long term selection experiment. Each flock consists of 100 breeding ewes (5 age groups), and a hogget flock of ewes and rams. Rams used as sires or replacement ewes are selected for entry in the Fl+ and Fl- flocks, solely on the basis of their clean fleece weight, measured at a shearing when the sheep are 15-16 months old. In the Fl+ flock, the 5 rams with the heaviest clean fleece weights are used as sires for the flock in the following year. In the Fl- flock, the rams with the lowest clean fleece weights are used. In the control flock, rams to be sires are selected randomly. The experiment commenced in 1951. The experimental details are described completely in Dun and Eastoe (1970). The results from the first 6 generations for direct and correlated responses to selection were presented by Pattie and Barlow (1974) and Barlow (1974) respectively.

Each wether was initially offered commercially prepared sheep pellets (Manildra Stockfeed Pty Ltd: 14.0% CP, 6% crude fibre, 5% fat) at a daily rate sufficient to maintain liveweight (lxM), based on an estimated ME value for the pellets of 11 MJ/kg DM and a maintenance requirement of 0.4 MJ/kg<sup>0.75</sup> liveweight (Oddy 1978). This schedule continued for 5 weeks. The sheep within each flock were then randomly divided to receive one of 2 rations. The sheep on the ‘Low’ (0.8xM) ration were given 80% of the quantity of commercial pellets given daily in the first period. The ‘High’ ration consisted of a 50:50 mixture of the commercial pellets and a pelleted mixture, containing 12.5% formaldehyde treated casein (fully described - experiment 2, Williams and Morley 1994). This mixture was provided to the sheep at twice the quantity provided to those on the ‘Low’ ration. It was considered to be 1.6xM. These 2 treatments continued for 4 weeks.

**Sampling and assay procedures**

Four samples of venous blood were collected into tubes containing sodium heparin during the final days of each period. On 2 days, samples were collected pre-feeding and 6-7 h post-feeding. The blood was assayed within 1 h for glutathione (Roberts and Agar 1971) and the haematocrit determined by centrifugation, in order to express the concentration of glutathione as mmol per L of red blood cells. The remaining blood was centrifuged to obtain a plasma sample, in which the total cyst(e)ine concentration was measured using a semi-automated procedure (Williams et al. 1986).

These data were subjected to analyses of variance using REG (Gilmour 1988). One wether from the R flock had an average GSH concentration of 1.07 mmol/L (sd = ± 0.02) during the first sampling period. This value was much lower (c. 5%) than those observed in the other sheep. The low concentration of GSH indicated that this sheep was a low GSH type (GSH<sup>h;</sup> Tucker and Kilgour 1970). Being genetically different from the other sheep, data from this sheep were excluded from the statistical analyses.

**RESULTS**

The average concentrations of cystine in the plasma samples collected from the sheep of the 3 flocks varied significantly during both feeding periods (Table 1). In the second period, the sheep consuming the Low ration had a lower average concentration of cystine in plasma than those on the High ration - 44.5 v 49.5 μmol/L (s.e.d. = 1.21 ; P < 0.01), with no significant interaction term.

**Table 1. Average concentrations (least square means) of cystine in plasma and of glutathione in erythrocytes for Fl+, R and Fl- animals during the pre-experimental and experimental periods**

<table>
<thead>
<tr>
<th>Feeding period</th>
<th>Fl+ n = 13</th>
<th>R n = 11</th>
<th>Fl- n = 8</th>
<th>s.e.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-experimental</td>
<td>37.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.2</td>
</tr>
<tr>
<td>μmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>experimental</td>
<td>40.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.5</td>
</tr>
<tr>
<td>Glutathione</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-experimental</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.13</td>
</tr>
<tr>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>experimental</td>
<td>3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.10</td>
</tr>
</tbody>
</table>

<sup>a</sup> A different superscript indicates a significantly different (P < 0.05) average value within periods.
There was significant variation among the 3 flocks in the level of GSH in the red blood cells in both feeding periods (Table 1), with the R sheep having the lowest concentration and the Fl- sheep the highest. Although the analysis indicated a significant effect of diet on GSH in the second period, with sheep on the Low ration having a lower GSH concentration in their red blood cells (3.43 vs 3.68 mmol/L; \( P < 0.01 \)), an interaction (\( P < 0.05 \)) between flock and dietary treatment prevented an unequivocal interpretation of the effect of diet.

The correlation between the plasma concentration of cystine and that of GSH in the red blood cells among sheep within flocks was small and non-significant, in both the IxM period (\( r = -0.06 \)) and the experimental period (\( r = 0.10 \)).

**DISCUSSION**

These comparisons have shown that the sheep in the 3 flocks maintain significantly different concentrations of cystine in their plasma. As we have demonstrated previously, the Fl+ sheep have a lower average concentration of cystine than the Fl- sheep. The present results contribute the additional relevant information that the sheep in the randomly selected control flock maintain an intermediate concentration of cystine, between those of the Fl+ and Fl- sheep. The results suggest therefore that the difference in cystine concentration observed when Fl+ and Fl- sheep were compared was not an anomalous or asymmetric response in one flock. Rather these results provide further evidence that there is an overall negative genetic relationship between the mass of wool produced and the concentration of cystine in plasma, as was also suggested by the results of Lee and Williams (1994).

The wool production of these sheep was not measured during this experiment because of the short duration of the periods of treatment, but the average greasy fleece weights at the previous shearing were 4.3, 3.8 and 1.2 kg for the Fl+, R and Fl- sheep respectively. The disparities in clean fleece weight would be even greater, as the clean scoured yield of wool from Fl- sheep is much lower than that of the other two flocks (Barlow 1974). Pattie and Barlow (1974) described and discussed the asymmetric responses in fleece weight in these flocks.

Although the differences in the concentration of cystine in plasma between these flocks represent correlated genetic responses to selective breeding for fleece weight, we can question whether the 2 traits are directly and causally related, as suggested by Black and Reis (1979), with the drive of the follicle population and resultant growth of wool, reducing the concentration of circulating sulphur amino acids. The responses in wool production are asymmetric (Pattie and Barlow 1974) and the Fl+ sheep exhibit a greater sensitivity to altered nutritional conditions in terms of their rate of wool production, leading to significant interactions between flock and diet for the rate of wool growth (Williams 1987). These observations contrast with the symmetric differences in plasma cystine between the flocks and the lack of any interaction between flocks and diets for this trait. These considerations suggest that any relationship between wool growth rate and the concentration of cystine in plasma would be quite complex.

On the other hand, these observed responses in plasma cystine suggest that this trait may be more closely related to the sulphur content of wool. This is lower in high producing sheep (Piper and Dolling 1966, Reis et al.1967), and reacts similarly in Fl+ and Fl- to abomasal infusions of SAA (Williams et al. 1972) which increase the content of sulphur in wool to its apparently maximum level (Reis 1967). Information on the symmetry of the responses in wool sulphur in the three flocks is limited to a report on sheep born in 1 year (McGuirk et al. 1984). The sheep of the Fl+, R and Fl- flocks produced wools with sulphur contents of 30.3, 32.5 and 35.4 mg sulphur/g wool respectively, again with the difference between Fl+ and R sheep in clean fleece weight (0.44 kg) being much less than this difference between R and Fl- (1.12 kg). Thus, the responses in the sulphur content of the wool appear to have symmetrically diverged about the value of the R flock. Some form of a direct general relationship between plasma cystine and wool sulphur would account for these observed changes in wool sulphur due to both genetic and dietary changes. The results presented by Lee and Williams (1996) also support this conclusion, but also suggest that the relationship between the two traits is not simple.

The comparisons involving GSH concentrations in erythrocytes included GSHH animals only. The R sheep had the lowest concentration of glutathione in their red blood cells, and Fl- sheep the highest concentration. These results generally agree with the findings of Hopkins et al. (1975) from a larger sampling that Fl- sheep had a greater concentration of GSH in their red blood cells than the Fl+ and R sheep, whose concentrations of GSH were similar.
Selective breeding for clean fleece weight has altered the concentrations of these two sulphur containing metabolites in blood, resulting in both cystine and glutathione having lower concentrations in sheep from the flocks with genetically greater rates of wool production. However, the very low correlations between GSH in red cells and cystine in plasma mean the former cannot be used to predict the concentration of cystine in plasma. Sumner et al. (1979) and Mata et al. (1995) have shown that a greater availability of methionine can increase the concentration of GSH in erythrocytes, but the responses vary with breed, season, and time of treatment. Mata et al. (1995) further indicated that this response among sheep was very variable.

REFERENCES