Indirect selection for adult fleece weight using canonical discriminant functions of blood metabolites

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ABSTRACT

The trial reported in this paper was undertaken to determine the potential of using multivariate analyses of blood metabolites to indirectly select for fleece weight. Blood samples from 41 Corriedale rams were analysed for 14 metabolites. A canonical discriminant function of creatinine, urea, magnesium and copper correctly classified 90% of the cull sheep (on the basis of greasy fleece weight).

While limitations to the interpretation of this trial are acknowledged, further work appears warranted, as the cost of analysing the blood metabolites is substantially cheaper than other proposed methods such as gene probes, or electrophoresis protein patterns to select for fleece weight at an early age.

Keywords Fleece weight; metabolites; canonical discriminant analysis.

INTRODUCTION

The ability to select sheep at an early age should improve net returns by increasing the carrying capacity of the farm. Selection of sheep on the basis of their fleece production at an early age is complicated by the effects of environmental factors such as age, birthtype, nutrition and age of dam on wool follicle maturation and production. When age and birth type are corrected for, the repeatability of fleece weight measured at 14-16 months of age and 3-5 months of age is 0.5-0.8 and 0.2-0.4 respectively, while heritability (h^2) estimates are 0.3-0.5 and 0.2-0.4 respectively (Mortimer, 1987).

Indirect selection for a trait or parameter(1) in 5-month-old sheep genetically correlated ($r_{G1,2}$) with adult fleece weight(2) will be more successful than selection for adult fleece weight on the basis of 5 month fleece weight(3) if:

$$h_1 r_{G_{1.2}} \sigma_1 > h_3 r_{G_{1.3}} \sigma_3$$

where σ_i is the standard deviation of trait i.

The possibility of using DNA probes in lambs to detect sheep with high fleece weight (Montogomery *et al.*, 1988) is attractive as $h_1 r_{G_{1.2}}$ is effectively 1. However the current high cost of this technology precludes its use as a routine method for screening stud rams. As most production traits are the net result of the activity of genes at a large number of loci, correlations between specific blood marker genes and wool growth would be expected to be low (Tucker, 1977). There are many genetic polymorphisms in farm animals (McDermid *et al.*, 1975) and some have been related to weight gain (Rahman and Konuk, 1977) and wool growth (Mayo *et al.*, 1970) in sheep. However their detection by electrophoresis is also relatively expensive.

Some blood metabolites have been related to wool production (Williams, 1979; Williams, 1987). Sheep with higher wool production tend to have lower levels of plasma cyst(e)ine (Williams *et al.*, 1972), reduced glutathione in the erythrocytes (Hopkins *et al.*, 1975), plasma urea (McClelland *et al.*, 1987) and lactate (Williams, 1987), while higher plasma levels of acetate and α-amino nitrogen have been found (G.M. Hough, unpublished). Higher wool or meat production has been associated with low red blood cell glutathione peroxidase activty (a selenium indicator, Atroshi *et al.*, 1981), high red blood cell K (Hopkins *et al.*, 1975) and plasma Cu (Wiener and Field, 1969). Williams, (1979) suggested zinc could also be important in controlling wool growth. None of the above metabolites are correlated highly enough with wool growth in grazing sheep to be used as a sole marker.

Sheep selected for fleece weight usually produce coarser wool with a lower sulphur content (McGuirk *et al.*, 1984), as the proportion of orthocortical cells containing high levels of low-sulphur proteins increases and the proportion of paracortical cells high in high-sulphur proteins decreases (Marshall *et al.*, 1985). Therefore electrophoresis of wool proteins could be used to indirectly select for wool production or specific wool qualities, though the cost-benefit of this approach is doubtful.

The introduction of autoanalyser systems has meant that some metabolites are now relatively inexpensive to measure. The metabolic profile test (Payne and Payne, 1987) is used to routinely screen

dairy herds for production diseases related to energy, protein and mineral metabolism. Rowlands *et al.*, (1973) suggested using the metabolic profile as a means of selecting cattle for growth. Seasonal and diurnal cycles and sampling and analytical errors are acknowledged sources of variation in measured metabolite levels (Payne and Payne, 1987).

The metabolic profile of sheep does not appear to have been used to select for fleece weight. Also multivariate methods of analysis, in particular discriminant or canonical discriminant analysis (Hope, 1968) of potentially discriminating metabolites have not been used to indirectly select for fleece weight. The objectives of these 2 methods of analysis is to maximise the difference between groups with particular properties, such as low and high wool production, in a population on the basis of a linear combination of variables, in this case metabolites. Farver *et al.*, (1980) used a discriminant analysis of blood variables to identify calves with a low potential for weight gain in a feedlot, in what appears to be the only reported study of multivariate analysis of metabolites as a selection method.

The study reported here was a preliminary trial to determine the potential of discriminant analysis to classify sheep by fleece weight.

MATERIALS AND METHODS

The data were derived from 41 11-month-old Corriedale rams. On 10 August 1987 blood samples were taken during the afternoon by venipuncture from the jugular. The rams had been shorn and fleece weighed 3 weeks earlier. Whole blood was analysed for glutathione peroxidase by the enzymatic method using cumene hydroperoxide as substrate and Hb by a cyanmethaemoglobin method (Boehringer Mannheim kit). After centrifugation, plasma was analysed for creatinine by the Jaffe method without deproteinisation (Boehringer Mannheim kit), cholesterol by the direct enzymatic method (BGU Biochemical Company kit), Ca by the O-cresolpthalein complex method (BGU Biochemical Company kit), P by the molydenum blue method (Lancer kit), Mg by a flurometric method, urea by an enzymatic method (BGU Biochemical Company kit), glucose by the hexokinase method (Boehringer Mannheim kit), total protein by the biuret method (Boehringer Mannheim kit) and β-hydroxybutyrate by an enzymatic method. All the above analyses, were carried out on a Mutlistat III Plus micro centrifugal analyser (Instrumentation Laboratory). Cu and Zn were analysed on an atomic absorption spectrophotmeter (Shimadzu-670).

The data were analysed by using discriminant and principal component analyses using the SAS statistical package (SAS, 1985) on a VAX mainframe computer. The variables chosen for the canonical discriminant function were those which had intermediate values for sheep with intermediate fleece weights. The sheep were graded into 3 classes, on the basis of fleece weight, corresponding to those which would be either kept as stud rams (7%), flock rams (44%) or culled (49%).

RESULTS

The values of the metabolic parameters for the 3 grades of sheep are given in Table 1. The metabolic parameters were correlated so the canonical correlation coefficient (>0.5) was higher than any individual correlation with greasy fleece weight. The blood metabolites with the highest correlations with fleece weight were protein ($r = -0.42$), glutathione peroxidase ($r = -0.39$), Mg ($r = -0.33$), Cu ($r = -0.32$), urea ($r = -0.29$) and creatinine ($r = -0.28$).

Canonical discriminant analysis appeared to have the most potential for classifying sheep into fleece weight grades. Canonical correlations increase as the number of variables in the discriminant function (index) increases. To minimise the number of blood parameters in the index and to rank both high and low producers, the approach taken was to include only those which had intermediate values in the medium fleece weight group. The inclusion of at least 3 of the parameters, creatinine, urea, Mg or Cu resulted in canonical correlations over 0.5. The indices used were: Index 1 = 0.05 Creatinine + 0.23 urea + 3.86 Mg − 0.3 Cu ($r = 0.55$); index 2 = 0.05 Creatinine + 4.95 Mg — 0.3 Cu ($r = 0.53$); Index 3 = 0.07 Creatinine + 0.31 urea — 0.32 Cu ($r = 0.52$) and Index 4 = 0.22 urea + 1.51 Mg — 0.29 Cu ($r = 0.51$). The mean index value for each group is shown in Table 1.

The success of these indices in correctly classifying individual sheep into their allocated fleece weight groups is given in Table 2. A graphic representation of the classification is shown in Fig. 1.

Plasma urea and Mg levels were not normally distributed. Log transformations of these parameters increased the canonical correlations by 0.1, but did not improve the classification results.

DISCUSSION

A high probability of correct classification of sheep into fleece weight groups was achieved with the indices, when only 3 of the parameters were used. All results were better than the chance probabilities of correct classification (48%, 44%, 7% for low, medium and high respectively). These results indicate that the indices have the potential to screen

off the elite high producing sheep as well as the cull rams. Urea, Mg and Cu are relatively inexpensive to measure and are analysed routinely in many laboratories. The correlations with fleece weight are probably due to increased nitrogen retention (urea), involvement with keratinisation of the fibre (Cu) and suint production (Mg).

There are a number of limitations to the interpretation of this study. First the blood was taken from 11-month-old sheep, whereas the blood factors must operate at an earlier age. The metabolic profile must also operate in a variety of habitats. Some indicators may only be useful when there is a specific deficiency, such as glutathione peroxidase when there is Se deficiency (Atroshi *et al.*, 1981) or Cu when liver Cu levels have run down (Wiener and Field, 1969). The indices may therefore need to be developed on an individual property basis.

A problem with the statistical interpretation is that the same data used to derive the functions were

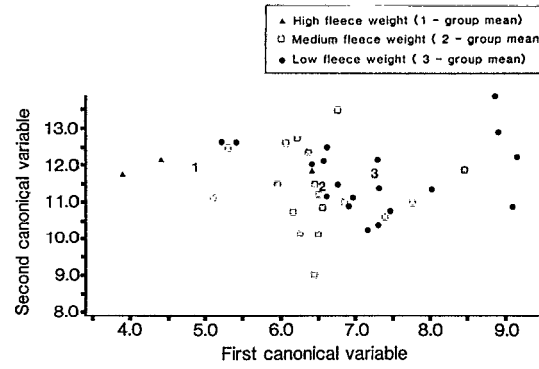

FIG. 1. Canonical plot of fleece weight based on a canonical discriminant function of creatinine, urea, Mg and Cu.

TABLE 1 Number, mean ± standard deviation of metabolic parameters and combined canonical discriminant indices in Corriedale ram hoggets graded into low, medium and high hogget fleece weight groups.

Parameters	Fleece weight group			
	Low	Medium	High	Total
Number of sheep	20	18	3	41
Creatinine (μmol/ℓ)	74.1 ± 7.8	70.4 ± 9.7	67.0 ± 0.0	2.0 ± 8.6
Urea (mmol/ℓ)	10.7 ± 2.0	10.3 ± 1.1	9.08 ± 1.59	10.4 ± 1.7
Glucose (mmol/ℓ)	4.00 ± 0.39	3.93 ± 0.41	3.93 ± 0.41	3.97 ± 0.39
β-Hydroxybutyrate (mmol/ℓ)	0.36 ± 0.09	0.36 ± 0.09	0.32 ± 0.08	0.36 ± 0.09
Protein (g/ℓ)	87.4 ± 4.5	82.4 ± 4.2	86.5 ± 6.1	85.1 ± 5.0
Albumen (g/ℓ)	28.2 ± 1.3	28.0 ± 1.4	28.6 ± 1.3	28.1 ± 1.3
Cholesterol (mg/ℓ)	0.43 ± 0.09	0.41 ± 0.07	0.49 ± 0.03	0.43 ± 0.08
Haemoglobin (g/ℓ)	0.11 ± 0.06	0.12 ± 0.05	0.11 ± 0.02	0.11 ± 0.07
Glutathione peroxidase (iu/gHB)	49.8 ± 10.9	39.6 ± 7.3	41.8 ± 15.6	44.7 ± 10.8
Calcium (mmol/ℓ)	2.57 ± 0.09	2.53 ± 0.10	2.60 ± 0.16	2.55 ± 0.10
Phosphorus (mmol/ℓ)	2.36 ± 0.25	2.34 ± 0.27	2.32 ± 0.26	2.35 ± 0.25
Magnesium (mmol/ℓ)	1.35 ± 0.12	1.28 ± 0.10	1.23 ± 0.11	1.31 ± 0.12
Copper (μmol/ℓ)	14.3 ± 2.3	15.0 ± 2.6	18.3 ± 2.5	14.9 ± 2.6
Zinc (μmol/ℓ)	8.90 ± 0.71	8.88 ± 0.94	9.04 ± 0.43	8.90 ± 0.79
Index				
1 (creatinine, urea, Mg, Cu)	7.26 ± 1.11	6.50 ± 0.79	4.90 ± 1.33	6.76 ± 1.17
2 (creatinine, Mg, Cu)	6.09 ± 1.09	5.34 ± 0.85	3.93 ± 1.21	5.70 ± 1.14
3 (creatinine, urea, Ca)	3.91 ± 1.10	3.31 ± 0.91	1.64 ± 1.09	3.48 ± 1.16
4 (urea, Mg, Cu)	5.09 ± 1.10	4.42 ± 0.79	2.94 ± 1.45	4.64 ± 1.13

TABLE 2 Correct classification (%) into groups using canonical discriminant indices.

Index	Fleece weight group			
	Low	Medium	High	Medium/High
1 (creatinine, urea, Mg, Cu)	75	67	67	90
2 (creatinine, Mg, Cu)	75	67	67	88
3 (creatinine, urea, Cu)	70	72	67	90
4 (urea, Mg, Cu)	65	61	67	83

used to verify the functions. A larger scale trial would enable data from half the sheep to be used to compute the indices and the data from the remaining half used to evaluate the discriminant functions. Alternatively each case can be classified according to the index computed from all the data, except the case being classified (Farver *et al.*, 1980). In the present study a random one-third of the sheep were classified after indices were computed from the remaining two-thirds of the data. Seventy one percent of the low producers were correctly classified as such.

The technique has shown enough promise to merit a larger scale trial in which blood metabolite levels in stud ram lambs will be compared with fleece weights of the same sheep as hoggets.

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