COMPARISON OF A MICROHISTOLOGICAL ANALYSIS OF FAECES AND ALKANE CONCENTRATIONS OF FAECES TO ESTIMATE THE BOTANICAL COMPOSITION OF THE DIET OF GRAZING SHEEP

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SUMMARY

The procedure to estimate pasture intake using a dosed alkane requires knowledge of the dietary concentration of an alkane pair, or of the botanical composition of the diet so that the dietary alkane concentrations can be estimated from the alkane profiles of the pasture species. This report compares the diet estimated using alkanes with that estimated using a microhistological method. The repeatability of the proportions of each botanical component in the diet estimated by each of the techniques ranged from 0 to 0.5. There were significant differences between methods, between sheep and between sampling days in the estimated composition of the diets. Overall, the herbage species selected in the diet by sheep grazing heterogenous pastures were poorly estimated. This is likely to have been as a consequence of insufficient differences in the concentrations of the 9 available alkanes in the large number of botanical components.

Keywords: diet selection, botanical composition, n-alkane, microhistological analysis

INTRODUCTION

Differences in the pattern of alkane concentrations between species (Chen *et al.* 1998) may allow the botanical composition of an animal's diet to be estimated from the faecal pattern of alkane concentrations, after adjusting for incomplete faecal recovery (Dove and Mayes 1991). The estimation of herbage intake using the alkane technique requires knowledge of the dietary concentrations of both the dosed alkane (e.g. C_{32}) and of a natural odd-chain alkane adjacent in chain length (e.g. C_{31} or C_{33}).

Oesophageally fistulated sheep may be used to determine diet composition in intensive grazing experiments, but both practical and welfare considerations restrict their use in extensive, pastoral grazing experiments (Le Du and Penning 1982). Additionally, the diet selected by oesophageally fistulated sheep may not be representative of all sheep within the flock for behavioural and experiential reasons (Arnold and Maller 1977; Parsons *et al.* 1994). Alkanes may offer a means of estimating diet composition of individual animals, and avoid the need for oesophageally fistulated animals in extensive grazing systems. The experiment described here compares the alkane-based estimates of the botanical composition of the diet with those obtained by identification of plant cells in faeces of sheep grazing a botanically diverse pasture.

MATERIALS AND METHODS

On 3 occasions over 7 days, faecal samples were collected from 12 medium wool adult Merino wethers. The wethers were grazing a paddock at the Agricultural Research and Advisory Station, Condobolin that included dryland lucerne, a small area of native grasses and forbs, and volunteer species within the lucerne pasture. Approximately 10 g DM of each faecal sample was frozen pending alkane analysis, and the remainder stored in 70% ethanol solution (v/v). Pluck samples of the species present were collected from a number of sites across the paddock and pooled. Some of each pooled sample was frozen pending alkane analysis, and the remainder stored in 70% ethanol solution to provide reference standards for microscopic identification of plant cell material.

Following freeze-drying, the concentrations of alkanes in both faecal and herbage samples were determined by gas chromatography using the method described by Dove *et al.* (1996). Faecal alkane concentrations were adjusted for incomplete recovery using mean literature values (Lee 2000). A principal components analysis was conducted using S-PLUS (Statistical Sciences 1993) to identify species with similar alkane concentration patterns. The botanical composition of the diet was estimated from the faecal alkane profile using the EatWhat software package (Dove and Moore 1995).

The preparation of reference material from the samples of species collected, and of faecal samples, was similar to that described by Storr (1961). Microscopic identification of species in faecal material was based on the epidermis of plant material recovered (Jarman and Phillips 1988). The representation of a species in the faecal material was determined as the area of epidermis of the species expressed as a percentage of the total area of identified epidermis particles.

The botanical composition estimates were analysed by least squares analysis of variance (REG; Gilmour 1988) fitting animal as a random effect, and sample time and method (microhistology *v.* alkane) as fixed effects.

The repeatability (t) between the methods in the estimation of each component's contribution to the diet was calculated from the between-animal variance (σ_B^2) and the sample variance (σ_W^2) as:

$$t = \sigma_B^2 / (\sigma_B^2 + \sigma_W^2) = ((MS_b - MS_w)/k) / (MS_w + (MS_b - MS_w)/k)$$

where k is the weighted mean number of records per animal, MS_b is the between-animal mean square, and MS_w is the sample mean square (Turner and Young 1969).

RESULTS

Over 90% of the variation in the alkane concentrations of the species was accounted for by the first 3 principal components, from which 9 associations of species were established. The estimates by each method of the botanical components of the diet based on the groups determined from the principal components analysis are shown in Table 1.

Table 1. Comparison of estimates of botanical composition (as a percentage) obtained from a microhistological analysis of faeces and from faecal alkane concentrations.

Botanical Component	Microhistology			Alkanes			Source of variation			
	Mean	s.d.	% samples	Mean	s.d.	% samples	Between method	Between sheep	Time	t
Danthonia spp.	13.2	8.0	100.0	0.0	0.0	0.0	**	ns	ns	0.08
Eragrostis sp	5.8	5.1	94.3	0.2	1.3	2.8	**	ns	ns	0.06
Lolium spp.	0.6	1.3	25.7	44.9	13.2	100.0	**	ns	**	0.13
Medicago sativa	47.5	13.7	100.0	36.0	21.0	97.2	**	**	**	0.50
Juncus spp., Stipa scabra	1.1	2.1	34.3	0.9	3.7	8.3	ns	*	ns	0.19
Vittadin spp.	8.9	6.1	100.0	9.9	5.7	97.2	ns	*	**	0.18
Aristida jerichoensis, Dicantheum sericeum, Enterapogon racemosa, Panicum spp.	13.3	8.9	100.0	0.0	0.0	0.0	**	ns	ns	0.08
Avena spp.	0.7	2.5	11.4	0.0	0.0	0.0	ns	ns	ns	0.01
Helipterum spp.	9.0	5.8	100.0	8.0	4.9	94.4	ns	ns	*	0.00

* P<0.05, ** P<0.01, ns not significant

There was a significant difference between the methods in the estimated dietary proportions of a number of botanical components. In particular, the *Lolium spp.* component was overestimated by the alkane optimisation procedure relative to the microhistology technique. The components comprising *Danthonia spp., Eragrostis spp.*, *Medicago sativa* and the 4 grass species (*Aristida jerichoensis, Dicantheum sericeum, Enteropogon racemosa,* and *Panicum spp.*) were significantly under-represented in the diets estimated by the alkane optimisation procedure relative to that observed by microhistology (Table 1). Although cells of *Danthonia spp.* and of the species in the 4-grass component were observed in all faecal samples, the alkane optimisation procedure did not include these components in any of the diet estimates.

Significant variation in diet composition was evident between animals and between sample collection times. The dietary proportions of *Lolium spp., M. sativa*, *Vittadin spp.*, and *Helipterum spp.* varied over time, while the proportions of *M. sativa, Juncus spp.*/*S. scabra* and *Vittadin spp.* varied between animals (Table 1). Only estimates of the dietary proportion of *M. sativa* were moderately repeatable between methods (Table 1).

DISCUSSION

Microhistological analysis of faecal material has been used to identify the botanical components of the diet selected by herbivores based on cuticular characteristics (Norbury and Sanson 1992) and to

quantify the composition of the diet (Croker 1959; Storr 1961). Estimates of the relative proportions of the dietary components obtained from the observation of the frequency of cuticular cells may be influenced by differential digestion of the components (Slater and Jones 1971). However, the presence of cells from a number of grass species (including *Danthonia spp.* and *Avena spp.*) in the faeces confirms their place in the diet, yet they were not included in any of the predicted diets (optimised from faecal alkane concentrations).

Both between-animal and temporal variation in the composition of the diet was confirmed. Between-animal and temporal variation in the level of intake is well documented (Sheehan *et al.* 1985; Vulich *et al.* 1990; Lee *et al.* 1995). Temporal variation in diet selection of sheep (Parsons *et al.* 1994) may be associated with changes in both time spent grazing and intake rate (Newman *et al.* 1994), and in sward characteristics (Penning *et al.* 1994). Variation in the proportional composition of the diet over time implies that individual alkane concentrations may vary between samples from the same sheep.

Estimates of the diet composition of sheep grazing complex heterogenous pastures are difficult, and not only because of the large number of species present. Variations in the age and in the proportions of plant parts within species both affect the alkane concentrations and distributions of alkanes in cuticular wax (Dove *et al.* 1996). It is possible that variation between parts of the same species will be enough to regard them as separate components of the pasture (Dove *et al.* 1996). The mathematical procedures available for diet estimation from faecal alkane concentrations require that the number of alkanes present equals or exceeds the number of species. It is possible to restrict the number of species included in the optimisation by eliminating some species either by grouping species shown to be similar (based on a statistical evaluation) or based on some other logical reason (eg. known to be unpalatable, or present in the sward in only minute quantities; Osoro *et al.* 1999). Some caution is required in such instances to avoid prejudicing the solution of the diet.

The restricted number of alkanes exacerbates the problem of the large number of species in complex pastures. The shorter chain length alkanes are less than ideal markers because the faecal recovery is low and there is limited information on that recovery (Lee 2000).

A number of factors may affect the estimation of diet composition from the faecal alkane concentrations. Error in characterising the alkane concentrations of individual species could be associated with sampling and/or analytical procedures. Although species accounts for most of the total variation in herbage alkane concentrations (Chen *et al.* 1998), there is additional variation between plant parts within species (Dove *et al.* 1996), between-samples within a site, and spatial variation (Lee and Nolan 2003). The extent to which the proportions of the plant parts consumed differed from those in the species samples collected contributes to error in characterising the species. Any errors in characterising the alkane profile of a species can influence the grouping of species following a principal components analysis, and hence the composition of the diet.

Analytical errors in measuring the concentrations of either faecal or herbage alkanes can lead to bias in the estimate of diet composition (Newman *et al.* 1998). However, similar analytical errors in both the faecal and herbage concentrations will tend to cancel out and have minimal effects on the estimated composition of the diet. The effect of errors in the estimate of dietary botanical composition on the intake estimate will depend on the relative differences in the alkane concentrations between the estimated diet and the actual diet.

Error in the correction for incomplete faecal recovery of alkanes, including deviations of the actual values from those assumed, and any variation in the actual recoveries between animals and over time may also affect diet estimates. Dove and Mayes (1996) presented data indicating the faecal recovery of individual alkanes is similar for each of the dietary components. However, Casson *et al.* (1990) reported variation in the faecal recovery of individual alkanes between pasture legume species (medics and subterranean clover) and varieties of medics that was apparently unrelated to digestibility. There is also limited evidence that the recovery of an alkane may vary with the level of intake (Ohajuruka and Palmquist 1991). Any effect of intake on faecal recovery will have little or no effect on the estimate of herbage intake if the recoveries of the dosed and adjacent odd-chain alkane remain similar. Errors in the recovery estimate, however, can lead to large errors in the estimate of diet composition, particularly when the error varies with chain length (Newman *et al.* 1998). Variation in faecal

recovery with intake and pasture species, and the consequences of error in the recovery values suggest that assuming a set of recovery values across pasture and genotypes, as was the case in this study, may be inappropriate.

Regardless of the mathematical procedure used to estimate the diet composition from faecal alkane concentrations, an accurate distinction of the dietary components requires that the patterns of alkane concentrations are sufficiently different between the species/components (Dove and Moore 1995). The small reduction in the residuals of the 3 best EatWhat solutions (< 2%) of the optimisation in this study indicated that the differences between the botanical components were not sufficient to satisfactorily resolve the diet. The poor agreement between the 2 techniques in estimating the diet is likely to have been as a consequence of insufficient differences in the concentrations of the 9 available alkanes in the large number of botanical components. Hence, alkanes alone are unlikely to provide good estimates of the composition of the diet when herbivores are grazing complex pastures.

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