ADVANCES IN THE ESTIMATION OF INTAKE AND DIET SELECTION IN THE GRAZING ANIMAL

H. DOVE

SUMMARY

Current methods for estimating pasture intake from a combination of estimates of faecal output and in vitro digestibility can be prone to error arising principally from errors in the digestibility estimate. Recent research indicates that the alkanes of plant cuticular wax, in combination with orally-administered synthetic alkanes, can be used to estimate intake in individual animals. Alkanes are not wholly recoverable in faeces, but this does not matter if the estimate is based on a pair of alkanes adjacent in chain length, since their recoveries will be similar. Alkane-based estimates of intake agree well with known intakes and have the added advantage of permitting estimates of diet selection in terms of plant species or plant parts. Techniques for estimating diet selection using alkanes are presented.

INTRODUCTION

Under Australian farming conditions, pasture remains the single most important source of nutrients for ruminant livestock. Despite this, researchers continue to be faced with the dilemma that, while estimates of individual animal performance are readily obtained, it is still difficult to estimate the herbage intake of individual animals. It is even more difficult to partition the intake of the animal into its component plant species or plant parts, even though we know that these can differ markedly in their nutritive value. Hence, we still experience difficulties in predicting, in quantitative terms, the response of animals to a given change in the amount or botanical composition of pasture. This paper will briefly review problems with existing methods for estimating diet selection and intake, and then present the results of some recent research in this area.

PROBLEMS IN THE ESTIMATION OF HERBAGE INTAKE

The problems associated with estimating intake in the grazing animal have been reviewed in detail elsewhere (Langlands 1975, 1987; Dove and Mayes 1991). Briefly, herbage intake has been calculated most often from the relationship between total intake, herbage digestibility and the resultant faecal output, as summarised in equation 1.

$$\text{Intake} = \text{Faecal output}/(1 - \text{Digestibility}) \qquad (1)$$

The total collection of faeces from the grazing animal is laborious, so that faecal output has usually been estimated from the dilution, in faeces, of a so-called 'external marker' administered orally to the animals. In the past, the marker of choice has been chromium sesquioxide (Cr_2O_3), and it is now possible to administer this marker in the form of intra-ruminal controlled-release devices (CRD). In order to estimate digestibility, the ideal approach would be to monitor the concentrations in herbage and faeces of an indigestible 'internal marker' occurring naturally in the plant. Unfortunately, no wholly satisfactory digestibility marker has been identified. As a result, herbage digestibilities are usually estimated using *in vitro* procedures which have previously been calibrated against

CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601

in *vivo* measurements of digestibility. Herbage samples for this procedure are usually collected with sheep fistulated at the oesophagus (OF sheep).

Errors in the estimation of intake using this Cr/ *in vitro* approach can arise either from errors in external marker delivery or from errors. associated with the estimate of digestibility. As Langlands (1975, 1987) has pointed out, the latter errors are of more concern, since small errors in the estimate of digestibility can result in much larger errors in estimated intake. Three sources of error are of particular concern,

1. *In vitro* digestibility procedures are ultimately only as good as the original *in vitro/in vivo* relationships upon which they are based. Frequently, these are established using only one class of animal (eg., wethers fed at maintenance) and their relevance to other classes of stock is questionable.

2. The *in vitro* procedure provides only one estimate for a given pasture sample. However, due to differences in the level of intake, the presence or absence of supplements or differences in parasite burden, the digestibility of the same herbage may differ substantially between individual animals.

3. Individual test animals may select a diet different from that selected by the OF animals.

Although the Cr/*in vitro* procedure is often described as providing estimates of herbage intake in individual animals, as a consequence of the first two of these sources of error, it in fact provides individual estimates of faecal output and a single, possibly misleading estimate of digestibility. To the extent that it does not provide truly individual herbage intakes, this method is not applicable in genetic studies of differences in the efficiency of utilisation of nutrients.

PLANT HYDROCARBONS - AN ALTERNATIVE APPROACH

Plant cuticular alkanes

The cuticular wax present on the leaf surface of most plants, including pasture species, is a complex mixture. In addition to saturated hydrocarbons (n-alkanes), it also contains long-chain fatty acids, long-chain alcohols, wax esters, secondary alcohols and several classes of ketones (see Dove and Mayes 1991 for further details). In chemotaxonomic studies and, more recently, in studies of the use of cuticular wax chemistry for estimating diet selection and intake, the alkanes have received most attention because they are present in the cuticular wax of most species, and because they are easily to analyse. The composition of the alkane fraction from a range of pasture species is shown in Table 1; several features deserve comment.

1. The carbon-chain lengths of the predominant alkanes are in the range C25 (pentacosane) to C35 (pentatriacontane).

2. Alkanes with odd-numbered carbon chains are present in much greater amounts than those with even-numbered chains. However, the small quantities of these even-numbered alkanes (not shown in Table 1) must still be taken into account when calculating intake (see below).

3. There are marked species differences in alkane concentrations and patterns which, as will be discussed below, allow a chemical approach to the estimation of diet selection in the grazing animal.

TABLE 1 Concentrations (mg/kg OM) of major alkanes in selected pasture grasses and legumes (T=trace quantity)

Species	Alkane					
	C25	C27	C29	C31	C33	C35
Perennial ryegrass	6.3	20.0	109.0	215.2	141.4	12.2
Annual ryegrass	29.7	82.9	195.7	298.1	47.1	T
Yorkshire Fog grass	41.2	55.1	101.3	97.7	19.9	T
Rhodes grass	-	24.0	51.4	95.2	89.1	26.5
Phalaris	31.0	40.9	49.7	34.6	4.2	T
Subterranean clover	3.6	15.5	250.4	74.3	9.6	T
Lucerne	13.0	54.8	206.9	103.5	7.6	T
White clover	4.0	86.3	219.3	237.6	19.3	T

Estimation of herbage intake using alkanes

The use of plant cuticular alkanes for estimating herbage intake was first proposed by Mayes et al. (1986) and is discussed at length by Dove and Mayes (1991). Initially, it was thought that natural alkanes might function as digestibility markers, but the results of Mayes and Lamb (1984) indicated that the faecal recovery of these alkanes was incomplete. For similar reasons, synthetic alkanes have incomplete recoveries and, with the probable exception of C36 alkane, cannot be used on their own as faecal output markers. However, Mayes et al. (1986) argued that the incomplete recoveries would not matter if a pair of alkanes, adjacent in chain length and similar in faecal recovery, were used. In effect, the errors arising from the incomplete recoveries of the natural and dosed, synthetic alkane would cancel out. This is perhaps more obvious if equation 1 is restated in terms of the alkane concentrations and alkane dose rate required to calculate intake (equation 2 below). The full derivation of equation 2 is given by Dove and Mayes (1991).

$$\text{Intake} = \frac{F_i}{F_j} D_j \Big/ \left(H_i - \frac{F_i}{F_j} H_j \right) \qquad (2)$$

where Fi and F_{ij} are the faecal concentrations of the odd-chain, plant and even-chain, dosed alkanes respectively, Dj is the dose rate of the synthetic alkane and Hi and Hj are the herbage concentrations of the odd-chain and even-chain alkanes respectively. Hence, when using a pair of alkanes to estimate intake, only the faecal ratio of the natural and dosed alkanes is needed. Moreover, if these concentrations are measured with similar bias as a result of similarly incomplete faecal recoveries, then the errors arising from incomplete recovery indeed cancel out.

Faecal alkane recoveries increase with increasing chain length to an observed maximum of 0.95 for C36 alkane (Dove and Mayes 1991). For the pair of alkanes most frequently used to estimate intake, C32 and C33, the means of published estimates of recovery are very similar (0.868±0.0175 and 0.872±0.0125 respectively). In consequence, in validation studies in which intakes calculated using this pair of alkanes have been compared with known intakes, the maximum observed discrepancy between known and estimated intake has been only 1.7% (Dove and Mayes 1991). It is worth emphasising that

even if there is a difference in the faecal recovery of the alkanes used to estimate intake, this will result in a smaller error in the estimated intake than an equivalent error in the *in vitro* estimate of digestibility. For example, Dove and Mayes (1991) calculated that if the faecal recoveries of the dosed and natural alkane pair differed by 3 percentage units, the error in estimating intake would be 4.9% and would be independent of the herbage digestibility. By contrast, an error of 3 percentage units in the *in vitro* digestibility would result in an error of 13% in the estimate of intake if the actual digestibility were 0.80. Moreover, even with a digestibility as low as 0.50, the error in estimating intake would be 5.7%.

The alkane approach thus results in accurate estimates of herbage intake, with the major advantage that the method automatically takes into account the differing herbage digestibilities occurring in individual animals, regardless of their level of intake, supplement intake or parasite burden. Since the alkane-based intake actually is an individual intake, the method lends itself to studies of the selection of animals on the basis of their efficiencies of utilisation of nutrients.

It is also possible to use the alkane procedure to estimate digestible OM intake, by including C36 alkane in the alkane mixture with which the animals are dosed. The faecal recovery of this alkane has consistently been measured at 0.95 (Dove and Mayes 1991), which allows its use as a faecal output marker. Digestibility can then be calculated from the estimates of faecal output and intake.

Administering synthetic alkanes to grazing animals

The successful use of the alkane procedure under field conditions requires that a convenient means be found for the dosing of synthetic alkanes. In previous work, synthetic alkanes have been given on a daily or twice-daily basis either in the form of alkane-impregnated shredded paper pellets (Mayes et al. 1986; Dove et al. 1989b) or as alkane-impregnated powdered cellulose contained in gelatin capsules (Dove et al. 1989a,b; Vulich et al. 1991). Comparisons of these modes of delivery have indicated no significant differences (Dove et al. 1989b; Vulich et al. 1991) though the gelatin capsules are more easily prepared.

After the commencement of dosing with either paper pellets or gelatin capsules, the faecal concentrations of dosed alkanes reach equilibrium after 5-6 days (Mayes et al. 1986; Dove et al. 1989b; Dove et al. 1991). Faecal concentrations may be erratic with once-daily dosing (Dove et al. 1991), but in sheep dosed twice-daily, temporal variation in faecal alkane concentration does not seem to be a problem (Dove and Mayes 1991). The situation with cattle is less certain. It must be emphasised that with the alkane procedure, it is only variation in the ratio of the faecal concentrations of natural and dosed alkanes which would be a cause for concern (see equation 2 above). As the data of Dove et al. (1991) indicate, this ratio is less prone to temporal variation than the absolute concentrations of alkanes.

If twice-daily dosing is used under field conditions; the labour required to produce alkane capsules or pellets rapidly becomes prohibitive, quite apart from the frequent disturbance which twice-daily dosing can cause to normal grazing patterns. Dove et al. (1991) recently reported on the performance of an intra-ruminal CRD for alkane delivery; the performance-of the devices is summarised in Table 2. The desired delivery rate was 40 mg/d of each alkane.

TABLE 2 Alkane delivery performance of intra-ruminal,controlled-release devices

Characteristic		Value
Linear release rate (mm/d)		1.23 (CV 4.1%)
Measured alkane contents (mg/mm)	C28	32.5
	C32	33.9
Resultant alkane release rates (mg/d)	C28	40.1
	C32	41.8

The faecal alkane ratios C28:C29 and C32:C33 were almost constant by the sixth day after CRD administration, both in intact sheep (Mayes et al. 1991) and in rumen-fistulated sheep from which the devices were removed every 2 days for measurement (Dove et al. 1991). The mean DM intake estimated from the C32:C33 alkane pair (913±58.4 g/d) was almost identical to the known mean intake of 914260.1 g/d. Further testing of these devices under field conditions is continuing. For the reasons discussed above, current versions of the CRD contain C36 alkane in addition to C32, to permit the estimation of faecal output and thus digestibility.

Comparison of alkane-based intakes with other field estimates

In ewes grazing phalaris pastures in late pregnancy, Dove et al. (1989a) compared herbage OM intakes estimated using the Cr/*in vitro* procedure with those estimated using C32 and C33 alkanes. A selection of their data are presented in Table 3.

TABLE 3 Comparison of herbage OM intakes (g OM/d) based on either the Cr/*in vitro* or alkane procedures

Group	Stocking rate	Intake:Cr/*in vitro*	Intake: Alkane
1	High	577	662
2	High	712	823
3	Low	1048	905
4	Low	1442	1316
	S.e.d.	123.8	119.3

Alkane-based intakes were significantly higher than those based on the Cr/*in vitro* procedure, when the level of intake was low (high stocking rate). At the low stocking rate, alkane-based intakes were significantly lower. These authors also measured faecal alkane recoveries via total faecal collection in a portion of the animals, so that estimates of herbage digestibility in individual animals could be calculated. In Figure 1, these are compared with the single *in vitro* digestibility value obtained for the herbage grazed by each group. At the high stocking rate, where intakes were low and the rate of passage of digesta therefore rapid, the digestibilities estimated in individual animals were almost all greater than the *in vitro* values, sometimes by more than 10 percentage units. The converse was true at the low stocking rates These data support the argument put forward by Dove et al. (1989a) that the alkane method was accommodating the differences in herbage digestibility generated by the different levels of intake at the two stocking rates.

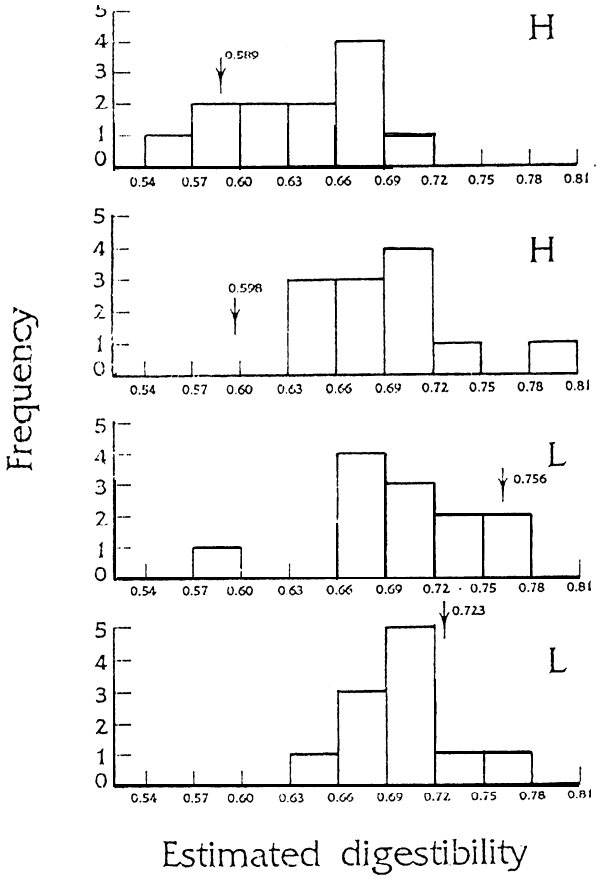

Fig. 1 Frequency distribution (i.e. numbers of animals) of pasture digestibilities estimated from pasture and adjusted faecal C33 alkane concentrations at high (H) and low (L) stocking rates. Arrows' show values for equivalent *in vitro* digestibilities. (From Dove et al. 1989a).

Dove et al. (1990) compared alkane-based and *in* vitro-based estimates of herbage intake in lactating ewes in which estimates of abomasal digesta flow and rumen microbial protein production were also made. Their results demonstrated that the proportion of apparently digested OM apparently disappearing across the stomach and the efficiency of rumen microbial protein synthesis were more consistent with published values when calculated using the alkane-based intakes.

These results, taken together with the comparisons of known intakes and those estimated using alkanes, suggest that the alkane procedure, embodied in equation 2, results in accurate estimates of intake. Moreover, these estimates seem more accurate, under field conditions, than those based on the Cr/*in vitro* approach, to the extent that they allow for differences in herbage digestibility between animals.

ESTIMATING DIET SELECTION IN GRAZING ANIMALS

Grazing ruminants can alter the composition of their diet by selecting different plant parts or plant species from a sward. Despite the importance of selection in terms of its effect on the nutritive value of the consumed diet, it has always proved particularly difficult to quantify or even identify the components of the consumed diet. The botanical composition of oesophageal samples has been estimated by microscopic examination (eg., Hamilton and Hall 1975), but such techniques are tedious and difficult. As an alternative, chemical methods have been attempted, such as the use of pinitol as a marker for legume

content (Smith 1982). However, this compound is water soluble and underestimates the legume content of chewed herbage because it leaches out in saliva (Forwood et al. 1987). Most chemical techniques also suffer from the disadvantages that they cannot identify individual plant species in the diet, and are not applicable to faecal samples.

Using alkanes to estimate botanical composition

Species differences in cuticular alkane concentration are evident in Table 1. Moreover, these between-species differences are frequently even more obvious when expressed as ratios of alkanes, as shown in Table 4.

TABLE 4 Alkane ratios in pasture grasses and legumes

Species	C29:C31 ratio	C29:C33 ratio
Perennial ryegrass	0.51	0.77
Annual ryegrass	0.66	4.16
Yorkshire fog grass	1.04	5.09
Phalaris	1.44	11.83
Lucerne	2.00	27.22
White clover	0.83	11.37
Subterranean clover	3.37	26.08

Recent work has shown that these differences in alkane concentrations or ratios can be used to estimate the species composition of herbage mixtures (Dove 1992) or of oesophageal extrusa and consumed herbage (Dove and Mayes 1991). The calculations can be done in several ways:

1. Simultaneous equations (eg., Dove 1992) in which case the number of alkanes and thus simultaneous equations must be equal to or greater than the number of plant species.

2. Ratios of alkanes, such as C29:C33, which, in a 'mixture of two species, change hyperbolically as the mixture changes from 100% of one species to 100% of the other. This approach can be used for calculating species content only when there are two species present, but as Dove (1992) demonstrated, the C29:C33 ratios of grasses and legumes are so different that they are still useful for ranking legume content, even when there are more than two species in the mixture.

3. Least squares optimisation procedures can be used to find the combination of component species which best explains (as distinct from uniquely fits) the alkane composition of the mixture (Dove and Mayes 1991; Dove et al. 1993). This approach is more algebraically robust than the use of simultaneous equations, since it is much less affected by small errors in estimated alkane compositions. It has the added advantage that it can be used when there are more plant species than alkanes.

The relationship between the known composition of mixtures of subterranean clover, perennial ryegrass and Yorkshire fog grass and the compositions calculated from simultaneous equations relating the alkane contents of the species and their mixtures (Dove 1992) is shown in Figure 2. The levels of all species were accurately estimated, with the largest mean discrepancy between estimated and known composition being 2.3% for perennial ryegrass.

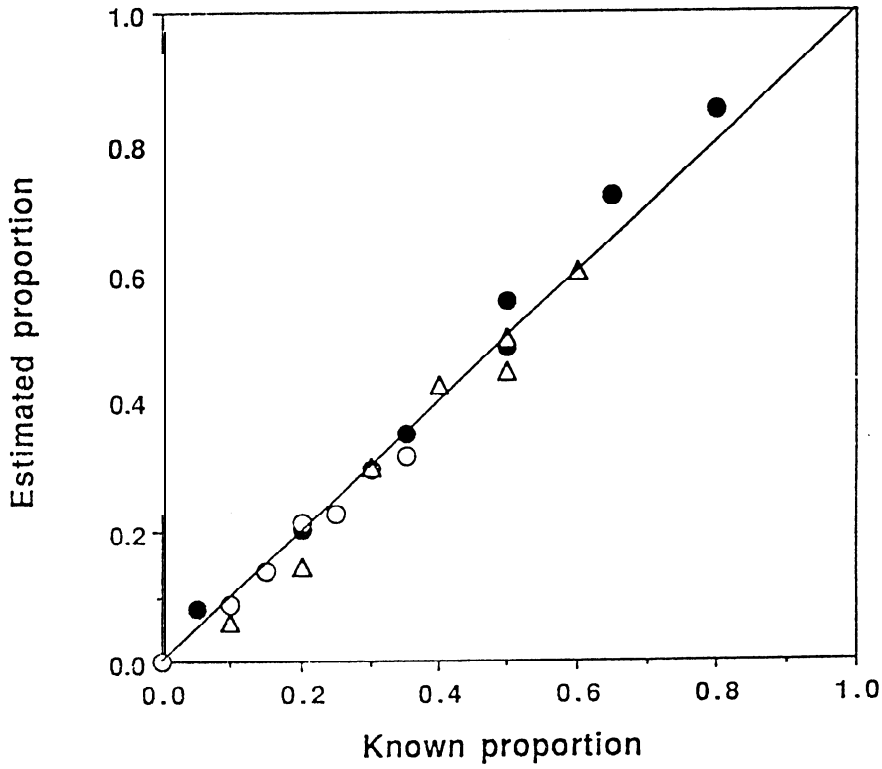

Fig. 2 Comparison of the known proportions of pasture species in a set of 6 mixtures, with those estimated from the alkane concentrations in the mixtures and the component species (O Subterranean clover cv. Larisa; ● Perennial ryegrass cv. Ellett; △ Yorkshire Fog grass).

TABLE 5 Comparison of the botanical composition of a sward with the estimated botanical composition of samples collected by sheep fistulated at the oesophagus (H.Dove, L. Cransberg and P.T. Doyle, unpublished data; cited by Dove and Mayes 1991)

	Plant species		
	Serradella	Balansa clover	Grass[A]
Sward composition	0.533	0.333	0.127
Oesophageal sample:			
Sheep 4	0.612	0.271	0.118
Sheep 5	0.528	0.307	0.165

Alkanes can also be used to estimate the botanical composition of chewed herbage such as oesophageal extrusa, since they are insoluble, intimately associated with the plant surface and are thus not leached out during mastication. Table 5 compares the botanical composition of a sward, determined by physical sorting of cut samples, with the botanical composition of oesophageal extrusa samples estimated from their alkane contents using a least squares optimisation procedure.

There is clearly a close correspondence between the botanical composition of the sward and those of the oesophageal samples. This may be because stocking rates in this study were high and little diet selection was possible. In the present symposium, Dove et al. (1993) present similarly derived data for a lush subterranean clover pasture and in their data, a greater degree of diet selection is evident.

Perhaps the most valuable application of alkanes in diet selection work is the estimation, from faecal alkane concentrations, of the composition of the diet selected by

individual, intact grazing animals. This uses the same algebraic approach outlined above for estimating the botanical composition of herbage, with the extra complication that the faecal alkane concentrations must be corrected for incomplete faecal recovery of alkanes. The required recovery corrections can either be obtained from companion indoor studies, or from total faecal collection within a subset of the grazing animals dosed with a mixture of synthetic, even-chain alkanes (Dove and Mayes 1991). Recoveries for the odd-chain, natural alkanes are obtained by interpolation.

Once faecal alkane concentrations are corrected for incomplete recovery, the proportions of different plant species in the diet can be estimated as described above. Least squares procedures are the most useful approach. If total herbage intake is estimated as earlier described (equation 2), then the intakes of different plant species can be calculated. This has not been possible before. Several validation studies of this approach have been conducted by R.W. Mayes and colleagues (cited by Dove and Mayes 1991) and an example is shown in Figure 3. Sheep in this study were fed diets with differing proportions of perennial ryegrass and white clover. The proportions of white clover were also estimated from the alkane compositions of the two pasture species and of the faeces from individual animals. The relationship between estimated and known proportions of white clover shown in Figure 3 did not differ significantly from the equivalence line.

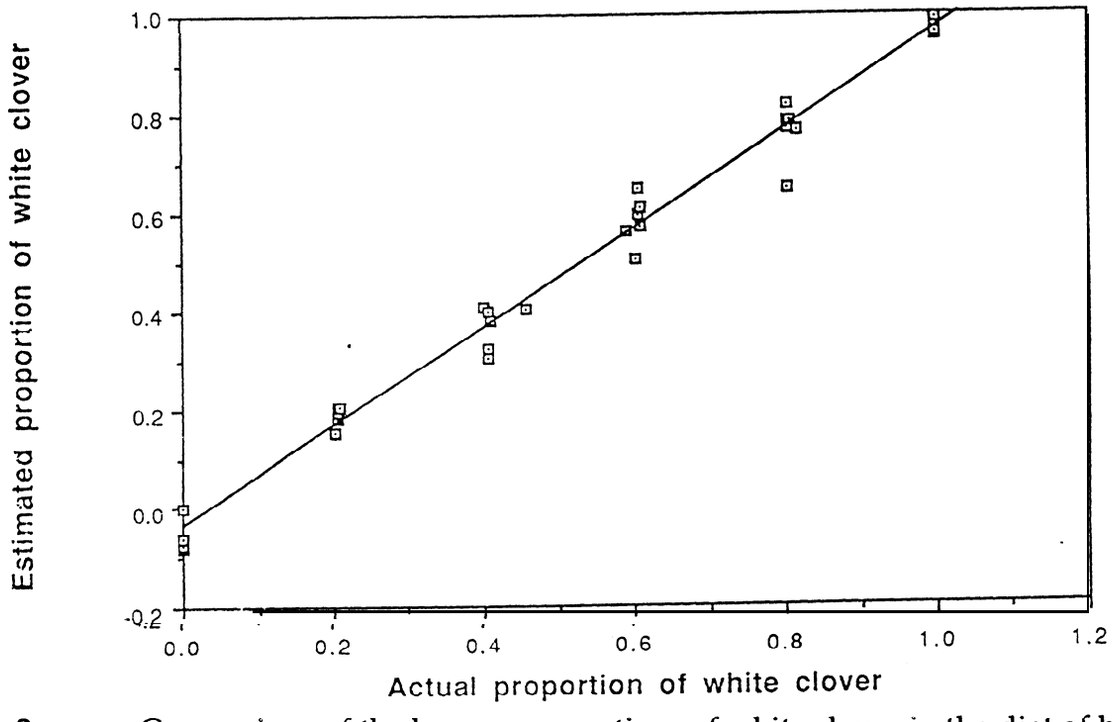

Fig. 3 Comparison of the known proportions of white clover in the diet of housed sheep with the proportions estimated from herbage and adjusted faecal alkane concentrations. The fitted regression is:

Estimated = 1.008 (Known) - 3.182 (r^2 = 0.988; P<0.001)

Such validation is, by definition, not possible in the grazing animal and indirect evaluations must be made. In the present symposium, Dove et al. (1993) present data for the species composition of OF samples and that of the diet selected by the same animals over a six-day sampling period. It should be noted that only one of these estimates (consumed diet) requires correction for incomplete faecal recovery. Once animals had become accustomed to the pasture (2 weeks) a close correspondence in botanical composition was observed between the diet selected at a single grazing and that selected

over the course of six days. This suggests that the procedures used for obtaining and using the faecal alkane corrections were valid.

We do not yet know how many plant species can be estimated in the diet on the basis of alkane patterns. However, Dove and Mayes (1991) presented data for the species composition of the diet of grazing/browsing goats, estimated from the alkanes in the eight plant species on offer and those in faeces. Algebraically speaking, all the plant species were successfully distinguished in the diet, though there was no 'benchmark' against which the estimates of diet selection could be assessed. However, the species which were estimated to be consumed in the greatest proportions and those which were not consumed were in agreement with behavioural observations and previous knowledge of the dietary preferences of goats in that environment.

Using alkanes to estimate the intake of plant parts

Although plant species is the main determinant of cuticular alkane patterns and concentrations, there are certainly also differences attributable to different plant parts (Dove and Mayes 1991; Laredo et al. 1991; Dove et al. 1992). For example, the differences in the alkane composition of leaf blade leaf sheath, stem and floral parts in senescing annual ryegrass (Dove et al. 1992) are shown in Table 6.

TABLE 6 Alkane (A) and alkene(E) concentrations (mg/kg OM) in different parts of annual ryegrass

Alkane/Alkene	Leaf blade	Leaf sheath	Stem	Floral parts
C27E	0	0	0	81.6
C27A	170.1	60.5	18.6	154.6
C29E	0	0	0	197.8
C29A	366.1	259.4	84.3	197.1
C31E	0	0	0	40.3
C31A	488.0	417.5	160.3	189.6
C33	140.8	48.5	19.9	22.3

Not only are differences in alkane composition evident between the plant parts, but the presence of alkenes (ie., alkanes with 1-2 double bonds) is evident in floral parts. This is also true of perennial ryegrass, though in this case, small quantities of alkene have also been detected in pseudostems (H. Dove and R.W. Mayes, unpublished data).

Such differences in alkane composition between the different parts of the plant can be a source of error in the estimation of diet selection if the samples of the various plant species collected for alkane analysis contain plant parts different from those collected by the grazing animal. However, such differences also permit the partitioning of the total diet into its component plant parts, using exactly the same approach as with plant species selection.. Indeed, such data were recently reported by Dove et al. (1992) for annual ryegrass swards, and the estimated selection in the grazing animals was in excellent agreement with the results of preferences tests conducted indoors.

CONCLUSIONS

The results presented indicate that the use of a combination of herbage and dosed, synthetic alkanes results in accurate estimates of intake which are truly individual in nature. The technique has now been applied on a routine basis in the field, in groups of

up to 80 animals (Dove et al. 1992). However, its widespread adoption will require the ready availability of an intra-ruminal alkane CRD. Work in this area is continuing.

The second major advantage offered by the use of alkanes is the opportunity to partition the total intake of the grazing animal into its component plant species or plant parts. Results on the use of alkanes to estimate diet selection look extremely promising. Moreover, we have yet to explore the possibility of using other cuticular wax components such as the long-chain fatty acids or alcohols, to improve estimates of selection. The future prospects for our being able to routinely estimate the intake of different plant parts from different plant species in the sward thus seem excellent.

ACKNOWLEDGEMENTS

The author acknowledges the generous support provided by the Wool Research and Development Corporation for the alkane studies described in this review.

REFERENCES

DOVE, H. (1992). Aust. I. Agric. Res. 43: 1711-24.

DOVE, H. and MAYES, R.W. (1991). Aust. I. Agric. Res. 42: 913-52.

DOVE, H., FOOT, J.Z. and FREER, M. (1989a). Proc. XVI Int. Grassld Cong. (Nice, France) pp. 1091-92.

DOVE, H., MAYES, R.W., FREER, M., COOMBE, J.B. and FOOT, J.Z. (1989b). Proc. XVI Int. Grassld Cong. (Nice, France) pp. 1093-94.

DOVE, H., MILNE, J.A. and MAYES, R.W. (1990). Proc. NZ Soc. Anim. Prod. 50: 457-59.

DOVE, H., MAYES, R.W., LAMB, C.S. and ELLIS, K.J. (1991). Proc. 3rd Int. Symp. Nutr. Herbivores (Penang, Malaysia) p.82.

DOVE, H., SIEVER-KELLY, C., LEURY, B.J., GATFORD, K.L. and SIMPSON, R.J. (1992). Proc. Nutr. Soc. Aust. 17: 149.

DOVE, H., FREER, M. and MOORE, A.D. (1993). In "Recent Advances in Animal Nutrition in Australia", pXXA, editor D.J. Farrell (University of New England, Armidale).

FORWOOD, J.R., STYPINSKI, I?, MAWHINNEY, T. and PATERSON, J.A. (1987). Agron. J. 79: 996-98

HAMILTON, B.A. and HALL, D.G. (1975). J. Br. Grassld Soc. 30: 229-35.

LANGLANDS, J.P. (1975). In "Digestion and Metabolism in the Ruminant", pp. 320-32, editors I.W. McDonald and A.C.I. Warner (University of New England, Armidale).

LANGLANDS, J.P. (1987). In "The Nutrition of Herbivores", PP. 363-90, editors J.B. Hacker and J.H. Ternouth (Academic Press, Sydney).

LAREDO, M.A., SIMPSON, G.D., MINSON, D.J. and ORPIN, C.G. (1991). J. Agric. Sci., Camb. 117: 355-61.

MAYES, R.W. and LAMB, C.S. (1984). Proc. Nutr. Soc. 43: 39A.

MAYES, R.W., LAMB, C.S. and COLGROVE, P.M. (1986). J. Agric. Sci., Camb. 107: 161-70.

MAYES, R.W., DOVE, H., LAMB, C.S. and ELLIS, K.J. (1991). Proc. 42nd Annual Meeting, Europ. Assoc. Anim. Prod. 1: 457-61.

SMITH, A.E. (1982). Agron. J. 74: 157-59.

VULICH, S.A., O'RIORDAN, EG. and HANRAHAN, J.P. (1991). J. Agric. Sci., Camb. 116: 319-23.