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Measurement of dietary nutrient intake in free-ranging mammalian herbivores

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Abstract

The nutrient intakes of mammalian herbivores depend on the amount and the nutrient content of the plant species and plant parts which they eat. We review the merits of oesophageal-fistulated (OF) animals, microhistological procedures, stable C-isotope discrimination and plant cuticular-wax markers as methods for estimating diet composition and intake in both ruminant and non-ruminant herbivores. We also briefly discuss methods based on grazing behaviour measurements or on H_2O or Na turnover, and methods for estimating supplement or soil intake. Estimates of intake in ruminants are often based on separate measurements of faecal output and herbage digestibility. We review this approach and emphasize that, under some circumstances, the applicability of *in vitro* digestibility estimates based on OF extrusa is questionable. We discuss how plant-wax marker patterns can be used to check whether OF and test animals are consuming similar diets, but also emphasize that a major advantage of the use of plant-wax markers is that this approach may obviate altogether the need for OF animals. Estimates of total herbage intake can be partitioned into the intakes coming from different plant species and/or parts, provided diet composition can be measured. Diet composition estimates based on C-isotope discrimination have the major disadvantage that they cannot be taken to species level. By contrast, microhistological methods can identify many plant species in extrusa, digesta or faeces, but often a large proportion of plant fragments remains unidentifiable. Plant-wax hydrocarbons show great promise as markers for estimating diet composition and intake. However, we suggest that to be applicable in complex plant communities there is a need with this method either to recruit a wider range of wax markers (e.g. alcohols, sterols, fatty acids) or to use it in combination with other methods. We suggest that, in turn, this generates an urgent need for research on statistical aspects of the combined use of markers or methods, in relation to the error structures of the data or methods being combined and the standard errors of the resultant estimates of diet composition and intake. We conclude by discussing the extension of intake and/or diet

Abbreviation: OM, organic matter.

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composition measurements to the measurement of nutrient transactions within the gut, particularly in relation to the supply of absorbable nutrients.

Herbivores: Nutrient intake

Introduction

Free-ranging domestic or wild herbivores exert choice in consuming the various parts of plants and species from the available vegetation. Since the concentrations of nutrients can vary considerably among the different dietary plant parts and species selected, this choice can markedly affect the herbivore's nutirent intake (Dove *et al*. 1999). Despite this, most quantitative studies of nutrient intake and utilization, especially with domestic livestock, have used housed captive animals, often fed on diets bearing little resemblance to those likely to be selected by grazing animals. Nutrient intake is the major determinant of nutritional status and production performance in farm livestock. Consequently, there have been numerous estimates of herbage intake by grazing ruminants, but few intake measurements in free-ranging pigs and horses. For all free-ranging domestic livestock, the number of diet composition estimations has been relatively small. In contrast, dietary studies with free-living wild herbivores have largely been confined to estimates of diet composition (Cornelis *et al*. 1999).

Mammalian herbivores, whether wild or domesticated, are significant components of natural or agricultural ecosystems. Not only will the wellbeing of a herbivore population be influenced by the nutritional status of its component individuals, but the type and quantity of the plant material they consume may affect the distribution patterns and numbers of plant and other animal species within the ecosystem. Hence, an understanding of the quantitative interaction between herbivores and plants is central to ensuring the long-term sustainability of these ecosystems. Furthermore, herbivores will influence the dynamic distribution of minerals and other nutrients within the ecosystem, through ingestion of plant material and redistribution by the deposition of excreta (for example see Pastor *et al*. 1993). The distribution of persistent pollutants in the environment, such as radionuclides and heavy metals, will similarly be affected (for example see Salt *et al*. 1992). The fact that the major factors influencing intake in free-ranging herbivores may differ greatly from those in housed animals emphasizes the need to measure intake, both of the complete diet and of its individual components, in grazing and browsing animals.

This present review is concerned with the assessment of what herbivores eat and their resultant intake of nutrients. We will not enter the current debate about why a herbivore selects a particular diet, or why it chooses to start or to finish eating (Day *et al*. 1998; Provenza *et al*. 1998). Nevertheless, many of the techniques discussed in this review are now sufficiently well developed that they can help to answer these questions. Similarly, most of the concepts may equally apply to the estimation of the intake of other substances in the diet, such as plant secondary compounds and environmental pollutants. Furthermore, some of the techniques for determining intake require prior knowledge of dietary composition. Intake of specific nutrients or other substances (for example see Mayes *et al*. 1994; Dove *et al*. 1999) can be determined from estimates of the intake of DM or organic matter (OM), and concentrations of the substance under investigation. Additional techniques may be required to estimate the digestible (or absorbable) intake of a nutrient or supply of a fermentation product such as microbial protein.

Determinations of intake and diet composition in free-ranging herbivores are generally difficult to undertake, and their errors are often large, mainly owing to the limitations of available measurement techniques. The measurement processes themselves may disturb the

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animals to the extent that their normal foraging behaviour may be compromised; this can be a major problem in rangeland, forest and other semi-natural environments. Furthermore, there is no true means of evaluating these techniques under free-ranging conditions, except by comparison with alternative, possibly inferior, methods. The indoor testing of such techniques, by feeding known quantities of vegetation of defined composition, generally provides an acceptable, but not absolute, validation.

Techniques for intake estimation have been extensively reviewed (for example see Langlands, 1987; Dove & Mayes, 1991). Most were developed for use in ruminant livestock, grazing simple grass swards which could be considered atypical compared with the conditions experienced by most of the world's large herbivores. On simple swards, animals may be constrained with respect to both choice of diet and intake of DM, and nutrient contents of herbage can be determined with reasonable accuracy. As the majority of free-ranging herbivores exist in heterogeneous environments, the determination of intake would usually necessitate an accompanying estimation of diet composition. In this present review existing methodologies for quantifying diet composition and intake in free-ranging herbivores are discussed and the potential ways of overcoming the limitations of these techniques will be considered. We also address the possibility of modifying such methods to permit their use in non-ruminants and wild herbivores, grazing or browsing in complex vegetation environments. Recent advances in measurement techniques, in particular in the use of various marker substances and sampling equipment, offer the potential for studying the utilization of certain nutrients by free-ranging herbivores; these will also be discussed.

Intake can be estimated from the depletion of a food resource through grazing or browsing. While conceptually simple, such plant-based methods are reliable only over a very short time scale because of continuing plant growth, and are thus not applicable for continuously grazed areas. Likewise, exclusion cages may give an indication of changes in vegetation cover, when ungrazed over an extended period, but they do not provide a reasonable measure of intake. Whilst such plant-based methods may be useful in helping to evaluate other measurement techniques (for example see Reeves *et al*. 1996), they have limited applicability and will not be discussed further in this review.

The main advantages of 'animal-based' methods over 'plant-based' methods are that separate estimates of intakes for individuals within a group can be made, although, for reasons mentioned below, such measurements may not always be completely independent of each other. Moreover, such estimates may be used for longer-term studies, such as continuously grazed systems of pasture management. For many years, the most widely used animal-based method for estimating intake, especially in pasture-based systems, has been from separate estimates of faecal output and diet digestibility. This method is applicable over a wide range of situations and is compatible with many nutritional measurements; it is also amenable to further development and application. We describe a number of other animal-based methods, including the use of measurements of foraging behaviour to derive an intake estimate (bite rate $\times$ bite size $\times$ biting time), and using estimates of the turnover of a dietary component (e.g. H_2O) within the animal. Before these animal-based techniques are addressed in detail it is apposite to consider first the measurement of diet composition. Methods used to estimate the intakes of supplementary feed and soil in free-ranging herbivores will be considered as special examples of the estimation of diet composition.

Approaches to the estimation of diet composition

Animal-based methods involve the measurement, in samples collected from animals, of chemical or physical components which can be uniquely associated with or partitioned between the

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respective plant species on offer. This approach includes methods based on the quantification of microscopic fragments of plant material in extrusa from oesophageal-fistulated animals, stomach contents, digesta or faeces. Other methods include the estimation of C-isotope ratios in such samples or in animal tissues (e.g. hair or wool) and the use of plant-wax components as markers.

The advantages and disadvantages of these various methods have been discussed extensively elsewhere (Holechek *et al*. 1982; Dove & Mayes, 1996, 1999; Cornelis *et al*. 1999) and only a brief description of some of their attributes will be presented here.

Use of oesophageal-fistulated animals for the estimation of diet composition

Whilst oesophageal-fistulated animals have been used mostly to obtain samples for *in vitro* estimates of herbage digestibility (see later), they have also been used to estimate the botanical composition of the consumed diet, especially in studies with domestic herbivores (for example see Coates *et al*. 1987; Salt *et al*. 1994; Dove, 1998). In most circumstances extrusa samples have been used to infer the diet selected by a separate group of intact animals grazing or browsing the same resource. The assumption that such extrusa samples represent the composition of the diet of the intact test animals may be questioned for the following reasons:

(1) extrusa samples are collected over a period of a few minutes, whereas the test animals may be grazing or browsing an area for days or weeks. Whilst repeated extrusa collections may give a more representative estimate of diet composition, the total period of extrusa collection is likely to remain much less than the representative grazing period of the test animals,
(2) the diet selected by oesophageal-fistulated animals may differ from that of the test animals, owing to the former animals having been surgically prepared, handled and managed differently or being in a different physiological state;
(3) the composition of extrusa may differ from that of the plant material ingested, owing to chewing, the addition of saliva and the possibility of plant solubles and small fragments bypassing the fistula.

There is no absolute way to test the validity of these assumptions. However, reasonable assessments can be made through comparisons using alternative techniques for the estimation of diet composition. Such approaches, relevant to procedures involving oesophageal-fistulated animals, are discussed later in this review. There are, however, other means of addressing potential limitations of the use of these animals. The development of a 'remote control' valve for the collection of oesophageal extrusa may help overcome the problem of the short collection period (Raats *et al*. 1996), since the valve permits samples of herbage to be collected throughout the day without disturbing normal grazing behaviour. The addition of saliva to ingested plant material during chewing precludes the use of extrusa samples for studies of Na and P status, unless correction is made for the salivary input (for example see Langlands, 1987). Saul *et al*. (1986) suggested that the water-soluble carbohydrate content of extrusa samples may not reflect that of the diet, owing to soluble material bypassing the fistula. For most other feed components (e.g. N and fibre fractions) and for digestibility, published data suggest there is little effect of chewing and that extrusa samples can be used to represent the dietary levels of these components (Saul *et al*. 1986; Langlands, 1987).

Microhistological procedures

Many variants of the procedure have been described but, in principle, all rely on the identification of plant tissue fragments in prepared samples of oesophageal extrusa, stomach contents

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or faeces (Holechek *et al*. 1982). The composition of the diet can then be described in terms of the proportion of (identifiable) fragments coming from each plant species. Calibrations may be required to convert the resultant diet composition to a DM basis. In most cases, epidermal cuticular fragments are observed and identified, though differences in opaline phytoliths between plant species have also been proposed as an identification aid (Matsumoto & Sugawara, 1997).

The method has been used extensively, especially with samples of stomach contents from dead wild animals (Holechek *et al*. 1982; Cornelis *et al*. 1999), in which such measurements would reflect mainly the composition of the last meal, and repeated measurements in the same animal are not possible. Also, the supply of suitable animals may be limited. Repeated samples of stomach contents for microhistological analyses could be obtained from ruminants fitted with a large rumen fistula (for example see Mohammad *et al*. 1995). Faecal sampling is much less restricted, allows repeated sampling and is also more appropriate for endangered animals and for domestic animals in which slaughter is too costly. Nevertheless, it has some major disadvantages, the main being the effect of differential digestion of fragments from different plant species. Moreover, as with almost all microhistological methods, the use of either stomach-contents or faeces samples can result in a large number of fragments which cannot be identified as being from particular species; this can render the method unreliable for quantitative estimation of diet composition. However, in such cases microhistological methods can still be useful in indicating the presence or absence of a particular plant species or plant part in the diet.

Stable carbon isotope discrimination

The ratio of the stable isotopes ^{13}C and ^{12}C in plant tissues differs between plants which have the C_3 and C_4 pathways of photosynthesis. This difference ($\delta^{13}C$) can be exploited to estimate the proportion of C_3 and C_4 herbage in a mixture (Jones *et al*. 1979; Tieszen *et al*. 1983). In tropical ecosystems, C_3 plants are represented principally by legumes, forbs and browse, whilst C_4 plants are represented mainly by tropical grasses and some forbs. This distinction has permitted the estimation of the proportion of these different plant types in the diet of herbivores, using the $\delta^{13}C$ value in extrusa from oesophageal-fistulated animals or in faeces (for example see Coates *et al*. 1987), or animal tissue (for example see Tieszen *et al*. 1983). The method cannot resolve diet composition to the species level, but estimation of the proportion of legume in the diet is still important, since the legume has a higher N content and may contribute much of the dietary N intake. Unfortunately the method is of limited use in colder areas of the world, where very few plants exhibit the C_4 photosynthetic pathway. The effect of differential digestion of different plant species must also be considered. When faeces samples are used, the method will tend to underestimate the intake of plants of high digestibility, since less of their C remains in faeces. Conversely, the use of animal tissues, such as wool or hair, which give a long-term indication of dietary ratios of C_3 and C_4 plants, will underestimate the dietary contribution of plants with lower digestibility.

Plant-wax markers for the estimation of diet composition

Plant cuticular waxes are complex mixtures of many classes of compounds, the composition of the mixture varying with different plant species or plant parts. Although such differences in wax composition could potentially be exploited to estimate diet composition using various

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compound types, to date only the *n*-alkanes of plant cuticular wax have been widely investigated as markers. In most higher plants, *n*-alkanes with odd-numbered C-chains in the range $C_{21}-C_{37}$ comprise most of the hydrocarbons of cuticular wax. Plant species differ in the composition of this fraction (for example see Dove, 1992; Mayes *et al*. 1994). Differences in alkane pattern can be used to estimate the proportions of different plant species or plant parts in herbage mixtures (Dove, 1992) or extrusa from oesophageal-fistulated animals (Salt *et al*. 1994; Dove *et al*. 1999) by calculating the combination of alkane profiles from the plants on offer which accounts for the observed alkane pattern in the mixture. The method is equally applicable for the estimation of the composition of digesta samples or the diet composition from faeces samples collected from either domestic animals (Salt *et al*. 1994; Dove *et al*. 1999) or wild species (Hulbert, 1993; Woolnough, 1998; MN Bugalho, RW Mayes and JA Milne, unpublished results). The recovery of alkanes in faeces is high but incomplete and, in ruminants, varies according to chain length. Thus when digesta or faeces samples are used, their alkane concentrations must be corrected for differences in recovery (Dove & Mayes, 1996; Dove *et al*. 2000).

With simple mixtures, calculations of dietary composition can be made using simultaneous equations (Dove, 1992), with the number of alkanes chosen as markers being equal to the number of dietary components. However, using a least-squares procedure allows the use of more alkanes than there are components (plant species and/or plant parts) in the mixture; this is because least-squares procedures search for the best fit rather than a unique solution (as obtained using simultaneous equations). Estimates of the species composition of herbage mixtures obtained from simultaneous equations were almost identical to those derived from least-squares procedures (Dove & Moore, 1995). Woolnough (1998) reached a similar conclusion in comparing alkane-based estimates of the diet composition in free-ranging populations of eastern grey kangaroos (*Macropus giganteus*) and northern hairy-nosed wombats (*Lasiorhinus krefftii*). A number of different algorithms have been used in the least-squares procedures. Mayes *et al*. (1994), using the 'Solver' routine within the 'Excel' spreadsheet program (Microsoft), minimized the sum of the squared discrepancies between the measured faecal proportions of individual alkanes (expressed relative to the total faecal alkane), and alkane proportions (of the total alkane) calculated from the alkane compositions of component plant species. Dove & Moore (1995) and Newman *et al*. (1995) used different non-negative least-squares procedures applied to faecal and plant alkane concentrations to estimate the intakes of plant components necessary to produce 1 kg faecal DM (or OM); diet composition could subsequently be calculated from these hypothetical intake values. Hameleers & Mayes (1998*a*) compared these three least-squares procedures and observed that they gave almost identical estimates of diet composition in dairy cows.

Plant wax alkanes, as markers to estimate diet composition, offer numerous advantages over alternative measurement methods. Estimates are conducted over, and are relevant to, a similar time scale to that required in nutritional studies. Sampling of dietary components and faeces is relatively simple and is potentially applicable to both domestic and wild herbivores, and chemical analysis is both straightforward and accurate. Furthermore, concurrent estimates of intake are possible (see later). However, the use of marker patterns (of which plant alkanes are but one example) to estimate diet composition is a relatively novel concept and there are a number of aspects which need to be addressed in order to evaluate and optimize the technique. In particular:

(1) more validations of the technique are required;
(2) procedures for statistical analysis of diet composition data need to be developed;

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(3) potential weighting procedures to optimize the discriminatory power of diet composition markers need to be explored;
(4) procedures need to be developed that allow marker systems to be used in situations where the number of potential dietary components exceeds the number of available markers.

To date, validations of diet composition estimates, carried out indoors by feeding known mixtures to livestock, have largely been confined to two-component mixtures (Dove & Mayes, 1991, 1996), though estimates of composition of herbage mixtures have involved up to ten species (Hoebee *et al*. 1998). Although not yet proven, the accuracy of diet composition estimates is likely to decline as the number of dietary components increases. There is clearly a need to make rigorous tests of the method using more complex dietary mixtures.

It is logical to assume that the most accurate estimates of diet composition will be obtained when all dietary components have similar total alkane levels, but where their patterns differ greatly. Multivariate statistical procedures such as principal components analysis or canonical variates analysis can indicate the degree to which alkane patterns differ between plant species on offer (Dove *et al*. 1999; MN Bugalho, RW Mayes and JA Milne, unpublished results), but further statistical procedures are required in order to establish confidence limits to resultant diet composition estimates. These will be affected by the nature of the various alkane patterns of the dietary components and by analytical error, sampling errors and intrinsic variation in the alkane compositions within individual dietary components.

For particular mixtures, the individual alkanes which differ most in relative concentration between components would be expected to be more effective as diet composition markers than those alkanes with similar levels in all components. Using existing algorithms, alkanes with the highest absolute concentrations have the greatest influence on the resultant diet composition estimate. In order to optimize the calculation procedure there is a case for adjusting the weighting of individual alkanes, such that those which differ more, in relative terms, between dietary components should have a greater influence on the diet composition estimate. It may also be beneficial to weight against those alkanes with greater analytical errors or within-component variability. An extreme form of weighting is the omission of certain alkanes from particular diet composition estimations; this can improve estimates of diet composition (Dove *et al*. 1999).

In theory, the maximum number of components that can be separated using alkanes is equal to the number of alkane markers available (about fifteen), but since many of the alkanes may be present at low concentrations, the practical limit may be less than fifteen. However, in complex associations such as rangelands or shrublands, there can be more than this number of plant species on offer to the herbivore (MN Bugalho, RW Mayes and JA Milne, unpublished results). To overcome this problem and extend the alkane procedure to complex plant associations, there are three possible options: (1) combine the discriminatory powers of this and other methods of estimating diet composition; (2) decrease the number of plant pools to be discriminated; (3) increase the number of markers with which to discriminate.

An example of the combination of methods is the successful use, by Salt *et al*. (1994), of both microhistological and alkane procedures to estimate diet composition in sheep which could choose to graze any combination of either *Deschampsia flexuosa* grassland or *Calluna vulgaris* heathland. A second possibility might be the combination of the alkane method with the discriminatory power of near i.r. reflectance spectroscopy for diet composition (Walker *et al*. 1998). A reduction in the number of plant pools to be discriminated could be based on preliminary information based on other techniques (e.g. observation of the animals, microhistological, near i.r. reflectance spectroscopy) which establish those plants not selected.

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Another option would be to group the individual plant species which had statistically indistinguishable alkane profiles (for example see Woolnough, 1998; MN Bugalho, RW Mayes and JA Milne, unpublished results). Resultant estimates of diet composition would thus be expressed in terms of the plant groups. For example, MN Bugalho, RW Mayes and JA Milne (unpublished results) grouped grass and forb species as a single 'herbage' component in studies with wild red deer which ingested mainly browse species. The acceptability of the diet composition estimates obtained after such grouping depends upon the purpose to which the results will be put. Provided the grouping does not adversely affect the accuracy of the actual determination of diet composition, then the results would be a valid estimate of, as in the above example, browse species plus a grouped 'herbage' component (MN Bugalho, RW Mayes and JA Milne, unpublished results). However, regardless of their having similar alkane profiles, the grouping of potential dietary components would not be appropriate if the individual components of the group had, for example, different N contents and the diet composition estimates were being used to estimate N intake. A third approach would be to increase the number of markers available for use in the calculation of diet composition. We have discussed in detail elsewhere the potential to use other plant wax compounds including alkenes, branched-chain alkanes, alcohols, sterols and possibly long-chain fatty acids as additional markers (Dove & Mayes, 1996, 1999). The long-chain alcohols of cuticular wax show particular promise, since they are often present in large amounts in plants which have low concentrations of alkanes (Dove & Mayes, 1999).

Comparison of diet composition estimates obtained using different techniques under field conditions

Under field conditions, one cannot validate estimates of diet composition but can only compare measurements made using different techniques. However, such information can be valuable with respect to technique assessment. The development of the alkane method, in particular, has allowed important reassessments to be made of the validity of using oesophageal-fistulated animals to sample grazed vegetation. The data in Table 1 represent one of the few comparisons (Dove, 1998) of the respective species composition of the herbage on offer, the diet of oesophageal-fistulated animals at a single grazing of that herbage, and the diet of the same animals over a period of 7 d. The extent to which these three compositions agreed depended on the nature of the pasture on offer.

On the clover-dominant pasture, the proportions of clover in the extrusa from oesophageal-fistulated animals, and the diet consumed by these animals over 7 d, were moderately but significantly higher than the sward clover content ($P < 0{\cdot}05$). There was also apparent selection against the weed species *Rumex acetosella* ($P < 0{\cdot}001$). By contrast, over 7 d the oesophageal-fistulated animals grazing the ryegrass-dominant sward consumed a diet which did not differ in overall species composition from that of the sward ($P > 0{\cdot}05$). However, the extrusa collected from a single grazing had a markedly different content of *Phalaris* ($P < 0{\cdot}001$), ryegrass ($P < 0{\cdot}001$) and *R. acetosella* ($P < 0{\cdot}05$).

This approach has allowed some assessment of the errors arising from the assumption that the single sample of extrusa from an oesophageal-fistulated animal represents the longer-term diet. The alkane method has also allowed reliable comparisons to be made between the botanical and nutrient composition of extrusa samples and the actual diet of intact test animals. Such comparisons have been made using microhistological procedures, but results have been variable, owing in part to difficulties with this technique. Coates *et al*. (1987) compared estimates

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Table 1. Comparison of the botanical composition (% dry matter) of subterranean clover-dominant or perennial ryegrass-dominant pastures ('herbage') with the botanical composition of the diet selected by oesophageal-fistulated sheep at a single grazing ('extrusa'), or by the same sheep over a period of 7 d ('diet')*

(Mean values for six oesophageal-fistulated sheep per pasture)

	Botanical composition (% dry matter)		
Pasture	Herbage	Extrusa	Diet
Clover dominant:			
Trifolium subterraneum	69·9	84·3	91·5
Lolium perenne	2·8	0	0·8
Rumex acetosella	22·5	8·8	5·1
Other grasses	4·7	6·9	3·4
Grass dominant:			
Phalaris aquatica	11·1	72·8	4·3
Lolium perenne	71·4	16·3	70·4
Rumex acetosella	13·4	8·6	2·8
Other grasses	4·2	2·4	22·6

* Recalculated from data presented by Dove (1998). Botanical composition of herbage by hand-sorting; botanical composition of extrusa and of the diet estimated from the pattern of alkane concentrations in herbage components, and extrusa or faeces samples (bulked: herbage, 18 or 26 October for clover-dominant or grass-dominant pasture respectively; extrusa 22 October; diet, 14–20 October). Calculations performed using EatWhat procedure of Dove & Moore (1995).

of the composition of extrusa collected by steers and the diet of cattle resident on tropical grass–*Stylosanthes* spp. pastures. Although estimates of the legume content of extrusa based on C-isotope and microhistological procedures were comparable, agreement between the legume content of extrusa and of the diet of the resident animals, estimated from the C-isotope ratio in their faeces, was poor. The authors therefore suggested that the assumptions concerning the use of oesophageal-fistulated animals should not be accepted without supporting evidence for their validity.

Dove *et al*. (2000) suggested that if oesophageal-fistulated and intact test animals were consuming similar diets, then the alkane compositions of extrusa should be similar to those in the faeces from test animals (corrected for incomplete recovery). Fig. 1 shows the relationship between the alkane composition (expressed as proportions of the total alkane content) of extrusa samples from adult oesophageal-fistulated wethers and that of faeces from pregnant ewes grazing the same grass-dominant pasture. The faecal data were corrected using recoveries obtained from total faecal collections in grazing wethers (Dove *et al*. 2000). The fitted regression through these data did not differ from the line of equality. This suggests that, in a grazing bout of 20–30 min, the oesophageal-fistulated wethers were selecting the same diet as that selected by the ewes over the 6 d faecal sampling period. Note that this is not absolute proof, since it is theoretically possible to consume diets of very similar alkane proportions but differing in species composition. This is more likely to be a problem with complex associations of plant species than with the simple swards used in this example. Although the results in Fig. 1 suggest that the oesophageal-fistulated animals and test animals consumed similar diets, there is ample evidence that this is not always the case (for example see Coates *et al*. 1987; Dove, 1998). In contrast to microhistological or C-isotope methods, this approach is equally applicable to the estimation of the composition of extrusa or consumed diets in terms of plant parts (Dove *et al*. 1999).

The estimation of intake from faecal output and diet digestibility

Intake can be estimated from the relationship which exists between the intake of a diet, its indigestibility (1 − digestibility) and the resultant faecal output. Conventionally, this approach

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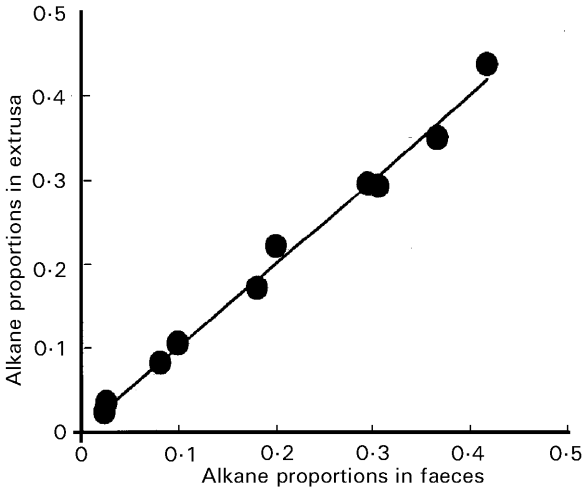

Fig. 1. Relationship between the alkane composition (concentration for each alkane expressed as a proportion of the total alkane content) of extrusa from oesophageal-fistulated wethers and that in the faeces of pregnant ewes grazing the same pasture ($y = 0{\cdot}987x + 0{\cdot}005$, $R^2 = 0{\cdot}993$; Dove *et al.* 2000).

is used to estimate the intake of DM or OM, but in principle can be applied to any nutrient or other substance in the diet. Because it is necessary to have a sample representative of the whole diet, in heterogeneous environments an accompanying estimate of diet composition would be required, unless it has been confidently established that a sample of mixed vegetation (e.g. extrusa) truly represents the diet of the test animal.

Faecal output

The principles of faecal output measurement in free-ranging animals are straightforward. Faecal output can be measured directly by total collection over a number of days, using bags attached to harnesses. The main concerns regarding this method are to ensure that no faeces are spilt and that the animals' normal behaviours are not compromised by the collection equipment or procedures. Less restrictive to the animals is the estimation of faecal output by dilution in the faeces of an external marker which is administered orally to the animal daily (or more frequently) over a period of 10–14 d. This allows the faeces to be sampled over a 5–7 d period, after the marker concentration has equilibrated in the faeces. Various markers have been used (Table 2), Cr_2O_3 being the most popular, but there has been recent concern over its potential carcinogenic properties. The main problems with the external marker approach relate to the properties of the markers themselves, especially incomplete faecal recovery. Diurnal variation in faecal marker concentration is another problem encountered (Dove *et al.* 2000). Attempts have been made to reduce this effect, either by careful selection of dosing and sampling times or through modifying the matrix in which the marker is dosed. More details about the use of daily-dosed external markers for estimation of faecal output are given elsewhere (for example see Le Du & Penning, 1982).

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Table 2. Some faecal markers used for the estimation of faecal output in herbivores (see Kotb & Luckey, 1972; Udén *et al.* 1980; Dove & Mayes, 1996)

Marker	Type	Analysis*	Recovery	Digesta association	Additional uses
Cr_2O_3†	Insoluble oxide	AA or XRF	Very high	None, dense	Supplement, CRD
TiO_2†	Insoluble oxide	AA or XRF	Very high	None, dense	
$BaSO_4$†	Insoluble salt	XRF	Very high	None, dense	Radiography
Ce, Dy, Er, Eu, Yt, Yb†	Soluble rare earths	AA or XRF	Medium/high	Mainly solid-phase	Digesta flow, rate of passage, supplement
Ru-phenanthroline†	Soluble complex	AA or XRF	High	Mainly solid-phase	Digesta flow, rate of passage
Cr-mordanted fibre†	Bonded to fibre	AA or XRF	Very high	Solid-phase	Rate of passage
Plastic particles	Insoluble polymer	Physical	Very high	None	Rate of passage, faecal identification
Artificial alkanes	Insoluble wax	GC	Medium/high	Mainly solid-phase	Intake, supplement, rate of passage, CRD
CrEDTA†	Soluble complex	AA or XRF	Medium/high	Liquid-phase	Digesta flow, rate of passage
CoEDTA	Soluble complex	AA or XRF	Medium/high	Liquid-phase	Digesta flow, rate of passage
Polyethylene glycol	Soluble polymer	Turbidity	High	Liquid-phase	Digesta flow, rate of passage

AA, atomic absorption spectroscopy; XRF, X-ray fluorescence spectroscopy; CRD, controlled-release device; GC, gas chromatography.
* Inductively-coupled plasma spectroscopy (ICP) can be used in place of analysis by AA.
†Radioactive forms have been used which have resulted in substantially simpler and more accurate analysis (by γ-spectroscopy).

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The degree of disturbance caused by the daily dosing and the potential diurnal variation in faecal marker concentration can be reduced by using intraruminal controlled-release devices which, after placement in the rumen, release marker at a predictable constant rate for a period of 3–4 weeks. Devices delivering Cr_2O_3 have been commercially available and have been evaluated in a number of studies (for example see Furnival *et al*. 1991*a*,*b*).

Repeated daily dosing can also be avoided by collecting a series of faeces samples following a single dose of marker; faecal output can then be estimated from the area under the resultant faecal concentration × time curve. Such areas have been assessed either by integrating a mathematical function derived from the curve (for example see France *et al*. 1988) or, for a complete curve, by calculating the area as the sum of a series of trapezia (Galyean, 1993), as shown in Fig. 2. The first method provides additional information on digesta kinetics, such as passage rate, but the times of faecal sampling (or defecations) are required and the required frequency of faecal sampling may cause unreasonable disturbance to animals. Using the 'trapezium' method, the time and frequency of faecal sampling are less important; even if the time sequence of the faecal samples is incorrect, errors in area estimation are small (typically $< 5\,\%$). Thus the 'trapezium' method would be more appropriate in situations where the frequency of faeces sampling is limited. Furthermore, for faeces collected from the ground, the 'trapezium' method could be used to estimate faecal output when the actual times of defecation are not known. Although there appear to have been no direct comparisons of faecal output estimates obtained from single and repeated daily doses of marker, it could be expected that variation in daily faecal output rate during the sampling period could lead to greater errors for the former method.

Estimation of digestibility

Obtaining reliable estimates of digestibility represents a greater problem than measurement of faecal output, because errors in the estimation of digestibility will always cause a larger error in the resultant intake estimate, especially at higher digestibilities (Langlands, 1987; Dove & Mayes, 1996). The main difficulty is in obtaining a digestibility estimate which represents the diet consumed by the grazing or browsing animal. Even on single-species grass pastures, where

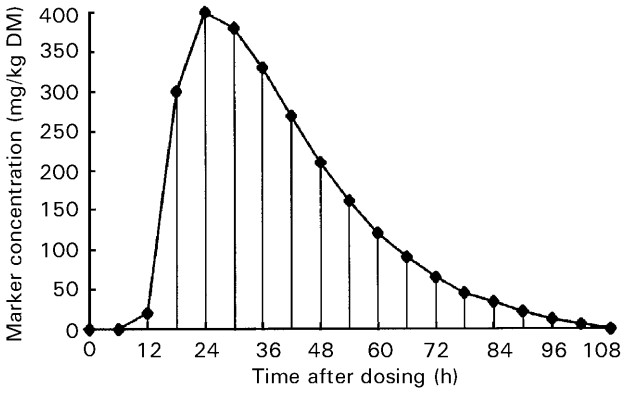

Fig. 2. Stylized representation of the 'trapezium' method for estimating the area under a faecal marker concentration *v*. time curve following a single administration of an external marker with a fixed sampling interval.

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the scope for variation in diet composition is limited, this is not easy. An approach, popular some years ago, is to use as an index of diet digestibility a component such as N, the faecal concentration of which is correlated with herbage digestibility (Le Du & Penning, 1982). Indoor trials with herbage closely similar to that of the experimental pasture are necessary to derive the relationship between the faecal concentration of the component and digestibility. Recently the faecal index method has been reassessed for a range of grass swards using a generalized regression equation with faecal N and faecal acid-detergent fibre as indices (Comeron & Peyraud, 1993). The most widely used method has been to measure digestibility *in vitro*. Although calibrations of *in vitro* × *in vivo* digestibility are necessary to obtain a predicted *in vivo* value, the number of indoor trials required to obtain such regressions is small. The original two-stage method of Tilley & Terry (1963) requires fresh rumen contents, which can be a disadvantage over cellulase–pepsin incubation. Bacterial cultures from ruminant faeces have also been used in place of rumen contents, (for example see Akhter *et al*. 1999).

These approaches, while in widespread use, have major disadvantages. Firstly, it has to be assumed that the same diet digestibility applies to all individuals in a group of animals grazing the same pasture; no allowance is made for individual-animal variation in digestibility, nor for the fact that digestibility is itself affected by intake (Le Du & Penning, 1982). Furthermore, the ages and physiological states of the animals used in the indoor calibration trials often differ from those of the test animals. Samples for *in vitro* analysis have frequently been provided by oesophageal-fistulated animals, the drawbacks of which have been discussed earlier. A further problem arises when consumed vegetation is only one of the components of a mixed diet, since the digestibility estimate required to determine intake should represent that of the whole diet. The difficulty in predicting whole-diet digestibility is further exacerbated when digestive interactions occur: for example, starchy components, such as cereal concentrates, can reduce the digestibility of roughages, such as herbage (for example see Dixon & Stockdale, 1999).

These problems can be largely overcome by using indigestible diet components as internal markers. Digestibility is estimated from the relative concentrations of internal marker in the diet and in faeces (i.e. 1 − diet : faeces concentration ratio). Since the faeces come from individual experimental animals, separate digestibility estimates for each animal are obtained. However, if the diet sample is considered, in terms of marker concentration, to represent material ingested by a group of test animals, the resultant digestibility estimates for the individuals are not strictly independent. With the internal marker method, the problem of obtaining a representative sample still exists; the scale of the problem compared with that incurred with the *in vitro* digestibility method depends upon the relative sampling variabilities for *in vitro* digestibility and the internal marker.

The internal marker method allows whole-diet digestibility to be determined when animals are receiving mixed diets, if the concentration of the internal marker in the complete diet can be estimated. When grazing animals are receiving feed supplements, the intake and the internal-marker concentration of the supplement must be known; this is not normally a problem as methods are available for estimating supplement intake (see later).

Some of the dietary components which have been investigated as internal markers are detailed in Table 3. Despite the potential advantages of using an internal marker to estimate digestibility, until recently the approach has been unsatisfactory, largely because the markers have failed to meet the criteria required of an ‘ideal’ marker (Kotb & Luckey, 1972). Many of the components used as internal markers have been characterized by inconsistent faecal recovery, mainly because most have not been discrete compounds. Their empirical nature means that it is uncertain whether a component measured in the faeces is the same entity as that measured in the diet. It is not clear whether low apparent recoveries in faeces are due to

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Table 3. Some internal faecal markers used for estimating digestibility in herbivores (see Kotb & Luckey, 1972; Penning & Johnson, 1983*a*,*b*; Smith, 1989; Dove & Mayes, 1996)

Marker	Type	Analysis	Recovery	Digesta association	Additional uses
Lignin	Fibre fraction	Extraction residue (empirical)	Variable	Solid-phase	Rate of passage*
Acid-detergent lignin	Fibre fraction	Extraction residue (empirical)	Variable	Solid-phase	
Indigestible ADF	Fibre fraction	Extraction residue (empirical)	Variable	Solid-phase	
Indigestible NDF	Fibre fraction	Extraction residue (empirical)	Variable	Solid phase	Rate of passage*
PIC	Fibre fraction	Extraction residue (empirical)	Variable	Solid-phase	
Acid-insoluble ash	Silicaceous	Extraction residue (empirical)	High	Solid-phase?	
Silica	Silicaceous	Various (discrete)	High	Solid-phase?	
Chromogen	Plant pigments	Colorimetric (empirical)	Variable	Uncertain	
Long-chain fatty acids	Plant-wax compound	Gas chromatography (discrete)	High	Mainly solid-phase	
Long-chain *n*-alkanes	Plant-wax compound	Gas chromatography (discrete)	Medium/high	Mainly solid-phase	Intake, diet composition, rate of passage*, digesta flow

ADF, acid-detergent fibre; NDF, neutral-detergent fibre; PIC, potentially indigestible cellulose.
* Radioactive forms have been used to estimate rate of passage.

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transformations of marker components in the gut, to absorption or to inconsistencies in analytical procedures. This may explain why a particular marker may be highly effective in some studies but useless in others.

With the exception of plant silica, for which soil contamination of analysed herbage can affect the reliability of digestibility estimates, it is only within the last 20 years that discrete compounds have been considered as internal markers for determining digestibility (Grace & Body, 1981). Whilst none of these compounds yet investigated could be considered to be an 'ideal' internal marker, the advantages over 'empirical' markers of having reliable analytical techniques and the ability to assess their behaviour in the digestive tract, whether through transformation or absorption, makes discrete markers potentially very useful in a wide range of situations. There is also potential for other plant compounds, not yet considered, to be effective internal markers.

Intake measurements using n*-alkanes as markers*

Plant wax *n*-alkanes were originally suggested as internal markers for estimating digestibility (Mayes & Lamb, 1984), since they are discrete compounds and are easily analysed. The concept of estimating intake by the concurrent use of two *n*-alkanes of adjacent chain length was first considered by Mayes *et al*. (1986*a*). In effect, a plant-wax (odd-chain) alkane is used as an internal digestibility marker, at the same time as a dosed even-chain alkane as a faecal-output marker. Whilst both digestibility and faecal-output estimates would be biased because of incomplete faecal recoveries, resultant intake estimates will be unbiased if the faecal recoveries of the two markers are the same. For plant odd-chain alkane, *i*, and dosed even-chain alkane, *j*:

$$\text{intake} = \frac{\text{dose rate}_j}{\frac{\text{faecal content}_j}{\text{faecal content}_i} \times \text{herbage content}_i - \text{herbage content}_j}.$$

Note that the presence of the even-chain alkane in the diet is taken into account. Using dosing regimens similar to that most commonly used for Cr_2O_3, numerous studies have demonstrated in sheep, cattle and goats that plant C_{33} and dosed C_{32} alkanes have very similar faecal recoveries and thus give estimates of herbage intake with very little bias (Dove & Mayes, 1991, 1996).

The alkane method for estimating intake offers a number of advantages over other techniques. It gives individual-animal intakes and can be used where animals are receiving feed supplements. Also, GC analysis allows both plant and dosed markers to be determined at the same time, which limits analytical error and bias. Since the ratio of the concentrations in faeces is used, it is not necessary to obtain absolute faecal concentrations. Direct comparisons of intake estimates using the alkane and *in vitro* digestibility–faecal output (Cr_2O_3) methods indicate the superiority of the alkane method for both cattle (Malossini *et al*. 1996) and sheep (Dove *et al*. 2000).

Direct comparisons of herbage intakes estimated using the alkane method and the *in vitro* digestibility–faecal output (Cr_2O_3) method have been conducted with sheep (Piasentier *et al*. 1995; Dove *et al*. 2000) and with dairy cows (Malossini *et al*. 1996). In both housed and grazing sheep, Piasentier *et al*. (1995) found that intakes estimated using the *in vitro*–Cr procedure were always higher than those estimated using $C_{31} : C_{32}$ alkanes, especially at higher intakes. Malossini *et al*. (1996) also reported an effect of level of intake on the discrepancy between the two methods, though in their case, when the daily intakes of dairy cows were

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above 14 kg OM, the *in vitro*–Cr procedure gave higher estimates whilst the alkane procedure ($C_{31}:C_{32}$) gave higher estimates below 14 kg OM/d.

More recently, Dove *et al*. (2000) demonstrated effects of both level of intake and physiological state (late pregnancy *v*. early lactation *v*. mid lactation) on the relationship between these methods in grazing ewes. They also showed that the major contributor to the discrepancy between the methods was the failure of *in vitro* estimates of digestibility to be representative of herbage digestibility *in vivo*. A recalculation of some of their data is shown in Table 4.

In ten of the twelve possible comparisons (four treatments, three stages), the *in vivo* and *in vitro* estimates of herbage digestibility differed significantly. Where the *in vivo* estimate was higher, the alkane-based estimate of intake was the higher of the two (positive discrepancy); when *in vivo* estimates were significantly lower, so also was the alkane-based estimate of intake (negative discrepancy). Where there was no significant difference between the two estimates of digestibility, there was no consistent difference in the discrepancy between the two estimates of intake (one positive, one negative). The presence or absence of supplement had no effect on these relationships. These observations are consistent with the explanation that the alkane procedure accommodated the difference in the digestibility estimates and resulted in a different estimate of intake (Dove *et al*. 2000). The significant differences in the *in vivo* and *in vitro* estimates of digestibility relate mainly to the effects of level of intake and physiological state on gut transit times (Dove *et al*. 2000). From the comparisons to date, it is clear that no simple relationship should be expected between herbage intake estimates based on alkanes compared with the *in vitro*–Cr procedure.

In common with other intake methods requiring the analysis of dietary samples, the feed sample must be representative, with respect to its alkane content, of the material ingested by the animals. As alkane concentrations can differ for different plant parts and plant species, care must be taken in sampling the vegetation for analysis. Although oesophageal-fistulated animals have been used to collect samples of ingested vegetation, for uniform swards, they have been shown to be unnecessary; hand-clipped grass samples are adequate. The concentrations of the predominant *n*-alkanes in hand-clipped herbage samples (75 m × 0·15 m area at 10 mm height above the soil surface) from a uniform *Lolium perenne–Phleum pratense* plot (0·48 ha) and in

Table 4. Comparison of the discrepancy between alkane-based ($C_{32}:C_{33}$) and *in vitro*–Cr-based estimates of herbage intake, and the difference between *in vivo* and *in vitro* estimates of the digestibility of the herbage consumed by grazing ewes (recalculated from the data of Dove *et al.* (2000))

State	Grazing pressure*	Supplement	Intake discrepancy (g OM/d)†	(*In vivo* − *in vitro*) digestibility‡
Pregnancy	High	−	136	0·068 ($P<0·05$)
		+	56	0·115 ($P<0·05$)
	Medium	−	− 109	− 0·041 ($P<0·05$)
		+	− 144	0·006 (NS)
Early lactation	High	−	134	0·052 ($P<0·05$)
		+	272	0·079 ($P<0·05$)
	Medium	−	410	0·101 ($P<0·001$)
		+	251	0·125 ($P<0·05$)
Mid lactation	High	−	− 55	− 0·074 ($P<0·01$)
		+	− 58	− 0·110 ($P<0·01$)
	Medium	−	397	− 0·016 (NS)
		+	346	0·043 ($P<0·05$)

OM, organic matter.
* High, 30.8 ewes/ha, mean herbage intakes 662–1218 g OM/d; medium, 17·1 ewes/ha, mean herbage intakes 904–1874 g OM/d.
†Intake estimated using $C_{32}:C_{33}$ alkane pair minus intake estimated using *in vitro*–Cr method.
‡Significance levels of differences between *in vivo* and *in vitro* estimates of herbage digestibility in parentheses.

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Table 5. Comparison of *n*-alkane concentrations (mg/kg dry matter) in extrusa samples from oesophageal-fistulated sheep grazing a *Lolium perenne–Phleum pratense* plot and hand-clipped samples taken from the same plot (data of G.R. Iason, unpublished results)

(Mean values and standard deviations)

Sample	*n*		C_{27}	C_{29}	C_{31}	C_{33}	C_{35}
Extrusa from oesophageal-fistulated sheep	5	Mean	30·8	56·7	89·5	89·3	13·8
		SD	4·24	5·61	6·28	10·56	1·34
Hand-clipped herbage	10	Mean	31·8	56·6	91·4	90·6	14·0
		SD	5·72	8·86	10·62	10·90	1·63

extrusa samples from oesophageal-fistulated sheep grazing the same plot are shown in Table 5 (GR Iason, unpublished results). It is clear that, in this study, differences between the two sample types were negligible.

The fact that faecal alkanes can also be used to determine diet composition may entirely remove the need to use oesophageal-fistulated animals; this could well be the greatest advantage of the alkane technique. In such cases the estimate of diet composition provides estimates of the alkane concentrations of the whole diet, with which intake can then be estimated.

Although Mayes *et al*. (1986*a*) were unable to find evidence of systematic diurnal variation in the faecal concentration ratios of dosed and herbage alkanes, such variation was observed in later studies. In dairy cattle, Dillon & Stakelum (1989) showed that the dosed alkane (C_{32}) was responsible for the diurnal variation. Thus, as with Cr_2O_3, the method of alkane dosing may influence the degree of diurnal variation in the faecal alkane ratio. Various carrier matrices for dosed alkanes have been tested, including shredded paper, powdered cellulose, paper stoppers and aqueous suspensions. The relative attributes of these have been discussed recently by Mayes & Duncan (1999). Alkane controlled-release devices have been developed to deliver even-chain alkanes for determining intake. They are designed to deliver alkane over a period of 20 d after insertion; faeces can be sampled after the first 7 d. The devices have been shown to be reliable (Table 6) and are now commercially available in sizes that allow intake estimation in adult sheep, and growing and adult cattle (Captec Alkane^{TM}, Captec (N.Z.) Ltd, Auckland, New Zealand).

Analogous to the use of a single dose of an external marker to estimate faecal output, dietary intake can be estimated using *n*-alkanes with the dosed alkane given as a single administration. Intake is calculated in the same way as for daily dosing except that the ratio of areas under the faecal excretion curves for the two alkane markers is used. This method has been validated in laboratory rabbits (Letso, 1996). It has not been tested in ruminants, but a modification of this procedure, developed for estimating both intake and diet composition over

Table 6. Effectiveness of controlled-release devices (CRD) as a means of administering C_{32}-alkane for the purpose of estimation of intake in sheep: discrepancies between actual intake of grass dry matter (DM) and intakes estimated using C_{32}- and C_{33}-alkanes as markers

Reference	Days after CRD insertion	*n*	Actual intake mean (g DM/d)	*Discrepancy (g DM/d)	
				Mean	SE
Mayes *et al.* (1991)	6–13	24	913·5	+0·2	16·50
Molle *et al.* (1998)	7–11†	5	666	+18	21·0
	14–18	5	717	−45	15·9
	21–25	5	690	−1	82·3

* Actual DM intake minus DM intake estimated using alkane markers.
†Same sheep and CRD used in all three measurement periods.

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a period of 6–48 h, has been evaluated in sheep (Duncan *et al*. 1999); in such an approach, not only the dosed alkanes but also dietary alkanes over the short grazing period (present at much higher concentrations than in the feed given before and after the experimental period) were considered as being 'single doses'.

The use of marker- and digestibility-based methods for estimating intake and dietary composition in free-ranging non-ruminant animals

Few attempts have been made to measure intake in free-ranging non-ruminant herbivores, compared with domestic ruminants, though theoretically the majority of available techniques could be used. However with a substantial increase in recent years in the outdoor rearing of pigs (considered herbivores for the purpose of this review) and with a growing interest in horse nutrition, there is a greater need to be able to determine intake of grazed vegetation by these animals.

Conventional faecal markers have been used in pigs and horses, primarily for estimation of diet digestibility and faecal output (for example see Moughan *et al*. 1991; Jagger *et al*. 1992; Todd *et al*. 1995) in housed animals. However, the concept of using an *in vitro* digestibility estimate for the purpose of intake determination in such animals has not been adopted. This is despite the fact that a number of *in vitro* methods have been developed, relevant to both pigs (Moughan, 1999) and horses (Miraglia & Tisserand, 1985; Lowman *et al*. 1999), to predict *in vivo* digestibility for the purpose of giving an indication of the nutritive value of the diet.

By contrast, the alkane marker technique for measuring intake has been tested in non-ruminants. Faecal alkane recovery measurements and intake validation trials have been carried out in horses (Dove & Mayes, 1996; O'Keefe & McMeniman, 1998). The alkane method was shown to be reliable, but unlike results in ruminants, the faecal recoveries of both dietary and dosed alkanes did not decrease with decreasing C-chain length. Gannon (1996) demonstrated that the alkane marker gave reliable intake estimates in pigs. However, Wilson *et al*. (1999) observed differences between the faecal recoveries of the natural (C_{33}) and dosed (C_{32}) alkanes, which led to bias in resultant intake estimates. Despite the obvious need to investigate discrepancies in validation results between different studies, estimates of herbage intake by free-ranging pigs have provided a useful insight into their nutrient supply (Gannon, 1996; MG Rivera Ferre, SA Edwards, RW Mayes, I Riddoch and D Hovell, unpublished results). Letso (1996) obtained reliable intake estimates in laboratory rabbits fed on pelleted grass meal, when the dosed alkane was administered either daily or as a single dose. These studies indicate that the alkane method can be used to estimate dietary intake in a range of non-ruminants and could probably be applicable to many other species. The method has recently been used in free-ranging elephants (IJ Gordon, personal communication).

Diet composition in wild non-ruminants has been studied by examination of stomach contents and faeces sampling using microhistological techniques (Wallace-Drees *et al*. 1986; Cornelis *et al*. 1999). The limitations of this technique are generally the same as those in ruminants. Plant-wax alkanes have been used as faecal markers to determine diet composition. The relative faecal recoveries of individual alkanes are similar in pigs (Gannon, 1996; Wilson, 1998), horses (Dove & Mayes, 1996) and hares (Hulbert, 1993), which suggests that in non-ruminants, in contrast to ruminants, recovery corrections are not required (Fig. 3). Using alkanes as diet composition markers, Hulbert (1993) characterized seasonal changes in the diet of mountain hares in the Scottish highlands. Alkanes have also been used to estimate diet composition in Australian marsupial species (for example see Woolnough, 1998).

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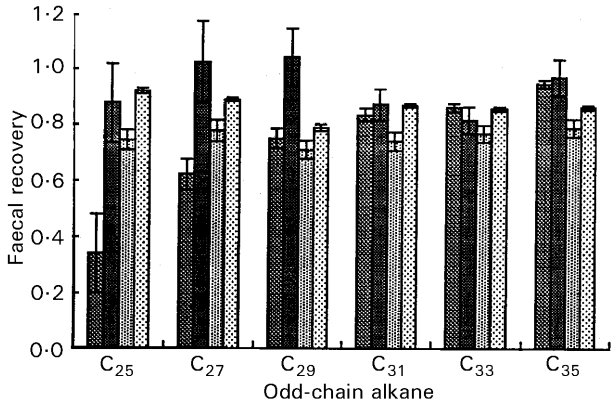

Fig. 3. Faecal recoveries of odd-chain *n*-alkanes (C_{25}–C_{35}) in: (▩), sheep (Dove & Mayes, 1991); (▩), mountain hares (Hulbert, 1993); (▩), pigs (Wilson, 1998); (▩), horses (D Cuddeford and RW Mayes, unpublished results). Values are means with standard errors represented by vertical bars.

Other animal-based methods of measuring intake

Estimating intake and diet composition from behavioural observations

At its simplest, the behaviour of a foraging herbivore can be directly observed and its intake rate obtained from the time spent consuming a food resource and/or the number of bites taken per unit time on that resource. Where there are several food types on offer, such as different vegetation patches or browsable tree species, this approach could be applied to obtain a measure of diet composition. A logical means of obtaining an estimate of intake from behavioural measurement is:

$$\text{intake (kg DM/d)} = \text{bite rate during feeding (bites/h)} \times \text{feeding time (h/d)} \times \text{bite size (kg DM/bite)}.$$

Such estimates of intake are likely to be crude for the following reasons: (1) the size of a 'bite' can vary greatly; (2) the observation period is likely to be relatively short (usually less than a day); (3) it may be difficult to make clear observations without disturbing the animals.

Because a herbivore may use different ingestive strategies for different plant species and plant parts, bite sizes are likely to vary greatly between the types of material eaten. Different techniques for measuring bite size may be required for different types of plant material. A simple 'plant-based' approach has been adopted with some success for browsing animals where the bite size can be assessed as the diameter of a bitten tree twig (Vivås & Sæther, 1987) or the distance from the end of the twig that leaves have been stripped. Bite size can be determined by measuring the relevant amount of material removed by an operator from similar intact twigs. Although relatively large numbers of measurements are required, the variability of bite size can be assessed.

More widely used animal-based methods, particularly for animals grazing high-quality swards, involve the use of oesophageal-fistulated animals or measurement of short-term live-weight change. When oesophageal-fistulated animals are used, the amounts of extrusa collected and the number of bites are monitored. Both the type of ingested plant material and bite size must be representative of the intact test animals, and there must be no loss of ingested material through swallowing or spillage. For determination of bite size by short-term weight change,

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animals are fitted with harnesses for total collection of excreta and the total number of bites taken over a grazing period of 1–2 h is recorded (Penning & Hooper, 1985). The animals are weighed immediately before and after the grazing period and corrections made for evaporative H_2O loss and production of CO_2 and CH_4. Such weight loss, the insensible weight loss, has been determined by preventing the same animals from feeding by penning outdoors, for a similar duration before or after the grazing period. Although the availability of highly accurate weighing machines has improved precision, several factors could still limit the accuracy of measurements. Estimates of insensible weight loss may not be strictly valid since there could be differences in metabolic rate, gut fill and fermentation rate between feeding and penned animals. However, such measurements are easier to make and probably less prone to error than a more direct alternative approach, such as using tracer techniques to measure H_2O turnover and CO_2 production rate. Furthermore the weight-change method could be unreliable in rainy or misty conditions when the animal's coat can take up water. Even dew on the vegetation may be a problem, as the herbage DM content may be extremely variable under such conditions.

Both of these animal-based methods give mean estimates of bite size representative of a short period of grazing. Gibb *et al*. (1998) showed that bite size can change throughout the day, and this needs to be considered when using behavioural techniques to obtain intake estimates which represent daily or longer-term values. Despite these reservations, it has been shown in goats that intake estimates obtained using short-term weight change to determine bite size and direct manual observation over short periods to measure bite rate were very similar to intakes obtained using C_{32} and C_{33} alkanes as markers (Sendalo, 1995). Whilst such work does not validate either intake-measurement technique, it does suggest that both approaches can give reasonable estimates of the true intakes.

In theory, the above limitations of using direct behavioural observations to determine intake can be overcome: continuous manual observations could be made over a period of many days taking care to minimize disturbance. It is largely because of the logistical impracticality of such an approach that automated devices to monitor foraging behaviour have been developed. Photographic and video-recording equipment have been widely used to observe animal locations and make simple behavioural observations about the animal (e.g. grazing, walking or lying down). However, the resolution of such techniques is such that it is rarely possible to measure bite rate or, unless the vegetation type is present in discrete patches, the plant species or plant part from which individual bites were taken.

Alternative approaches in which the animals carry the monitoring equipment have been developed over many years. A simple device for recording grazing activity in sheep or cattle on short swards, in terms of the lowering of the head to represent grazing, has been available for many years (Vibracorder, Kienzle Apparate GmbH, Villingen, Germany; Allden, 1962); tilt switches have also been used. Since such devices are not capable of recording individual bites, equipment has been developed to record jaw movements by various means, including pneumatic balloons, strain gauges and, more recently, a C-packed rubber tube which, upon stretching, exhibits a change in its electrical resistance (Penning, 1983). Microphones to monitor the sound of jaw activities have also been used (Laca *et al*. 1994). The outputs from these transducers have been recorded as analogue signals on magnetic tape, or digitally to a fixed computer via a radio link or a data recorder mounted on the animal. These devices have allowed the ingestive behaviour of animals to be monitored continuously for periods of 24 h or more. Since jaw movements are not restricted to biting activity, there is a need to distinguish biting, chewing and, in the case of ruminants, rumination activities from the recordings. The tedium of making such interpretations has recently been removed by the development of

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computer software to automate the process. Rutter (1999) has recently reviewed automated techniques for monitoring ingestive behaviour.

Intake methods using automated monitoring equipment have generally been restricted to domestic livestock grazing high-quality pastures. Under such conditions, for example dairy cattle on perennial ryegrass swards, this approach has been effective in improving understanding of the effects of pasture-management strategies on animal performance. For the equipment to be used in a wider range of scenarios, such as in forest and rangeland environments, further development work is required. As the ingestive behaviour of a herbivore will vary according to the nature of the plant being harvested, the interpretative software for discriminating jaw activities may need much revision to cope with animals in heterogeneous environments. Existing equipment is probably too fragile to allow reliable data acquisition in many semi-natural environments. However, for a number of years, devices using radio transmitters or global positioning systems, developed to locate wild animals, have been highly successful. With modern electronic technology it is feasible to make more robust equipment for monitoring ingestive behaviour and to increase the number of measurement variables, their accuracy and their duration (Rutter, 1999). It is unfortunate that, for reasons of cost and logistics, the number of animals which could be simultaneously fitted with monitoring equipment is always likely to be limited.

Intake estimates based on turnover rates

Dietary intake has been estimated in free-ranging ruminants from determination of their water turnover rates by sequential blood sampling following a single injection of $^{3}H_2O$ (Benjamin *et al*. 1975; Silanikove *et al*. 1987). Under arid conditions when no drinking water was available, H_2O turnover rate was assumed to be the same as H_2O intake and DM intake could be calculated from knowledge of the DM content of the diet. The method can also be used when the intake of drinking water is known. Under less arid conditions, many herbivores can be expected to meet their requirements for H_2O entirely from the diet and so, in theory, the H_2O-turnover method could be used to determine intake. However, there is potential for dietary moisture content to vary considerably and collecting representative samples of vegetation could be difficult, especially with the incidence of rain or heavy dew. Potential restrictions on the use of $^{3}H_2O$, due to its radioactivity, could be avoided by using $^{2}H_2O$ instead (Rogers *et al*. 1985). Despite the number of estimates of energy expenditure made in free-ranging herbivores by the 'double-labelled water' method, which involves measuring the turnover rates of ^{2}H and ^{18}O in the body-H_2O pool (Nagy *et al*. 1999), the potential to use the ^{2}H data to estimate dietary intake has not been exploited.

A similar approach, but without the problems associated with free H_2O consumption and variation in dietary moisture content, is to estimate intake from the turnover rate of Na in the animal and the dietary Na content (Green, 1978). Na from the diet is completely absorbable from the gut (Farley & Robbins, 1997) and thus equilibrates with the body-Na pool. Na-turnover rate can be determined by measuring the radioactivity of blood plasma or serum following a single injection of ^{22}Na as a tracer. This method has been quite widely used to measure dietary intake in carnivores but its use in herbivores has been limited; the method has been tested in rabbits (Green & Dinsmore, 1978), reindeer (Staaland *et al*. 1982) and cattle (Silanikove *et al*. 1987). Its accuracy is higher for diets of relatively high Na content (Farley & Robbins, 1997); unfortunately, the diets of most herbivores have relatively low Na levels. As with $^{3}H_2O$, the use of ^{22}Na as a radioactive tracer may be restricted for legal and safety reasons.

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^3H_2O and ^2H_2O have also been used to obtain estimates of milk intake both in domestic species (for example see Dove, 1988) and wild herbivores (for example see Krockenberger *et al*. 1998). By concurrently administering ^3H_2O to the dam and ^2H_2O to the offspring, milk intake can be estimated from the total body-H_2O turnover of the offspring and the proportion of this coming from milk (Holleman *et al*. 1975; Dove, 1988). The difference between the two represents mainly the intake of H_2O in vegetation and drinking water. Krockenberger *et al*. (1998) recently estimated the foliage intake of young koalas (*Phascolarctus cinereus*) from the 'non-milk' component of their total H_2O turnover. This requires an estimate of the foliage DM content and assumes that the free H_2O intake is negligible. In koalas, this assumption is reasonable (Krockenberger *et al*. 1998).

Estimating intake of components other than grazed vegetation

In certain circumstances, free-ranging herbivores may consume components additional to grazed or browsed vegetation, such as dietary feed supplements, milk in young herbivores, and ingestion of soil, both intentional or circumstantial. These can have a large effect on the total intake of nutrients and other substances. Thus there may be a need to determine concurrently the intake of both vegetation and the additional component.

Estimating supplement intake

In agricultural production systems, supplementary feeding is commonplace and a knowledge of the supplement intake of individual grazing animals would greatly facilitate progress in understanding the response to supplements. The possibility of associative effects of cellulosic (herbage) and starchy (cereal supplement) diet components on digestibility has been mentioned earlier. However, the effects of feeding of grain supplements on the voluntary intake of herbage (substitution) are usually larger than those on digestion itself (Dixon & Stockdale, 1999), implying that the substitution effect relates to factors in addition to the effect of the supplement on the rate of digestion of the herbage. This highlights the need for methods of measuring intake of both vegetation and supplement that do not depend upon estimates of *in vitro* digestibility, but which accommodate the level of whole-diet digestibility occurring in individual animals, whatever their supplement intake.

Methods for estimating supplement intake have been reviewed recently by McLennan (1999). Most have been based on the concept of labelling the supplement with a marker and, depending on the type of marker, monitoring its concentration in either faeces, blood plasma or body H_2O. The size of the pool in which the marker is distributed must also be quantified in some way. A collation of some published marker-based estimates of supplement intake is given in Table 7.

Accurate estimates of supplement intake in penned animals were obtained with Cr_2O_3 (Dove & Coombe, 1992; Table 7), while Curtis *et al*. (1994) found that in grazing animals consuming lupin seed from a self-feeder, the summed individual intakes were within 5 % of the weight of lupins added to the feeder. By contrast, Dove & Oliván (1998) found that when C_{38}-alkane was sprayed on sunflower meal as a marker, supplement intake was significantly underestimated (Table 7), because of the low faecal recovery of the C_{38}-alkane. In free-ranging animals faecal output can be determined using methods described earlier, such as total col-

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Table 7. Collation of some published estimates of the accuracy of marker-based estimates of supplement intake in sheep

Reference	Marker	Sample*	Regression†, $y = mx + b$			Regression differs from $y = x$?
			m	b	r^2	
Dove & Coombe (1992)	Cr_2O_3	Faeces	1·05	− 4·50	0·963	No
Dove & Oliván (1998)	C_{38} alkane	Faeces	0·84	− 7·37	0·989	Yes
Dove & Oliván (1998)	Alkane mix‡	Faeces	0·99	10·66	0·991	No
Kahn (1994)	Lithium	Plasma	1·00	0·03	0·98	No
Juwarini *et al.* (1981)§	^3H_2O	Body water	0·97	10·81	0·990	No
Dove *et al.* (1995)	^3H_2O	Body water	1·07	− 12·28	0·957	No
Dove (1984)	[^{3}H]gypsum	Body water	1·07	− 19·58	0·973	No
Dove & Coombe (1992)	[^{3}H]gypsum	Body water	0·97	8·86	0·997	No

* Sample in which marker actually analysed.
† y = supplement intake estimated using marker; x = known supplement intake. See original publications for standard errors of regression variables.
‡ Supplement intake estimated from estimate of supplement proportion in total intake.
§ Calculated from data in Juwarini *et al.* (1981).

lections with bags and harnesses or from dosing with another marker such as C_{36}-alkane (Dove & Oliván, 1998).

An alternative is to use markers which can be monitored in the blood. Juwarini *et al.* (1981) labelled whole grain with ^3H_2O, by soaking oven-dried wheat in ^3H_2O and allowing it to air-dry. Dove *et al.* (1995) used this method with oat grain and, in both studies, measurement of the accumulation of ^3H in the body-H_2O pool resulted in accurate estimates of supplement intake (Table 7). To prevent evaporative losses of ^3H_2O, Dove (1984) mixed plaster of Paris with ^3H_2O to form a stable marker ([^{3}H]gypsum; $CaSO_4.2H_2O$) which was then incorporated into the supplement (10 g/kg). This proved an excellent marker for estimating supplement intake (see Table 7). Like the method of Juwarini *et al.* (1981), it requires estimates of ^3H_2O dilution space and H_2O turnover rate in the animals (for details see Dove, 1984). In theory, ^3H_2O could be replaced by non-radioactive ^2H_2O.

Suharyono *et al.* (1991) and Kahn (1994) determined supplement intake by monitoring Li levels in blood plasma 4–14 h after offering animals a single feed of supplement containing LiCl. Having assumed that Li distribution volume was proportional to live weight, the product (Li concentration × live weight) for each animal was expressed as a proportion of the sum of these products across all animals; this gave an estimate of the proportion of the total supplement offered which was consumed by each animal. Although the method gives accurate estimates of supplement intake (see Table 7), care is needed to avoid feed aversions due to high dietary levels of LiCl (Burritt & Provenza, 1989). To avoid this, Kahn (1994) suggested that supplement labelling should aim to result in daily intakes of about 50 mg LiCl/kg live weight. Another possible disadvantage of this approach is that, in more extensive feeding situations, a single day may not be sufficient time to obtain an estimate of the supplement intake for all individual animals.

Another method of estimating supplement intake is to consider the supplement as one component of a mixed diet and to determine the diet composition from the patterns of *n*-alkane in the faeces and in the dietary components, using the method described earlier. Intake of both basal diet and supplement can then be determined from the same analysis by reference to dosed C_{32} -alkane. To use this approach, the supplement must have alkanes at reasonable levels with a profile that differs from that of the basal diet. This is so with forage-based supplements (e.g. hay, silage) and some grains such as wheat or oats. Hameleers & Mayes (1998*b*) determined intakes of grass silage and grazed herbage in cattle by this method. Supplements which have no plant wax, such as legume grains and extracted oilseed meals, could be sprayed with alkane

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mixtures. Dove & Oliván (1998) sprayed sunflower meal with beeswax, which has high levels of alkanes. This allowed the proportions of supplement and the roughage component (chaff) in the diet to be estimated. By reference to dosed alkanes, derived total and supplement intakes agreed very closely with known values (Dove & Oliván, 1998; Table 7).

Estimating soil intake

As mentioned earlier, free-ranging herbivores often ingest appreciable quantities of soil, either by choice or inadvertently; this may provide useful quantities of both trace elements and macro-elements (Dewes, 1996; Grace *et al*. 1996). Furthermore, many environmental pollutants, including radionuclides, heavy metals and organic compounds, accumulate in soil but their uptake by plants is limited (Jones, 1991; Webber *et al*. 1994). In these circumstances the ingestion of soil can be a major route of transfer to the animal (Beresford & Howard, 1991; Abrahams & Thornton, 1994).

One method of estimating inadvertent soil intake is to determine the degree of contamination of vegetation with soil and to measure vegetation intake. Another method is to estimate the output of soil in faeces. In the first method, great care is necessary in obtaining a sample of vegetation which is representative in terms of soil contamination. Various methods have been used to assess the soil content of a vegetation sample (for review see Hinton, 1992). Most have used elements which are present in the soil but are not taken up by plants; the degree of soil contamination can be estimated from the concentrations of the marker element in the vegetation sample and a representative sample of soil. The most widely used element has been Ti, but others, such as Sc, have also been employed. In comparative studies, Hinton *et al*. (1995) observed that differences in estimates of soil contamination obtained from different markers were due to differences in the distribution of the markers across different soil particle sizes. Thus soil particles adherent to vegetation may not have the same marker content as that of a bulk soil sample. It is possible that a different approach to measure soil contamination of vegetation, such as careful washing (Hinton, 1992), may be more accurate.

Total soil intake (including intentional ingestion) can be determined from the faecal concentration of an indigestible component, which is present in soil but not in vegetation. Ti has been the most popular marker (for example see Mayland *et al*. 1975) since TiO_2 is completely recovered in faeces (for example see Jagger *et al*. 1992). Acid-insoluble ash has also been used but has tended to overestimate faecal soil content, due to the presence of silica (the major component of acid-insoluble ash) in plant material (Mayland *et al*. 1975). To quantify the amount of soil excreted in faeces an estimate of total faecal output is required. Using Ti and dosed alkanes it has recently been shown that, in adult pigs at pasture, soil can comprise up to 50 % of faecal DM (MG Rivera Ferre, SA Edwards, RW Mayes, I Riddoch and D Hovell, unpublished results). No validation studies have been carried out in which faecal outputs of soil have been compared with known intakes. Thus, even if the Ti method gives reliable estimates of excretion rate of soil mineral matter, it is not certain whether such values give a true indication of soil intake. Faecal recoveries of soil OM are not known and it is possible that the amounts of soil residing in the gut may vary over
a considerable period.

Estimating intake of digestible nutrients and digestion end-products

In carrying out nutritional studies with free-ranging herbivores there are many instances in which knowledge of the intake of absorbable nutrients is more useful than that of the gross amounts ingested. For some nutrients, the estimation of digestibility in conjunction with an

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estimate of total intake would suffice but, for others, different methods would need to be employed.

As mentioned earlier, some of the methods for determining intake require estimates of whole-diet digestibility. Such methods can thus provide estimates of digestible DM or digestible OM intake; the latter is particularly useful as it closely approximates digestible energy intake. As mentioned earlier, plant-wax alkanes can be used as internal markers to provide digestibility estimates, but require correction for incomplete recovery (Mayes & Lamb, 1984); uncertainty in prediction of such recoveries may limit the accuracy of results.

An alternative method is to estimate digestibility in free-ranging animals from a knowledge of intake and faecal output, the latter being measured by total collection or an indigestible external marker. If intake is estimated using dosed C_{32}-and dietary C_{33}-alkanes as markers, the dosed C_{32}-alkane could, at the same time, provide an estimate of faecal output, but requires correction for incomplete recovery. A more satisfactory marker, because it has a faecal recovery of at least 95 %, is dosed C_{36}-alkane (Mayes *et al*. 1986*b*; Dove & Oliván, 1998). This alkane has been incorporated into the commercially available alkane controlled-release device for the express purpose of permitting estimates of faecal output and hence digestibility.

In ruminants, the influence of diet and rumen microbes on the supply of absorbable energy and protein cannot be adequately assessed by merely estimating the digestibilities of dietary OM and protein. The fermentation processes in the ruminant foregut have been quantified in housed animals, surgically prepared with cannulas in the rumen and abomasum or duodenum, by using intraruminally infused digesta-flow markers, such as CrEDTA and Ru-phenanthroline complexes, and microbial markers (e.g. [^{35}S]sulfate). The development of portable pumps for infusion of markers (for example see Corbett *et al*. 1976) and automatic digesta sampling equipment (Evans *et al*. 1981) extended such measurements to grazing animals (for example see Beever *et al*. 1986; Cruickshank *et al*. 1992). The natural alkanes in the diet have potential as markers for determining digesta flow through the duodenum, as no losses occur in the forestomachs and they remain attached to digesta particles (Mayes *et al*. 1988; Samaniego, 1996). With associated intake estimates they allow estimations of digesta flow to be made without the need for rumen cannulation or infusion equipment. However, dietary alkanes have not yet been used to measure digesta flow in free-ranging ruminants.

The need for surgical intervention to quantify rumen digestion of OM and microbial protein represents a major disadvantage of the approach. Not only are there ethical concerns regarding the health and well-being of surgically prepared animals but also their maintenance is labour intensive. However, microbial protein supply can be estimated without the need for cannulated animals by measuring the urinary excretion rate of purine derivatives (predominantly allantoin). These compounds originate primarily from the nucleic acid present in microbial material which, after leaving the reticulorumen, is digested and absorbed in the small intestine. Microbial protein supply can be estimated from relationships previously obtained between microbial protein leaving the reticulorumen and the urinary excretion rate of purine derivatives. Whilst this method could be used in free-ranging ruminants, it is necessary to collect urine samples and measure urinary output (Mayes *et al*. 1995).

Although equipment has been developed for the direct measurement of output and sampling of urine in free-ranging sheep (for example see Chambers *et al*. 1976) and cattle (for example see Betteridge & Andrewes, 1986), none has been entirely successful. Markers such as inulin, *p*-aminohippuric acid and CrEDTA could be used to estimate urinary output, since they are quantitatively excreted in the urine following intravenous infusion. This method has been tested in man (Bingham & Cummings, 1983) and caged mink (Wamberg *et al*. 1996) and, in theory, could be used in herbivores. Further simplification of the measurement of urine output

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would be possible if markers could be administered orally; their absorption from the gut and subsequent excretion would have to be quantitative. Experimental evidence suggests that salicyclic acid (Pagella, 1998) and orcinol (Martin *et al*. 1983) may be suitable markers, provided that the dose rate is too low to affect the animals physiologically. Without the need for dosing, creatinine has been used as an endogenous urinary marker to give an indication of urinary output. Although urinary creatinine output is relatively constant within individuals, it may not be sufficiently predictable to allow accurate estimates of urinary excretion rates (Faichney *et al*. 1998).

For a number of nutrients, especially trace and macro minerals, the extent of their endogenous excretion in faeces is sufficiently high that the apparent digestibility (absorption coefficient) gives a poor indication of transfer across the gut wall. This also applies to a range of pollutant radionuclides and heavy metals. With the availability of isotopic tracers it is possible to quantify endogenous faecal excretion of a range of essential mineral elements and pollutants, and hence to determine the extent of 'true' absorption. One approach, considering Ca for example, is to administer ^{45}Ca intravenously at the same time as conducting Ca balance measurements (for example see Martz *et al*. 1999); this allows the endogenous faecal excretion of Ca to be estimated. Various modifications of this approach to quantify the true absorption of radio-labelled Cs have been discussed by Mayes *et al*. (1996). The methodology now exists for determining the intakes of 'truly absorbable' elements by free-ranging animals. Howard *et al*. (1993) attempted to estimate the intakes of truly absorbable ^{137}Cs and ^{90}Sr in dairy cattle grazing contaminated pasture close to the Chernobyl nuclear power plant. The tracer radionuclides ^{134}Cs and ^{85}Sr were administered intravenously by portable infusion pump; herbage intakes and faecal outputs were determined using alkanes as markers. The relative outputs of the tracer radionuclides in milk and faeces enabled the endogenous faecal excretion and hence intakes of truly absorbable ^{137}Cs and ^{90}Sr to be determined.

Possible future directions in the development of methodologies for estimating intake in free-ranging herbivores

Recent technological advances in the areas of microelectronics, data processing and analytical chemistry offer considerable potential for improving our ability to estimate intake in free-ranging animals. Aspects that we consider as likely key areas are described below:

(1) developments in the use of markers: compared with the substances potentially available for use as markers, very few have been exploited for this purpose. In particular, the concept of using plant compounds as markers for characterizing diet composition is relatively new. With modern methods of chemical analysis the scope for using a wide range of plant compounds as diet composition markers is considerable. It should soon be possible to make accurate measurements of diet composition in conditions where animals have the opportunity of selecting highly complex diets.

    There is also potential to exploit, as markers, compounds that are excreted in urine. In addition to the use of urinary allantoin to predict microbial protein synthesis, hippuric acid has the potential of being used to study interactions between the fermentation of starch and fibre, since much of it originates from the rumen digestion of lignocellulose (Mayes *et al*. 1995). Urinary metabolites may be used to identify plant species eaten by herbivores, since breakdown products of many plant secondary compounds appear in the urine;

(2) combination of different measurement techniques: the ability to estimate nutrient intake in free-ranging herbivores could be much improved through the concurrent use of different

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methodologies. Examples have already been mentioned but there are many more. For instance, Garcia *et al*. (1999) estimated intakes of maize silage and pasture grass in grazing cattle using a combination of the $\delta^{13}C$ and alkane techniques. Using faecal and urinary markers together allows both digestible intake and microbial protein synthesis rate to be determined simultaneously, thus giving an insight into protein–energy relationships in the rumen. Furthermore, the estimation of urinary output, dietary intake and faecal output would enable various nutrient (e.g. N and minerals) balance studies to be conducted in free-ranging animals (Mayes *et al*. 1995). The use of marker methods together with behavioural monitoring techniques can also be exploited. For example, global positioning system techniques can be used to monitor both the location of an animal and when the animal occupied that location. This information not only can be used to give an indication of the vegetation ingested by the animal but also can indicate the location of faeces and the time that defaecation occurred;

(3) intake measurements in herbivores inhabiting semi-natural environments: through many of the advances described above it is likely that much progress will be made in the ability to determine intake in animals, particularly wild herbivores, foraging within a range of extensive habitats. In conjunction with global positioning systems monitoring, the development of controlled-release devices to administer markers over a sustained period offers the opportunity not only to estimate intake and faecal output but also to monitor individual ruminants within a group; a unique combination of marker administered via a controlled-release device to each animal would enable faeces to be identified. Although controlled-release devices are not presently available for non-ruminants, it may be possible to use single doses of exogenous markers to identify faeces and estimate intake.

Both faeces and urine (samples retained in snow) have been used to assess nutritional status in wild herbivores. Levels of N and diaminopimelic acid (an amino acid synthesized by bacteria in the gut) in faeces have been used to assess the nutritional status of wild deer (for example see Kucera, 1997). Urinary allantoin : creatinine and urea : creatinine ratios have been used for the same purpose (Vagnoni *et al*. 1996; Moen & DelGuidice, 1997). Although, to date, these markers have been used as crude indices of nutritional status, it is possible that, in combination with intake and diet-composition data, these and similar markers could provide a means of obtaining more quantitative nutritional information, in particular about the protein and energy status of the animal;

(4) beyond mammalian herbivores: despite the title of this present review, it is likely that most of the described techniques to determine dietary composition and intake are not restricted to mammals or to herbivores. For example, limited studies have been carried out on the use of alkanes as markers in farmed fish (Gudmundsson & Halldorsdottír, 1995), chickens (Hameleers *et al*. 1996) and farmed ostriches (Orr, 1998). Furthermore, it is possible that the cuticular hydrocarbons, which are abundant in insects, could be used as markers to measure intake and diet composition in insectivores, such as bats.

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