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Methodology for studying vegetation of grazing lands and determination of grazing animal responses

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SUMMARY – Information on vegetation of grazing lands and animal grazing responses is critical to our understanding of livestock production and our ability to manage both animal and plant resources to optimise the productivity of grazing lands. A battery of methods has been developed to tackle these objectives. Methods concerning vegetation productivity of grazing lands include forage production, cover, density, forage quality and utilisation. The techniques available for measuring these attributes of vegetation are often laborious and subject to numerous errors. Techniques that work well under one set of grazing land conditions may be totally inadequate under another set of conditions. Also measurements taken only on the vegetation and extrapolated as conclusions for animal performance lead to erroneous results, since animals express selective grazing and use the forages in a different way from what is estimated by laborious techniques. The level of production of animals grazing these vegetation types is dependent upon their ability to obtain a diet adequate to meet their nutrient requirements for maintenance, growth and reproduction. This in turn is regulated by tactical decisions made by the animal about, for example, diet selection and how long to search between bites. These decisions influence the rate of food intake and nutrient composition of the diet. It is clear that efforts have been intensified towards better prediction of these two last important parameters using, for example, n-alkanes and NIRS techniques. These techniques may be suitable for grazing lands hosting a mixture of plant species, while, other simple techniques may be used for pastures and ranges dominated by one species. The situation becomes complicated when herbaceous species and shrubs are available at the same time in the grazing land. Lastly, techniques developed for measuring rate of intake, foraging behaviour and diet composition are discussed with respect to their accuracy, feasibility, rapidity and cost. It is concluded that there is an increasing awareness of the necessity to understand the foraging strategy of domestic livestock in the development of efficient management systems to meet agricultural and environmental goals. Standardisation of developed techniques is recommended if the exchange and valorisation of findings between different laboratories are targeted.

Keywords: Techniques, vegetation description, grazing animals, grazing lands.

RESUME – "*Méthodologie d'étude de la végétation et du comportement des animaux sur parcours". La connaissance de la nature et de l'état de la végétation sur un parcours et la maîtrise du comportement de l'animal sont nécessaires pour définir une meilleure stratégie de conduite des animaux et de gestion des ressources pastorales. De nombreuses méthodes directes et indirectes ont été développées pour atteindre ces objectifs. Parmi les techniques permettant de caractériser la végétation dans les parcours on cite l'estimation de la biomasse disponible, du taux de recouvrement, de la densité et de la valeur alimentaire et du degré d'utilisation des ressources pastorales par l'animal. De nombreuses techniques sont compliquées et sujettes à des erreurs. Par ailleurs, l'extrapolation des résultats des mesures prises sur le végétal pour interpréter les performances animales n'est pas souvent recommandée. Les animaux sur parcours manifestent un comportement de sélectivité et d'utilisation des ressources fourragères très variable. Le niveau de production des herbivores sur parcours dépend de la composition et de la qualité nutritionnelle de la ration qu'ils prélèvent. Pour atteindre cet objectif l'animal engage des décisions tactiques pour la sélection de sa ration et le temps qu'il doit consacrer pour aboutir à la composition et la qualité de la ration recherchées. La détermination de l'ingestion et de la composition de la ration sont donc deux paramètres très importants pour une gestion du parcours et une conduite efficace des animaux. Ainsi de nombreuses méthodes ont été développées pour estimer ces deux paramètres, dont celles qui reposent sur l'utilisation des n-alcanes et la spectroscopie en infra-rouge (NIRs). Ces deux dernières techniques sont intéressantes dans le cas d'animaux sur des parcours associant différentes espèces végétales. Des techniques plus simples pourraient être appliquées pour des parcours renfermant une seule espèce végétale. La tâche devient très compliquée quand il s'agit de parcours regroupant à la fois des plantes herbacées et des arbres et/ou arbustes. Les méthodes développées pour la prédiction de l'ingestion, de la digestibilité et de la composition de la ration sont discutées dans cet article en relation avec leur degré de précision, faisabilité, rapidité et leur coût. En conclusion, il ressort qu'une attention particulière devrait être accordée à l'étude des mécanismes permettant de comprendre le rôle de la stratégie fourragère définie par l'animal sur parcours dans le développement de schémas de gestion efficaces et permettant un équilibre agro-*

environnemental durable. Toutefois, il semble opportun de penser à la standardisation des techniques de laboratoire si un échange scientifique fructueux entre les chercheurs de différents pays est ciblé.

Mots-clés : *Techniques, végétation, réponse de l'animal, parcours.*

Introduction

In a FAO's report (1995), it was mentioned that livestock use 3.3 billion hectares of grazing lands such as rangelands (i.e. grasslands, shrublands, savannas, tundra and open forest lands), pasturelands and grazed forests. To most effectively manage vegetation and grazing animals for livestock production with care to avoid overuse and destruction of natural resources, we need information concerning vegetation ecology and an understanding of plant-animal interactions. Measurements or estimations of vegetation characteristics, such as weight, cover, density, and nutritional value and of foraging behaviour are vital to achieve this knowledge. Although in the past 40 years several new techniques have been developed or old techniques have been modified, the problem concerning what is the best technique in terms of accuracy and precision stays open.

The objectives of this paper are to: (i) review and evaluate the most common techniques used for the determination of vegetation characteristics of grazing lands and grazing animal responses; and (ii) to make recommendations for research needs for rangeland and livestock managers.

Measurement techniques of vegetation

Terms such as biomass, herbage, browse, forage, cover and density are used to describe the ecological condition of grazing land and its ability to contribute nutrients to a grazing animal. Biomass is one of the most important characteristics of grazing land vegetation and it is defined as the total amount of living plants and animals above and below ground in an area at a given time (Range Term Glossary Committee, 1989). According to the same source, herbage is "herbs taken collectively or total above ground biomass of herbaceous plants regardless of grazing preference or availability"; browse is "that part of leaf and twig growth of shrubs, woody vines and trees available for animal consumption"; and forage is "browse and herbage which are available and may provide food for grazing animals or be harvested for feeding". Availability and acceptability should be included in the definition of forage, describing the portion of the forage production that is available and accessible for use by a specified kind or class of grazing animals. Cover is "the plants or plant parts, living or dead, on the surface of the ground or the area of ground cover by plants of one or more species", while density "the number of individuals per unit of area".

It is obvious that the estimation of the above vegetation parameters, especially forage production, are very useful to project stocking rates and feed requirements for specific time periods (i.e. annually, grazing season, rotation cycle, etc.). In addition, the degree to which a forage meets the nutritional requirements of a specific kind and class of animal (i.e. forage quality; Allen and Segarra, 2001) is always important to be taken into account. Therefore, a battery of methods has been developed to tackle these objectives.

Forage production

Clipping and weighing

Weight is the most important expression of herbage production. Clipping is probably the most common method for determining herbage weight for pasturelands and grasslands where the vegetation is primarily herbaceous and is stratified into relatively homogeneous types. The herbage weight is determined in representative small plots (quadrats), which can be varied in size and shape (e.g. rectangular, quadrangular, and circular; for details see Cook and Stubbendieck, 1986). Herbage weight from such quadrats is multiplied by a given factor to obtain kg/ha. Quadrangular quadrates with area of 250 cm^2 are the most common used for herbage determination. Several types of hand clippers and shears are used for plant material harvest of quadrats and the clipping is applied either on individual species or on the whole herbage indiscriminately. The clipping height is usually 5 cm above the ground and separation into live and dead components is frequently needed.

Browse production is based on current twig and leaf growth available to animals, ordinarily within 1.5 m of the ground. Measurements of browse are done on representative plants of each present woody species in the rangeland, which are cut at 10 cm height above ground and the collected material is hand-separated into browse material (leaves and twigs up to 2 mm diameter) and branches and all of them are weighed after oven drying at 70°C. Browse is expressed in kg DM per plant; whether the number of each woody species per unit area is known browse can be expressed in kg/ha, as well. However, in shrublands composed of both woody plants and an undergrowth of herbaceous plants, measurements have to be done on both herbage and browse. This, can most of the time be achieved with clipped plots. For example, in studies dealt with kermes oak (*Quercus coccifera* L.) shrublands quadrangular quadrats either 0.5 x 0.5 or 1 x 1 m were used (Papanastasis and Liacos, 1983; Papachristou and Nastis, 1993; Papachristou, 1997) for the determination of available forage. The disadvantage of this method is that it is tedious and time consuming.

Estimation and double sampling

To overcome the disadvantage of the previous method, weight estimate method has been developed which is much faster than clipping and weighing and permits more intensive sampling (Pechanec and Pickford, 1937; Reese *et al.*, 1980). This method presupposes extensive training with actual clipping to adjust estimates and to improve accuracy. Disadvantages include the need to develop estimation skills, a high degree of concentration needed by the estimator, lack of accuracy and variation among observers (Shoop and McIlvain, 1963). Estimators tend to underestimate quadrats with high herbage weights and overestimate those with low herbage weights.

To improve the weight estimation technique some researchers (Cook and Bonham, 1977; Ahmed and Bonham, 1982) used the double sampling, in which a small proportion of the estimated plots are independently clipped and weighed. From this small sample the relation between estimated and actual weight is calculated and used to adjust the estimate of the large sample. The double sampling method was widely used in shrublands for the determination of their forage production (Platis and Papanastasis, 2003).

Indirect methods

A huge effort has been done for determining herbage weight from other easily measured variables which might be related to herbage weight. Precipitation is one of these variables used as the independent variable to predict herbage weight (Papanastasis, 1982).

In some cases, annual or seasonal precipitation accounts for a large proportion of the variation in annual herbage production. However, data collected over a period of years with nearly average precipitation, may not be reliable to predict herbage weight during an atypical year. The disadvantage of this prediction method is that several years of data are needed with a wide variation in total precipitation. Therefore, several combinations of months may be tested to develop those with the strongest relationship.

Also, cover found to be related to herbage weight and was used as the independent variable (Payne, 1974). However, it is easier to estimate cover of compact plants with regular outlines than those with irregular outlines (Goebel *et al.*, 1958; Reppert *et al.*, 1962). The disadvantage of this method is that it presumes that cover can be measured easily and accurately but this does not at all time happen (see Cook and Stubbendieck, 1986).

For shrubs, browse weight estimates instead of clipping and weighing may be an alternative, which will permit more adequate sampling of browse. Several studies have been conducted to estimate individual twig weight for different species. Shafer (1963) simply determined the average weight of twigs of several eastern hardwood species and then counted the number of twigs on each plant. Several other workers have developed regression equations expressing the relationship between twig length and or diameter and twig weight (Basile and Hutchings, 1966; Lyon, 1970; Halls and Harlow, 1971; Ferguson and Marsden, 1977; Dean *et al.*, 1981; Provenza and Urness, 1981; Bartolome and Kosco, 1982). Most of these studies show a relatively strong relationship between twig length and diameter and twig weight. Once the relationship has been established, the researcher need only measure twig lengths or diameters to be able to predict twig weight. In some cases, additional measurements such as main stem diameter and number of twigs improve the relationship (Schuster, 1965). While these methods provide a means of estimating twig weight, they do not provide an estimate of standing crop per unit area. It is still necessary to extrapolate these data to an individual plant and to an area basis. Also, one needs to be sure that the equations hold from year to year and from site to site when growing conditions might be different (Ruyle *et al.*, 1983).

Another approach for woody species has been to measure one or more dimensions of the canopy and to relate these measurements to canopy biomass. In some cases simple measurements, such as canopy diameter, are sufficient as in the case of true mountain mahogany *(Cercocarpus montanus)* (Medin, 1960). In other cases, additional measurements may be needed (Hutchings and Mason, 1970). Ludwig *et al*. (1975) devised formulas for the volumes of several south-western shrubs and related shrub canopy volume to weight. In many cases, higher coefficients of determination can be obtained if data are transformed to logarithms (Harniss and Murray, 1976; Rittenhouse and Sneva, 1977; Uresk *et al.*, 1977; Whisenant and Burzlaff, 1978; Bryant and Kothmann, 1979). These methods appear promising; however, density also has to be determined to calculate biomass per unit area.

Recently, Platis and Papanastasis (2003) investigated the spatial distribution of available forage in kermes oak shrublands and its relationship with the shrub cover measured on the ground and in aerial photographs. They used aerial photographs of high resolution (1:6,000) to estimate shrub cover and correlated this to ground measurement. Estimated cover had a high correlation ($r = 0.96$) with ground measurements in open, moderate and dense shrub stands. The regression of available forage with shrub cover measured on the ground and on aerial photographs produced non-linear equations with high coefficients of determination ($R^2 > 0.70$) only when shrub height up to 0.5 m irrespective of the cover class was considered. It seems that shrub cover measured on aerial photographs can be used to predict shrub cover on the ground as well as available forage in kermes oak shrublands with not too tall individuals; thus reducing labour and time consuming sampling.

Cover

Cover (area occupied) has often been used as a primary attribute of vegetation in ecological or rangeland studies. Cover can be used as a basis for comparison among plants of differing life forms and is a non-destructive measurement. Permanent sampling units can be established and repeated measurements taken. Basal cover has often been used to evaluate herbs while canopy or aerial cover has commonly been used for woody plants. In the following lines of this section only four of the existing cover techniques will be briefly discussed.

Estimation techniques

Estimation techniques are perhaps the simplest of the techniques developed to determine cover. Cover can be estimated for all the individual species or for selected species or forage categories (e.g. grasses, forbs and browse) directly in percentage by using quadrats, grids or other devices (see Cook and Stubbendieck, 1986). For example Cook and Bonham (1977) described a frame divided into small grids for sampling salt-desert shrub vegetation. Each grid equalled 5 cm by 5 cm cells or 0.25 % cover when used on 1 m^2 quadrats. The observer counted the number of cell grids directly over the plants which summed into actual cm^2 of cover or relative percentage cover of the whole. The disadvantage of this method is that training and adjustments of estimates are difficult. However, it may be easier to estimate percentage cover by classes rather than absolute coverage. For example, Daubenmire (1959) suggested the following six classes: 0-5, 5-25, 25-50, 50-75, 75-95, and 95-100 % cover.

Line intercept

The line intercept method consists of stretching a tape or line through the vegetation. This tape (say 25 m length) is marked into intercepts (0.25 m) and cover is estimated by the number of intercepts, which are partially or fully covered by either the vegetation as whole or a single species (for details see Cook and Stubbendieck, 1986). This method is often considered one of the most reliable methods for determining cover and is often used for comparing other methods. The main drawbacks to the line intercept method are the time required to conduct the sampling and the difficulty to stretch a line between two points in tall and dense woody vegetation.

Point sampling

Cover can be estimated by pins operating as physical points and using the percentage of pins that touch the vegetation as a measure of the cover of that vegetation (Park, 1973). Because the point is dimensionless its size affects the cover estimation; however, this error can be minimized by sharpening the pins (Long *et al*., 1972) and calculating corrections factors (Warren-Wilson, 1960). The point frame usually contains ten pins spaced 5 cm apart. The pins pass through two holes in the frame and are sharpened to a point. Each pin is lowered and the species which the pin touches is recorded. After all ten pins have been lowered and data recorded, the point frame is moved to a new location. Thus, each frame can be located randomly, but their pins are located systematically with respect to each other.

Loop procedure

This method was developed by Parker (1951) and was further refined by Driscoll (1958), and Parker and Harris (1959). More specifically, transects (e.g. 25 m long) are set up in vegetation and 100 recording (i.e. per 25 cm) are done per transect with a 2 cm loop. This method was widely used in rangeland studies although an overestimation of the actual cover up to 8 times could occur depending on species and location (see Cook and Stubbendieck, 1986).

Density

Density determinations are useful when one is more interested in the number of individuals rather than cover or biomass, such as in evaluations of seedlings. Density can be determined by the use of quadrats or distance techniques (Cook and Stubbendieck, 1986).

Forage quality

Forage quality may be determined from the vegetation directly or as expressed in animal products (Beaty and Engel, 1980). Palatability, total digestible nutrients, energy values, and individual nutrients and chemical components, rate of passage and intake are all factors determining the degree to which the forage is able to meet the nutritional demands of the animal. Measurements of forage quality may be obtained from field grazing trials, laboratory analyses, or a combination of both (see for details in section "Studying responses of grazing animals" of this paper).

Forage utilisation

Utilisation is defined as the amount or percentage of the current growth of forage which have been removed by grazing animals and can be applied to single plants, plant groups or the grazing land as a whole (Heady, 1975). The determination of the forage utilisation is one of the most important works in the whole field of rangeland management. Based on forage utilisation data, the regulation of animal stocking rates can be achieved in a way that allows plants to recover from grazing and the whole rangeland to restore to its previous condition. Over the years considerable effort has been devoted to the development of methods of measuring forage utilisation and developing utilisation standards and proper factors for each of the important forage plants and rangeland types under various grazing conditions. A brief description of the most important techniques is given below.

Cage comparison method

Grazing is excluded from plots by cages or enclosures. These plots and adjacent similar ones (paired plots) are clipped and the difference in weight represents the percentage of forage consumed. Two similar plots are selected on the basis of composition, growth and utilisation. One of these may be placed randomly and the second is selected to pair with the randomly selected one. After the two plots are selected, a coin is tossed to indicate which unit to cage. At the end of each sampling period new areas are selected as before. Both utilisation and forage production may be measured by this method.

This method has been widely used in rangeland and pastureland studies for a number of years (see Cook and Stubbendieck, 1986). The plots have been of various sizes with smaller ones to have advantage for the collection of larger sample numbers while larger ones have less border effect from the cages. The disadvantage of this method is that differences in growth on the protected and grazed areas may distort the calculated utilisation.

Weight before and after grazing

Difference in plant unit weight, before and after grazing, forms the basis for this method. Plant units consist of distinct, recognisable parts such as a stem, a twig of currently growth or a whole plant. The sampling unit may also be paired quadrats and protected plots are necessary where growth occurs during the grazing season. Percentage utilisation is determined by collecting a given number of specific plant units before grazing and a similar number after grazing. This method works well where forage is grazed for short periods and regrowth is minimal.

Ocular estimate-by-plot

This method is an estimate of the amount of forage that is removed by grazing. These estimates are made on plots small enough that the entire plot is clearly visible from one point. In training, the field workers clip plots to estimate grazing, then clip the remaining plant material and uses the weight of both clippings to calibrate the estimate of forage removal. This method is suitable for grasses, forbs, and shrubs. The disadvantage is that estimates rather than objective measurements are used.

Ocular estimate-by-average of plants

This method is based on estimates of weight removal from individual plants, instead of the entire forage on plots as with the previous method. These estimates are then weighted and averaged by species to obtain plot ratings, although slightly less rapid than the ocular estimate-by-plot method, there is less personal error since each observation is confined to a single plant and it is possible to obtain an adequate sample of every species of interest.

Estimates of shrub utilisation

Several methods have been devised to determine utilisation of woody species, but these plants pose special problems. For example, some researchers (see Cook and Stubbendieck, 1986) use procedures similar to before-and-after grazing method mentioned earlier with the plant unit being a twig. Twigs can be tagged at the end of the growing season and measured. The same twigs are re-measured before growth the next year. The disadvantage of this method is that a certain percentage of reduction in twig length may not represent a similar percentage of utilisation by weight. However, several studies have shown a high correlation between twig length and weight (Ferguson and Marsden, 1977; Dean *et al.*, 1981; Provenza and Urness, 1981; Bartolome and Kosco, 1982).

Studying responses of grazing animals

Free-ranging herbivores develop a foraging strategy to extract an adequate supply of nutrients. They perform short- and long-term tactics to obtain adequate diet composition and intake level. The short-term tactic refers to the adjustment of, for example, the bite rate and size, the movement rate and selection criteria. In the rangeland, the animal makes continuous decisions on the sites where to be present to find requested forages and to overcome nutrient requirements. Decision for location corresponds to long-term tactic. In addition to these tactics, several external factors may play an important role in the foraging strategy. These include mainly specific plant morphology and physiology, social and physical environment, vegetation structure, the presence of parasites and the physiological state of the animal. Therefore, key variables in this foraging strategy are the species composition of the consumed diet, the total amount consumed and the extent of digestion of plant cell wall material. These parameters have been extensively studied in housed intact and surgically prepared animals, but they have been difficult to study in grazing and browsing animals. Therefore, many attempts have been made to develop or to refine experimental protocols allowing for better determination of faecal output, diet composition and its intake and digestibility. Since direct measurement of these parameters is difficult in the field, scientists have developed indirect methods based on the use of internal and external marker substances. The appropriate methods used by authors are selected on the basis of their accuracy for predicting a parameter, minimum labour involved and the cost dictated mainly by the type and amounts of chemicals used and by the type of analytical equipment requested. Several other criteria are considered while choosing among these methods. For studying vegetation, not destructive and less time consuming methods are preferred. For studying animal response, methods not causing animal disturbance and surgical intervention are the most used. In the following sections we will try to discuss the opportunity of the main direct and indirect methods reported in the literature.

Estimating forage intake

Intake is probably the most important variable determining animal performance and voluntary intake is generally correlated with the amount of nutrients that can be extracted from feed. The measurement of forage intake serves firstly as a means of explaining differences in animal performance between two species or cultivars, secondly as the basis for a hypothesis that attempts to provide a more general explanation for such differences between species or cultivars, and thirdly as an aid to explain variation in animal performance associated with different grazing regimes and management practices. Estimating the feed intake of a grazing animal must by definition rely upon techniques imposing minimal disturbance to the normal grazing activity of that animal. Plant-based methods described above impose little interference on the animal per se, but their application is limited to specific grazing situations and they can only provide an estimate of the mean intake of a group. The animal-based techniques, whilst of necessity requiring some interference with the grazing livestock, are potentially usable in a wide range of grazing circumstances and allow some examination of between-animal variation. Specific techniques have been developed to estimate short-term and long-term intake.

Gordon (1995) reported in his review four techniques which could be used to estimate short-term intake, and these are: (i) mimic by an observer of the bite size by visual observation of bite and hand plucking similar vegetation and measures the bite rate of a tame free-ranging animal; (ii) determination of short-term changes in live weight before and after free-grazing; and (iii) determination of the number of boluses by an animal while feeding which is strongly correlated with forage intake, and d) collection followed by weighing of extrusa from oesophageally-fistulated animals over a known time period and the simultaneous recording of bite rate.

Numerous methods have been developed for measuring long-term (daily) intake rate. The most common method is based on a direct approach which calculate intake from estimates of faecal output and diet digestibility and this is obtained after simple manipulation of the digestibility relationship:

Digestibility (D) = [Intake (I) – Faecal output (F)] / Intake (I); or, I = F / (1 – D)

Measurement of I is therefore dependent upon accurate estimation of F and D. It should be noted that error in estimation of F leads to equivalent error in I but that error in D, mainly when D is greater than 0.50, leads to a proportionately larger error in (1 - D) and consequently in intake. Methods of estimating F and D are described in the following sections.

Faecal output

Total collection

Harnessing animals and fitting dung bags to collect all the faeces voided can measure total faeces output. Harnesses, dung bags and urine separators (for female animals) should be fitted to the animal several days before a collection period. This acclimatisation also allows adjustments to be made to ensure that the harnesses fit properly and that faeces are not lost from the bags. To give reasonable estimate of faeces it is recommended to collect faeces over a minimum of five days. The frequency with which dung bags should be emptied is determined by the volume of fresh faeces produced.

Twice daily is generally recommended but once daily may be sufficient when intake is very low, whilst three or four times daily may be necessary for dairy cows producing large volumes of faeces. Different shapes and sizes of faecal harness have been used on different animal species and attempts have been made seeking the confort of these animals and the accuracy of estimating faecal production. Yiakoulaki and Nastis (1998) have, for example, designed a modified faecal harness which allow minimal faecal losses and fit well on goats without disturbing their mobility, bipedal stance, and consequently their grazing behaviour. Additionally, the disadvantages of total collection outlined in Table 1 encouraged researchers to develop indirect methods based on the use of markers.

Table 1. Some advantages and limits of the main methods used to determine intake, faecal output, and diet digestibility and diet composition on free-ranging animals

Parameter	Technique	Advantages	Limits
Faecal output	Faecal bags	- Amount of faeces actually voided - No chemicals - Give rapid results	- Tedious - Problem with diet low in dry matter - Distortion of hind legs due to weight of faeces in bags - May affect animal behaviour - Not practical with high size animals
	Orally-dosed markers (Cr_2O_3)	- Amount of faeces actually voided	- Rectal grab sampling - Diurnal variation in faecal chromium concentration - Animal disturbance
	n-alkanes	- Easy to analyse	- Alkanes not completely recovered in faeces - Hydrocarbons could be absorbed in the small intestine
Forage intake	Weighing animals	- No need for laboratory analyses (chemicals and specific apparatus) - An estimate of fresh forage consumed could be obtained	-Large investment (equipment and sophisticated computer software) - Still not used in practice - Need to account for weight loss (defaction and urination)
	Difference in forage mass (HM)	- No need for laboratory analyses (chemicals and specific apparatus)	- Assumption that the decline in HM is entirely consumed - Not applicable for woody species - How to consider pasture growth and senescence?
	Bite counting	- Operator training for bite count observations is minimal and requires little specialist expertise	- Time consuming - Difficult at night - Unreliable (inter-observer variation)
	Esophageal fistulas	See below	- Incomplete extrusa recovery - May affect grazing behaviour
Diet digestibility	48 h-*in vitro* and *in situ* techniques	- Less time than *in vivo* digestion trial	- Not adequate for tannin-rich - Forage consumed by penned animal is often not similar to that consumed by grazing animal - Same digestion degree between penned and grazing animals
	Internal markers (lignin, silica, etc.) NIRS	- No use of external markers (cost) - Rapidity, accuracy	- Diurnal variation of lignin concentration - Soil contamination (silica) - Sophisticated equipment - Database for calibration

Table 1 (cont.). Some advantages and limits of the main methods used to determine intake, faecal output, and diet digestibility and diet composition on free-ranging animals

Parameter	Technique	Advantages	Limits
Diet composition	Hand-plucking	- Sample free from salivary contamination	- Tractable animals which can be approached closely
	Esophageal fistulas	- Accurate representation of diets (selective grazing/browsing)	- Requirement for surgery - Biases due to short time of collection period
	Micro-histological analyses of rumen content (plant epidermal fragments)		- Laborious - Prone to error because of the differences in the digestibility of different component species and of their plant parts - Unequivocal identification of some species (dicots) is difficult
	Micro-histological analyses of faecal samples	Short-term (single defaction) and long-term (series of faecal samples bulked over a number of days) estimates of diet composition could be made	- Forage species passed in the faeces are often not proportional to those consumed - Destruction of some plant species during preparation
	n-alkanes in faeces	- Analysis easy and precise	- Plants (species or parts) low in alkanes could not be identified
	Bite size and count	- Requires little equipment	- Operator training in plant identification - Low precision of identification when forage species make up less than 2% of the diet
Sampling for intake, digestibility and diet composition determination	Hand-sampling	- Little equipment and time	- This procedure is subjective - May not correspond to the actual plant parts consumed by the animal
	Esophageal fistula	- Little physiological disturbance compared to rumen fistula - Used in large and small size animals	- Salivary contamination of forage (interactions tannins-salivary proteins) - Chemical changes during mastication and salivation - Incomplete recovery

Estimation of faeces production using markers

Faecal output may be determined by analysing bulked samples of faeces taken once or twice daily from the rectum for a marker with which the animal is dosed once or twice daily. The ideal marker should be: (i) quantitatively recovered in the faeces; (ii) non-toxic; (iii) readily analysed by physical or chemical methods; and (iv) be present only in small amount in the original diet. Faeces production can be estimated from the following equation:

Daily faeces produced (g) = Weight of marker given (g/d) x Recovery of marker in faeces / Marker concentration of marker in faeces (g/g)

Currently the most widely used marker is chromic sesquioxide (Cr_2O_3). Following oral dosing of this marker, the faecal concentrations reach equilibrium in most animals after 5-6 days; thereafter faeces samples can be collected by rectal grab sampling or removal from the ground. It is provided to the animal through: (i) commercially produced gelatine capsules containing 1 g or 10 g Cr_2O_3 in an oil base; (ii) paper impregnated with Cr_2O_3; or (iii) where animals are individually fed known quantities of a feed (usually concentrates) it is possible to incorporate the marker into this feed. The possible errors involved in estimating faecal output from daily or more frequent dosing with Cr_2O_3 have been extensively discussed in previous reviews (e.g. Dove and Mayes, 1991). Intraruminal, controlled release device which deliver Cr_2O_3 at a constant rate have been used by numerous authors to predict faecal production in much larger numbers of animals with less labour involved when compared to the daily dosing technique. However, concern has still been expressed about the constancy of the chromium release under varying dietary conditions. The accuracy of this marker has been more questioned with regard to estimation of diet digestibility. The use of plant wax components has been suggested as an alternative to better estimate intake by grazing / browsing animals (Dove and Mayes, 1995).

Recently, Landau *et al.* (2003) demonstrated the efficient use of Polyethylene glycol (PEG) as marker of faecal output in goats. In addition to the relative accuracy of this technique, PEG can be used at the same time to deactivate tannins in tree and shrub foliage which are abundant in Mediterranean rangelands. These authors showed also that PEG concentration in goat faeces could be predicted by the near-infrared reflectance spectroscopy (NIRS).

The wax of the plant cuticle is a complex chemical mixture. Although hydrocarbons are usually minor components they appear to be ubiquitous to the cuticular wax of higher plants. The predominant hydrocarbons of most plants are n-alkanes which usually occur as mixtures, ranging in chain length from 21-37 carbon atoms. Plant alkanes consist of predominately (90%) odd-numbered chains of 25-35 carbons. The waxes are relatively indigestible, and, as chain length increases, the percent recovery in the faeces increases. Interest devoted to n-alkanes could be explained by the fact they can be used to provide estimates of both digestibility and faecal output for the calculation of intake. Forage species contain variable quantities of alkanes, and the concentration of C33 may be too low in some species for use as the internal marker. This can require the use of a shorter chain length with a lower percent recovery in the faeces and possibly result in errors in calculation of intake. Mayes *et al.* (1986) developed a double alkane procedure for estimating intake. In this approach animals are dosed known quantities of an even-chain alkane and intake is estimated from the daily dose rate and the dietary and faecal concentration of the dosed even-chain alkane and a natural, odd-chain alkane adjacent in chain length. Intake is calculated using the following equation: Intake = $[F_i/F_k) \times D_k] / [H_i – (F_i/F_k) \times H_k]$

Where H_i and F_i are the herbage intake and faecal concentrations of the odd-chain alkanes, H_k and F_k are the equivalent concentrations of the even-chain, dosed alkane (of which there will be a small amount in forage) and D_k is the daily dose of the even-chain alkane. Mayes *et al.* (1986) suggested in this method that incomplete faecal recoveries would not matter provided the method employed a pair of alkanes which were similar in recovery. To reduce the labor required for daily or more frequent dosing of animals with alkanes, an intraruminal alkane controlled release device has been developed. The release rates of alkanes were shown to be constant and were within 1.5-4% of the nominal release rates. Studies on stall-fed animals have showed that the alkane procedure for estimating dietary intake is reliable. However, absolute validation of the method with grazing / browsing animals is virtually impossible to achieve, because alternative methods with which to compare the technique may be no more reliable, or possibly inferior. The main precaution required in the use of the method is to ensure that the diet sample, in terms of its alkane concentrations, is representative of that consumed by the experimental animals. For uniform sown pastures, this is relatively easy to achieve by hand-gathering or by collecting extrusa samples from esophageally-fistulated animals.

Diet digestibility

Compared to faecal output, the determination of forage digestibility is prone to larger errors since it cannot be measured directly *in vivo* in the grazing/browsing animals, which have the opportunity of selecting a diet of different quality to that of the total vegetation. Therefore, a wide range of indirect methods has been developed. Major amongst these are the *in vitro* digestibility procedures, the use of internal markers and the faecal index technique.

The in vitro *digestibility procedures*

These procedures involve the incubation of a diet sample with buffered rumen liquor, followed by acid pepsin digestion, or with buffered cellulase preparations. The two-stage method developed by Tilley and Terry (1963) to measure the 48-h *in vitro* digestibility of extrusa samples or hand plucked samples is the most frequently used to assess the digestibility of the diet consumed by the grazing/browsing animals.

The use of internal markers

Several major plant components have been suggested as potential markers, and these are mainly lignin, chromagen and silica. The digestibility is estimated from the ratio of the concentration of one of these indigestible markers in the feed to that of the marker in the faeces. Serious doubts are cast on the suitability of this method for a number of reasons; some of them are reported in Table 1.

The faecal index method

This method requires a conventional indoor *in vivo* digestibility trial to be performed with forage similar to that being grazed, and that a faecal component is related to *in vivo* digestibility; this component needs not be indigestible. The concentration of this component is then assessed in faecal samples from the grazing animals and the diet digestibility predicted from the relationship derived indoors. Nitrogen is the faecal component most frequently used and the technique is at its best when local regressions are produced for each specific set of circumstances, since the between forage factors are the major source of variation in the parameters of the regression. To minimize error in the estimation of the digestibility of grazed pasture the nitrogen regression must be derived with material similar to that selected by the animal when grazing. The faecal index method is of little use under continuous grazing management or in swards where opportunities exist for widespread selection between plants or parts of plants. It is at its best under a strip-grazing management in which the forage removed by the grazing animal is equivalent to that harvested for the indoor digestibility trial. Deviation from this condition will increase the likelihood of error or bias in the estimate of digestibility. Obviously, it would be difficult to use this method in rangeland conditions mainly when wood species dominate.

Near-infrared reflectance spectroscopy (NIRS)

This technique has shown great promise for analysis of forage nutritive quality. Strong correlations exist between NIRS and the various components of forage nutritive value. The success of this technique is dependent on accuracy of measurements of known samples used to calibrate the instrument and on adequate wavelength selection, prediction equation and data processing. It has been widely used to assess the chemical composition and digestibility of common roughages, mainly herbaceous species. However, only few studies have been carried out on shrub species. Faecal NIRS equations have been developed by Leite and Stuth (1995) to predict crude protein and digestible organic matter of the diet of free-ranging goats. Coefficients of determination of these two parameters were 0.94 and 0.95, respectively. Meuret *et al*. (1993) concluded that NIR spectroscopy is an adequate technique for the prediction of the nutritive value (chemical composition and *in vitro* digestibility) of Mediterranean foliages from trees and shrubs with reliability similar to that obtained from classical fodder analysis procedures.

Diet composition

In addition to intake and digestibility, the range manager is concerned with the composition of the diet selected by the animal. Knowledge of the proportion of each range species in the diet will facilitate calculation of the nutritive value of the diet and therefore supplementary strategy could be decided. Moreover, pressure on several high palatable range species could be identified and management strategy of the rangeland could be defined. Simple to complicate methods have been used to assess diet composition of grazing/browsing herbivores. Procedures for estimating diet composition in herbivores and their advantages and limits have been reviewed by numerous authors (e.g. Holechek *et al*., 1982; Dove and Mayes, 1995). Briefly, the range of these techniques include mainly:

(i) Direct observation of foraging behavior to determine feeding times and to identify the different plant species selected by the animal.

(ii) Examination of stomach contents after slaughtering animals.

(iii) Examination of extrusa samples from esophageal-fistulated animals.

(iv) Microscopic examination of plant cuticle fragments in faecal samples. This technique has been widely employed for determining the botanical composition of the diet of domestic and wild herbivores.

(v) The use of plant wax alkanes.

Since the last few years there is a continuous interest devoted to the last technique (i.e. use of n-alkanes) as it allows better prediction of diet prediction of grazing/browsing animals than most of the above techniques. A major potential of the use of n-alkanes method is that some 8-15 possible markers are available for estimating diet composition, thus making the characterization of complex diets feasible. The dietary components may be separate plant species or cultivars, different plant parts or even plant communities. Alkanes have been used to determine diet composition from extrusa and from faeces samples. However, the main pitfall of this method is that plant or part of plants low in alkanes could not allow an adequate determination of diet composition.

Technique of choice for better assessment of free-ranging animals

Actually, recommending a specific technique as the best for estimating faecal output, intake, and diet digestibility and composition is not an easy task. As shown in Table 1, all techniques reviewed in this paper have advantages but also have limits. Disadvantages of each technique may be judged on the basis of its accuracy, labor and cost involved, and interference with animal behavior. Unfortunately, only few comparative studies on free-ranging/browsing ruminants involving different techniques have been carried out to assess the nutritive quality of diets. The lack and often the difficulty in introducing in the experimental design a direct technique as control to measure for example diet digestibility renders the decision on the best indirect technique somewhat impossible.

The accuracy of each technique in predicting the nutritive value of diets selected by the animal depends firstly on how samples of plants consumed by the animal were obtained. For example, extrusa samples collected from esophageal-fistulated animals are contaminated with saliva; this affects the accuracy of digestibility determination in the case of tanniniferous plant species. Hand-plucked samples of vegetation could also be unrepresentative for the plant parts actually consumed by the animal. In addition to sampling problem, results obtained with indirect methods within the same trial are often different. The problem seems less acute when estimation of faecal output is considered. Indeed, Dove *et al*. (2000) concluded that estimates of faecal production by ewes on *Phalaris*-dominated pasture were similar among the two markers used, i.e. Cr_2O_3 and n-alkanes. However, estimates of forage intake based on the use of C33/C32 alkanes were more accurate than Cr_2O_3. Meuret *et al*. (1985) reported lower intake by goats on *Quercus pubescens*-dominated shrubland estimated by Cr_2O_3 dosing (2.16 kg DM/day) than that estimated by direct observation of animals (2.61 kg DM/day). The DM intakes and the digestible energy in the diet consumed by sheep on *Cenchrus ciliaris* estimated by total faecal collection (harnesses) and Cr_2O_3 in faecal samples were quite similar (Sankhyan *et al*., 1999). Using different techniques for diet sampling, the later authors obtained different values of DM intake which were 74.2, 49.6, 64.2 and 39.2 g/day/kg $W^{0.75}$, respectively with clipping, mouth grab, hand-plucking and esophageal extrusa techniques. Several comparative studies have shown that results obtained on stall-fed animal are different from those obtained on free-ranging animal for the same parameter. Piasentier *et al*. (1995) noted a great deviation for OM intake by ewes on fescue meadow between confining and free-grazing conditions. Ben Salem *et al*. (unpublished data) recorded a higher DM intake of *Atriplex nummularia* by lambs housed in metabolic cages (465 g/day) than that estimated by bite counting and weighing (374 g/day). It is clear from these results and others reported in the literature that all methods described in the current paper are prone of errors, but depending on the objective and degree of accuracy targeted, several techniques are still widely used.

Conclusions

Studying vegetation and animal responses under rangelands conditions is a difficult and complex task. A wide range of direct and indirect methods are available, however, the choice of appropriate ones depends on numerous factors specific to vegetation or animal behavior and some of these factors may concern both plant and animal. In general, accuracy, cost-effective, less labor and time-demanding methods are the main criteria considered by scientists. For rangeland characterization, these factors include grazing land conditions, vegetation structure, and the familiarity with the used method. For the animal, the choice between techniques may vary according to animal species and physiological state. Studies reported in the literature dealing with the estimation of the nutritive quality of the diet consumed by free-ranging ruminants have been carried out mostly in monospecies pastures. However, limited data are available for shrubland conditions and herbaceous-woody species mixed rangelands. Adaptation or development of new techniques, which fit with these conditions, should be encouraged. The multipurpose use of several techniques like n-alkanes and NIRS (estimating faecal output, intake, digestibility and diet composition) are considered by numerous scientists as favorite techniques for the assessment of the diet quality. Caution should be taken when the estimation of the nutritive value of tanniniferous plant species is scheduled. This starts from the choice of sampling method to avoid, for example, saliva contamination to the use of adequate method to determine the nutritive value of range species. One would be ambitious to use urinary markers coupled with faecal markers to better describe the process of digestion and nutrient supply in grazing/browsing ruminants. Finally, for possible comparison of results between different laboratories, the standardization of methods used for rangeland description and animal response study is highly recommended.

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