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Review

Techniques Measuring Digestibility for the Nutritional Evaluation of Feeds

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ABSTRACT

One of the most significant factors, which determine the nutritive value of a feed is its digestibility. Digestibility data can offer an insight into the proper feeding of animals. Of the various techniques that have been used to date the total collection is the most reliable method of measuring feed's digestibility. However, it is time consuming, and expensive. The time and expense involved in digestion experiments can be economized by the use of indicator method where total feces are not collected. This method is the most useful in evaluating digestibilities of feedstuffs fed to captive wild animals. The digestibility of a feedstuff may also be predicted from chemical composition of the feed. This process involves development of multiple regression equations relating various chemical components to *in vivo* digestibility. *In vitro* digestibility techniques provide a quick, inexpensive and precise prediction of *in vivo* or conventionally determined digestibility in ruminants. Nylon bag technique is however, quite useful for evaluating kinetic aspects of digestion in ruminants.

Key Words: Techniques; Digestibility; Nutrition; Feeds

INTRODUCTION

Nutritive value of feeds is determined by a number of factors, including composition, odor, texture and taste (Schneider & Flat, 1975). These factors are generally measurable in the case of the animal as digestibility and intake. Digestibility is simply a measure of the availability of nutrients. When digestibility is combined with intake data, one can make an accurate prediction of overall nutritive value. Of the two factors, intake is relatively more important than digestibility in determining overall nutritive value because highly digestible feeds are of little value unless consumed by the animal in question. However, digestibility usually provides a fairly reliable index of nutritive value because more digestible feeds are normally consumed to a greater extent than less digestible feeds. Only that portion which is soluble or is rendered soluble by hydrolysis or some other chemical or physical change can be taken up into the circulation and assist in supplying the animal body with material for building and repair of tissue or supply the energy necessary for body functions. In addition, measures of digestibility are somewhat easier to obtain than measures of intake and thus, considerable effort has been made by animal nutritionists to develop effective means of determining digestibility. This paper review different techniques used for the estimation of digestibility and factors that affecting it.

Total collection technique. The total collection (conventional digestion trial) is the most reliable method of measuring a feed's digestibility. Unfortunately, however, it is somewhat time consuming, tedious, and costly. Basically, the feed in question is fed in known quantities to an animal. Usually, the animal is restrained in an individual cage so that a quantitative collection of feces can be made. Accurate records of feed intake, refusals and fecal output are kept, and a sub sample of each (usually 10% of daily output in the case of feces) is retained for analysis. When estimates of nitrogen balance are desired, urine output is also measured. Three animals per feed are required as a minimum. The animals are usually allowed from 7 to 21 days (d) to adjust to the feed, followed by a collection. Samples can then be dried, ground, and analyzed for the nutrients of interest. Digestibility of any given nutrient can be calculated as follows:

$$\text{Nutrient digestibility (\%)} = \frac{\text{Nutrient intake - Nutrient in feces}}{\text{Nutrient intake}} \times 100$$

The most common arrangement for collecting the excreta of animals for digestibility experiments is through the use of metabolic crates. A metabolic crate is actually a stall or box large enough for the animal set on legs from 50 cm to 1 m high. It is so planned as to permit the quantitative collection of feces and urine. However, a common criticism of digestion estimation by total collection technique is that feed intake by animals is sometimes abnormally low and erratic. This lack of appetite is in many cases attributable to the fact that the animal may be too nervous or frightened to eat, resulting from the close confinement made necessary by the very nature of the equipment used. It is important that the experimental animals must be sufficiently comfortable during the adjustment period. The space allowed to the animal must be large enough to permit considerable freedom of movement. But conducting a digestion experiment may normally entail appreciable annoyance to the animal. Some individual animals are temperamentally

unsuited to be used in such experiments and are too nervous to be used in digestion trials. Mostly captive wild animals fall into this category. Even though conventional digestion trials are the standard with which all other measures of digestibility are compared, the values obtained still vary ± 1 to 4 % as a result of animal-to-animal variation, sampling procedures and analytical errors.

Difference technique. Calculation of digestibility of a nutrient in a test diet is based upon the assumption that digestibility of a mixed diet is equal to the summation of the proportions of the diet supplied by each ingredient when fed alone. The digestibility of a nutrient in the test feed stuff being fed in form of mixed feed is calculated as follow.

$$\text{Digestibility of nutrient in test feed (\%)} = \frac{(A)-(B)\,(C) \times 100}{D}$$

A = Digestibility of nutrient in total diet; B = Digestibility of nutrient in basal diet (usually already determined when fed alone; C = proportion of total nutrient in diet supplied by basal diet (D) proportion of total nutrient in diet supplied by test feed.

Marker technique. There has been considerable interest among animal nutritionists in methods of reducing the time and expense involved in digestion experiments by the use of methods where total feces are not collected and weighed but are merely analyzed. This departure from the former method of determining digestibility has been designated as the indicator or index method (Kotb & Luckey, 1972). In this method, in addition to the chemical analysis of the usual proximate nutrients, the content in the feed and feces of an indigestible reference substance is determined. The substance may be a natural constituent of the feed (internal indicator) or it may be added to the feed (external indicator). Substances used for this purpose include ferric oxide, chromic oxide, lignin, silica, chromogen, acid-insoluble ash (Van Keulen & Young, 1977) and indigestible acid detergent fiber (Waller *et al*., 1980). A good marker must be strictly non absorbable, must not affect or be affected by the gastrointestinal tract or its microbial population, must be physically similar to or intimately associated with feed material and its method of estimation in digesta samples must be specific and sensitive and not interfere with other analyses. A characteristic of this method is that the digestibility is calculated from the relation between the nutrients and the indicator substance in the feed and in the feces. This method had been called a qualitative method, although this name is not strictly accurate. The digestion coefficient is computed by using the change in the ratio of each nutrient with reference to the special indigestible substance in the feed and in the feces. An example of this is the determination of the digestibility of the dry matter of a feed by the following equation:

$$\text{Digestion coefficient of dry matter} = 100-100 \times \frac{\text{\% Indicator in feed DM}}{\text{\% Indicator in fecal DM}}$$

By chemical analysis of a suitable feed sample, the ratio of the concentration of the inert substance to that of any nutrient in the feed can also be established. A similar ratio can be determined in the feces and the digestibility can be calculated without weighing either the feed consumed or the feces produced. Thus, if the percentage of any nutrient in this feed and feces is known and the percentage of the indicator substance is also determined in the feed and feces, the digestibility of that nutrient can be found by means of the following formula:

$$\text{Digestion coefficient of a nutrient} = 100-100 \times \frac{\text{\% Indicator in feed} \times \text{\% Nutrient in feces}}{\text{\% Indicator in feces} \times \text{\% Nutrient in feed}}$$

When determining the coefficients of digestibility of nutrient by the indicator method, it is assumed that the reference substance passes through the alimentary tract at a uniform rate. If its rate of excretion during the day is inconsistent, special sampling plans is followed to adjust for diurnal variation. If on the other hand, the ratio of the indicator and the nutrients is the same throughout 24 h period, only a small amount of feces collected at any time of the day or night should be sufficient to give an estimate of digestibility. However, it is unwise to collect only one sample for digestibility determinations. The animal should always be allowed an adequate preliminary period to adjust to the feed. The fecal (grab) samples should be collected for several consecutive days and pooled for subsequent analyses. This method of determining digestibility will hopefully avoid much of the time, labor and expense involved in conducting digestion trials. Also, this method would appear to be the most useful in evaluating digestibilities of feedstuffs fed to captive wild animals.

Prediction technique. Alternative measure of digestibility is the prediction of digestibility from chemical composition of the feed in question. This process involves development of multiple regression equations relating various chemical components to *in vivo* digestibility. Generally, the digestibility estimates obtained from prediction equations are not as precise as one might desire (±3 to 4 % of values obtained from conventional trials), and at the present time, *in vitro* digestibility measurements are more extensively used to estimate digestibility than are prediction equations based on chemical composition.

***In vitro* technique.** *In vitro* digestibility techniques provide a quick, inexpensive, and precise prediction of *in vivo* or conventionally determined digestibility in ruminants. The *in vitro* procedure does a better job of prediction than chemical composition because it accounts for all factors affecting digestibility, whether known or unknown, which is not possible with current chemical methods. As indicated previously, the *in vitro* procedure is quite simple, but nonetheless subject to a number of variables that may influence the results obtained. Basically, a small sample of feed (~0.5 g) is weighed into a 50 mL centrifuge tube. McDougall's buffer (based on the composition of sheep saliva) and ruminal fluid from a donor animal are added, and the tube is allowed to incubate for 48 h at 39^0C. The fermentation is then stopped, tubes are centrifuged, and supernatant fluid discarded. Acidified pepsin is added, and the tube is allowed to incubate for another 48 h at 39^0C.

Finally, the contents are filtered, and the residue is dried and weighed. It produces values that are numerically similar to *in vivo* values for many types of forage. However, the method requires fistulated animals to obtain rumen fluid and long incubation periods. It is also thought to have poor reproducibility, partly due to variability in rumen fluid composition and activity. To avoid the latter, similar herbages should be compared in the same experiment if possible and standards should be included in the run to enable subsequent adjustment of digestibility results to values for the fixed standard.

The technique is based on the premise that the final residue is similar to the feces voided by animals eating the forages. This assumption is not strictly true, because metabolic fecal N, which is present in *in vivo* but not *in vitro* residues, can cause lower protein digestibility *in vivo*. Also, the *in vitro* indigestible residue may contain bacterial residues and other substances, which would have been digested in the distal parts of the digestive tract *in vivo*.

Gas technique. The gas production technique was developed to predict *in vivo* digestibility by simulating the *in vivo* fermentation of feedstuffs. The gas production technique and its variants are superior to digestibility and degradability techniques because they account for contributions from soluble and insoluble feed fractions while providing information on the dynamics of forage fermentation. Additionally, when nutrient content is not limiting, gas production measures microbial growth. However, the use of the gas production technique as an index of the nutritive value is hampered by the dependence of total gas production on sample size, sample form and the composition of the end products of fermentation. A marked shift in the proportions of volatile fatty acids produced can occur when feeds with different composition are fermented and the ratio of fermented to degraded carbohydrate and yield of gaseous products per mole of hexose fermented are not constant.

The production of gas by the reaction of fermentation end products with the buffer also complicates interpretation of gas production profiles; especially as such indirect gas production is rarely accounted for. Caution is therefore required when interpreting gas production profiles and accounting for the end products of the fermentation should ensure the validity of any interpretations. An additional problem in using gas production measurements to estimate ruminal fermentation is that the profile must be described with an 'appropriate' model to enable the estimation of the parameters of the curve. Several better-fitting models have been recently proposed (Merchen, 1988).

Nylon bag technique. Another method of estimating digestibility of feeds is the nylon bag technique. In this procedure, nylon bags (~5 cm x 15 cm) are filled with 2 to 3 g of the feed in question and incubated in the rumen of a cannulated animal. Generally, bags are secured to a weighted cord to prevent floating in the rumen and to ensure adequate exposure to microbial digestion. Bags are then removed, washed under tap water, dried and the weight of residue determined. An empty bag should be incubated that serve as a blank. One should consider the pore size of the nylon material, which should be small enough to prevent passage of feed from the bag, but large enough to permit microbial entry. A pore size of 50 µ or less is desirable. Furthermore, the sample to bag size ratio is quite important, and a ratio of ~10 mg/cm^2 of bag surface is probably adequate. One disadvantage of the nylon bag technique is that fewer samples can be run at one time than with the Tilley and Terry method, and a donor animal with large diameter cannulae is desirable. Nylon bag (or *in situ*) techniques, are, however, quite useful for evaluating kinetic aspects of digestion in ruminants. Through the use of multiple incubation times and computer models, rates of nutrient digestion can be estimated.

FACTORS AFFECTING DIGESTIBILITY

Workers over the years have investigated more than 50 different factors that might influence the efficiency of digestion. Some of the most important factors as related to both captive wild and domesticated animals are outlined below.

Feed intake. The plane of nutrition is one of the primary factors that affect digestibility of any feed. Experiments have showed that livestock usually, digest a larger percentage of the nutrients in their feed when fed restrictedly than when they receive full feed (Okin & Mathison, 1991; Faichney, 1993; Poppi *et al*. 1981a). Most data indicate some depression in apparent digestibility as level of intake is increased. This may be due to a more rapid movement of feed through the tract, thus allowing less time for digestion and absorption.

Particle size. Much data exist indicating that forage digestibility is depressed by grinding to a very fine particle size (Galloway *et al*., 1993; Alwash & Thomas, 1974; Firkins *et al*., 1986). Fine grinding also apparently increases rate of passage that consequently reduces the digestibility.

Chemical composition. One of the most significant factor, which affect digestibility is the chemical composition of the feeds (Poppi *et al*., 1981b; Luginbuhl *et al*., 1994; Sarwar *et al*., 1985). Digestibility of one feed is believed to differ from that of a similar feed because each may contain different contents of certain chemical entities, particularly since some of these diminish the opportunity for the digestive enzymes to come in contact with their respective substrates. On the other hand, digestibility of complete feeds can be enhanced by the additions of relatively small quantities of specific nutrients such as protein or soluble carbohydrates.

Feed processing. Processing of feedstuffs is conducted in an attempt to enhance digestibility (Faichney, 1986; Sarwar *et al*., 1992). Changes in physical form can influence digestibility of the dry matter, energy, protein or any of the organic substances in feed products. Such processes as drying, grinding, pelleting and wafering all act to generally

affect digestibility. Chemical, biological treatments and chopping improve the digestibility of fibrous feeds (Sarwar *et al*., 1994).

Climate. The digestibility was higher at higher temperature than in a cold environment which may be due to higher mean retention time of the feedstuff in the digestive tract (Faichney, 1986). In some studies (Kennedy *et al*., 1976; Kennedy & Milligen, 1978; Kennedy, 1985), sheep exposed to cold (0ºC) had a lower digestibility than controls in warmer temperatures (22ºC). Increased reticulo-rumen motility in the sheep exposed to cold temperature (Kennedy, 1985) may be responsible for the decreased mean retention time. Increasing passage rate in such circumstances could serve as a strategy for increasing dry matter consumption to meet demands for higher energy imposed by cold climate (Merchen, 1988). Neural and endocrine regulation of ruminal contractions in animals exposed to cold have also been reported, but the precise mechanism is still to be determined (Kennedy *et al*., 1980).

Age. It is generally felt that animal individuality affects digestibility more than age. However, older animals appear to better digest some nutrients (e.g., fiber, minerals) than do the young of their species. The evidence available indicated that, in general, age itself makes little or no difference in the ability of animals to digest nutrients. In the case of ruminant species, the young cannot digest much roughage until their digestive tracts, especially their rumens, are developed. Also the ability of old animals to digest feed is often impaired by poor teeth, which makes adequate chewing of their feed difficult. Declining health might further adversely affect digestibility at an advanced age. However, the digestibility of feed by younger animals may often be influenced more by the presence of parasites.

Exercise. Although some workers have found that exercise hastens the process of digestion, it is generally considered to be a factor of minor importance. Other factors such as frequency of feeding, amount of water ingested and animal species may also affect digestibility but the data are contradictory and work remains to be done on these relationships.

CONCLUSIONS

Of the various techniques that have been used to date, the total collection technique is the most reliable method of measuring a feed's digestibility. The time and labor expense involved in the *in vivo* digestibility technique can be minimized by the use of indicator method. The digestibility of a feedstuff may also be predicted from chemical composition of the feed in question. This process involves development of multiple regression equations relating various chemical components to *in vivo* digestibility. *In vitro* digestibility techniques provide a quick, inexpensive, and precise prediction of *in vivo* or conventionally determined digestibility in ruminants. Nylon bag technique is however, quite useful for evaluating kinetic aspects of digestion in ruminants.

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