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Standardization of Analytical Methodology for Feeds

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In vivo digestibility-intake trials are expensive, time consuming, and require relatively large amounts of feed. Although there have been attempts to develop techniques to use small animals as pilot animals for evaluation of ruminant feeds, none have achieved widespread acceptance. Therefore, the sheep has become, in effect, a pilot ruminant because the extensive data obtained with sheep are extrapolated to cattle. Such extrapolations should be made with caution because important differences can, and do, occur. Cattle digest roughages more efficiently than do sheep and the differences between values determined with cattle, compared to those obtained with sheep, increase as digestibility decreases. Although insufficient data are available to adequately compare voluntary intakes between cattle and sheep, most experienced investigators feel there can be significant differences. Available evidence indicates that relative differences in feed value between herbages are similar for sheep and cattle although absolute values may differ for most feeds. However, such correlations become unreliable for low quality feeds (e.g. digestibilities below 45-50%). Specific suggestions for digestibility and intake assay procedures are discussed.

In most cases the energy from a given ration that is available for the metabolic processes of the animal it is fed to (available energy) is primarily responsible for establishing the level of production that ration will support. Or, conversely, the animal feeder first considers the available energy required for the particular level of production desired (maintenance of breeding animals, gestation, lactation, growth, finishing for slaughter, etc.), then balances the other nutrients, with supplements where necessary, to the required available energy level. In practical feeding operations, rations are developed on the basis of the best estimates, or predictions, that are available for the nutritive value of the feeds to be used and for those that might be required as supplements.

The available energy value of a roughage is usually very closely related to its overall feeding value, so much so that the two terms are often used interchangeably. Roughage available energy is also, however, the most difficult to estimate or predict. Although there has been considerable work undertaken, and progress is being made, there is not yet a laboratory analytical technique that will estimate the available energy value of a roughage satisfactorily over a wide range of conditions and substrates. Therefore animal feeding data are still required for refined estimates of the available energy value of roughages. It has been established that determinations of digestibility and voluntary intake provide estimates of available energy value that are reasonably reliable and go a long way toward bridging the gap between analytical laboratory estimates and actual performance.

This paper will be limited to discussions pertaining to estimating the available energy of "roughages" for ruminants. For the purpose of this paper, roughages will mean feedstuffs derived from the vegetative and more fibrous portion of plants including forages, herbage, waste products high in cellulose, etc.

Principles and Application of a Pilot Ruminant

Feeding experiments with large ruminants are very expensive because of the high cost of facilities and animals, and the relatively large amounts of feed required. The need for a pilot animal for roughage evaluation work is self-evident. Such a pilot animal should be small, so that costs are minimized, and should provide data that can be reliably extrapolated to domesticated ruminants. Although there have been attempts to develop techniques to use meadow voles, rabbits, insects, etc. as pilot animals, none of these attempts have achieved widespread acceptance. Therefore, most of the work is carried out with sheep. In such work, the sheep is the primary animal insofar as

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there are large sheep populations in many parts of
the world and the estimates of feeding value ob-
tained with sheep are directly applicable to those
populations. However, the sheep can also be con-
sidered a pilot ruminant because only limited
data are obtained with cattle per se. Instead,
values obtained with sheep are extrapolated to
cattle.

Before considering the reliability of the sheep
as a pilot ruminant for cattle, a very brief synopsis
of some basic principles is offered. First, the im-
portance of the feeding value of a roughage de-
creases as the proportion of concentrate in the
ration increases. In high concentrate diets, the
value of a roughage is often due to its fibre con-
tent, which is required to maintain rumen func-
tion, rather than to its nutritive value. Secondly,
voluntary intake is a very important index of
roughage feeding value in feeding regimes where
roughage is fed to appetite, but it is not, of course,
of any importance when roughages are fed at
levels below appetite (e.g. maintenance feeding,
feeding a fixed quantity of roughage with con-
centrates, etc.). Thirdly, although for a given
herbage species there is often a good correlation
between digestibility and voluntary intake, the
interrelationships break down when comparing
different species, or mixtures of herbage species.
It has been amply demonstrated (Heaney 1970)
that either digestibility or intake can be highly
misleading if used alone and must, therefore, be
considered together when comparing herbage
species (or evaluating mixtures). Lastly, it must
be remembered that even in vivo measurements
of digestibility and intake are not constants in
themselves, but can vary with changes in the phy-
sical form (e.g. grinding and pelleting) of the
roughage, physiological state of the animal, etc.

Application of Sheep Data to Cattle

Because both sheep and cattle are ruminants,
both are highly refined domestic species, and gen-
erally speaking they are fed similar feeds, feed
value estimates are often freely interchanged be-
tween the two species. Such extrapolations
should be made with caution, however, because
important differences can, and do, occur.

Digestibility

On the average, cattle tend to digest roughages
more efficiently than do sheep (Playne 1978). For
high quality roughages, having dry matter digesti-
bilities exceeding 55-60%, the differences are
small (usually 1–3 percentage points) and can
probably be safely ignored for practical purposes.

As digestibilities decrease, however, the differ-
ences between values determined with sheep,
compared to those of cattle, increase. For low
quality roughages cattle digestibilities can be
significantly higher than those obtained with
sheep. In addition, the spread of points also be-
comes greater at low digestibilities and the corre-
lation between cattle digestibility values and
sheep digestibility values diminishes. Thus, the
available evidence indicates that there are abso-
lute differences between sheep and cattle diges-
tibility values that increase as digestibility de-
creases. However, relative differences in digesti-
bility between herbages are reasonably consistent
regardless of whether they are determined with
sheep or with cattle except for mature, low qua-
ity materials of low digestibility (less than 45%).
In these latter cases the correlation between sheep
and cattle data becomes unreliable.

Intake

The use of the measured voluntary, or ad libi-
tum, intake of a herbage as one of the parameters
characterizing its feeding value is a comparatively
recent innovation. To date there have not been
efficient comparative studies to provide sufficient
data to adequately evaluate comparative intakes
between sheep and cattle. Most experienced in-
vestigators feel there can be significant differ-
ences. Generally, sheep tend to be more sensitive
than cattle and more likely to have lower intakes
of feeds such as poor quality silages. On the other
hand, there appears to be good agreement be-
tween sheep and cattle for both acceptability and
intake of the grasses and legumes usually used for
pasture and/or conserved forage.

In summary, available evidence to date indi-
cates that feeding value estimates obtained with
sheep can be applied to cattle and used with confi-
dence provided reasonable precautions are ob-
served. Relative differences in feed value between
herbages are similar for the two animal species
even though absolute values may differ. The like-
likelihood of erroneous conclusions resulting from
an extrapolation of sheep data to cattle are mini-
mal for most herbages. The possible exception to
this generalization is for very low-quality, high
cellulose materials such as cereal straws, etc. For
these types of low-quality herbages the correla-
tion between sheep and cattle data diminishes to
the point where extrapolation can be misleading.
There is a need for further research in this area.

Measurement of Intake and Digestibility

Determination of apparent digestibility is based
on the principle that the difference between the
quantity of a nutrient consumed and the quantity voided in the feces represents the amount apparently digested and absorbed during passage through the digestive tract. Similarly, measurement of voluntary intake is based on the premise that intake measurements can be accurate and repeatable for a given herbage. To obtain accurate and repeatable values, relatively standard techniques have evolved. For this discussion, the technique for indoor trials conducted in digestion or metabolism stalls will be considered. Design of the digestion stall, per se, will not be discussed. Many variations of plans exist for stalls that suitably restrain sheep, so that they can be individually fed, and have suitable equipment for collection of uncontaminated feces and/or urine (e.g. Cammell 1977). Such equipment is equally effective for housing sheep for determinations of voluntary intake. In practice, it is often standard procedure to measure the digestibility and intake together, either simultaneously or sequentially, for the two determinations. Each requires a suitable preliminary period followed by a suitable measurement period, both of which must be carried out under standardized, controlled conditions. When proper criteria are met, digestibility and intake determinations are, for all practical purposes, biological assays. That is, the resulting values are unique and repeatable, provided the proper procedures and conditions are followed. They are not affected by a change in animals, subsequent periods (i.e. time), time of the year, etc.

**Digestion Trials**

The basic principle underlying the digestibility determination is quite simple; namely, the difference between what is fed and what is voided in the feces represents what is digested. There is, however, a mixing of subsequent feedings in the digestive tract so that the feces collected do not derive precisely from the feed that was carefully weighed and sampled. Particularly in the ruminant, each feeding is thoroughly mixed with varying proportions of several earlier feeds and the feces voided on a given day derive from feed that was ingested over several days. It is imperative, therefore, that stabilized conditions be established so that meaningful averages are measured. As a result, digestion trials consist of a preliminary period and a measurement period.

**Preliminary Period**

The preliminary period must be sufficiently long to accomplish three objectives. First, residues from the previous feedstuff must be given time to be expelled from the digestive tract; second, the rumen microflora must be given time to adjust to the new feed; and third, the animal must become accustomed to consuming the new feed. The recommended length of this period depends, to some extent, upon the nature of the feed being assayed and the experimental regime. Most studies have shown that 7–10 days are sufficient when ordinary forages are fed, there is not a major change in feed type from that preceding the trial, and where a constant level of feeding below the ad libitum level is used. Although 7–10 days does not completely clear the digestive tract of previous feeds, and there may not have been a complete readaptation of the microflora, errors caused by these factors will be minute and, usually, undetectable. Longer preliminary periods are desirable when “problem” feeds (such as silages, straws, alkali-processed materials, ground and pelleted forages, etc.) are assayed or when the feed to be assayed is drastically different from the previous feed. In these latter cases the preliminary period should be at least 14–21 days.

**Measurement Period**

The measurement period must be long enough to ensure that the collected feces accurately represent the residues from the feed that was fed. “Endpoint” errors, caused by the length of time between the last defecation and the start or finish of the measurement period, decrease in direct proportion to the length of the measurement period. At least 7 days are required to minimize such endpoint errors when digestibility is determined at constant daily feed intake. If digestibility is to be determined at an ad libitum level of feeding, the measurement period must be extended to at least 10–14 days because the endpoint errors are further affected by the unavoidable daily fluctuations in food intake. Any feed that is uneaten will usually have a different composition from that which was offered. Therefore, such uneaten feed must be weighed, sampled, and analyzed to accurately correct the feed offered.

**Intake Trials**

The productive value of livestock rations depends on their ability to provide available nutrients to the animal. For monogastric animals consuming high energy, concentrate feeds, the homeostatic mechanisms tending to regulate intake to meet the animal’s energy needs are of major importance. The high roughage diets fed to ruminants do not, however, contain sufficient available energy to meet the animal’s needs (except for those few high-quality forages having digestibili-
ties above 65-68%). For roughages, therefore, voluntary intake has come to be recognized as one of the inherent factors related to quality and is, in itself, an indication of roughage feeding value. Although the use of the measured intake of roughage as one of the important parameters characterizing its feeding value is a comparatively recent innovation, assay procedures have been developed that are adequate to provide satisfactory measurements. Precise knowledge about the effects of some variables has not yet been determined but, generally speaking, when there is doubt, possible errors can be minimized by providing safety factors in the form of longer trials.

**Preliminary Period**

A preliminary period is required for intake measurements for essentially the same reason as for digestibility measurements, but the need to achieve a relatively stabilized intake of a newly introduced roughage is almost always the most important factor determining minimum length of the preliminary period. Characteristically, relatively wide fluctuations in daily feed consumption are encountered when a new roughage is first introduced to a ruminant. Under a regulated feeding regime these fluctuations can be reduced to a minimum, but not eliminated, and can be stabilized at a reasonably constant level so that an accurate measurement of intake can be undertaken. For common forages a preliminary period of at least 10 days is required (Blaxter et al. 1961). For “problem” feeds such as silages, straws, processed low-quality feeds, etc., longer periods are required because it takes longer for a stabilized intake to be reached. Usually, however, 2–3 weeks is sufficient. In addition, experience has shown that there can be serious carry-over effects (Heaney and Pigden 1972) whereby the intake of a subsequent roughage can be significantly affected by the previous “problem” roughage. At present there is little precise information regarding the length of period of this type of compensatory intake, but a minimum of 3 weeks and preferably 4 weeks is recommended as a preliminary period whenever this type of problem is suspected.

**Measurement Period**

Most investigators agree that, provided the preliminary period has been sufficient and effective, a measurement period of at least 7 days is adequate to minimize errors due to the residual daily fluctuations in consumption.

**Level of Feeding**

Because the objective is to measure true ad libitum intake, each animal must have access to feed at all times and the feed allowance must be sufficient to provide some feed weighback every day. Conversely, the actual amount of weighback should be controlled so that the opportunity for selection is minimized. Otherwise, the higher quality portions will be consumed, the lower quality ones rejected, and the resulting measurement values will be in error insofar as they will measure the feed that was selected and not the entire feed that was intended to be assayed. Only when selection is impossible, e.g. pelleted feeds, can the aforementioned principles be disregarded. Available evidence (Heaney 1973) indicates that an average weighback of up to 15% causes only negligible errors in measured intake. Conversely, if weighbacks are kept at an average of only 1–2%, the intake values tend to be depressed. Therefore, it is usually recommended that weighbacks should be about 10%. The inevitable daily fluctuations in consumption make it difficult to maintain a precise level of weighback but experienced feeders have no difficulty keeping daily weighbacks within a range of 5–15%. Feeding at this level minimizes any errors due to selection but assures that true voluntary intake is measured. If digestibility is to be measured at an ad libitum level of feeding it is even more important to control daily weighbacks because the evidence shows that digestibility is affected much more by selection than is intake.

**Number of Animals**

The precision of any biological assay procedure is a function of many factors including analytical errors, techniques to ensure collection of representative samples, and inherent animal to animal variability. For intake and/or digestibility assays, inherent animal to animal variability is usually the major factor determining the ultimate precision that can be attained although in some cases obtaining representative samples for analysis can also be a major problem (e.g. Cammell 1977). The standard deviation of an individual dry matter digestibility coefficient is usually between 1.0 and 1.3 percentage units (Forbes et al. 1946; Raymond et al. 1953). Statistically, using three sheep per digestibility measurement will give an 80% chance, at the 5% level of significance, of detecting digestibility differences of 3–4%, and four to six sheep should reliably detect differences of 3% (Fig. 1). Increasing the number of sheep further results in only minimal increases in precision.

Intake measurements, on the other hand, are less precise (Heaney et al. 1968). The standard deviation of an individual measurement is usually
between 7 and 10 intake units (g consumed/W0.75/day). Statistically, about 12 sheep are required to detect differences of 10 intake units (i.e. 80% chance at 5% significance). Increasing the number of sheep above 15 results in only marginal increases in precision, but decreasing the number below 10 rapidly decreases the precision of intake measurements (Fig. 1).

Considering these statistical implications, it is usually recommended that a minimum of three sheep be used if only digestibility is to be measured and six sheep be used if intake, or both intake and digestibility in the same trial, is to be measured. In practice most investigators have found these minimum numbers will allow detection of digestibility differences of 2–3 percentage units and intake differences of 6–10 intake units in most cases. It must be recognized, however, that there will be instances when such differences will not be significant when the suggested minimum numbers are used. Nevertheless, the above recommended minimum numbers are a reasonable compromise between cost and precision.

Fig. 1. Number of animals required to obtain an 80% chance that the digestibility or intake difference will be significant at the 5% level (intake from Heaney et al. 1968; digestibility from Raymond et al. 1953).