Use of Indicator Methods in Range Digestion Trials. A Review

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The nutrition of range livestock is necessarily an important consideration not only of the individual rancher but also the technical range manager. Of the environmental factors which affect animal productivity, probably the most important is that of nutrition. An increased productivity per animal generally means a better ranch income to the operator and is definitely concerned with stabilizing the livestock industry which is one of the goals of range management.

As range livestock operations are generally quite extensive in nature and as low productive ranges are commonly utilized, the nutrition of range livestock is often found to be marginal or submarginal. In contrast with farm livestock production, economics do not allow for range livestock to be kept continually on a high plane of nutrition, but an optimum does exist below which an animal's productivity is apt to be curtailed.

During the past 35 years considerable work has been done on the chemical analysis of bulk samples of the more common range plants. Although this is an index to the value of different species, it is not entirely satisfactory. Cook and Harris (1951) reported that nutritive value as measured by chemical content alone is not a reliable index to the availability of the various nutrients contained in the forage unless accompanied by digestibility determinations or balance trials. This necessitates the more extensive use of digestion and metabolization trials.

Kennedy and Dinsmore (1909) in their early work in Nevada showed farsightedness in expressing concern that reseeded plants must not only seed themselves but must be valuable from the standpoint of nutrition. Also, the decreased livestock weight gains and percent calf crop which accompany overgrazing are due primarily to a lowered plane of nutrition of the grazing animals.

Ruminant Digestion Trials

Schneider (1947) reported that the first recorded digestion trials were carried on by Henneberg and Stohman in 1864 in Germany and that since then over 25,000 digestion trials have been reported from all classes of animals. However, most of these trials have been carried out with either monogastric animals or with ruminants fed the normal farm roughages and concentrates. Because of their size and ease in handling, sheep have been used in ruminant digestion trials to a much greater extent than cattle.

Although the anatomical and physiological differences in the digestive system in cattle and sheep differ only to a limited extent, variations between the digestive abilities of these two species have been recognized for many years. However, the direction and extent has not been agreed upon by different research workers.

Watson, *et al.*, (1948) and Jordan and Staples (1951) reported that in general the differences in ability of sheep and cattle to digest the various nutrients were not significant. However, Cipolloni, *et al.*, (1951) and Forbes (1950) reported a greater accuracy when digestibility data were obtained with cattle and sheep separately and listed in separate digestibility coefficient tables. In addition, Smith, *et al.* (1956) in recent studies in Utah found that the ability of deer to digest fibrous material was very similar to that of sheep.

For practical purposes and until more digestibility data by species is available, some interspecific application of digestibility data could probably be justified in grazing animals.

Under strictly range conditions where the animals were allowed to graze and select free choice from the native species, only a limited number of digestion trials have been carried out. Some of the reasons for the great lack of experimental work with range livestock can be attributed to: (1) the difficulty of determining the exact amounts of the different species and plant parts consumed, (2) the lack of proven handling techniques, and (3) the large expense involved in conducting range digestion trials.

The original method of determining digestibility was essentially that of determining the difference between total amount of a nutrient appearing in the ingesta and the amount appearing in the feces. This is based upon the definition that the digestibility coefficient of a nutrient is the percent originally ingested that does not appear in the feces. This method necessitates total feces collection and is still considered the most accurate for the most part. Metabolization trials require the additional collection of urine.

Total feces collection by use of fecal bags is not too well adapted to grazing trials as the fecal bags are apt to affect the grazing behavior of the animals and the fecal bags and harness may be damaged. Although feces collection has been found to be particularly difficult with female animals, Balch *et al.* (1951) reported an apparatus for the separate collection of both feces and urine in metabolization trials with grazing cows which worked successfully.

Many variations in handling techniques of the grazing animals during digestion trials have been used. Although many grazing and forage consumption problems are common to digestion trials on both tame pastures and range areas, some feedlot techniques have been found unadapted to grazing trials on range lands.

Harris, *et al.* (1952) have given in detail the techniques found to be particularly useful with range sheep. Primary emphasis was placed upon the use of herding and of portable pens. Bohman and coworkers (1955) found that variations in techniques from those used with sheep were necessary for use with cattle. Because of the lack of gregariousness in cattle and the high cost of fencing, portable corrals and feeding chutes were improvised which also proved to be satisfactory for weighing, blood sampling and the feeding of supplements.

Proposed Indicators: Their Chemical Composition, Indigestibility and Determination

As total collection is "long, tedious, and expensive" (Reid, *et al.*, 1950), research personnel began early to seek an indirect method of determining digestibility. This has been partially answered by the use of an inert reference material or "indicator." The digestibility coefficient of a nutrient can then be found by determining the ratio of the concentration of the indicator to that of the nutrient in both the feed and the feces. By the use of these methods, digestibility of a nutrient can be obtained theoretically without measuring either the total dry matter intake or feces output, thus eliminating the need for total feces collection. By formula this can be shown as follows where X equals nutrient digestibility:

X = 100 — 100 (percent indicator in feed ÷ percent nutrient in feed) × (percent nutrient in feces ÷ percent indicator in feces.)

With chromogen "percent indicator" is changed to "indicator content."

Reid, *et al.* (1950) reported the following as features of a successful indicator method:

1. A reference material which occurs naturally (except in certain exogenous indicators) and in a measurable quantity in the feedstuff, which is indigestible and therefore completely recoverable in the feces, and for which there is a simple, accurate and rapid procedure for chemical analysis.
2. The recovery of the reference material from the feces must not be influenced by treatment of the feed, stage of maturity, or by irregular passage of the indicator through the gut.
3. The equilibrium of the reference substance in the feed must be established soon after feeding is begun in order that short-time trials may be used.

According to Maynard (1951), the reference material should have no pharmacological action on the digestive tract. Reid (1952) stated that, in testing any indicator, comparison with the results of a conventional (total collection) trial is required.

Three indicators that have been used successfully under range or semi-range conditions are: (1) lignin, (2) chromogen, and (3) fecal nitrogen. Other indicators which have proven less adaptable or have not been tried sufficiently under range conditions are: (4) forage protein, (5) silica, (6) methoxyl group, (7) iron oxide, (8) chromic oxide or chromium sesquioxide, (9) barium sulfate, (10) titanic oxide, and (11) anthraquinone violet.

Lignin

Lignin, a collective name given to the indigestible constituent in plant cells, has been commonly used as an indicator of digestibility. Cook and Harris (1951) working with winter range plants in Utah found that the lignin ratio technique gave very satisfactory results.

However, it is now being questioned whether the lignin is actually indigestible. Lignin quality as well as quantity varies between plant species, between age classes within species, and between individual plant parts. Apparent lignin digestibility of from —10 percent to 30 percent have been reported. Ellis, *et al.* (1946) considered the apparent digestibility to be due largely to an inefficient method of determination and proposed a standard 72 percent H_2SO_4 method of determination.

In studies in Utah in which winter browse plants were fed to deer, Smith, *et al.* (1956) found that the digestion values determined by the lignin ratio differed markedly from those from conventional analysis. It was reported that a skilled chemist was unable to make consistent lignin analyses from the same samples. These authors concluded that, although it was not clear whether the apparent lignin digestibility was due to actual digestibility or to inability to accurately isolate the lignin, the lignin ratio was invalid for computing the digestibilities of native forages high in woody material.

Efforts have been made to isolate certain chemical compounds from within the lignin fraction which might prove to be truly indigestible and easily determined. Richards and Reid (1952) investigated the methoxyl fraction of lignin as a reference material but found that this did not prove satisfactory.

Chromogen

Reid, *et al.* (1950) proposed the use of pigments naturally occurring in the plant and which absorb light at 406 mμ for measuring digestibility and dry matter intake of roughages. The exact nature of these pigments being unknown at the time, they were given the name of "chromogenic substances" or "chromogen(s)." Chromogen was found to be widely distributed in plants, and a quantitative relationship was found between the chromogen content of the ingested forage and the feces excreted.

Reid, *et al.* (1952) reported a modification of their original chromogen ratio procedures adapted to use with grazing animals. This improvement was brought about by prediction of forage chromogen content from that in the feces rather than by direct determination. The following relationship between the chromogen-dry matter ratio of feces voided and that of the forage actually consumed was proposed from their experimental data:

$Y = .0925X + 137.3 \log X - 242.12$ where Y is the units of chromogen per gram of forage and X is the units of chromogen per gram of feces. The correlation coefficient between the computed chromogen concentration and that predicted by formula was $.995 \pm .001$.

Cook and Harris (1951) found that in browse plants high in ether extract and essential oils, chromogen recovery was markedly inadequate. This unfavorable finding, together with others, has initiated a concerted effort to determine the actual digestibility and composition of the chromogen fraction.

Irwin, *et al.* (1953) made a study of the actual components of chromogen and found these could be grouped under three classes: (1) carotenoids, (2) xanthophylls, and (3) chlorophylls. Carotenoids and xanthophylls were found to be very unstable and absorbed in the digestive tract. Because of the dynamic nature of the chlorophylls, no one chlorophyll pigment could be used as an indicator. Pheophytin, a decomposition product of chlorophyll, was found to be the most significant plant pigment in cow feces.

Davidson (1954) in trials with sheep fed grass hay found apparent digestibilities of the various plant pigments in the digestive tract as follows: carotenoids, 2-12 percent; xanthophyll, 10-20 percent; chlorophyll a, 81-87 percent; chlorophyll b, 77-82 percent; pheophytin a, 22-52 percent; and pheophytin b, —36 percent to 17 percent.

Kane and Jacobson (1954) reported trials in which pheophytin proved satisfactory as an indicator. They recommended the pheophytin method should have a greater accuracy over the use of other plant pigments since only one pigment determined the optical readings.

In studies on the use of chromogen as an indicator, Smart, *et al.* (1954) found that the introduction of copper into the porphyrin ring of chlorophylls and pheophytins by treatment with cupric chloride stabilized these substances against acid, alkali and light while at the same time largely destroying the carotenoids. They compared their modification with the original chromogen method of Reid, *et al.* (1950). In trials with winter leaves of switchcane, 55 percent of the chromogen was recovered while 100 percent of the copper derivatives of chlorophyll was recovered. In spring leaves the corresponding recoveries were 82.9 and 99.3 percent. These authors found Reid's (1950) method worked well only when the principal pigments were the chlorophylls and their degradation products. This modification of the chromogen method may prove to be of real improvement over previous procedures.

Other Indicators

Lancaster (1949a) proposed a method of determining digestibility of pasture forage consumed by the use of fecal nitrogen concentration. This author, working with sheep, found a constant of $.83 \pm .102$ gm. of nitrogen was excreted per 100 gm. of pasture organic matter ingested. Lancaster reported that the summation of data from 52 trials showed that the variation in digestibility estimation by the feces nitrogen method was less than that obtained by conventional trials.

Gallup and Briggs (1948) also reported a constant relationship between fecal nitrogen and dry matter intake. Lancaster (1949b) modified his original method by dividing forages on the basis of their protein contents. To the forages containing less than 15 percent protein he gave a constant of $.67 \pm .120$ gm. and to forages with 15 percent or more protein $.80 \pm .081$ gm.

In contrast, Forbes (1949) could find no constancy in fecal output per 100 gm. of dry matter intake but found rather an increase in this ratio as the protein content of the ration increased. Soni, *et al.* (1954) found that the fecal nitrogen method appeared to give as good an indication of dry matter digestibility as the chromogen method. They reported the fecal nitrogen method was simple and convenient and called for further study.

Silica has been found particularly inaccurate as an indicator of digestibility in grazing trials as animals pick up large quantities of dirt in normal grazing. Chromium oxide, although not a naturally-occurring plant component, has found use as an added or exogenous indicator which can be fed in the concentrates or administered directly by capsule or drenching. Its use in grazing trials is primarily for the purpose of determining dry matter intake rather than nutrient digestibility.

Feces Sampling and Diurnal Variation

If the indicator becomes evenly distributed throughout the ingesta and passes through the digestive tract in a similar manner without stratification, one "grab" sample of fecal material is theoretically enough to accurately determine nutrient digestibility and dry matter consumption by use of the indicator methods. In other words, the indicator methods are truly successful only if they greatly decrease the sample size or number of samples of feces necessary to give reliable digestibility data.

However, Reid (1952) reported diurnal variation to be a problem in all indicators and that added exogenous indicators such as chromium oxide appeared to be less uniformly excreted than naturally occurring indicators.

Attempts have been made to set up daily time schedules of feces sampling to coincide with the time when the indicator content of the feces approaches or is equi-distant from the 24-hour mean. Although this has proven partially successful, diurnal variation remains a serious problem in the practical use of the indicator methods.

Special Problems in Determining Dry Matter and Nutrient Consumption

The minimum factor which must be determined manually under all indicator methods at present is the nutrient content of the ingested forage. However, the actual forage which animals consume under grazing conditions and its nutrient content is extremely difficult to determine. Cook, *et al.* (1948b) reported this lack of knowledge of the composition of the grazing animal's diet to be due to:

1. Difficulty of collecting representative samples of vegetation because of soil, site and seasonal variations.
2. Difficulty of finding what species and portions of plants are actually consumed.
3. Difficulty in interpreting the nutritive content of the ingested forage.

Clipping techniques have been commonly utilized in determining nutrient intake, but Crampton and Jackson (1941) reported that the manual sampling of pasture plots by mowing, clipping or even plucking, cannot, on theoretical grounds alone, be expected to simulate grazing with any certain degree of accuracy. However, Cook and Harris (1951) utilized a hand plucking technique with satisfactory results by paying close attention to and taking the plucks in close proximity to the grazing animals.

Reid (1952) reported that ordinary digestion trials in which forage is cut and hand fed to animals yield atypical results when applied to grazing conditions because the barn-fed animals do not select the same amount, species and plant parts as do the grazing animals. This inability of investigators to exactly simulate the forage consumed by unrestricted grazing animals has possibly been the greatest obstacle in the advancement of range livestock nutrition.

Cook, *et al.* (1948b) indicated that the determination of the botanical composition of a sheep's diet by multiplying the species composition of the flora by the percent of each species consumed at the end of the season was too general and that utilization of stomach analyses was unsatisfactory. These authors recommended a "before-and-after grazing" system of clipping to determine nutrient and dry matter intake by difference as well as botanical composition of the grazing sheep's diet.

Hardison, *et al.* (1954) proposed an estimate system of determining the nutrient composition and digestibility of herbage consumed by grazing animals based on data from similar barn feeding experiments and the fecal excretion properties of the grazing animals. Garrigus and Rusk (1939) developed a dry matter-defecation ratio procedure based on a constant relationship between the dry matter consumed and that defecated. This latter has received common usage in the United States with caged animals but necessitates total collection.

It has been commonly noted that grazing animals select certain plants over certain others and leaves over stems. Cook, *et al.* (1948a) in studies with grazing sheep found that intensity of grazing and abundance of palatable plants were the major factors controlling the forage sheep's diet. Cook, *et al.* (1948b) reported that since sheep selected largely leaves and stem tips, the quality of the ingested forage was much greater than the bulk chemical analysis would suggest.

Hardison, *et al.* (1951) reported that forage hand clipped and fed to steers was only 91.6 percent as digestible as that actually consumed by grazing steers. In later trials with grazing steers Hardison, *et al.* (1954) found that the average diet contained 23.3 percent more crude protein, 35.3 percent more fat, 25.1 percent more ash, but 16.8 percent less crude fibre than the clipped whole herbage consumed by barn-fed animals.

Indicators may be used in grazing trials with total collection to determine dry matter consumption as follows where Y equals pounds of dry matter consumed:

Y = pounds dry matter excreted × percent indicator in the feces ÷ percent indicator in ingesta

Reid, *et al.* (1952), however, suggested a double sampling procedure adapted to grazing trials in which a natural occurring plant indicator is employed for determining digestibility and an exogenous indicator is added to the animal's ration in known quantities to be used as an index of total quantity of feces voided per unit of time. This procedure, if proven successful, would eliminate the use of both hand sampling and fecal bags for determining dry matter consumption. These authors recommended the simultaneous use of chromogen and chromium sesquioxide.

Kane, *et al.* (1953) found that the simultaneous use of an internal and an exogenous indicator gave excellent results in measuring both dry matter consumption and digestibility. The following formulas were utilized by these authors:

1. Dry matter digestion = 100—100 (indicator content in the feed ÷ indicator content in the feces)
2. Dry matter consumption = (total amount of external indicator in feed ÷ amount external indicator in feces sample) × (amount of dry matter in feces sample ÷ percent dry matter indigestible)

Hardison, *et al.* (1953) reported that this double sampling procedure gave accurate results and that the correlation coefficient of the predicted dry matter consumption by this method and that determined conventionally was .981. Double sampling procedures are now in common use and will undoubtedly continue to play an important part in grazing trials. Raymond and Minson (1955) reported that fecal production data estimated by chromic oxide may allow more valid estimates of daily herbage intake than do total collection data.

Summary

Although the nutrition of range livestock is of great importance to both ranchers and range managers, only a very limited number of digestion trials have been completed under range conditions. Many problems have been met in determining the actual nutrient and botanical content of a grazing animal's diet.

The use of inert reference materials such as lignin, chromogen and fecal nitrogen in determining nutrient digestibility and dry matter consumption has given great impetus to this field of endeavor. However, the use of lignin has been questioned because of its apparent digestibility. The value of chromogen as an indicator of digestibility, on the other hand, has been enhanced by the ability of research personnel to stabilize the chlorophyll fraction by the use of copper compounds.

An aid of particular importance is the double sampling technique in which an added indicator is used in determining dry matter consumption and a natural indicator in determining digestibility. Yet, further improvement and refinement in the use of indicator methods under range conditions is deemed necessary.

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