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Title DETERMINATION OF INTAKE AND DIGESTIBILITY OF PASTURE FORAGES USING CONVENTIONAL AND INDICATOR TECHNIQUES

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Digestibility experiments were carried out with dairy heifers to determine the digestibility of orchard grass, Dactylis glomerata, and also to evaluate the accuracy of various indicator techniques in pasture digestibility experiments. Chromic oxide was used as an external indicator to predict fecal dry matter output in three digestion trials. Cut grass was fed in one of the trials and corn silage in the other two. The internal indicators fecal nitrogen and protein of feed and feces were studied in two of the trials in which one conventional and one grazing trial were involved. The experimental animals consisted of six approximately two year old Holstein heifers and six Jersey heifers of the same ages. All animals were in about the fifth month of pregnancy.

In the experiments, five grams of chromic oxide in gelatin capsules were fed to the animals twice a day at 7 A. M. and 4 P. M.

Solka-floc, which is a pure cellulose material, was used as a carrier for chromic oxide. Fecal grab samples were collected from the rectum of grazing animals at 6 A. M. and 4 P. M. The chromic oxide recoveries in the feces of the stall fed animals fed orchard grass ranged from 98. 4 to 99. 2 percent, averaging 98. 8 percent. The average difference between the predicted and actual fecal dry matter output was 23. 1 g. This magnitude of difference yielded a 1. 01 percent error of prediction. However, chromic oxide recoveries for the silage trials were considerably lower, estimated as 79. 9 and 74. 9 percent for trials III and IV respectively. These low recoveries of chromic oxide resulted in a rather high error of prediction of fecal dry matter output with silages.

The average errors of prediction for the grass forage dry matter intakes using fecal nitrogen and protein indigestibility techniques were found to be 1. 4 and -6. 4 percent, respectively. The respective differences between the average actual and predicted forage dry matter intakes were 86. 1 and -405. 2 g. Based on the results of this study the fecal nitrogen index technique appears to be superior to the protein indigestibility technique.

The digestion coefficients of ether extracts were found to be higher for the conventional trial with pasture than for that of the grazing trial, while the reverse was true for the nitrogen free extracts. The average TDN and digestible energy values for orchard

grass dry matter in the conventional trial were found to be 67. 1 and 68. 0 percent, respectively. The difference in estimations of the TDN and digestible energy values between total collection and fecal nitrogen technique was 0. 5 percent, which may be regarded as a reasonable error in digestibility experiments. The TDN and digestible energy values of the grazing trial were slightly higher than that of the conventional trial. The digestible energy determinations were highly correlated with the calculated TDN values.

It was concluded that chromic oxide and fecal nitrogen may be used effectively in pasture digestibility experiments. More research is needed to establish their use as reliable indicators with single feed stuffs, such as silages, or when combined in complete rations.

DETERMINATION OF INTAKE AND DIGESTIBILITY OF PASTURE FORAGES USING CONVENTIONAL AND INDICATOR TECHNIQUES

by

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INTRODUCTION

Tremendous advancement has been achieved in the field of animal nutrition in the last two decades. The evaluation of feeding stuffs has enabled a considerable part of this progress. The "more accurate" and the "more rapid" methods for determining the nutritive value of feeding stuffs have been one of the unique goals of the animal scientist.

An extensive survey (Jennings, 48, p. 22) showed that green pasture supplies the largest part of the feed source for dairy cattle, beef cattle and sheep in the United States. Moore (75, 14 p.) reported that pastures provide the cheapest total digestible nutrients for animals. The advantages of using pastures have attracted the attention of animal nutritionists and many experiments have been conducted to find a satisfactory technique to evaluate the intake and digestibility of pastures, particularly under grazing conditions.

More difficulties have been encountered in determining the digestibility of pastures than any other feeding stuff due to the difficulty in resembling selective grazing in the conventional digestion trial. To overcome this difficulty and also to reduce the heavy costs of digestion trials a series of techniques dealing with the digestible

or indigestible constituents of plants have been used for the evaluation of pastures. There may be many variable factors affecting the results of any biological study. Accordingly, a number of techniques based on different indicators have been proposed for determining the digestibility of pastures. These techniques have been studied at several experiment stations by many workers. Some of the results are in agreement while others are not. More research work is needed to develop workable techniques and procedures.

This thesis presents the results of studies to determine the consumption and digestibility of orchard grass pasture by dairy cattle using chromic oxide and fecal nitrogen as external and internal indicators. Results obtained by this method were compared with the results of conventional digestion trials carried out simultaneously. The first part of the thesis consists of the evaluation of the accuracy of the chromic oxide technique to determine fecal dry matter output of dairy cattle. Three digestion trials were carried out using dairy heifers. The first group, consisting of three Holstein heifers, was fed only orchard grass while the second and third groups, which were Jersey heifers, were fed silages. The results obtained from total collection technique were compared with the calculated findings using chromic oxide as an indicator.

Grass consumption and nutritive value of pasture were studied by grazing trial using fecal nitrogen and the protein content of feed

and feces as internal indicators. A comparison between total digestible nutrients and digestible energy values of the pasture was also included in the study.

REVIEW OF LITERATURE

The first procedure developed among the various measures for estimating the nutritive value of feeds was a chemical analysis. The value of chemical analysis in animal nutrition was appreciated even 100 years ago (Crampton and Lloyd, 20, p. 13). Henneberg and Stohmann at the Weende Experiment Station in Germany devised a scheme for the routine description of animal feedstuffs in 1864. It is now commonly known as the Weende analysis or proximate analysis. At the time of these pioneer workers the nutritionally important components of protein were not recognized, fats were considered to be nonspecific sources of energy and vitamins were not known. They were mostly interested in carbohydrates. They believed that the carbohydrates could be grouped into: a) starches and sugars, and b) a coarse fibrous fraction. Chemical analysis of feeds was necessary, but it was not enough unless the digestible portion of the nutrients could be determined. According to Schneider (90, p. ix) the first recorded digestion trials were carried on by Henneberg and Stohmann in Germany and since that time over 25, 000 digestion trials have been reported in different countries of the world. Most of these trials have been carried out with ruminant animals fed many different kinds of roughages and concentrates. However, because of their size and ease in handling, sheep have been used in ruminant digestion trials

to a much greater extent than cattle. In spite of similarities in the anatomical and physiological conditions of the digestive system in cattle and sheep some variations between the digestive abilities of these two species have been reported. However no agreement has been established by investigators. Thus, Jordan and Staples (50, p. 236-243) reported that no significant difference in the digestive abilities between sheep and cattle was detected. Forbes (29, p. 231-237), however, suggested that separate digestibility coefficient tables for cattle and sheep be used. Separate tables for cattle and sheep are given in the book Feeds of the World by Schneider (90, 299p.)

The term "digestion" includes all the physiological activities and biochemical changes which food undergoes within the digestive tract to prepare it for absorption and use in the body. The "digestion coefficient" of a substance may be defined as the percentage of a nutrient consumed in the ration which is absorbed so that it does not appear in the feces.

Total Collection Method

In general digestion coefficients of nutrients in feeds have been determined by "standard" or "conventional" techniques. These involve the complete record of the nutrients consumed and total collection of the feces. This method of determining digestibility, although it is considered to be one of the most accurate techniques, requires

special metabolism stalls or fecal collection bags to collect feces and urine output separately. It also removes the animal from its natural environment. Various types of digestion cages have been designed for laboratory animals, sheep and swine (Bratzler, 8, p. 592-601 and Hansard et al. 39, p. 88-96). Ritzman and Colovos (89, 16 p.) developed a metabolism stall which incorporates a moving endless belt carrying feces up to a hopper but separating urine by down-flow. Different types of metabolism stalls for use in total collection trials with steers have been described by Horn et al. (46, p. 20-24), Nelson et al (77, p. 504-510) and Briggs and Gallup (9, p. 479-482). Hobbs et al. (43, p. 563-570) have also developed equipment which separates the urine from feces voided by either steers or heifers.

Total feces collection by use of fecal bags is not too well adapted to grazing trials. The fecal bags tend to annoy the animals resulting in an effect on the grazing behaviour of the animals and also the fecal bags and harnesses may be damaged. For steers and wethers, collection bags are attached to the animal so that feces are collected separately from urine. Collection bags designed by Garrigus and Rusk (36, p. 453-458) have been commonly used with steers. Although separate collection of feces and urine has been found to be particularly difficult with female animals, Balch et al. (3, p. 98-101) reported a successful apparatus for the separate collection of feces and urine in metabolism trials with grazing cows.

Grazing Trials

Besides the expense and labor involved in conventional digestion trials with cut grass such trials do not necessarily give the actual digestibility values of the pasture. The assumption has to be made that the herbage represents that which grazing animals would eat on the pasture. However, when grazing, animals select grass according to varieties and the more nutritious leafy parts of the plants in preference to stems.

Jonston-Wallace and Kennedy (49, p. 190-197) reported that cattle will select leafy herbage in an immature stage of growth and usually shorter plants rather than taller herbage of the same species. Tall herbage is generally more mature, stemmier and otherwise of a somewhat different physical and chemical composition than shorter plants. Factors other than height may influence animals to graze the shorter herbage.

Hardison et al. (41, p. 89-102) studied the degree of herbage selection under various conditions by grazing steers. The diet selected was higher in crude protein, ether extract and mineral matter, but lower in crude fiber than the whole herbage available for consumption. Grazed herbage was more digestible than cut herbage from the same source fed to steers in digestion stalls. When grazing was restricted to small areas containing less herbage the extent of selection

was reduced, whereas in larger areas with large quantities of herbage the degree of selection was increased. Although it was demonstrated that grazing animals select more digestible plants, no information was obtained on what factors cause animals to graze discriminately. These authors concluded that the chemical composition of clipped herbage does not represent the chemical composition of herbage selected by grazing animals and the digestibility of clipped and hand fed herbage may be quite misleading for the estimating the value of pasture under grazing conditions.

Indicator Techniques

As indicated the conduct of a digestion trial is a laborous, expensive and time consuming procedure. To overcome these handicaps nutrition scientists have searched for an indirect method of estimating digestibility. The problem has been partially solved by the use of an inert reference substance or "indicator" which naturally occur in or can be added to the feeding stuff being examined. The digestibility coefficient of a nutrient can then be found by determining the ratio of the concentration of the indicator in the dry matter of the feed to that which appeared in the feces. An ideal indicator for digestibility studies has been specified by Maynard and Loosli (70, p. 303) as:

> It should be totally indigestible and unabsorbable, have no pharmacological action on the digestive tract, pass through the tract at a uniform rate, be readily determined

chemically, and preferably be a natural constituent of the feed under test.

The estimation of the dry matter intake and digestibility requires two simultaneous indicators. One indicator should be internal, that is one that occurs naturally in the forage such as lignin, plant pigments etc. The second indicator must be an external one, that is to be added to the ration in known amounts. Reid et al. (83, p. 60-71) considering the difficulties involved in the use of various indicator techniques suggested that the ideal method should possess the following features:

1. It should employ a reference material which occurs naturally and in a measurable quantity in the feed stuff; which is indigestible and therefore, completely recoverable in the feces; and for which the chemical analysis is simple, accurate and rapid.

2. The recovery of the reference substance from the feces must not be influenced by treatment of the feed (curing methods, heat, etc), by stage of maturity or by irregular passage of the indicator through the gut.

3. The equilibrium of the reference substance in the feces with that in the feed must be established soon after feeding is begun in order that short time trials may be used.

The procedure of estimating digestibility is based upon the determination of the concentration of the nutrient (s) and indicator both in feed and in feces without measuring either the total feed intake or fecal output. The calculation of digestibility by an indicator method is done using the following formula:

$$\text{Digestibility} = 100 - \left[100 \frac{\%\text{indicator in feed}}{\%\text{indicator in feces}} \times \frac{\%\text{nutrient in feces}}{\%\text{nutrient in feed}}\right]$$

Lucas (67, p. 301-302) developed a formula, actually a modified form of the above formula, for obtaining apparent digestibility by the indicator procedure as follows:

$$d^* = 100 - \frac{r^* p C_i}{p_i C} \quad \text{in which}$$

d^*	apparent digestibility
r^*	assumed percentage recovery of indicator
p	percentage of the nutrient in the feces
C_i	percentage of the indicator in the feed
p_i	percentage of the indicator in the feces
C	percentage of the nutrient in the feed

The indicator methods used for the measurement of digestibility may be considered in two forms:

1. The ratio technique, which deals with a naturally occurring, indigestible indicator and requires the determination of the indicator in the herbage and in the feces.

2. The fecal index technique, in which the indicator does not necessarily need to be indigestible and is measured only in the feces.

The following results have been obtained from the use of various indicator substances in the digestibility experiments.

Silica

Gallup and Kuhlman (33, p. 665-669) studied the effectiveness

of silica as a reference material in digestibility studies with heifers and found that naturally occuring silica in feed served as a satisfactory index of digestibility. The coefficient of digestibility for protein, using silica as an indicator, was in close agreement with those obtained from conventional trials. However the same workers (34, p. 889-894) in a latter study with silage reported that approximately 15 percent of the silica was metabolisable and was not a dependable indicator for the estimation of the digestibility of the nutrients. Druce and Willcox (23, p. 188-192) also worked to evaluate silica in digestibility studies and concluded that the amount of silica recovered in feces was too variable to be used as an indicator.

Iron Oxide

Bergeim (4, p. 29-33) added iron oxide to feed for the purpose of indirect determination of digestibility and obtained successful results. Bergeim's experiment was one of the earliest using an indicator in digestibility studies. The feasibility of the technique stimulated other workers to conduct addional experiments. However the first results obtained were not very encouraging. Moore and Winter (74, p. 297-305) and Knott et al. (60, p. 553-556) reported that iron oxide was unsatisfactory for determining digestion coefficients. Large variations in the amount of iron oxide passing through the digestion system made it unreliable as a measure of digestibility.

Lignin

When an indigestible portion of feeds was sought as a possible measure of its digestibility, lignin attracted the attention of research workers in the field. In theory, the lignin ratio technique is based on the indigestibility of lignin. Experiments in the field of animal nutrition hold conflicting opinions about the applicability of the lignin technique. Crampton and Jackson (19, p. 333-339) reported that lignin ratio technique was not a reliable measure of digestibility. Ellis et al. (26, p. 285-297) proposed a "72 percent H_2SO_4 method" for the determination of lignin and using this method they showed that lignin was not digested by cattle, sheep or rabbit. Forbes et al. (27, p. 298-305), Swift et al. (96, p. 432-444) and Kane et al. (51, p. 583-596) reported satisfactory results using lignin as an indicator, but Davis et al. (22, p. 285-288) and Bondy and Meyer (6, p. 248-256) did not get satisfactory results.

Forbes and Garrigus (30, p. 354-362 and 31, p. 531-539) studied the lignin ratio technique and found very close association between organic matter digestibility and the lignin content of the forage. Their conclusion was that for purposes of predicting organic matter digestibility of forages the best measure was the lignin content of the forage. Kane et al. (52, p. 492 and 54, p. 325-332) and Luiting (68, p. 333-342) did not obtain satisfactory results and reported that

incomplete recoveries of lignin made it unreliable to calculate the digestion coefficients by the lignin ratio technique.

Smith et al. (93, p. 142-145) found that the digestibility values determined by the lignin ratio differed markedly from those of conventional trials. They reported that an experienced chemist was not able to obtain consistent results from the same samples analyzed at different times. The digestibility values of lignin ranged from 5.8 percent to 32.0 percent for different species of plants. They concluded that although it was not clear whether these apparent digestibility values of lignin were due to actual digestibility or to inability to accurately isolate the lignin, the lignin ratio technique was not satisfactory for estimating the digestibilities of native forages.

According to Reid (84, p. 1334-1339) and McCullough (71, p. 219-222) the possible explanations for the inconsistent recoveries of lignin are: 1) the lignin of one plant may be chemically different from that of another, 2) the composition of lignin may vary with the stage of growth, 3) the lignin ingested by an animal may differ from that voided and, 4) the different methods of lignin analysis may measure different plant constituents, since lignin is not a specific chemical substance.

Methoxyl Group

The chemical determination of lignin is quite laborious and

requires tedious procedures to remove interfering substances and as a consequence the results are often quite variable. As plants mature there is an increase in methoxyl radicals on the lignin molecule. Therefore the methoxyl group might be used as an indicator for the digestibility of forages. Richards and Reid (87, p. 595-602) tested this possibility and found that forage methoxyl group was digested and the digestibility decreased as the plant matured. There was highly significant negative correlation between the methoxyl content of feces of the grazing animals and the digestible dry matter content of the forage. They expressed the opinion that the methoxyl content of feces might be a useful indicator of predicting digestibility of forage dry matter. Anthony and Reid (2, p. 1715-1722) studied the relationships between the digestibility and methoxyl content of forages and also between forage digestibility and the fecal methoxyl content. Although they found that methoxyl was highly correlated with forage digestibility, but due to possible errors in methoxyl determinations their conclusion was not positive for the use of methoxyl group in predicting forage digestibility.

Various Dyes

Various dyes have been employed as indicators of digestibility in ruminants. Corbin and Forbes (18, p. 574-580) used anthraquinone violet as dye stuff and obtained 100. 5 percent recovery in the

feces of the animals. In grazing studies, total fecal dry matter output could be determined by means of dye concentration in feces of the animals which were given known amounts of the dye at frequent intervals. In spite of these positive results dyes have not been used to any great extent in digestion studies.

Plant Chromogens

Reid et al. (83, p. 60-71) were the first workers to use plant pigments as an indicator in digestibility studies. They proposed that the plant pigments so-called chromogenic substances might serve as indigestible internal indicators to estimate forage digestibility. Their report was based on 36 conventional digestion trials using wethers, bulls, and steers. The spectrophotometric examination of 85 percent acetone extracts of forages and of the feces excreted by animals consuming the respective forages indicated that some substances absorbing light at 406 mμ wave length were completely recoverable in the feces. Since the actual properties of these substances were not known, the authors used the terms "chromogen(s)" and "chromogenic substances". The average rate of recovery of the chromogenic substance(s) in the feces of the animals fed the forages was 100. 5 percent. The results obtained from the chromogen indicator techniques were in very close agreement with those obtained by the conventional methods.

Reid et al. (85, p. 255-269) in a later study modified their original chromogen ratio procedures. They found that the use of Na_2CrO_4 was not an accurate standardization material and extracts containing very large or very small quantities of chromogens showed rather marked errors. From a study of 18 pasture forage mixtures ranging in dry matter digestibility from 51.6 to 74.0 percent, the authors established a mathematical equation for the relationship between the chromogen-dry matter ratio of feces voided and that of forage actually consumed. This relationship is expressed by the following equation:

$$Y = 0.0925\,X + 137.3\,\log X - 242.12$$

where "Y" is the units of chromogen per gram dry matter of forage and "X" is the units of chromogen per gram of fecal dry matter.

The digestibility of forage dry matter may be estimated by measuring fecal chromogen concentration, calculating the chromogen concentration of the consumed forage from the above equation and putting these values into the following formula:

$$\text{Dry Matter Digestibility} = 100 - \left[100 \frac{\text{Units chromogen/g forage dry matter}}{\text{Units chromogen/g feces dry matter}}\right]$$

The results obtained from the chromogen indicator technique agreed with those obtained from conventional digestion trials. The

digestion coefficients of dry matter as determined by the conventional and chromogen techniques for pasture grass at the vegetative stage were 72. 9 and 73. 3 percent respectively. Varying results have been obtained using chromogens in digestibility studies since that time. In addition to the reports of the inventors of the technique many research workers (Hardison and Reid, 40, p. 35-52; Kane et al., 55, p. 637-644; Soni et al., 94, p. 474-479; Miller et al., 72, p. 15; and others) have reported satisfactory results using sheep, cows and steers and under stall feeding and pasture conditions.

Cook and Harris (13, p. 565-573), however, reported that the chromogen technique of determining digestibility of winter range forages was not satisfactory in sheep due to inadequate recovery of chromogens in the feces. Irvin et al. (47, p. 545-551) studied the actual components of the chromogens and classified them into three groups: 1) carotenoids, 2) xanthophylls and 3) chlorophylls. They reported that carotenoids and xanthophylls were very unstable and too digestible to be used as indicators in digestibility determinations. Because of the unstable nature of the chlorophylls no one pigment could be used as an indicator. Pheophytin, a decomposition product of chlorophyll was found to be the most stable plant pigment and the need of more research was suggested.

Kane and Jacobson (56, p. 672) reported that pheophytin gave very satisfactory results in digestibility trials. They recommended

that the pheophytin method should have a greater accuracy over the use of other plant pigments. However, Riley's (88, p. 60) findings were not encouraging for the use of pheophytin. The average recovery of pheophytin in feces was found to be 75. 6 percent for pasture trials and 46. 3 percent for silage trials.

Oldfield et al. (79, p. 1259) reported that there was considerable diurnal variation in the excretion of chromogens. Kennedy and Lancaster (58, p. 56-62), Kennedy et al. (59, p. 627-638), Greenhalgh and Corbett (37, p. 371-376), Brisson (12, p. 435-438) and Marten (69, p. 3796-3797) did not find satisfactory results in digestibility studies using plant chromogens as indicators. Wheeler (97, p. 74) explained his results stating "coefficients were so variable as to be almost meaningless."

Chromic Oxide

Chromic oxide has been used extensively as an external indicator to estimate fecal dry matter output of animals since the first report by Edin in 1918. Edin et al. (24, p. 166-171) reported the detailed procedure of chromic oxide technique in a later publication. In previous discussions it was mentioned that the fecal output of grazing animals may be measured by the total collection from animals using collection bags and harnesses or may be estimated by the administration of indicator materials. Using an indicator the fecal dry

matter output of an animal may be estimated by the following equation:

$$\text{Fecal Dry Matter Output(g DM/day)} = \frac{\text{Indicator consumed (g/day)}}{\text{Indicator concentration of feces (g/g DM)}}$$

The feasibility of Cr_2O_3 as a means of measuring the fecal output of a grazing animal is dependent upon the quantitative recovery of the material in the feces. To be a successful indicator a material should be recovered in the feces at least at a consistent rate if not completely recovered. Proper corrections then can be made for the estimation of the output. The adequacy of consistent recovery of Cr_2O_3 might be directly related to three factors:

1. Diurnal excretion of chromic oxide,
2. Processing of feces for the chemical analysis,
3. Accuracy of chemical analysis.

Diurnal variation in the excretion of chromic oxide was reported by many workers. Reid (84, p. 1334-1339) found that satisfactory estimates of the amount of feces voided per unit of time may be obtained by the analysis of partial collections of feces. However, Kane et al. (53, p. 263-273) reported highly variable results between the A. M. and P. M. fecal samples of dairy cows and concluded that such a variation in excretion indicates that the time of sampling is very important in a digestibility study. Hardison and Reid (40, p. 35-52) employed a detailed fecal grab sampling plan and found that the Cr_2O_3

content of the feces voided at intervals of the day is extremely variable. However the nature of the Cr_2O_3 excretion pattern indicated that the amount of fecal output could be estimated from samples of feces taken at any time, provided that the rate of recovery of chromic oxide is known. They chose 6 A. M. and 4 P. M. as daily fecal sampling times and they obtained excellent results. The mean rates of recovery of chromic oxide from the feces taken from grazing steers at 6 A. M. and 4 P. M. were 71. 8 and 129. 3 percent respectively. The average daily recovery of chromic oxide was found as 99. 95 percent.

Smith and Reid (92, p. 515-524) reported no difference in the accuracy of the estimated output of feces between the administration of chromic oxide in capsules and in concentrates. There was an intra-day variation in the excretion of chromic oxide and average minimum excretion was at 2 P. M. and average maximum excretion was at 12 midnight. Lancaster et al. (64, p. 117-126) using dairy cows also reported daily variation in the excretion of chromic oxide. They reported lower concentrations of chromic oxide in the afternoon samples. Their conclusion was that the fecal output could be predicted as accurately from morning grab samples alone as from A. M. and P. M. samples combined.

Hardison et al. (42, p. 11-17) studied the effect of certain factors on the excretion pattern of chromic oxide and found that the

variability in its excretion was much greater when chromic oxide was given once daily than when it was given twice daily. The animals excreted Cr_2O_3 at certain periods of the day from 91 to 111 percent. Average maximum concentration of chromic oxide was found at 12 noon and a minimum at about 10 P. M. Putnam et al. (81, p. 1723-1729) conducted an experiment to study the effects of the feeding schedule and various ratios of forage to concentrates upon the excretion pattern of chromic oxide. There was considerable variation in the relative Cr_2O_3 recovery at any sampling time. However neither the feeding schedule nor the proportion of roughage to concentrate had an influence on the recovery of Cr_2O_3.

Davis et al. (21, p. 152-159) and Gacula et al. (32, p. 261-270) reported considerable variations in the chromic oxide content of the feces samples taken at various hours of the day, regardless of whether Cr_2O_3 was administered once or twice daily. However twice daily administration of Cr_2O_3 appeared to lessen the variation in its excretion. Brisson et al. (10, p. 90-94) administered chromic oxide in once, twice and in six equal doses at four hour intervals and searched the chromic oxide excretion pattern from grab samples of feces. When the frequency of Cr_2O_3 administration was increased to six times a day no significant differences were detected between the concentrations at the various sampling times.

Stevenson (95, p. 339-345) studied the possible errors in the

estimation of total recovery of chromic oxide from representative feces samples and obtained 97. 4 percent recovery. The low rate of recovery was attributed to a loss of chromic oxide when the dry feces were ground. A difference of 2. 7 percent was found in the recovery of chromic oxide between two milling operations. The recovery of chromic oxide in grab samples taken at 6 A. M. and 4 P. M. was 92. 7 percent.

Elam et al. (25, p. 1199) used chromic oxide in a pelleted ration fed to Hereford heifers and found a significant time-concentration variation of fecal chromic oxide. Variations appeared to be related to the feeding schedule and the magnitude of excretion. Steers fed a predominantly roughage pelleted ration twice daily gave better results. Bradley et al. (7, p. 1199) reported that chromic oxide concentration in feces varied from 57 to 208 percent and from 73 to 155 percent when it was administered in capsule and as a part of a pelleted ration respectively.

Pigden and Brisson (80, p. 185) and Brisson and Pigden (11, p. 1200) reported the use of chromic oxide in the so-called "sustained-release pellet" form. Maximum deviation from relative nine day average concentration was found as 92 to 107 percent for confined animals and 86 to 112 percent for field animals. The chromic oxide concentration expressed as percent of the respective daily mean concentration was 100. 3 percent at 7 A. M. and 100. 5 percent at 4 P. M.

Differences in the concentrations of chromic oxide in different times of the day were not statistically significant and total recovery was found as 100. 4 percent.

Corbett et al. (14, p. 1014-1016) reported that the irregular excretion of chromic oxide administered in gelatin capsules is due primarily to the rapid passage of a large proportion of each dose through the digestive system with only a small portion being mixed in the reticulo-rumen with the ingesta. He suggested that diurnal variation in the excretion of chromic oxide could be reduced if it is administered as a component of a specially made paper which contains 35 to 40 percent chromic oxide on a dry basis. In a later experiment Corbett et al. (16, p. 289-299) administered the chromic oxide impregnated papers as a single sheet of paper and also paper shredded into strips of about 2 X 120 mm. They found superiority of shredded paper over single ones explaining the release of chromic oxide from various sites in the rumen rather than from one position. Greenhalgh and Runcie (38, p. 95-103) also used chromic oxide impregnated and shredded papers and reported satisfactory results.

Kane et al. (57, p. 1359-1366) used radioactive chromic oxide as a digestibility indicator in dairy cattle by making direct comparisons with total collection and chromic oxide ratio technique. The use of radioactive chromic oxide ($Cr_2^{51}O_3$) saved time and labor and gave about the same degree of precision as that may be obtained

from the normal Cr_2O_3. On the other hand segragation of animals and disposal of excreta containing radioactive isotopes and also condemnation of the milk and meat products of the experimental animals by the Atomic Energy Commission and Food and Drug regulations, made it impractical to use it in digestibility experiments.

Fecal Nitrogen

After a series of experiments Raymond (82, p. 937-938) found a relationship between the nitrogen content of fecal organic matter (FOM) and the nitrogen content of the dry matter of grass fed to sheep. He expressed his findings in the following equation:

Percent of N in feed DM = 0.795X percent N in FOM +0.14

Lancaster (62, p. 31-38) found that a constant of 0.83 $\pm$ 0.102 gram of nitrogen was excreted per 100 gram of pasture organic matter consumed. He postulated that nitrogen excreted in sheep feces per unit intake of pasture organic matter is constant and consequently he proposed a method of determining digestibility of pasture forage consumed by the use of fecal nitrogen concentration. The forage organic matter consumption was estimated by the following formula:

$$\text{OM consumed per 100 g of OM in feces} = \frac{100\,n}{C}$$

where n is percent of nitrogen in feces organic matter and C is a constant.

Gallup and Briggs (35, p. 110-116) also reported an apparent relationship between fecal nitrogen and dry matter intake. They proposed that the total fecal nitrogen excretion of steers is so related to their dry matter intake that when either of these quantities is known the other one can be estimated by the use of an approximate factor. This factor would vary for different feeds but for any given roughage it would remain fairly constant over a relatively wide range of variations in consumption.

Lancaster (63, p. 330) in a later report modified his previous method by dividing forages on the basis of their protein content. For forages with less than 15 percent protein on the dry basis he proposed 0.67 ± 0.120 as constant and for forages with 15 percent or more protein he suggested 0.80 ± 0.081. These data were based on 153 digestibility trials which were carried out by investigators on pastures in England, the United States, South Africa and New Zealand.

Forbes (28, p. 19-23), however, reported that there was no constancy in fecal nitrogen output per 100 gram of dry matter intake. When the protein content of the feed was increased the fecal nitrogen excretion per 100 gram of dry matter was also increased. When a protein supplement was added to increase the protein content of a hay ration to 13 percent the fecal nitrogen excretion increased to 0.86 gram per 100 gram dry matter intake.

Soni et al. (94, p. 474-479), Schneider et al. (91, p. 25) and

Miller et al. (72, p. 16) reported that the fecal nitrogen method appeared to give quite satisfactory results in determining digestibility and called for further study. They also found no diurnal variation in the estimation of digestibility.

Lancaster (65, p. 15-20) further modified his original method (62, p. 31-38) for use with cattle instead of sheep in calculating the digestibility of grass from the nitrogen content of their feces. The data for the modification of the method were based on 22 digestion trials. Feed to feces ratio or intake factor (Y) is obtained from the following equation:

$$Y = \frac{X}{C}$$

where X is the nitrogen percentage of fecal organic matter and C (a constant) is the nitrogen excretion in feces per 100 gram grass consumed. The efficiency of this formula for predicting intake factor would depend on the constancy of C. The derived formula for the intake factor is:

$$Y = 0.97X + 1.02 \pm 0.39$$

For practical purposes this formula was simplified to:

$$Y = X + 0.9$$

This method for the determination of the digestibility of forages has

been tested in several countries by many workers (Kennedy and Lancaster 58 p. 56-62; Kennedy et al., 59, p. 627-638; Greenhalgh and Corbett, 37, p. 371-376; Marten, 69, p. 3796-3797; Wheeler, 97, p. 51 and others) and have indicated that the fecal nitrogen technique is the internal indicator of choice. The fecal nitrogen technique allows a more accurate prediction of feed to feces ratio than does chromogen and also nitrogen is simply determined by a well standardized chemical procedure. There is a problem in its use due to the fact that the concentration of nitrogen in feces excreted during a period of 24 hours follows an unpredictable rhythmic variation. Also fecal nitrogen contains not only undigested feed protein but some body proteins, derived from metabolic excretions (Brisson, 12, p. 435-438; and Corbett and Greenhalgh, 17, p. 173).

Protein

Mitchell (73, p. 159-173) studied the evaluation of feeds on the basis of digestible nutrients and found out that there was some relationships between the chemical composition of a feed and its digestibility. He expressed the relationship for feeds containing five percent or more protein on the dry basis by the following equation:

$$D = 42.64 (P - 5)^{0.215}$$

in which D is the apparent digestibility of the protein and P is the

protein content of the dry matter of the feed.

Forbes (29, p. 231-237) studied the possibility of use of protein as an indicator in forage digestibility studies. The data obtained from protein indigestibility method were compared with the data obtained from conventional digestion trials. The digestibilities of forages were found similar based on two different methods, namely, protein indigestibility and conventional techniques, using steers as experimental animals. His conclusion was that the method may be used with a satisfactory degree of accuracy for the determination of digestibility of forages under grazing conditions.

Holter (44, p. 1934-1935) and Holter and Reid (45, p. 1339-1349) studied the relationship between the concentrations of crude protein and apparent digestible protein in forages when using forage and fecal protein as indicators of digestibility. Their conclusion was that due to the simplicity and accuracy of the chemical determination, protein could be used effectively as an indicator under many practical grazing and group feeding conditions.

MATERIAL AND METHODS

Three conventional digestion trials and one grazing trial constitute the material of this study. Conventional digestion trials were conducted to obtain reference information for the grazing trial, in which external and internal indicators (chromic oxide and fecal nitrogen) were employed to determine the digestibility and consumption of pasture forages.

Six purebred two year old Holstein and three purebred Jersey heifers as shown in Table 1, were used in these digestion trials. Both the Holstein and Jersey heifers were approximately five months pregnant.

Table 1. Description of the Experimental Animals

Trial	Animal No.	Breed	Age Months	Pregnancy Months	Weight lb.
I	H759	Holstein	23	5.5	1035
	H760	Holstein	23	5.5	1020
	H762	Holstein	23	4.5	920
II	H764	Holstein	23	5.5	1030
	H765	Holstein	22	4.5	1107
	H766	Holstein	22	5.0	970
III	J 464	Jersey	21	5.0	745
	J 465	Jersey	21	3.5	694
	J 466	Jersey	21	6.0	718
IV	J 464	Jersey	22	6.0	756
	J 465	Jersey	22	4.5	732
	J 466	Jersey	22	7.0	760

Trials I and II

Trial I consisted of a conventional digestion trial with cut pasture forage and Trial II was a grazing trial carried out simultaneously. Three Holstein heifers (H-764, H-765, H-766) were randomly assigned to the conventional digestion trial while the other three heifers (H-759, H-760, H-762) were used in the grazing trial. The numbers in sequence for Trial I and Trial II were purely by chance.

The heifers had been kept on pasture without supplementation of concentrates for one month prior to the trials. This pretreatment eliminated any possible interference of previous concentrate feeding or change over effects. The heifers were place on Pasture No. 4 on July 19, 1963. Pasture No. 4, a permanent pasture located on the Oregon State University Dairy farm, was an orchard grass, Dactylis glomerata, pasture. The grass was at the early bloom of the second cutting stage of growth, and about eight inches tall. The pasture was irrigated before the heifers were placed on it. Adequate acreage reserved for the experiment was divided into two plots by an electric fence. One plot was used by the three heifers while the other was used to cut forage for the conventional digestion trial heifers. Iodized salt and water were available to the animals at all times.

All six heifers were maintained on a similar grazing pasture for five days then the three digestion stall heifers were taken to the

barn and fed cut grass in stanchions for three days. Grass was cut by a power mower twice daily and carried to the barn in paper sacks. The heifers were fed four times daily--morning, noon, afternoon and night. After three days feeding of cut grass in the barn, the heifers were placed in the digestion stalls.

Chromic oxide was used as an external indicator to estimate the fecal dry matter output. Five grams of chromic oxide in gelatine capsules were administered by a balling gun to both the grazing and conventional digestion trial heifers at 7 A. M. and 4 P. M. Chromic oxide administration was started seven days before the first fecal collection day so its excretion in the feces was assumed to have reached a constant level (Miller et al., 72, 20 p.).

Chromic oxide capsules were prepared according to the following procedure: Approximately two parts of chromic oxide dispersed on one part of Solka-floc*, used as a carrier material, was mixed throughly adding water until it became a slow flowing paste. This mixture was placed in an electric oven at a temperature of 96°C. After 24 hours the surface of the mixture was crushed by a spatula and then put back into the oven again. The chromic oxide and Solka-floc mixture was dried in the oven until there was no difference

*Solka-floc is a purified wood cellulose product of the Brown Company, Berlin, New Hampshire.

between two subsequent weighings over three hours. The mixture was cooled, crushed and sifted through a metal 20 mesh sieve. Chromic oxide concentration of the mixture was determined as discussed later. Five grams of the chromic oxide mixture were weighed into Number 10 gelatine capsules.

Digestion stalls located in the nutrition unit of the University Dairy Farm installations were used in these studies. These digestion stalls are constructed so that the excreted feces and urine fall on an endless belt approximately two feet wide and five feet long. This belt is driven in an uphill motion by a roller at each end with one roller being about six inches higher than the other. The urine flows to the lower end of the belt and drains into a pan placed under the lower roller and the feces is carried to the higher end and dropped into another pan. This mechanical set up provides separate collection of urine and feces. Protective side boards can be placed along with the belt to save any splashes of feces which might be encountered. The manger and sides of the stalls are adjustable to the size of the animal so that the rear of the animal would be very close to the moving belt and droppings on the floor can be avoided.

The animals were fed in the stalls as they were fed in stanchions. It was desired to feed each animal in the digestion stalls the maximum amount of feed which she would completely consume. The previous three days feeding period in the stanchions gave an accurate

estimate of the amount of grass each heifer would consume per day. The heifers were fed weighed amounts of grass four times a day. Any refusal of grass was weighed back in the morning and was subtracted from the amount of feed which was given in the previous 24 hours. The grass was usually almost completely consumed by the heifers. However if the refusal exceeded five pounds, it was sampled for analysis to determine whether it had been a selective refusal. On one occasion the power mower did not work and grass was clipped by a pasture clipper. Two of the heifers refused a large portion of the grass on that day consuming only about one-half of their usual intake.

A sample of feed was taken from the forage fed in the afternoon prior to the first day of collection period. The sample was placed in a plastic sack and stored in a refrigerator overnight. The next morning a sample of feed was taken in the same manner, mixed with the sample taken in the previous evening and called the grass sample of the first day. This sample was taken to the Department of Agricultural Chemistry along with the fecal sample of the first day for drying. This feed sample was also considered as a representative feed sample for the grazing animals. This sampling procedure was repeated during the one week of the experimental collection period. The feed samples were dried and composited into one sample for chemical analysis and for energy determination by the Parr oxygen bomb calorimeter.

The feces pans were emptied and rinsed 24 hours before the first fecal sampling time. The next morning the feces pan from each heifer was weighed and ten pounds of water was added to facilitate mixing in order to obtain a more uniform and representative sample from the 24 hour collection of feces. A sample of about three pounds of feces was taken for chemical analysis.

The process of collecting feed and feces samples were continued for five days, Monday through Friday, giving a total of five samples of feed and five samples of feces from each heifer.

In the grazing trial conducted simultaneously with the three Holstein heifers to determine the consumption and digestibility of pasture forages chromic oxide and fecal nitrogen were used as internal indicators. Fecal samples were collected as "grab" samples from the rectum twice daily at 6 A. M. and 4 P. M. At these times the heifers were brought into the barn for administration of chromic oxide capsules and for grab sampling of feces. Approximately one pound of fecal sample was taken from each heifer each time and put into a plastic cup with the lid being closed tightly allowing no air in the cup. The sample was immediately frozen in a deep freeze inasmuch as the drying facilities were not adequate to dry all of the conventional and grazing trial fecal samples at once. At the end of the five day collection period, the accumulated frozen samples were taken to the Department of Agricultural Chemistry and dried in a

steam heated oven at the temperature of 80°C.

Trials III and IV

These two trials were conducted to investigate further the value of the external indicator, chromic oxide, as a means of accurately estimating fecal dry matter output of dairy cattle when fed silages alone. Three purebred Jersey heifers at about two years of age were used in these two trials. Actually the trials were planned with four heifers but one of the heifers developed a uterine infection on the first day of placement in the digestion stalls for the Trial III and was eliminated from the experiment. Two of the heifers--J-465 and J-466--were fed corn ensiled alone while the third--J-464--was fed corn ensiled with 200 pounds of millrun per ton of forage. Two wood-stave silos of about 100 tons capacity each at the University Dairy Farm were used for the two kinds of silage.

After a 12 day preliminary feeding period the heifers were put in digestion stalls on February 17, 1964. Chromic oxide administration in gelatin capsules was started seven days before the first collection day, and was given twice a day, 7 A. M. and 4 P. M. totalling ten grams of chromic oxide per day as in the previous trials. Samples of about four pounds of each kind of silage were taken daily for a period of five days, dried at the department of Agricultural Chemistry, and composited for proximate analysis. Feces was sampled

from 24 hour collection periods for each heifer for a period of five days. Daily fecal samples were dried for proximate and for chromic oxide analysis.

Trial IV was a cross-over design of Trial III. In this trial the two kinds of silage were reversed for the experimental animals. Thus one of the heifers, J-464, received corn silage without millrun while the other two heifers received corn silage with millrun. The same procedures as in Trial III were followed concerning the preliminary period, chromic oxide administration, silage sampling, fecal sampling and drying of samples.

The modified method of Bolin et al. (5, p. 634-635) was used to determine the chromic oxide concentration in dry feces samples. Approximately a one gram sample was weighed and transferred into 100 ml pyrex volumetric flask and 15 ml oxidizing reagent, as suggested by Bolin et al. (5, p. 634-635), was added so that adherent particles were washed down into the flask. Samples were placed on a multiple unit hot plate, which initially was cold and they were allowed to digest. During this process a point of rapid and vigorous oxidation takes place and the brown color of the mixture turns into light green in about 35 minutes. At this stage adherent material should be swirled down. Oxidation of chromic to chromate occurs in about ten minutes. The samples were allowed to digest until virtual disappearance of white fumes from the flask, accompanied by strong

reflux action in the neck of the flasks. The samples were cooled, diluted with H_2O, cooled again, diluted to volume, mixed throughly by inversion and allowed to stand overnight for SiO_2 to settle completely. A portion of supernatant solution was withdrawn by a volumetric pipette for spectrophotometric reading and read at 430 mμ wavelength using distilled water as blank.

For the gross analysis of the forage and feces the methods described by the Association of Official Agricultural Chemists (1, p. 367-385) were used. These analysis included dry matter, crude protein, ether extract, crude fiber and ash. A Parr plain coat oxygen bomb calorimeter was used to determine the gross energy of feed and feces. The statistical methods given by Li (66, 568 p) were used for the statistical analysis of the data.

RESULTS AND DISCUSSION

Evaluation of Chromic Oxide

a. Pasture Forage Trials. In digestibility experiments we need to know the amount of feed consumed and the amount of feces voided. It is not a problem to get this information from conventional digestion trials, but in case of indicator techniques there are a number of factors influencing the estimation of the amount of feed intake and fecal output. For the estimation of fecal dry matter output, a so called external indicator is used. In this study chromic oxide was used as the external indicator. A conventional digestion trial was conducted to determine the rate of recovery of chromic oxide as a reference source for the grazing trial. Table 2 shows the percentage recoveries of chromic oxide and the estimated and measured fecal dry matter outputs. The average values for the five days collection period are given in this table. The recovery of chromic oxide in the feces ranged from 98. 4 to 99. 2 percent and averaged 98. 8 percent for the three heifers. These results are in agreement with most of the previous reports (Putnam et al., 81, p. 1723-1729; Smith and Reid, 92, p. 515-524; Corbett et al., 15, p. 266-276; Stevenson, 95, p. 339-345 and others).

Measured fecal dry matter (FDM) outputs of the three stall-fed

Table 2. Chromic Oxide Recoveries in Feces and FDM Outputs of Stall Heifers

Animal No.	Recovery of Cr_2O_3 %	Measured FDM g.	Predicted FDM g.	Difference g.	Deviation %
764	99.0	1833.1	1852.5	19.4	1.06
765	99.2	2135.0	2152.9	17.9	0.84
766	98.4	1913.5	1945.5	32.0	1.67
Average	98.8	1960.5	1983.6	23.1	1.01

heifers averaged 1960.5 grams whereas the estimated fecal dry matter outputs using chromic axide averaged 1983.6 grams, a difference of 23.1 grams. The high percentage of recovery of chromic oxide in the feces resulted in a very small difference between measured and estimated FDM outputs. Deviations or errors of the average estimated FDM from the average measured FDM ranged from 0.84 to 1.67 percent for the individual heifers. The over-all average deviation for the trial was calculated as 1.01 percent. Daily recoveries of chromic oxide and as a consequence daily FDM outputs were not estimated, because the daily samples were composited into one sample.

Estimated FDM outputs of the grazing trial heifers are shown in Table 3. Estimated FDM outputs were corrected taking into account the percentage deviation of estimated FDM from measured FDM for the conventional digestion trial. This deviation resulted

Table 3. Fecal Dry Matter (FDM) Outputs of the Grazing Trial Heifers

Day	759		760		762	
	Estimated	Corrected	Estimated	Corrected	Estimated	Corrected
	g.	g.	g.	g.	g.	g.
1	2959.1	2924.0	2874.8	2840.7	2792.2	2759.0
2	3001.5	2965.9	2958.8	2923.7	2962.9	2927.7
3	2831.6	2798.0	2720.8	2688.5	2642.4	2611.0
4	2751.5	2718.8	3075.2	3038.7	2664.2	2632.6
5	2549.7	2519.4	2820.4	2786.9	2617.7	2586.6
Average	2818.7	2785.7	2890.0	2856.2	2735.9	2703.9

from 1.01 percent incomplete recovery of chromic oxide in the excreted feces. The average FDM output of the grazing trial was found at 2781.9 grams. The average FDM output of the pastured heifers was 42 percent higher than that of the conventional trial heifers. This situation will be discussed later.

b. Silage Trials (Trials III and IV). Excellent recoveries of chromic oxide in feces were obtained for Trial I in which only cut grass was fed to the animals. It was desired to test further the recovery rate of chromic oxide when it is used in the silage digestion trials. Chromic oxide recoveries, FDM outputs of the heifers and percent errors of the estimations for Trials III and IV are shown in Tables 4 and 5. Two kinds of silages were used in each of the trials. To eliminate any individual effects of the heifers on the digestibility,

Table 4. Cr_2O_3 Recoveries and FDM Outputs of the Heifers of Trial III.

Heifer/day	Recovery of Cr_2O_3 %	Measured FDM g.	Estimated FDM g.	Difference g.	Deviation %
464/1	69.7	1567.0	2247.0	680.0	43.4
2	67.6	1396.4	2066.3	669.9	48.0
3	83.6	1519.2	1816.6	297.4	19.6
4	81.2	1534.8	1890.6	355.8	23.2
5	69.2	1306.4	1886.5	580.1	44.4
Mean	74.3	1464.8	1981.4	516.6	35.3
465/1	96.5	1192.6	1236.1	43.5	3.6
2	72.4	859.5	1186.8	327.3	38.1
3	93.0	1026.8	1104.0	77.2	7.5
4	95.8	1169.7	1220.6	50.9	4.4
5	75.9	1076.8	1418.5	341.7	31.7
Mean	86.7	1065.1	1233.2	168.1	15.8
466/1	73.0	1214.2	1664.1	449.9	37.0
2	80.8	1186.3	1469.0	282.7	23.8
3	87.2	1172.0	1344.0	172.0	14.7
4	71.9	1093.8	1520.7	426.9	39.0
5	81.2	1294.2	1593.9	299.7	23.2
Mean	78.8	1192.1	1518.3	326.2	27.4
Trial Mean	79.9	1240.7	1577.6	336.9	27.2

the silages were reversed for the animals in Trial IV.

First of all it was tested whether there was any difference or not between the recoveries of chromic oxide in the feces of the animals which were fed the two kinds of silages during Trials III and IV. The analysis of variance showed that feeding of two kinds of silages had no effect on the chromic oxide recoveries.

In Trial III, as seen in Table 4, the daily recoveries of chromic

Table 5. Cr_2O_3 Recoveries and FDM Outputs of the Heifers in Trial IV.

Heifer/day	Recovery of Cr_2O_3 %	Measured FDM g.	Estimated FDM g.	Difference g.	Deviation %
464/1	85.1	1477.6	1736.8	259.2	17.5
2	66.1	1246.6	1887.0	640.4	51.4
3	70.6	993.4	1406.7	413.3	41.6
4	49.6	621.6	1254.5	632.9	101.8
5	67.3	782.2	1161.6	379.4	48.5
Mean	67.7	1024.3	1489.3	465.0	45.4
465/1	86.7	1871.9	2159.9	288.0	15.4
2	75.5	1597.7	2117.2	519.5	32.5
3	74.6	1543.9	2069.3	525.4	34.0
4	67.2	1407.0	2092.7	685.7	48.7
5	70.6	1381.0	1956.6	575.6	41.7
Mean	74.9	1560.3	2079.1	518.8	33.2
466/1	104.1	2066.0	1984.6	81.4	3.9
2	76.4	1718.6	2248.5	529.9	30.8
3	84.8	1787.0	2108.3	321.3	18.0
4	76.8	1726.6	2249.1	522.5	30.3
5	67.9	1287.4	1896.6	609.2	47.3
Mean	82.0	1717.1	2097.4	380.3	22.1
Trial Mean	74.9	1433.9	1888.6	454.7	31.7

oxide in the feces varied from 67.6 to 96.5 percent. Heifer 464 had the lowest mean chromic oxide recovery with a value of 74.3 percent. Heifers 465 and 466 had mean recoveries of 86.7 and 78.8 percent respectively. The average rate of recovery during the trial was 79.9 percent. Percent errors in the prediction of the FDM outputs were inversely proportional to the percent recoveries of chromic oxide in the feces with the measured FDM always lower than the estimated

FDM. The percent of error was calculated as the deviation of the amount of estimated FDM output from the measured FDM output. Percent errors for Trial III ranged from 3. 6 to 48. 0 percent. The highest mean error was found for heifer 464 as a result of the lowest mean chromic oxide recovery in the feces. The average error for three heifers during the trial was found to be 27. 2 percent.

As seen in Table 5, variations in the recoveries of chromic oxide and as a result in percent errors for the estimated FDM outputs were larger in Trial IV than in Trial III. Recoveries of chromic oxide ranged from 49. 6 to 104. 1 percent and errors ranged from 3. 9 to 101. 8 percent. Heifer number 464 had the lowest, 67. 7 percent, mean recovery of chromic oxide as she did in the previous trial. In fact on the second day of the collection period her feed consumption decreased and she did not recover by the end of the collection period. The other two heifers, 465 and 466, had mean recoveries of 74. 9 and 82. 0 percent, respectively. Average recovery for the trial was calculated as 74. 9 percent.

The analysis of variance of the silage trial data is given in Table 6. Neither among day nor among heifer variations were statistically significant for Trial III. In Trial IV the situation was different. Among day variations were highly significant ($P < 0.01$) and also there were significant ($P < 0.05$) differences in the among heifer variations. When Trials III and IV were combined significant

differences ($P < 0.05$) were found in both among and within heifer variations. However, as mentioned before, one of the heifers developed a digestive disturbance during Trial IV. Her feed consumption and fecal output dropped to less than one-half of the usual and also the chromic oxide recoveries were proportionately low. When the data from heifer 464 were excluded from the analysis of variance no differences were obtained for among and within heifer variations. It was then apparent that these significant differences came from the unreliable data of heifer 464.

Table 6. Analysis of Variance of the Recovery of Chromic Oxide for Trials III and IV.

Trial	Source of Variation	SS	DF	MS	F
III	Days	400.8	4	100.2	1.523
	Animals	397.4	2	198.7	3.020
	Error	526.6	8	65.8	
	Total	1324.8	14		
IV	Days	1339.6	4	334.9	9.821**
	Animals	508.4	2	254.2	7.454*
	Error	272.8	8	34.1	
	Total	2120.8	14		
III & IV[a]	Days	683.9	4	171.0	2.253
	Animals	535.8	4	134.0	1.765
	Error	1214.5	16	75.9	
	Total	2434.2	24		

[a]Data from heifer 464 were not included.

As is seen by comparing the data in Tables 2, 4, and 5 there are large differences in the recoveries of chromic oxide between grass and silage digestion experiments. Very high recoveries were obtained in the Trial I, in which cut grass was fed to the animals while low recoveries were obtained in the Trials III and IV in which silage was fed to the animals. Many experiments have been published on the use of chromic oxide in forage and in grazing experiments. In most of these experiments high recoveries of chromic oxide in feces and as a result small errors of prediction for FDM outputs have been reported (Hardison and Reid, 40, p. 46; Putnam et al. 81, p. 1723-1729; Reid, 84, p. 1334-1339; Soni et al., 94, p. 474-479; and others). On the other hand there are only a few reports on the use of chromic oxide in silage digestion trials. The mean difference between the predicted and actual FDM outputs in two silage digestibility experiments was 382 grams per animal daily. The data of heifer 464 were not included in this average. The average recovery of chromic oxide was found to be 79.3 percent.

Miller et al. (73, p. 18) used chromic oxide with four types of roughages including silage and hay plus silage and obtained 78.5 per cent recovery of chromic oxide. Although they did not report the recovery rates for each roughage, they showed high statistically significant differences among roughages. Oldfield et al. (79, p. 1259) obtained 93.2 percent recovery using rush-sedge mountain meadow hay as feed source for steers. Wheeler (97, p. 45) found only 81

percent recovery in an experiment with steers.

The reason for low recovery rates of chromic oxide is not very clear. Since the same chemical standards and procedures were used in both silage and grass digestion experiments, differences should not be due to analytical procedures. A different mill was used for the grinding of dried feces obtained from the silage experiments. Stevenson (95, p. 339-345) reported 2.7 percent difference in the recovery rate between two milling processes. It was believed that the milling process might be a small contributing factor to the differences in recovery rates of chromic oxide and between the two types of digestibility experiments. Sampling techniques may be considered as causing some of the error, but the main reason for the low recovery of chromic oxide in the digestive tract may be the acid character of the silage enhancing retention.

Prediction of Feed Intake

Feed intakes of the animals in conventional digestion stalls were predicted using the fecal nitrogen index technique (Lancaster, 65, p. 15-20) and the protein indigestibility technique (Forbes, 29, p. 231-237). The results are shown in Table 7. As seen in the table, relatively close agreements were obtained between the estimated dry matter (DM) intakes using the fecal nitrogen index technique and actual DM intakes, the differences for the three heifers ranging from

-441 to 647 grams. The percentage error in the predictions from the actual DM intake of the three heifers were -7. 0 percent, + 10. 4 percent and + 0. 8 percent. There was a 86. 1 gram difference per animal daily between the average actual and predicted forage dry matter intakes. This magnitude of difference or deviation resulted 1. 4 percent error of prediction. When no consideration was given to the sign of the deviations an average error of 6. 1 percent was found. This error might be designated as the gross deviation of predicted DM intake from actual DM intake.

Table 7. Actual and Predicted Daily Forage Dry Matter Intakes of Stall-fed Animals for Five Day Period.

Animal No.	Actual DM Intake g.	Fecal Nitrogen Index			Protein Indigestibility		
		Intake g.	Deviation g.	Error %	Intake g.	Deviation g.	Error %
764	6316. 2	5875. 5	-440. 7	- 7. 0	5535. 6	-780. 6	-12. 4
765	6249. 5	6896. 2	+646. 7	+10. 4	6467. 7	+218. 2	+ 3. 5
766	6364. 2	6416. 7	+ 52. 5	+ 0. 8	5711. 0	-653. 2	-10. 3
Average	6310. 0	6396. 1	+ 86. 1	+ 1. 4	5904. 8	-405. 2	- 6. 4
Gross Deviation			380. 0	6. 1		550. 7	8. 7

Protein indigestibility as an index of predicting forage DM intake resulted in greater deviations. Differences between actual and predicted forage dry matter intakes ranged between -780. 6 and 218. 2 grams. The range of percent errors of prediction was from -12. 4 to

3. 5. Average predicted forage dry matter intake was 405. 2 grams less than actual intake. This amount of average deviation gave -6. 4 percent error of prediction for the trial. The average gross deviation was calculated as 8. 7 percent.

Fecal nitrogen index technique gave an average of five percent less error of prediction; in other words, five percent more accurate prediction than that of the indigestible protein technique. The difference between gross deviations of the two techniques was 2. 6 percent in favor of the fecal nitrogen index technique. There are only limited data published concerning the accuracy of the "protein indigestibility" method. Forbes (29, p. 231-237) as an originator of the method, reported close agreement between the digestibilities obtained by protein indigestibility and conventional techniques. Kane et al. (55, p. 637-644), Holter (44, p. 1934-1935) and Holter and Reid (45, p. 1339-1349) reported satisfactory results. In this present experiment the data indicated that protein indigestibility technique was not as accurate as the fecal nitrogen technique. The 1. 4 percent error for the trial in predicting forage dry matter intake by nitrogen index technique is in agreement with the results reported by Kennedy and Lancaster (58, p. 56-62), Kennedy et al. (59 p. 627-638), Greenhalgh and Corbett (37, p. 371-376), Brisson (12, p. 435-438), Marten (69, p. 3796-3797), Wheeler (97, p. 68) and others. This small error of prediction may enable us to say that using the fecal nitrogen

technique we can predict the forage dry matter consumption of grazing animals within an error of 1.4 percent. Our assumption for the above statement is that the same constancy was involved in the grazing trial. The constant is that the amount of nitrogen excreted in feces per 100 grams of grass consumed on the organic matter basis. The constant (C) calculated for the conventional digestion trial was 0.74. This constant agrees very closely to that of Lancaster (63, p. 330) which was 0.76 for 153 digestion trials.

The predicted forage dry matter consumptions of the grazing animals are shown in Table 8 using the previously discussed fecal nitrogen and protein indigestibility indexes and a so-called "simultaneous dry matter digestibility" index. Predicted dry matter intakes using simultaneous dry matter digestibility technique were based on the percent dry matter digestibility values of the forage for the stall-fed animals. The average dry matter intakes using the three techniques were found to be 9435, 9057, and 8949 grams, respectively. The intake values estimated by simultaneous dry matter digestibility were smaller than the values of the other two techniques. The actual average DM intakes of the stall-fed animals, as shown in Table 7, were 6310 grams which falls between the predicted average values of 6396 and 5905 grams using fecal nitrogen and protein as indicators. If the dry matter digestibility values were the same in stalls and on pasture, the predicted intake values using the simultaneous dry

matter digestibility technique would fall between the intake values of nitrogen and protein indicator techniques. As shown in Table 8, this was not found to be true.

Table 8. Estimated Forage Dry Matter Intakes of Pastured Heifers

Animal No.	Fecal Nitrogen Index g.	Protein Indigestibility g.	Simultaneous DM Digestibility g.
759	9576.8	9227.0	8961.9
760	9593.9	9161.8	9188.6
762	9136.3	8782.3	8698.7
Average	9435.7	9057.0	8949.7

The intake values of the nitrogen and protein techniques need to be corrected according to the reference information in Table 7. The nitrogen technique values would be 1.4 percent smaller and protein technique values would be 6.4 percent larger than the values given in Table 8. Even these corrections do not change the interpretations of the results. Two of the possible explanations of these variations may be as follows: 1) Animals on pasture select the plants and also prefer the more nutritious leafy parts of the plants rather than the stemmy sections (Hardison et al., 41, p. 89-102 and Jonston-Wallace and Kennedy, 49, p. 190-197). This condition tends to increase digestibility of actually grazed pasture. The lower digestibility values used as reference information resulted in smaller predicted forage

dry matter intakes on pasture. 2) The dry matter digestibility of one of the heifers in the digestion stalls was 4. 5 percent lower than the other two heifers resulting in a low average digestibility value for the conventional trial used as the reference material for the grazing trial. The higher indigestibility value gave a smaller estimated forage dry matter intake for the grazing animals. The analysis of variance showed a highly significant ($P< 0.01$) difference among the methods of estimating forage dry matter consumption.

It would appear from our studies that the fecal nitrogen technique is the most accurate method to estimate the forage dry matter intake of grazing animals, since it gave the lowest error of prediction, only 1. 4 percent, compared to the conventional digestion trial. This deviation may be regarded as negligible for grazing trials.

Average corrected forage dry matter intakes for the grazing trial animals using the fecal nitrogen technique was calculated as 9305 grams. The actual consumptions of the stall-fed animals averaged 6310 grams. On the average, the pastured heifers consumed 2995 grams more forage dry matter than that of the stall-fed heifers. It was believed that this increased feed consumption was due primarily to the requirements for the extra physical activities on pasture. On a percentage basis, the pastured animals consumed 47 percent more forage dry matter than the stall-fed heifers. Reid et al. (86, p. 88-94) calculated 40 percent additional maintencance requirement

for the expenditure of energy utilitzed by grazing activities on pasture than that for cows fed in the barn. The New Zealand Department of Agriculture (78, p. 19-23) reported that an additional requirement of 50 percent was found for grazing animals at the Ruakura Animal Research Station. Our finding for this experiment is in agreement with the above reports, but it does not agree with Kromann et al.'s (61, p. 450-453) results in which the authors did not find any difference between the digestible energy requirements of cows on pasture and in a dry lot.

Estimations of Digestibility

The proximate analysis of orchard grass and fecal samples are given in Table 9. As seen in the table, grass dry matter contained 15.07 percent crude protein and 27.7 percent crude fiber. This indicates that the pasture could be classified as of good quality. The forage protein content of the pasture was higher than the values reported by Schneider et al. (91, p. 39) and Morrison (76, p. 1028) for bloom stage orchard grass. However, the protein was lower than the values given by Kane et al. (55, p. 637-644). The average crude protein and ether extract concentrations of the grazing trial fecal samples were higher than that of the conventional trial. The NFE concentrations for the conventional trial was 5.1 percent higher than that of the grazing trial while the crude fiber concentrations were the

same.

Table 9. Proximate Analysis of Feed and Fecal Samples.

Trial	Sample	C. Protein %	E. Extracts %	C. Fiber %	NFE %	Ash %
Conventional	Grass	15.07	3.69	27.7	44.3	9.2
	764	13.64	7.08	22.4	38.4	18.5
	765	13.69	7.21	22.7	39.7	16.7
	766	13.49	6.90	23.1	38.2	18.3
	Average	13.60	7.06	22.7	38.8	17.8
Grazing	759	14.97	9.47	24.4	32.0	19.2
	760	14.49	9.64	22.3	34.8	18.7
	762	14.68	9.96	21.1	34.3	19.9
	Average	14.71	9.69	22.6	33.7	19.3

The digestion coefficients of the grass nutrients calculated from the conventional and the grazing trial data are given in Table 10. The estimation of digestibility in the conventional trials was carried out using total collection and fecal nitrogen techniques. The digestion coefficients of orchard grass in this experiment were in agreement with those reported by Kane et al. (55, p. 640) but higher than those of Schneider et al. (91, p. 26). The digestibility values estimated by the fecal nitrogen technique were higher than that of total collection. However, the differences were relatively small, being less than 0.5 percent for crude protein and nitrogen free extract and 0.9 percent for ether extract. Heifer number 765, as estimated by the total collection method, had lower digestibility values of the nutrients than

the other two heifers. In fact these lower digestibility values resulted in lower digestion coefficients for the total collection method than that of the fecal nitrogen technique. The digestibility values for heifer 765 were approximately the same as the digestion coefficients of the other two heifers using the fecal nitrogen technique. There was no apparent explanation of the lower digestibility values for heifer 765. She seemed healthy during the experiment and consumed as much feed as the other heifers, but excreted more fecal dry matter. However, the magnitude of predicted dry matter intake by the fecal nitrogen technique made the digestibility values of nutrients as large as the values for the other two heifers.

Table 10. Digestion Coefficients of the Nutrients.

Trial	Method	No. of Animal	C. Protein %	E. Extracts %	C. Fiber %	NFE %
Conventional	Total Collection	764	73.7	44.3	76.6	74.8
		765	69.0	33.2	72.0	69.4
		766	73.1	43.8	75.0	74.1
		Average	71.9	40.4	74.5	72.8
	Fecal Nitrogen	764	71.7	40.1	74.8	72.8
		765	71.9	39.5	74.6	72.3
		766	73.3	44.2	75.2	74.3
		Average	72.3	41.3	74.9	73.1
Grazing	Fecal Nitrogen	759	71.1	25.4	74.4	78.9
		760	71.4	22.2	76.1	76.6
		762	71.2	20.1	77.5	77.0
		Average	71.2	22.6	76.0	77.5

The digestion coefficients of the nutrients in the conventional and in the grazing trials using the fecal nitrogen technique in both cases would give a fairly representative basis for the comparisons between the trials. In general the digestion coefficients of crude protein and crude fiber were the same, whereas ether extract was 18.7 percent higher in the conventional trial, while NFE was 4.4 percent higher in the grazing trial. This situation may indicate that the digestibilities of carbohydrates were higher on the pasture and digestibilities of fats and proteins are higher in the digestion stalls. Hardison et al. (41, p. 96) and Kane et al. (55, p. 637-644) reported that digestion coefficients of all the nutrients were higher for grazed herbage on pasture than cut herbage in digestion stalls. They explained it by the consumption of more nutritious plants on pasture. In this experiment the higher digestion coefficients of nitrogen free extract for grazed herbage may be explained in the same way.

The calculated total digestible nutrients values of orchard grass as fed are given in Table 11, and the values of total digestible nutrients and digestible energy, on a dry basis, are given in Table 12. The average TDN values of orchard grass pasture, as feed basis, for conventional and grazing trials were found to be 17.0 and 17.2 percent, respectively.

Table 11. Total Digestible Nutrients of Orchard Grass as Fed to the Animals.

Conventional Trial		Grazing Trial	
Animal No.	TDN %	Animal No.	TDN %
764	17.5	759	17.3
765	16.2	760	17.1
766	17.3	762	17.2
Average	17.0	Average	17.2

Table 12. Calculated Digestible Values of Orchard Grass-Dry Basis.

		Total Collection		Fecal Nitrogen	
Trial	Animal No.	TDN %	Dig. Energy %	TDN %	Dig. Energy %
Conventional	764	69.2	70.1	67.2	67.9
	765	63.9	64.4	66.8	67.8
	766	68.3	69.6	68.5	69.8
	Average	67.1	68.0	67.5	68.5
Grazing	759	----	----	68.4	70.0
	760	----	----	67.6	69.4
	762	----	----	68.0	69.8
	Average	----	----	68.0	69.7
	Adjusted Av.	----	----	67.6	69.2

The estimations of the TDN and digestible energy values on dry basis for the conventional trial were made using both the total collection and the fecal nitrogen index methods. Average TDN values for the conventional trial were found to be 67. 1 percent by the total collection and 67. 5 percent by the fecal nitrogen techniques. Here again, as a result of lower digestion coefficients of the nutrients a 4. 8 percent lower TDN value was obtained for heifer 765 than for the other heifers with the total collection technique. However, greater predicted forage dry matter intake by the fecal nitrogen technique made the TDN value higher for the heifer when using fecal nitrogen as an index material. An average of 0. 4 percent difference between the TDN values of the total collection and fecal nitrogen techniques, indicates that one may rely upon the use of fecal nitrogen as an indicator of forage digestibility.

The average TDN values of the conventional and grazing trials, using the fecal nitrogen technique in both cases. was found to be equal. Since the same method was used, it was assumed that the same factors are involved in the estimations of the TDN values for the conventional and grazing trials. The estimated TDN value for the grazing trial was corrected using the results of the total collection method as a reference information. The adjusted average TDN value for the grazing trial was found to be 67. 6 percent, which is almost identical to that of the total collection method.

The digestible energy values of orchard grass were estimated using the Parr Plain Coat Oxygen Bomb Calorimeter. Average digestible energy values for the stall-fed animals using the total collection and fecal nitrogen techniques were found to be 68. 0 and 68. 5 percent, respectively. The difference between the two estimations is very small. The average digestible energy value of the grazing trial was 69. 2 percent which was 1. 2 percent higher than that of the conventional trial using the fecal nitrogen technique. The adjusted digestible energy value for the grazing trial may be regarded as the actual digestibility value of the pasture.

Very close agreements were found between the TDN and digestible energy values for individual animals and also for the trials. The correlation coefficient between the TDN and the digestible energy values was calculated as 0. 92 using the fecal nitrogen data which was highly significant at the one percent ($P < 0.01$) level.

SUMMARY AND CONCLUSIONS

An evaluation of chromic oxide as an external indicator showed that the recovery of chromic oxide in the feces is sufficiently high to be used in forage digestibility studies. Ten grams of chromic oxide were fed to the three heifers twice daily at 7 A. M. and 4 P. M. The recovery of chromic oxide in the feces was 98. 8 percent. The high rate of recovery of chromic oxide was in agreement with the specifications of an indicator given by Maynard and Loosli (70, p. 303). The recovery rates of chromic oxide ranged from 98. 4 to 99. 2 percent. There was 23. 1 grams per animal daily difference between the predicted fecal dry matter output using the chromic oxide ratio technique and the actual fecal dry matter output, which resulted in a 1. 01 percent error of prediction. We may conclude that the fecal dry matter output of the grazing animals could be calculated within an error of 1. 01 percent, and since the rate of error was known, the predicted values could further be corrected.

A grazing digestibility trial was carried out simultaneously with the conventional trial and the predicted average fecal dry matter output was found to be 2814. 9 grams using chromic oxide as an indicator. The corrected value was calculated to be 2781. 9 grams.

The accuracy of chromic oxide as an indicator was also studied in silage digestion trials. The recovery rates of chromic oxide were

low for the two silage digestion trials, the mean recovery rates being 79. 9 and 74. 9 percent for Trials III and IV, respectively. As a result of low recovery rates, the errors of prediction were high, estimated as 27. 2 percent for Trial III and 31. 7 for Trial IV. There is not a clearcut explanation for the low recovery rates of chromic oxide with silage feeding.

The predicted forage dry matter intakes of the stall-fed animals, using fecal nitrogen index (Lancaster, 65, p. 15-20) and protein indigestibility (Forbes,29,p.231-237) techniques, were compared with the actual intakes. The percent errors of prediction of the nitrogen index and protein digestibility techniques were calculated as 1. 4 and -6. 4 percent, respectively. When no consideration was given to the signs of deviations, the respective errors were calculated as 6. 1 and 8. 7 percent. The precision of the nitrogen index technique was superior to the protein indigestibility technique. The difference between the average forage dry matter intake values of actual and nitrogen index techniques was found to be 86. 1 grams per animal daily.

The average predicted forage dry matter intakes of the pastured heifers using nitrogen index, protein indigestibility and simultaneous dry matter digestibility techniques were found to be 9435. 7, 9057. 0, and 8949. 7 grams, respectively. It was expected that the average intake value of the simultaneous dry matter digestibility technique would fall between the average intake values of the other two techniques,

since this was the case for the conventional trial. The assumption made for the previous statement was that the dry matter digestibilities of orchard grass were the same on pasture and in the digestion stalls. However, lower dry matter digestibility for one of the heifers in the digestion stalls resulted in lower dry matter intake estimations for the pastured heifers. Fecal nitrogen appeared in this experiment to be the internal indicator of choice due to smaller error of prediction.

The average estimated dry matter intakes of the pastured heifers was 2995 grams greater than that of the stall-fed heifers. On a percentage basis, the dry matter consumption was 47 percent higher on pasture. This additional feed intake appears to be required for the energy expenditure of the grazing activities.

The digestion coefficient of ether extracts was higher for the conventional trial than that of the grazing trial, while the reverse was true for NFE. Particularly, digestion coefficients of the ether extracts for the conventional and the grazing trials were found to be 41. 3 and 22. 6 percent, respectively, using fecal nitrogen technique in both cases.

There was 0. 4 percent difference in TDN values between the total collection and the fecal nitrogen techniques in the conventional digestion trial. The difference of 0. 4 percent in the estimation of the TDN values is quite acceptable and less labor and equipment are needed in the use of the fecal nitrogen technique. The TDN values

of orchard grass dry matter using the fecal nitrogen technique in conventional and grazing trials were found to be 67. 5 and 68. 0 percent, respectively.

The difference in the digestible energy values between the total collection and fecal nitrogen techniques of the conventional trial were found to be 0. 5 percent. The digestible energy values of orchard grass in the conventional and grazing trials were found to be 68. 5 and 69. 7 percent, respectively. The small average differences in estimated TDN and digestible energy values between conventional and grazing trials is in favor of the latter and may be attributed to selective grazing on pasture. The digestible energy determinations showed a high correlation coefficient of 0. 92 with the calculated TDN values.

BIBLIOGRAPHY

1. Association of Official Agricultural Chemists. Official Methods of Analysis. 8th ed., Washington, D. C., Association of Official Agricultural Chemists, 1955. 1008 p.

2. Anthony, W. B. and J. T. Reid. Methoxyl as an indicator of the nutritive value of forage. Journal of Dairy Science 41:1715-1722. 1958.

3. Balch, C. C., S. Barlett and V. W. Johnson. Apparatus for the separate collection of feces and urine from cows. Journal of Agricultural Science 41:98-101. 1951.

4. Bergeim, O. Intestinal chemistry. IV. A method for the study of food utilisation or digestibility. The Journal of Biological Chemistry 70:29-33. 1926.

5. Bolin, D. V., R. P. King and E. W. Klosterman. A simplified method for the determination of chromic oxide when used as an index substance. Science 118:634-635. 1952.

6. Bondi, A. and H. Meyer. Lignins in young plants. Biochemical Journal 43:248-256. 1948.

7. Bradley, N. W. et al. Use of the chromic oxide method for determining digestible energy and protein in complete pelleted steer rations. Journal of Animal Science 17:1199. 1958.

8. Bratzler, J. W. A metabolism crate for use with sheep. Journal of Animal Science 10:592-601. 1951.

9. Briggs, H. M. and W. D. Gallup. Metabolism stalls for steers and wethers. Journal of Animal Science 8:479-482. 1949.

10. Brisson, G. J., W. J. Pigden and P. E. Sylvestre. Effect of frequency of administration of chromic oxide on its fecal excretion pattern by grazing cattle. Canadian Journal of Animal Science 37:90-94. 1957.

11. Brisson, G. J. and W. J. Pigden. Chromic oxide in a sustained-release pellet to reduce variation in its diurnal excretion pattern in cattle. Journal of Animal Science 17:1200. 1958.

12. Brisson, G. J. Grazing intake and behaviour studies. Indicator methods for estimating amount of forage consumed by grazing animals. In: Proceedings of the 8th International Grassland Congress, Reading, 1960. Oxford, Alden Press, 1960. P. 435-438.

13. Cook, C. W. and L. E. Harris. A comparison of the lignin ratio technique and the chromogen method of determining digestibility and forage consumption of desert range plants by sheep. Journal of Animal Science 10:565-573. 1951.

14. Corbett, J. L., J. F. D. Greenhalgh and A. P. MacDonald. Paper as a carrier of chromium sesquioxide. Nature 182:1014-1016. 1958.

15. Corbett, J. L. et al. Excretion of chromium oxide and polyethyleneglycol by dairy cows. British Journal of Nutrition 12:266-276. 1958.

16. Corbett, J. L. et al. Excretion of chromium sesquioxide administered as a component of paper to sheep. British Journal of Nutrition 14:289-299. 1960.

17. Corbett, J. L. and J. F. D. Greenhalgh. Measurement of the quantities of herbage consumed by grazing animals. In: Society of Chemical Industry Monograph No. 9: Symposia on production and use of grass, Dublin, 1959. London, Society of Chemical Industry, 1960. p. 167-180.

18. Corbin, J. E. and R. M. Forbes. Dye as a reference material for determining the digestibility of a ration with lambs. Journal of Animal Science 10:574-580. 1951.

19. Crampton, E. W. and I. R. C. Jackson. Seasonal variation in chemical composition of pasture herbage and relation to its digestibility by steers and sheep. Journal of Animal Science 3:333-339. 1944.

20. Crampton, E. W. and L. E. Lloyd. Fundamentals of Nutrition. San Francisco, W. H. Freeman and Company, 1959. 494 p.

21. Davis, C. L., J. H. Byers and E. E. Luber. An evaluation of the chromic oxide method for determining digestibility. Journal of Dairy Science 41:152-159. 1958.

22. Davis, R. D., C. O. Miller and I. L. Lindall. Apparent digestibility by sheep of lignin in pea and Lima-Bean vines. Journal of Agricultural Research 74:285-288. 1947.

23. Druce, E. and J. S. Willcox. The application of modified procedures in digestibility studies. Empire Journal of Experimental Agriculture 17:188-192. 1949.

24. Edin, H., G. Kihlen and S. Nordfeldt. A summarised description of "Edin's indicator method" for the determination of the digestibility of feeds and feed mixtures. Lantbruks-Högskoans Annaler (Annaler Royal Agricultural College Sweeden) 12:166-171. 1944.

25. Elam, C. J., P. A. Putnam and R. E. Davis. The fecal excretion pattern of chromic oxide when administered to Hereford heifers in a completely pelleted ration. Journal of Animal Science 17:1199. 1958.

26. Ellis, G. H., G. Matrone and L. A. Maynard. A 72 percent H_2SO_4 method for determination of lignin and its use in animal nutrition studies. Journal of Animal Science 5:285-297. 1946.

27. Forbes, R. M. et al. Variation in determination of digestive capacity of sheep. Journal of Animal Science 5:298-305. 1946.

28. Forbes, R. M. Some difficulties involved in the use of fecal nitrogen as a measure of dry matter intake of grazing animals. Journal of Animal Science 8:19-23. 1949.

29. Forbes, R. M. Protein as an indicator of pasture forage digestibility. Journal of Animal Science 9:231-237. 1950.

30. Forbes, R. M. and W. P. Garrigus. Some relationship between chemical composition, nutritive value, and intake of forages grazed by steers and wethers. Journal of Animal Science 9: 354-362. 1950.

31. Forbes, R. M. and W. P. Garrigus. Some effects of forage composition on its nutritive value when cut and fed green to steers and wethers as determined conventionally and by the lignin ratio. Journal of Animal Science 9:531-539. 1950.

32. Gacula, M. C. Jr., A. B. Lopez and L. L. Calo. A survey of the use of chromic oxide as indicator for measurement of dry matter intake of grazing cattle in the Philippines. Philippine Agriculturist 44:261-270. 1960.

33. Gallup, W. D. and A. H. Kuhlman. A preliminary study of determination of the apparent digestibility of protein by modified procedures. Journal of Agricultural Research 42:665-669. 1931.

34. Gallup, W. D. and A. H. Kuhlman. The composition and digestibility Mung-bean silage, with observation on the silica ratio procedure for studying digestibility. Journal of Agricultural Research 52:889-894. 1936.

35. Gallup, W. D. and H. M. Briggs. The apparent digestibility of prairy hay of variable protein content, with some observations of fecal nitrogen excretion by steers in relation to their dry matter intake. Journal of Animal Science 7:110-116. 1948.

36. Garrigus, W. P. and H. P. Rusk. Some effects of the species and stage of maturity of plants on the forage consumption of grazing steers of various weights. 1939. p. 453-458. (Illinois, Agricultural Experiment Station. Bulletin 454)

37. Greenhalgh, J. F. D. and J. L. Corbett. The indirect estimation of the digestibility of pasture herbage I. Nitrogen and chromogen as fecal index substances. Journal of Agricultural Science 55:371-376. 1960.

38. Greenhalgh, J. F. D. and K. V. Runcie. The herbage intake and milk production of strip and zero grazed dairy cows. Journal of Agricultural Science 59:95-103. 1962.

39. Hansard, S. L. et al. The design and operation of metabolism units for nutrition studies with swine. Journal of Animal Science 10:88-96. 1951.

40. Hardison, W. A. and J. T. Reid. Use of indicators in the measurement of the dry matter intake of grazing animals. Journal of Nutrition 51:35-52. 1953.

41. Hardison, W. A. et al. Degree of herbage selection by grazing cattle. Journal of Dairy Science 37:89-102. 1954.

42. Hardison, W. A. et al. Fecal chromic oxide concentration in 12 dairy cows as related to time on frequency of administration and to feeding schedule. Journal of Nutrition 58:11-17. 1956.

43. Hobbs, C. S., S. L. Hansard and E. R. Barrick. Simplified methods and equipment used in separation of urine and feces eliminated by heifers and steers. Journal of Animal Science 9:563-570. 1950.

44. Holter, J. A. The use of forage and fecal protein as indicators of forage digestibility. Ph. D. thesis. Ithaca, Cornell University, 1959. 103 numb. leaves. (Abstracted in Dissertation Abstracts 20:1934-1935. 1959)

45. Holter, J. A. and J. T. Reid. Relationship between the concentrations of crude protein and apparently digestible protein in forages. Journal of Animal Science 18:1339-1349. 1959.

46. Horn, L. H., Jr., M. L. Ray and A. L. Neumann. Digestion and nutrient balance stalls for steers. Journal of Animal Science 13:20-24. 1954.

47. Irvin, H. M. et al. The role of plant pigments in digestion trial studies. Journal of Animal Science 12:544-551. 1953.

48. Jennings, R. D. Relative use of feeds for livestock including pasture by states. Washington, D. C., 1955. 59 p. (U. S. Dept. of Agriculture-ARS Statistical Bulletin 153)

49. Jonston-Wallace, D. B. and K. Kennedy. Grazing management practices and their relationship to the behaviour and grazing habits of cattle. Journal of Agricultural Science 34:190-197. 1944.

50. Jordan, R. M. and G. Staples. Digestive comparisons between steers and lambs fed prairie hays of different quality. Journal of Animal Science 10:236-243. 1951.

51. Kane, E. A., W. C. Jacobson and L. A. Moore. A comparison of techniques used in digestibility studies with dairy cattle. Journal of Nutrition 41:583-596. 1950.

52. Kane, E. A. et al. Comparative digestion studies on orchard grass. Journal of Dairy Science 34:492. 1951.

53. Kane, E. A., W. C. Jacobson and L. A. Moore. Diurnal variation in the excretion of chromium oxide and lignin. Journal of Nutrition 47:263-273. 1952.

54. Kane, E. A. et al. A comparison of various digestion trial techniques with dairy cattle. Journal of Dairy Science 36:325-332. 1953.

55. Kane, E. A. et al. The estimation of the dry matter consumption of grazing animals by ratio techniques. Journal of Dairy Science 36:637-644. 1953.

56. Kane, E. A. and W. C. Jacobson. An improved method of using plant pigments as an indicator of digestibility. Journal of Dairy Science 37:672. 1954.

57. Kane, E. A., W. C. Jacobson and P. M. Damewood Jr. Use of radioactive chromium oxide in digestibility determinations. Journal of Dairy Science 42:1359-1366. 1959.

58. Kennedy, W. K. and R. J. Lancaster. Comparison of fecal nitrogen for the estimation of feed to feces ratio of pasture fed cattle. Proceedings of the New Zealand Society of Animal Production 17:56-62. 1957.

59. Kennedy, W. K., A. H. Carter and R. J. Lancaster. Comparison of fecal pigments and fecal nitrogen as digestibility indicators in grazing cattle studies. New Zealand Journal of Agricultural Research 2:627-638. 1959.

60. Knott, J. C., H. K. Murer and R. E. Hodgson. The determination of apparent digestibility of green and cured grass by modified procedures. Journal of Agricultural Research 53:553-556. 1936.

61. Kromann, R. P., J. H. Meyer and J. L. Hull. Energy requirements of grazing steers. Journal of Animal Science 20:450-453. 1961.

62. Lancaster, R. J. The measurement of feed intake by grazing cattle and sheep. I. A method of calculating the digestibility of pasture based on the nitrogen content of feces derived from the pasture. New Zealand Journal of Science and Technology 31:31-38. 1949.

63. Lancaster, R. J. Estimation of digestibility of grazed pasture from feces nitrogen. Nature 163:330. 1949.

64. Lancaster, R. J., M. R. Coup and J. C. Percival. Measurement of feed intake by grazing cattle and sheep. Part III. Marker technique for investigating the feces output of grazing cows. New Zealand Journal of Science and Technology 35:117-126. 1953.

65. Lancaster, R. J. Measurement of feed intake of grazing cattle and sheep. V. Estimation of the feed to feces ratio from the nitrogen content of the feces of pasture-fed cattle. New Zealand Journal of Science and Technology 36:15-20. 1954.

66. Li, Jerome C. R. Introduction to statistical inference. Ann Arbor, Michigan, Edwards Brothers, Inc., 1961. 568 p.

67. Lucas, H. L. Algebraic relationships between digestion coefficients determined by the conventional method and by indicator methods. Science 116:301-302. 1952.

68. Luitingh, H. C. The use of chromium oxide and lignin as indicators for the purpose of determining intake and digestibility of mixed rations by beef steers. Journal of Agricultural Science 56:333-342. 1961.

69. Marten, G. C. An investigation of the chromogen-chromic oxide and nitrogen-chromic oxide indicator and the TDN requirement methods in evaluating pasture forages for dairy cows. Ph. D. thesis. Minneapolis, University of Minnesota, 1961. 142 numb. leaves. (Abstracted in Dissertation Abstracts 22:3796-3797. 1962)

70. Maynard, Leonard A. and Loosli John K. Animal nutrition. 5th ed., New York, McGraw-Hill Book Co., Inc., 1962. 533 p.

71. McCullough, M. E. Symposium on forage evaluation: III. The significance of and techniques used to measure forage intake and digestibility. Agronomy Journal 51:219-222. 1959.

72. Miller, W. J. et al. The effect of feeding frequency, type of roughage and method of administration on the diurnal excretion of chromic oxide, chromogens, nitrogen, dry matter and ash. Athens, 1957. 20 p. (Georgia. Agricultural Experiment Station. Technical Bulletin, N. S. 14)

73. Mitchell, H. H. The evaluation of feeds on the basis of digestible and metabolisable nutrients. Journal of Animal Science 1:159-173. 1942.

74. Moore, L. A. and O. B. Winter. Rate of passage of inert materials through the digestive tract of bovine. Journal of Dairy Science 17:297-305. 1934.

75. Moore, L. A. Importance of quality in the utilization of forage. Washington, D. C., 1955. 14 p. (U. S. Department of Agriculture-ARS 52-2)

76. Morrison, Frank B. Feeds and feeding. 22nd ed., Clinton, Iowa, The Morrison Publishing Co., 1959. 1165 p.

77. Nelson, A. B. et al. A modified metabolism stall for steers. Journal of Animal Science 13:504-510. 1954.

78. New Zealand Department of Agriculture. Animal Research Division Annual Report, 1956-1957. Wellington, 1957. 50 p.

79. Oldfield, J. E. et al. Chromium oxide, chromogen and protein excretion patterns of beef steers when fed mountain meadow hay. Journal of Animal Science 15:1259. 1956.

80. Pigden, W. J. and G. J. Brisson. Note on a chromium oxide pellet to provide uniform release of this indicator in the rumen of cattle. Canadian Journal of Animal Science 37:185. 1957.

81. Putnam, P. A., J. K. Loosli and R. G. Warner. Excretion of chromium oxide by dairy cows. Journal of Dairy Science 41: 1723-1729. 1958.

82. Raymond, W. F. Evaluation of herbage for grazing. Nature 161: 937-938. 1948.

83. Reid, J. T. et al. A new indicator method for determination of digestibility and consumption of forages by ruminants. Journal of Dairy Science 33:60-71. 1950.

84. Reid, J. T. Indicator methods, their potentialities and limitations. In: Proceedings of the Sixth International Grassland Congress, Pennsylvania State College, 1952. Washington, D. C., National Pub. Co., 1952. p. 1334-1339.

85. Reid, J. T. et al. A procedure for measuring the digestibility of pasture forage under grazing conditions. Journal of Nutrition 46:255-269. 1952.

86. Reid, J. T., A. M. Smith and M. J. Anderson. Difference in the requirements for maintenance of dairy cattle between pasture and barn feeding conditions. In: Proceedings of the Cornell Nutrition Conference for Feed Manufacturers, Buffalo, New York, 1958. Ithaca, Cornell University, 1958, p. 88-94.

87. Richards, C. R. and J. T. Reid. The use of methoxyl groups in forage and fecal materials as an index of the feeding value of forages. Journal of Dairy Science 35:595-602. 1952.

88. Riley, Leonard William. An evaluation of the pheophytin method for determining the dry matter digestibility of forages consumed by ruminants. Master's thesis. Corvallis, Oregon State University, 1957. 69 numb. leaves.

89. Ritzman, E. G. and N. F. Colovos. An automatic method for collecting solid and liquid excreta from cows in digestion experiments. Durham, 1932. 16 p. (New Hampshire. Agricultural Experiment Station. Technical Bulletin 52)

90. Schneider, B. H. Feeds of the world, their digestibility and composition. Morgantown, Agricultural Experiment Station, West Virginia University, 1947. 299 p.

91. Schneider, B. H., B. K. Soni and W. E. Ham. Methods for determining consumption and digestibility of pasture forages by sheep. Pullman, 1955. 42 p. (Washington. Agricultural Experiment Stations. Technical Bulletin 16)

92. Smith, A. M. and J. T. Reid. Use of chromic oxide as an indicator of fecal output for the purpose of determining the intake of pasture herbage by grazing cows. Journal of Dairy Science 38:515-524. 1955.

93. Smith, A. D., R. B. Turner and G. A. Harris. The apparent digestibility of lignin by mule deer. Journal of Range Management 9:142-145. 1956.

94. Soni, B. et al. Diurnal variation in the estimates of digestibility of pasture forage using plant chromogens and fecal nitrogen as indicators. Journal of Animal Science 13:474-479. 1954.

95. Stevenson, A. E. Measurement of feed intake by grazing cattle and sheep. VIII. Some observations on the accuracy of the chromic oxide technique for the estimation of feces output of dairy cattle. New Zealand Journal of Agricultural Research 5:339-345. 1962.

96. Swift, R. W. et al. Digestibility of rations for ruminants as affected by proportion of nutrients. Journal of Animal Science 6 :432-444. 1947.

97. Wheeler, Robert Roderick. Evaluation of various indicator techniques in estimating forage intake and digestibility by range cattle. Ph. D. thesis. Corvallis, Oregon State University, 1962. 85 numb. leaves.