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THE DEVELOPMENT AND APPLICATION OF FECAL INDEXES FOR PREDICTING DRY MATTER INTAKE, DRY MATTER DIGESTIBILITY AND FECAL DRY MATTER OUTPUT

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

N, Stanford Hall

June 1978

1358329

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to the faculty and staff of the University of Tennessee, College of Agriculture for providing support during his course of study.

The author is especially grateful and appreciative to his major professor, Dr. J. W. Holloway, for his friendship and direction during the course of graduate study and for his genuine interest and knowledge of animal nutrition which motivated the author during his work.

The author is grateful to Dr. Haley Jamison for his encouragement and assistance throughout his college education as well as serving on the graduate committee.

The author is appreciative to Dr. C. C. Chamberlain for serving on the graduate committee and criticism of the manuscript.

Many others who aided in completion of this research and to whom the author is grateful are: Roger Munsinger and Marvis E. Fryer for their interest and assistance in the laboratory analyses; Mrs. Viola Gibbons for her excellent work in preparing the tables and figures; Dr. M. J. Montgomery for his assistance in obtaining the fecal samples and digestion trial results; the staff of the University of Tennessee Computer Center for making available the computer facilities used in the analyses of this data.

The author is grateful for the friendship and support shown by Jim Hopper, Johnny Beaty, Bob Montgomery, Randy Lyle, Lee Telega, Rick Estell, Bill Brown, Bob Nutt, Bob Jones and all his fellow graduate students during the course of study.

A special thanks to Miss Jean Ann Haston for her aid, interest, encouragement and moral support in all the author's endeavors.

The author wishes to thank his parents, Mr. and Mrs. Donnie Hall, for their financial support, encouragement, and of most importance, their love, while completing this study.

ABSTRACT

Analytical analyses were conducted upon thirty-six fecal samples obtained from two digestion trials to determine Dry Matter, Nitrogen, Acid Detergent Fiber, Acid Detergent Lignin and Urobilinogen. In addition, individual *in vivo* Digestible Dry Matter was calculated from total fecal collection procedures. A technique was developed for quantitative urobilinogen analysis of dried fecal samples. From these independent variables cited, and the independent variable animal weight, equations were developed for predicting Fecal Dry Matter Output, Dry Matter Intake, Digestible Dry Matter, and Digestible Dry Matter Intake. A prediction equation was developed which accounted for over 43 percent of the variation in Fecal Dry Matter Output ($P < 0.0003$). The equation included the independent variable Urobilinogen, Acid Detergent Lignin, and Animal Weight. It was found that 78 percent of the variation in Dry Matter Intake ($P < 0.0001$) could be explained by an equation containing the independent variables Nitrogen, Acid Detergent Lignin, and Urobilinogen Cubed. An equation including the independent variables Nitrogen, Acid Detergent Lignin, Acid Detergent Fiber, and the Natural Log of Urobilinogen explained 67 percent of the variation in Digestible Dry Matter ($P < 0.0001$). Digestible Dry Matter Intake was found to be the most predictable of all the dependent variables studied. Almost 83 percent of the variation in Digestible Dry Matter Intake ($P < 0.0001$) was accounted for by an equation containing the independent variables Nitrogen, Acid Detergent Fiber, and Urobilinogen Cubed. The large amount of explainable variation in the dependent variables considered in this study was achieved by using

different regression equations for each dependent variable to be predicted. It was concluded, therefore, that no one fecal index could best explain the variation in all dependent variables of interest. Rather, several fecal indexes must be employed if more than one dependent variable is to be predicted. The value of fecal indexes in the evaluation of grazed forages is supported by the results of this study.

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CHAPTER I

INTRODUCTION

Numerous researchers have attempted to develop fecal indexes for predicting the digestibility of forage grazed by pastured animals. A procedure for accurately predicting digestibility of grazed forages, dry matter intake of grazing animals or their fecal output would be extremely valuable in the study of forages in the form grazed by animals. The lack of a reliable method by which grazed forages can be accurately evaluated has been primarily responsible for the relatively small amount of information regarding the true nutritive value of pastures.

Many techniques have been employed to determine intake, digestibility, and fecal output of grazing animals. However, these techniques, in many cases, restricted the grazing habits of the animals. Techniques requiring (1) following the animal as it grazes, (2) confining of the animal, (3) harnessing collection bags to the animal (4) esophageal fistulazation, and (5) rumen evacuation, result in animal restrictions that may interfere with normal gazing habits. The magnitude of this interference is almost impossible to measure. It is the belief of this writer that valid conclusions cannot be obtained from grazing trials unless the animal is completely normal and undisturbed while grazing.

Development of a technique for estimating quantity or quality of forages grazed that requires only analysis of excreted feces would be desirable. Such a technique would not stress the animal and its grazing

habits need not be altered. Therefore, this study involves developing and evaluating fecal indexes for predicting dry matter intake, digestibility of consumed forage, and fecal dry matter output.

Variables to be considered in developing fecal indexes include (1) Nitrogen (2) Acid Detergent Fiber (3) Acid Detergent Lignin and (4) Urobilinogen--a natural excretion product of all normal animals.

CHAPTER II

REVIEW OF LITERATURE

Pasture is generally considered to be the most economical of livestock feeds and is becoming increasingly important in a world which is demanding more of our tillable land to be used in the production of grains for human consumption. Even so, research dealing with the utilization of forages in the form grazed by animals has lagged behind the research conducted on other kinds of animal feed. This lag can be attributed largely to the lack of a reliable method for estimating consumption and digestibility under grazing conditions (Schneider et al., 1955). Furthermore, Schneider et al., 1955, emphasized that the problem of measuring the degree of selective grazing has contributed largely to insufficient data on the subject.

I. IMPORTANCE OF SELECTIVE GRAZING

The problem of accounting for animal selectivity under grazing conditions has been recognized by numerous researchers (Erizian, 1932; Garrigus and Ruck, 1939; Hubbard, 1952; Hardison et al., 1954; Ivins, 1955; Heady and Torell, 1959; Weir et al., 1959; Weir and Torell, 1959; Blaser et al., 1960; Lesperance et al., 1960a; Corbett et al., 1960; Arnold, 1960; Van Dyne and Torell, 1964; High, 1966; Conrad, 1966; Bredon et al., 1967; Wang, 1968; Kiesling et al., 1969; Barth and Kazzal, 1971; Coppock et al., 1974).

Hardison et al. (1954) defined selectivity as a situation where animals select a diet of a chemical composition different from that of the whole, clipped herbage available to them.

For many years, some researchers have thought that animals select their diet upon the basis of an inherent "nutritional wisdom" (Ivins, 1955; Arnold et al. 1964). Specifically, Arnold et al. (1974) included among the animal factors which affect forage preferences: (1) special senses; (2) response to changing plant composition; and (3) nutritional wisdom. However, Conrad (1966) and Coppock et al. (1974) reported results suggesting that selectivity is a result of palatability and taste differences rather than nutritional wisdom. Balch (1954), Shellenberger and Kesler (1961) and Hurd and Blaser (1962) pointed out that the portion of the plant selected may be more a reflection of accessibility to the animal than to an inherent sense of superior nutritive value. Other researchers such as Van der Kley (1956) showed that cattle preferred the protein rich portions of herbage and Simkins et al. (1969) found differences in preference even among pelleted feeds.

In an attempt to relate selectivity to inheritance, Bell and Williams (1959) used monozygotic twins which were compared to unrelated sets of animals. They found a much wider range in selection between sets of twins than among members of unrelated sets of animals. In a separate study, Bell (1959) stated that all animals respond to four basic tastes: salt, sour, sweet, and bitter.

Hardison et al. (1954) first reported that the quantitative degree to which selective grazing occurs. They explained that the

degree of selectivity was reduced by restricting grazing to a small area containing small amounts of forage, while availability of large quantities of forage increased the degree of selection expressed.

In regard to total intake, Van Dyne and Heady (1965) stated that sheep spent more time selecting a high quality diet in late summer than did cattle. They reported sheep to consume more forage in mid-summer than late summer, while forage intake by cattle was inversely proportional to herbage availability. Furthermore, they discovered that cattle forage intake was 5 percent greater in late summer than in mid-summer. Forage intake by cattle was less affected by lack of herbage than was forage intake by sheep, but cattle diets contained less crude protein than sheep diets during the summer.

Selectivity was found to be greater among cattle and sheep during the early part of the grazing period than later in the season when available forage decreased (Elam and Davis, 1961; Lesperance et al., 1960a; Van Dyne et al., 1960a; Van Dyne et al., 1964). According to Lofgreen et al. (1957) sheep grazed more selectively than cattle on irrigated pasture, while Cook et al. (1963) and Van Dyne et al. (1964) found that averaged over the summer, cattle grazed significantly more forage per pound of body weight than did sheep. Although most researchers reported selectivity to be more pronounced when there was an abundance of forage available to the animal, which is usually the case earlier in the grazing season, Lofgreen and Meyer (1956) reported selective grazing among dairy cattle to be more evident with mature than with immature forage. Perhaps they were referring to a situation

where all the plants were more mature in a particular sward, thereby becoming unpalatable to the grazing animal which was forced to apply a higher degree of selection.

Ridley et al. (1963) reported that the botanical composition of pastures was not highly correlated with that of the forage selected by cattle. Furthermore, Shumway et al. (1963) observed that crude protein content of grazed samples averaged 11.22 percent in comparison with a protein content of 6.17 percent in clipped samples. Barth and Kazzal (1971), also found grazed samples to be higher in protein than clipped samples from the same pasture. This indicated that the animals exerted a substantial degree of selection for material higher in protein than that of whole clipped samples which were available to them. Weir and Torell (1959) found that sheep consistently selected forage higher in crude protein and lower in crude fiber than that obtained by hand clipping. The sheep in their study selected forage containing 4.2 percent more protein and 3.5 percent less crude fiber than was found in hand clipped samples from the same source. General agreement with these results has been found by several researchers (Meyer et al., 1957; Pieper et al., 1959; Bryant et al., 1961; Bredon et al., 1967; Wang, 1968; and Keisling et al., 1969). The possibility of routine adjustments for such selection differences was rejected by Nevens (1927) when he noted that no two cows exhibit the same preference, and the choice of several cows changed suddenly and then persisted.

Putnam et al. (1964) evaluated the relationship of cattle size and selectivity. They reported that larger cattle spent less time

consuming an equivalent amount of feed than did small cattle. Taylor (1959), Putnam (1964) and NRC (1964) reported that sheep become more sedentary as they become fatter, and feed intake as a percent of body weight tends to decline as lambs approach a higher degree of finish. Such a relationship would indicate that grazing animals may change their grazing habits, in regard to selective grazing, as they change in total energy balance.

The effect of diet composition on diet preference in lambs was studied by Glimp (1971) and Stronzinski et al. (1972). These researchers found a change in diet preference over time for a higher portion of intake from higher energy diets. The animals in their study changed from little discrimination among diets during the first two weeks, to an increasing proportion of daily intake from a higher concentrated diet as their trial progressed. Here again, the possibility of a grazing animal changing its pattern of grazing and degree of selectivity as its energy balance changes, is foreseen.

II. TECHNIQUES AND PROBLEMS ASSOCIATED WITH MEASURING SELECTIVE GRAZING

Interest in understanding why an animal chooses to consume particular plants and/or plant parts, can be traced back as far as 1749, when Hesselgren reported an experiment in which he offered hundreds of plant species to sheep, cattle, goats, horses, and pigs. He found goats and sheep were less, and swine more discriminating than the other species he studied. He concluded than no common factor explained the acceptance or refusal of a particular plant by livestock. Furthermore, he stated

that plants characterized as "aromatic" were not relished, and that even sheep did not discriminate between poisonous and non-poisonous plants. Although his study was a crude attempt at evaluating selectivity, it was one of the many methods used in seeking the answer to an extremely difficult question.

Hand Plucking

Researchers tried to reduce the effects of selective grazing on intake measurements by scrutinizing one or several unrestrained animals as they grazed, then trying to pluck samples representative of what the animal was selecting. The technique, known as "hand plucking," has been one of the most extensively used (Erizian, 1932; Crampton and Purdy, 1941; Saltonstall, 1948; Hubbard, 1952; Schneider et al., 1953; Hardison et al., 1954; Weir and Torell, 1959).

Hand plucking is accomplished by observing which plants or portions of plants the animal grazes and then sampling comparable ungrazed material (Cook, 1964). The samples collected are assumed to represent the diet chosen by the animal. Upon analysis of these samples, an idea of the chemical constituents of the ingested forage can be derived.

This method, as well as clipping the general area grazed, has been criticized by numerous researchers (Levy, 1927; Davies and Trumble, 1934; Tinney et al., 1937; Brandt and Ewalt, 1939; Crampton and Forshaw, 1939; Garrigus and Ruck, 1939; Schneider et al., 1955; Corbett and Greenhalgh, 1960; Rusoff and Foote, 1962; Cook, 1964; Harris et al., 1967). A summary of the inadequacy of hand plucking was offered by

Brandt and Ewalt (1939) when they stated that manual sampling of pasture plots, or even hand plucking, cannot, on theoretical grounds alone, be expected to simulate actual grazing with any degree of accuracy. Also concerning such attempts involving hand plucking, Corbett et al. (1960) and Garrigus and Ruck (1939) stated that methods for estimating grazing intake by pasture sampling are generally very imprecise, and inadequate, in so far as they do not yield direct information concerning the nutritive value of the herbage consumed. Robinson et al. (1937), Jones et al. (1937) and Cook et al. (1958) concluded that hand plucking is totally inadequate in estimating the diet of grazing animals. However, such criticism should be more qualified, if the pasture sampled was completely homogenous in makeup, immature in growth, and overall uniform in composition. In such an instance, hand plucking would serve as a useful tool in analyzing grazed forage. Nevertheless, the unlikeliness of such a pasture must be realized, and such methods may, in general, be inaccurate.

Cage Method

A method similar to hand clipping is known as "harvesting before and after grazing" or "cage method." Cook (1964) described this procedure as follows: Several plots in the pasture are protected from grazing by portable cages so that ungrazed material can be harvested at the end of the grazing period. The forage enclosed in the cage is the source of the "before-grazing" samples. Uncaged forages from the same area are likewise harvested at the end of the experiment to obtain the "after-grazing" samples. The differences in chemical composition of

these samples provide estimates of the chemical content of the forage selected by the animal.

However, Davies and Trumble (1934) presented a report in which they emphasized that chemical methods of separating the "Nutritive fractions" may not be valid because these fractions are not chemical entities and vary in composition with the source and nature of the herbage. In addition, a chemical description of pasturage according to Crampton and Forshaw (1939) has not been proven to be a reliable index of nutritive value as represented by gains in weight of grazing animals. Any proof of a definite chemical nature of the herbage, to which animal performance can be linked, has yet to be proven and printed according to the literature review of this writer.

Rumen Evacuation Method

The rumen-evacuation method has been used to obtain samples which represent material consumed. Descriptions of this method are offered by several researchers (Saltonstall, 1948; Ridley et al., 1963; Shumway et al., 1963; Van Dyne and Torell, 1964; Harris et al., 1967). These researchers agreed that a grazing animal has the ability to select a diet higher in crude protein and lower in crude fiber than samples clipped from the same pasture. Even so, problems associated with this method are numerous (Cook, 1964). The list of problems include: risk to the animal's life, very unpleasant for attendants working with the rumen fistula, rumen leakage, salivary contamination, loss of microbial population in some cases, incomplete recovery of ingested material may effect results calculated, and contamination of material by the rumen

wall itself; to list some of the most recognized shortcomings of this method. Lesperance et al. (1960a) stated, concerning the rumen evacuation method in particular, and all fistual approaches in general, that the amounts of ether extract and protein were found to be the same as in the feed. Significant changes were noted in the amount of crude fiber, nitrogen free extract, and energy. They added phosphorous and calcium to the list of minerals which are significantly affected by saliva contamination, which unavoidably occurs when utilizing the rumen evacuation technique.

Esophageal Fistula Technique

A technique which has been widely used in the study of selectivity is the esophageal fistula. This method was developed by Torell (1954) and modified by Cook et al. (1958) and Van Dyne and Torell (1964), and most recently by Bohman et al. (1967). Esophageal fistulas have been used throughout the United States and the world (Bath et al., 1956; Cook et al., 1958; Weir et al., 1959; Cook and Stoddart, 1961: Van Dyne and Torell, 1964; Blackstone et al., 1965; Cook et al., 1965; Hoehne et al., 1965; Grimes et al., 1965; Arnold et al., 1966; High, 1966; Torell et al., 1967; Guthrie et al., 1968; Kiesling et al., 1969; Jefferies and Rice, 1969). These researchers found what had generally been proclaimed by other researchers using various other methods--that animals consistently select forages higher in crude protein and lower in crude fiber than that obtained by hand plucking from the same pasture source.

Van Dyne and Heady (1965) pointed out that while their studies and those of others indicate the esophageal fistula to be a valuable

method, there are many unanswered questions concerning its usage. Van Dyne and Heady (1965) hypothesized that fasted animals yield different results from those which are grazing within their normal habits. Another is the matter of differences between morning and afternoon grazing. Van Dyne and Heady (1965) reported that in most studies employing the esophageal fistula, samples were taken only once a day, generally in the morning. Their study showed many differences in dietary composition between morning and afternoon grazing: thus biased estimates were a possibility in these experiments. They stated that differences between morning and afternoon samples could be due to hunger after the night's fast, to changes in moisture content of the herbage during the day, to temperature changes, or to any combination of these variables. They concluded that whatever the cause, time of grazing is an important factor in the study of dietary botanical composition, and that sampling should be conducted throughout the day rather than at the same time daily.

Still other precautions must be taken when interpreting data collected from esophageal fistulated animals. Corrections to eliminate the effects of saliva contamination (Barth et al., 1956; Van Dyne and Torell, 1964) and for partial mastication and rumination (Hoehne et al., 1965; Barth et al., 1969a) must be made.

Possibly the most limiting factor in the utilization of the esophageal fistula is the establishment of the devise in the animal. Many animals die shortly after such an operation. Torell (1954) reported success in only one out of four sheep. Lesperance et al. (1959) reported using four steers, all of which died within a year, and some only lived

a few weeks. Similar losses were noted by Cook et al. (1958), McManus et al. (1962), and Nelson (1962).

Chromogen Technique

A need for a method which would accomplish the same goal as that realized from the more tedious and less accurate methods discussed previously was sought by Reid et al. (1950). They concluded that since animals graze selectively, it is impossible, except by chance and in the case of a few plant species, to sample manually in such a way that the composition of the hand-chosen sample is representative of the forage consumed by grazing animals Due to this, these researchers gave attention to characteristics of fecal composition in an effort to develop a measure that would serve as a substitute for forage sampling. Their idea was to employ naturally occurring plant pigments collectively known as "chromogens," which are undigestible pigments in the plant which produce color in solutions and can be quantitated spectrophotometrically. Chromogens appearing in the feces were hoped to reflect the nature of the grass actually consumed thereby amounting to forage sampling. Furthermore, such a relationship would eliminate the need for analyzing grass for chromogen. These researchers found a curvilinear relationship existed between the chromogen concentration of the feces voided and the computed chromogen concentraction of the consumed forage.

Work along these same lines was being carried out by Kane et al. in 1951, by McCullough et al. in 1951, by Hardison et al. in 1951, and then by Richards and Reid in 1952. These workers reported satisfactory results by the use of a mathematical relationship between

the chromogen dry matter ratio of the feces voided and that of the forage consumed.

However, Reid et al. (1950) had precluded his own work with doubts as to the feasibility of using plant chromogens in such a manner. He stated that a weakness in the usefulness of the chromogen method may lie in the fact that the concentration of chromogen throughout the plant is not uniform. In fact, the level of chromogen in the leaves of most plants was found to be significantly higher than that found in the stems. Thus selectivity could not be accurately measured by the use of this method.

Fecal Nitrogen Index

Hobbs et al. (1945), Gallup and Briggs (1948) and Stallcup et al. (1975), presented data which indicated the possibility of using fecal nitrogen in calculation of the dry-matter intake of grazing animals. Gallup and Briggs (1948) based their assumption upon four observations: (1) In their experiments on the digestibility of praire hay, fecal nitrogen excretion per 100 grams of dry matter intake was relatively constant; (2) When a protein supplement was added to the hay, fecal nitrogen excretion increased regularly; (3) Calculations from Morrison's tables revealed total fecal nitrogen output to be constant from animals consuming alfalfa hay with protein content varying from 12 to 16 percent; and (4) calculations based upon a summary by Mitchell, of Morrison's data, indicated that the feeding of roughages resulted in an excretion of 0.67 grams of fecal nitrogen per 100 grams of dry matter intake, when the protein content of the roughage varied from

8 to 16 percent and the excretion of 0.76 grams of fecal nitrogen when the protein content varied from 16 to 24 percent.

Mitchell (1924) found a constant relationship between the feed consumption of rats and metabolic fecal nitrogen. Raymond (1948) found a relationship between fecal nitrogen, forage nitrogen, and digestibility of dry matter. He showed that the percent nitrogen in the diet of sheep could be predicted from the percent nitrogen in the feces.

Lancaster (1949a,b) found different relationships between fecal nitrogen and organic matter digestibility for forages which varied in percent protein. Lancaster (1949a,b) and Breirem (1952) suggested different constants should be developed from conventional trials in which the level of nitrogen is similar to that which is expected in grazing studies to be conducted. The concept of "local" regression equations derived from the same growth of herbage that is being grazed should be used in such studies, concluded Corbett (1960) and Langlands et al. (1963).

Many equations have been developed which relate digestible organic matter and fecal nitrogen, and the feed-to-feces ratio and fecal nitrogen. A linear regression with variables, digestibility and fecal nitrogen was found by Raymond et al. (1954). However, Kennedy, et al., (1959) found a logarithmic relationship between the feed-to-feces ratio and fecal nitrogen, and suggested that Lancaster (1954) had found a linear relationship because of a smaller range of data. Kennedy et al. (1959) also concluded that fecal nitrogen provided a

more reliable index than fecal pigments for estimation of the feed-to-feces ratio. Yet, they added that application of the two methods simultaneously might better represent the ratio.

Fecal nitrogen index regressions have been found to vary significantly with the season of the year (Minson and Raymond, 1958; Greenhalgh and Corbett 1960; Greenhalgh et al., 1960; Minson and Kemp, 1961; Langlands et al., 1963). Lambourne and Reardon (1962 stated that this seasonal bias was an artifact of differences in the leaf-to-stem ratios between herbage clipped for conventional digestion trials and that actually consumed by selectively grazing animals.

Pearce, et al. (1962), Vercoe, et al. (1962), and Lambourne and Reardon (1963) concluded that even "local" regression equations did not eliminate errors involved in the fecal index techniques associated with the selectivity of the animals for different plants and plant parts.

Greenhalgh et al. (1966) found little difference between the prediction equation derived from top and bottom cut herbage. This discrepancy can be attributed to the fact that Greenhalgh et al.(1966) employed cattle and the other investigators used sheep, which are considered to be more selective grazers (Streeter, 1969). Langlands et al. (1963) further emphasized such complications when he pointed out that if sheep are employed rather than cattle in a digestibility trial aimed at determining the usefulness of fecal nitrogen indexes, simply because sheep are more easily attended in such trials than are cattle, the data obtained cannot be used in regard to grazing cattle.

Glover et al. (1961) pointed out that the direct proportionality between metabolic fecal nitrogen and feed consumption proposed by Mitchell (1924), was later modified by Schneider (1934). Who showed that body weight also influences metabolic fecal nitrogen. When rats were used as experimental animals Blaxter and Mitchell (1948) contended that the fiber content of the feed also determines the ratio of metabolic fecal nitrogen to dry matter consumption.

Forbes (1949) emphasized the difficulty involved in the use of fecal nitrogen as an index for grazing animals. He stated, concerning dried roughages, that there is a steady increase in fecal nitrogen per 100 grams of dry matter intake as the protein content of the ration increases. He further concluded that the ratio was too variable to be of practical use in digestion studies with fresh grass, and that data was too scanty to warrant definite conclusions beyond the fact that total fecal nitrogen varies too widely to be of practical use.

Hutchinson (1958) suggested that only part of the metabolic fecal nitrogen (M.F.N.) was directly related to dry matter intake, the remainder consisting of a constant endogenous fraction. He found that the nitogen index relationship existed only between 8 and 45 grams of nitrogen intake per day in sheep. In addition, Hutchinson (1958) pointed out that there may be differences between individual animals in the digestibility calculated: fecal nitrogen relationships arising, for example, from variation in metabolic fecal nitrogen excretion such as was indicated by the results obtained by Ashton (1962) or from parasitic infestation by Spedding (1954).

The assumption that fecal nitrogen is related to metabolic fecal material, which in turn is related to the feed-to-feces ratio, seems weak (Streeter, 1969) if one notes the work of Virtanen (1966), who showed that fecal nitrogen is mainly of bacterial origin rather than metabolic. The fraction of fecal nitrogen which is not bacterial may well be present in the lignin molecule (Van Soest, 1967). The nitrogen content of lignin has been shown to vary with the plant species from which the lignin was isolated (Bondi and Meter, 1948), therefore, the consumption of a variety of plant species by grazing animals would result in different relationships between fecal nitrogen and the feed-to-feces ratio.

Stronzinski et al. (1972) concluded that metabolic fecal nitrogen is affected by the energy balance of the animal, as well as by the digestibility of the ration. Stallcup et al. (1975) cautioned that nitrogen's relation to intake departs from linearity as nitrogen intake varies. Consequently, such a method may not be applicable to wide useage especially in regard to forages, on the basis that a given pasture may change in its content and thereby vary in its nitrogen concentration.

Finally, the question of diurnal excretion of fecal nitrogen has not been absolutely answered. Soni et al. (1954) found no significant diurnal variation in the excretion of fecal nitrogen. However, Brisson (1960) found coefficients of variation in the concentration of nitrogen in fecal grab samples to be 7.5 percent, and Lambourne and Reardon (1963) reported a diurnal variation in fecal grab sample nitrogen to be 7.0 percent.

Vera (1973) examined the usefulness of fecal nitrogen as an internal indicator and summarized the criticisms of such a technique as follows: the bias introduced by difference in intake between grazing and housed animals; the fact that most fecal nitrogen is of bacterial origin and as such does not necessarily hold a causal relationship to digestibility; the differences in the type of relation between nitrogen and digestibility introduced by selective grazing; and that the complicating factor in the use of nitrogen as an indicator is the level of crude protein in the diet.

III. SUMMARY

Although many studies have yielded precise information and seem to be reliable estimates of selectivity, there still remains the question of their accuracy concerning normal, free-grazing animals. Furthermore, a question remains as to what degree the feeds examined and feces analyzed represent an entity, or in other words, does one constitute the other. With such a question remaining, there is an indication of a real need to develop a procedure which actually measures the effect of selectivity. With the phenomenon of selective grazing in mind, many investigators in recent years have felt that the only reliable way to conduct pasture studies is by using the animal as the basic unit. A report by the Joint Committee of American Society of Agronomy, the American Dairy Science Association, the American Society of Animal Production, and the American Society of Range Management recommended that only the animal should be used for the determination of forage quality (Anonymous, 1952). Such a conclusion indicates that the animal and its ability to selectively graze, must be the basis for our study.

CHAPTER III

PROSPECTS OF NEW METHODS FOR EVALUATING GRAZED FORAGES

There appears to be more promise in a method which approaches the measurement of selectivity by analysis of the grazing animal's fecal excreta. Such an approach would allow the animal to maintain its normal state, without being disturbed to any degree. Previous attempts in most cases have involved the use of a reference substance known as an "indicator."

External indicators such as chromium sesquioxide (Cr_2O_3) have been employed extensively (Chanda et al., 1951; Schurch et al., 1950; Dansky and Hill, 1952; Hardison and Reid, 1953; Lloyd and McCay, 1954; Clawson et al., 1955; Smith and Reid, 1955; Lambourne, 1957a; Bisson et al., 1957; Holmes et al., 1961; Johnson et al., 1964; Christian et al., 1965; and Hopper, 1977). Such external indicators when administered to animals eliminates the need for total fecal collections.

Reid et al. (1950) listed the necessary features of an indicator substance as follows: (1) it should employ a reference material which is indigestible and 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, by state 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.

Such restrictions are not easily overcome by a substance. Furthermore, the animals must be administered the substance in some form which also will not violate the restrictions (Crampton and Lloyd, 1951; Pigden and Brisson, 1957; and Yadava et al. 1973).

An additional restriction or qualification which would allow utilization of a substance in the study of selectivity is that the substance must not disturb the grazing animal either by its presence of its administration. Due to this additional qualification, manually administered indicators do not fulfill the requirements needed if the grazing animal is not to be subjected to any change in its natural habits. Therefore, another substance should be found which first fulfills Reid's restrictions, then secondly, does not disturb the animal.

To meet such ammended restrictions, one would have to utilize a substance which is naturally present in the animal's body and excreted at a constant rate, without variation due to feed intake, maturity of ingesta, and composition of ingesta. Such a substance could be termed a "naturally occurring internal biological indicator."

Since such a substance would have to be excreted at a constant rate in the feces, the first step in locating such a substance would be a thorough examination of the feces.

Bile, a significant percentage of the feces, is known to be secreted continually (Guyton, 1971) by the liver. Unlike other gastrointestinal secretions, bile is stored in the gallbladder until needed in the gut. The gallbladder empties the bile into the intestine in response to cholecystokinin, a hormone extracted from the intestinal

mucosa by fats in the intestine. This in turn passes by way of the blood, to the gallbladder, and causes a rhythmic contraction of the gallbladder. However, these contractions alone are not sufficient to cause emptying of the gallbladder, because the sphincter of Oddi, which constricts the common bile duct where it empties into the small intestine, normally remains tonically contracted despite gallbladder contractions. But, every time a peristaltic wave passes over the duodenum, the wave of relaxation immediately ahead of the peristaltic contraction relaxes the sphincter of Oddi at the same time that it relaxes the gut wall, thus allowing small squirts of bile to flow into the duodenum.

Concerning grazing animals, the fat content of their diet remains relatively stable so that any change in fat content of the herbage would be a continual occurrence and within a limited range. This would indicate a rather constant flow of bile into the small intestine.

Analysis of the bile found in the small intestine reveals a number of substances synthesized by the liver itself as well as substances which were formed elsewhere in the body. Among these is a substance known as bilirubin. The origin of bilirubin is described by Guyton (1971) as follows: When the red blood cells have lived out their lifespan, approximately 125 days in the adult cow, and have become too fragil to exist any longer in the circulatory system, their cell membranes rupture, and the released hemoglobin is phagocytized by reticuloendothelial cells throughout the body. Here, the hemoglobin is first split into globin and heme, and the heme ring is opened to give a straight chain of four pyrrole nuclei, which is the substance

from which the bile pigments are formed. The first pigment is biliverdin but this is rapidly reduced to bilirubin, which is gradually released into the plasma. The bilirubin is combined with plasma proteins and then eventually absorbed into the liver cells where the bilirubin is removed from the protein and conjugated with other substances that make it become highly soluble. It is in the soluble form that the bilirubin is excreted into the bile.

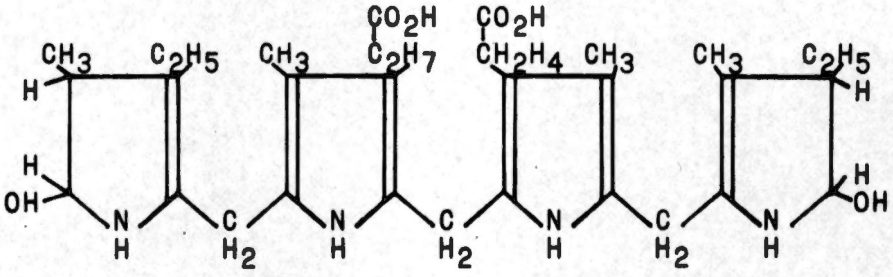
As bile is secreted into the small intestine, the bilirubin it contains is converted by bacterial action mainly into the substance urobilinogen which is also highly soluble. Finally, in the feces, the urobilinogen is oxidized to urobilin. Chemical formulas of these excretion products are presented in Figure 1.

Lemberg et al. (1938) explained the changes which take place involving bilirubin with the aid of structural formulas (Figure 1) as follows: When bilirubin is reduced it yields a leuco-compound $C_{33}H_{44}O_6N_4$ called mesobilirubinogen (Formula III, Figure 1), the prefix "meso" indicating that the vinyl side chains of bilirubin have become reduced to ethyl. The shorter name mesobilinogen, which is also in use, is preferable, because it is in better harmony with the fact that this substance is the chromogen of other bile pigments of the meso-type, as well as, of mesobilirubin. The oxidation of mesobilinogen by atmospheric oxygen produces a yellow pigment, urobilin-IXa (Formula IV, Figure 1).

The presence and quantification of bilirubin and its oxidation products, urobilinogen and urobilin have been accomplished by a number of researchers in human and animal medicine (Malloy and Evelyn, 1937;

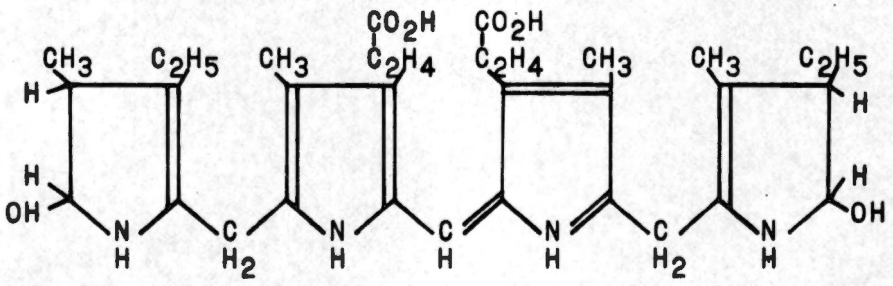
STRUCTURAL FORMULA FOR BILIRUBIN DERIVATIVES

I



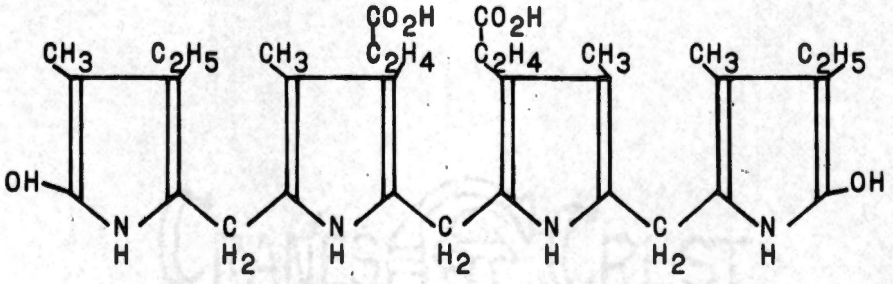
UROBILINOGEN (stercobilinogen)

II



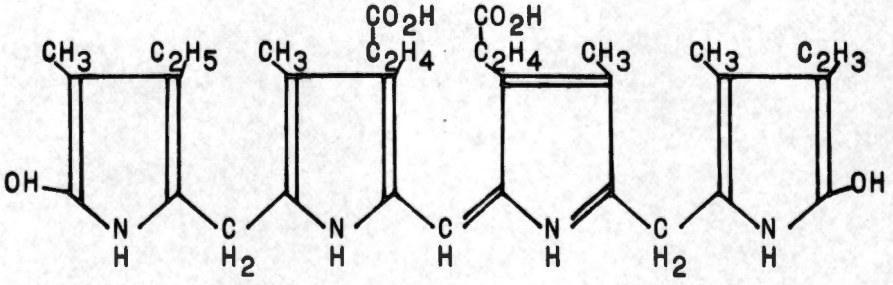
UROBILIN (stercobilin)

III



MESOBILINOGEN (mesobilirubinogen)

IV



UROBILIN IX_a

Figure 1. Structural formula for bilirubin derivatives.

Watson 1938; Schwartz et al., 1944; Gardner, 1955; Henry et al., 1961; Watson et al., 1962; Watson 1965; Watson et al., 1966; Levy et al., 1968; With, 1968; Cornelius, 1970; Johnson et al., 1970; Schwartz et al., 1971).

The application of such a substance in the study of intake and digestibility and thereby selectivity, is theoretically possible. On the basis that if the normal red blood cell life span is 125 days in adult cows, a relationship should exist as outlined by Schwartz and Bracho (1972) as follows: (1) The total circulating blood volume in a 650 kg cow is approximately 36 liters (Johnson and Schwartz, 1970). With 10.8 grams of hemoglobin per 100 ml, this blood volume would contain approximately 3,890 grams of hemoglobin; (2) Quantitative degradation of 1 gram of hemoglobin yields 36 mg. of urobilinogen; (3) With an average life span of 125 days, the daily degradation of 0.8 percent of the total hemoglobin should yield 1,120 mg of urobilinogen per day.

If this value of 1,120 mg of urobilinogen per day is accurate, and can be adjusted for weight of the animal, the utilization of urobilinogen measurements should be applicable in determining fecal output. Whereas the amount of feces increases or decreases, the percentage of urobilinogen would inversely respond.

Returning to our hypothesis that such a substance could be used in determining intake, we muct check Reid's qualifications and see if urobilinogen will meet these restrictions. His first requirement was that the substance be indigestible and recoverable in the feces. Urobilinogen is indigestible in so much as it is an end product,

unaffected, once excreted by the digestion process. A chemical analysis for urobilinogen must be simple and accurate if it again is to satisfy Reid's description of an indicator. The analysis will be presented later in this paper and shown to be easily done with little experience or specialized equipment. Reid stated that the substance must not be influenced by treatment of the feed, stage or maturity, or passage of feed through the gut. Urobilinogen, being derived from material excreted in the small intestine, would not be affected by any of these restrictions. Reid's final requirement, that the substance is quickly established in the feeding regime, is easily met by urobilinogen, due to its presence from birth in the animal's excreta. Finally, the additional requirement imposed upon an indicator substance used in the study of selective grazing was that its application would not disturb the animal in any manner. Without question, any substance which is normally excreted from an animal in the feces, would not violate this final restriction.

Therefore, we have described a substance which seemingly meets all the requirements necessary in a selectivity study utilizing fecal output as a basis. The testing of the hypothesis that urobilinogen can be utilized in such a manner, and the development of a fecal index involving not only urobilinogen, but other fecal constituents which may or may not have been employed previously in such investigations, is the subject of this research.

CHAPTER IV

MATERIALS AND METHODS

Analytical analyses were conducted upon thirty-six fecal samples obtained from two digestion trials to determine: (1) Dry Matter (DM); (2) Nitrogen (N); (3) Acid Detergent Fiber (ADF); (4) Acid Detergent Lignin (ADL); and (5) Urobilinogen (UROB). In addition, individual *in vivo* Digestible Dry Matter (DDM) was calculated from total fecal collection procedures.

I. CONDUCTION OF DIGESTION TRIALS

Dried fecal samples were obtained from two digestion trials conducted at the Dairy Experiment Station, Lewisburg, Tennessee, in 1974 and 1975. The 1974 trial included 18 healthy, gestating Jersey heifers, ranging in body weight from 273.5 to 369.2 kilograms, with a mean body weight of 321 kilograms. The 1975 trial employed 18 different gestating Jersey heifers ranging in weight from 285.8 to 402.8 kilograms, with a mean body weight of 341 kilograms.

The heifers in both trials were fed in a 3 X 2 factoral arrangement of treatments. Each trial allowed a seven day adjustment period, which was followed by a seven day preliminary feeding period, during which daily intake was recorded. The heifers were fed during the final five day collection period on the basis of 100 percent of their preliminary intake, six ration treatments as follows: (1) Corn Silage, alone; (2) Corn Silage plus 3 pounds of 44 percent soybean meal; (3) Forage Sorghum Variety 102F, alone; (4) Forage Sorghum Variety 102F,

plus 3 pounds of 44 percent soybean meal; (5) Forage Sorghum Variety 33, along; and (6) Forage Sorghum Variety 33, plus 3 pounds of 44 percent soybean meal. The two sorghum varieties were chosen solely on the basis of total sorghum seed sales in the state of Tennessee--Variety 33 and 102F recording the highest volume of sorghum seed sales in the state the previous year (1973).

During the five day collection period, total feces was collected, of which a sample of approximately 1 percent of each day's fecal output was frozen. These daily aliquots were combined at the conclusion of the trial and dried in a forced air draft oven at 60° C for subsequent chemical analyses.

Intake and fecal output data from the digestion trials are presented in Table 1. Also in Table 1 are the calculated in vivo DM digestibility values. From this table it is seen that Fecal DM Output (FDMO) had a small standard deviation of only 0.26 kg/day. FDMO ranged from 1.39 kg/day to 2.63 kg/day, with a coefficient of variation of 13.64 percent. Dry Matter Intake (DMI) had a standard deviation of 1.14 kg/day and a coefficient of variation of 21.05 percent DMI. ranged from 3.83 kg/day to 7.76 kg/day.

DDM was the most variable estimate having a coefficient of variation of 29.02 percent and a standard deviation of 6.12. DDM over all treatments ranged from a minimum mean value of 56.06 percent in Treatment 3, to a maximum mean value of 72.95 percent in Treatment 2.

Similar intake values are desirable in a trial aimed at comparing rations, such being the original goal of these trials. However, small variations in DMI and FDMO are not as desirable in studies whereby these

TABLE 1

DATA COLLECTED FROM DIGESTION TRIALS

INDEPENDENT VARIABLE	Treatment 1		2		3		4		5		6		OVERALL	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Number of heifers	7		6		6		6		5		6			
Digestible Dry Matter Percent	65.38	2.70	72.95	2.67	56.06	4.80	64.35	1.89	59.30	3.35	62.18	3.43	63.54	6.12
DM Intake (Kg/day)	5.28	0.77	7.08	0.62	4.27	0.32	5.79	0.85	4.17	0.26	5.68	0.63	5.41	1.14
Fecal DM Output (kg/day)	1.82	0.26	1.91	0.23	1.85	0.17	1.97	0.20	1.70	0.20	2.15	0.32	1.91	0.26

variables are to be used in developing prediction equations for intake and fecal output. Rather, trial results having a greater amount of data variation would be preferable, in that more confidence could be placed upon prediction equations based on those variables. Consequently, it is noted here, that more variation in DMI and FDMO, as well as a larger number of experimental units, would have been desirable for the present research. Nevertheless, this research was an initial study in developing fecal indexes involving fecal constituents studied previously, as well as fecal urobilinogen, which prior to this work had not been studied as a component of fecal indexes.

II. ANALYTICAL ANALYSES OF FECAL SAMPLES

Chemical analyses for all fecal constituents estimated, excluding urobilinogen, were preformed according to techniques as follows: (1) DM (A.O.A.C., 1975); (2) N (A.O.A.C., 1975); (3) ADF (Van Soest, 1963); and (4) ADL (Van Soest, 1963).

III. TECHNIQUE USED FOR FECAL UROBILINOGEN ANALYSIS

The analysis for urobilinogen in this study was a variation of the technique used by Schwartz and Bracho (1972) which was a modification of an earlier method developed by Watson (1936). A brief explanation of the theoretical basis of this analytical procedure is given in the following paragraph: Freshly excreted feces contain oxidized forms of bilirubin, collectively known as the urobilins (Schwartz et al., 1944). Bilirubin is not normally found in the fresh feces due to the action of micro flora

in the bovine gut which reduce it to urobilinogen (Cornelius, 1970). Bilirubin only appears in the feces in conditions preventing its reduction to urobilinogen such as rapid passage through the tract (diarrheas) or suppression of bacterial action. Bacterial action can be suppressed through the clinical use of broad spectrum antibiotics (Cornelius, 1970). Upon contact with atmospheric oxygen, the urobilinogens are immediately oxidized into at least two forms, urobilin IXa, optically inactive, and L-stercobilin (Figure 1, Lemberg et al., 1938; and Watson, 1936). These urobilins, which contain both optically active and inactive forms of bilirubin, are not easily quantitated and therefore must be reduced to the urobilinogen form for quantitative determinations.

A stepwise description of the method used in this study is as follows:

(1) A 5 gram portion of dry, finely ground feces is added to a clean dry mortar.

(2) 20 ml of a 90 ml volume of distilled water is added to the feces in the mortar. This small amount of water is allowed to wet the entire amount of feces in the mortar so to eliminate any dust loss from the feces sample.

(3) Additional small amounts of the 90 ml volume of water are added over approximately five minutes with mixing and further grinding with a pestle.

(4) Eventually all but 10 ml of the 90 ml volume of water is added and mixed thoroughly.

(5) The feces (5 grams) and water (80 ml) mixture is allowed to stand for approximately 5 minutes while the next two steps are performed.

(6) 40 ml of 20 percent solution (W/V) of ferrous sulfate (20 grams of ferrous sulfate: 92 ml water, prepared within an hour of use) is added with constant swirling to 40 ml of a 10 percent NaOH solution in a dry suction flask no smaller than 500 ml in volume.

(7) After thorough mixing of the $FeSO_4$ mixture, the fecal mixture, still in the mortar, is again stirred briefly and brought into solution as much as possible.

(8) The mortar contents are now poured into the suction flask.

(9) The mortar and pestle are quickly rinsed clean with the remaining 10 ml of distilled water from the original 90 ml volume, this too, is now poured into the flask.

(10) Air is immediately evacuated from the flask by water suction, swirling the mixture constantly for approximately 30 seconds or until appreciable foaming begins. This is done to limit reoxidation of urobilinogen at the air surface (Schwartz and Bracho, 1972).

(11) The mixture is sealed and set aside in the dart at ambient room temperature for 2 hours.

(12) After 2 hours, the mixture is transferred into centrifuge tubes and spun at 10,000g for 15 minutes in a refrigerated centrifuge.

(13) After centrifugation, the supernatant portions in each tube, free of opalescence, are carefully pooled into a clean flask, being extremely careful not to pour any of the solid material into the flask.

(14) Colormetric analysis must be determined immediately after centrifugation.

Colorimetric Analysis

1. Direct analysis of the pooled supernatant is performed by adding a 3 ml portion of the liquid to two 50 ml beakers.
2. To the first beaker containing 3 ml of supernatant, 3 ml of a modified Ehrlich's reagent (1.4 gm. p-dimethylaminobenzaldehyde, 300 ml concentrated (12N) HCL, and 200 ml distilled water) is added.
3. After mixing by gentle swirling, 6 ml of saturated aqueous sodium acetate is added. The characteristic color development of urobilinogen will correspond to the amount present. Color may range from a very faint pink case, if very small amounts of urobilinogen are present, to a darker red color if larger quantities are present. Experience with dried fecal samples has taught this researcher not to expect any more than a very faint pink color seen by looking down the top of the cuvet containing the mixture.
4. Absorbance is immediately read using a spectrophotometer (Model DU, Beckman Inst., Inc., Palo Alto, Calif.) at a 565 nm and a slit width of 0.15 mm.
5. The blank for the color intensity reading is made in the same manner as in previous steps 1--3, but the sodium acetate and Ehrlich's reagent are first mixed and then added to the supernatant in the second beaker. This initial neutralization of the strong acid in the Ehrlich's reagent prevents formation of the colored urobilinogen aldehyde (Schwartz and Bracho, 1972).

IV. CALCULATION OF UROBILINOGEN

The early literature indicated that the standard for this analysis required a supply of crystalline bilirubin which could be reduced to urobilinogen. However, the poor availability of this substance and in turn, the procedure of converting bilirubin to urobilinogen was found

to be very difficult, if not impossible by inexperienced, minimally equipped laboratory workers. This fact, and the commercial unavailability of pure urobilin or stercobilin, has led to adoption of artificial reference substances for determination of these pigments in biologic specimens by the urobilinogen-aldehyde reaction (Henry, 1961).

Phenolphthalein, phenol red (also known as phenolsulfonphthalein or PSP), bromoauric acid and a mixture of Pontacyl dyes have been used as artifical standards for this purpose (Terwin, 1925; Heilmeyer, 1931; Watson 1936; Schwartz et al.. 1944).

Henry et al. (1961) compared these artificial standards and concluded the following: Phenolphthalein is not desirable as a standard because of color instability and a spectrum differing somewhat from that of urobilinogen aldehyde. The Pontacyl dye mixture also has a spectrum dissimilar to that of urobilinogen-aldehyde, making standardization with this solution dependent upon the instrument used. PSP, on the other hand, has a spectrum identical with that of urobilinogen-aldehyde between 530 and 570 mu and results using this dye as a standard are independent of the instrument used in this wave length region.

Henry et al. (1961) found the relationship of PSP and urobilinogen-aldehyde to be as follows: a 0.20 mg/100 ml solution of PSP in 0.05 percent NaOH = 0.346 mg/100ml urobilinogen (the concentrations refer to the final solutions read in the spectrophotometer).

For the present analysis, 0.05 percent NaOH solutions were prepared ranging from 0.6 to 8.0 mg PSP/ml. The corresponding absorbancy

readings followed Beer's law in that as each concentration of PSP/ml increased, the absorbance reading increased proportionally. Data obtained from spectrophotometric analysis of these solutions appears in Table 2 and Figure 2.

The standard curve produced was used in calculating mg urobilinogen per gram of feces as follows: Urobilinogen (mg/ml)= -0.00056552 + 7.2086976 (Asborbancy)

$(R^2 = 1.0)$

$$\text{Urobilinogen (mg/g)} = \frac{\left[\begin{array}{l}\text{Urobilinogen mg/ml X ml} \\ \text{solution from which} \\ \text{absorbancy was taken}\end{array}\right.}{\text{grams feces in analyses}}$$

EXAMPLE: UROB mg/g = (UROB mg/ml X 175ml)/5 grams feces.

TABLE 2

STANDARD CURVE DERIVED BY UTILIZATION OF PHENOSULFONPHTHALEIN (R^2=1.00)

PSP mg/ml	UROB Equivalent mg/ml	Absorbance
0.6	1.04	0.144
0.8	1.38	0.192
1.0	1.73	0.240
2.0	3.46	0.480
4.0	6.92	0.960
6.0	10.38	1.440
8.0	13.84	1.920

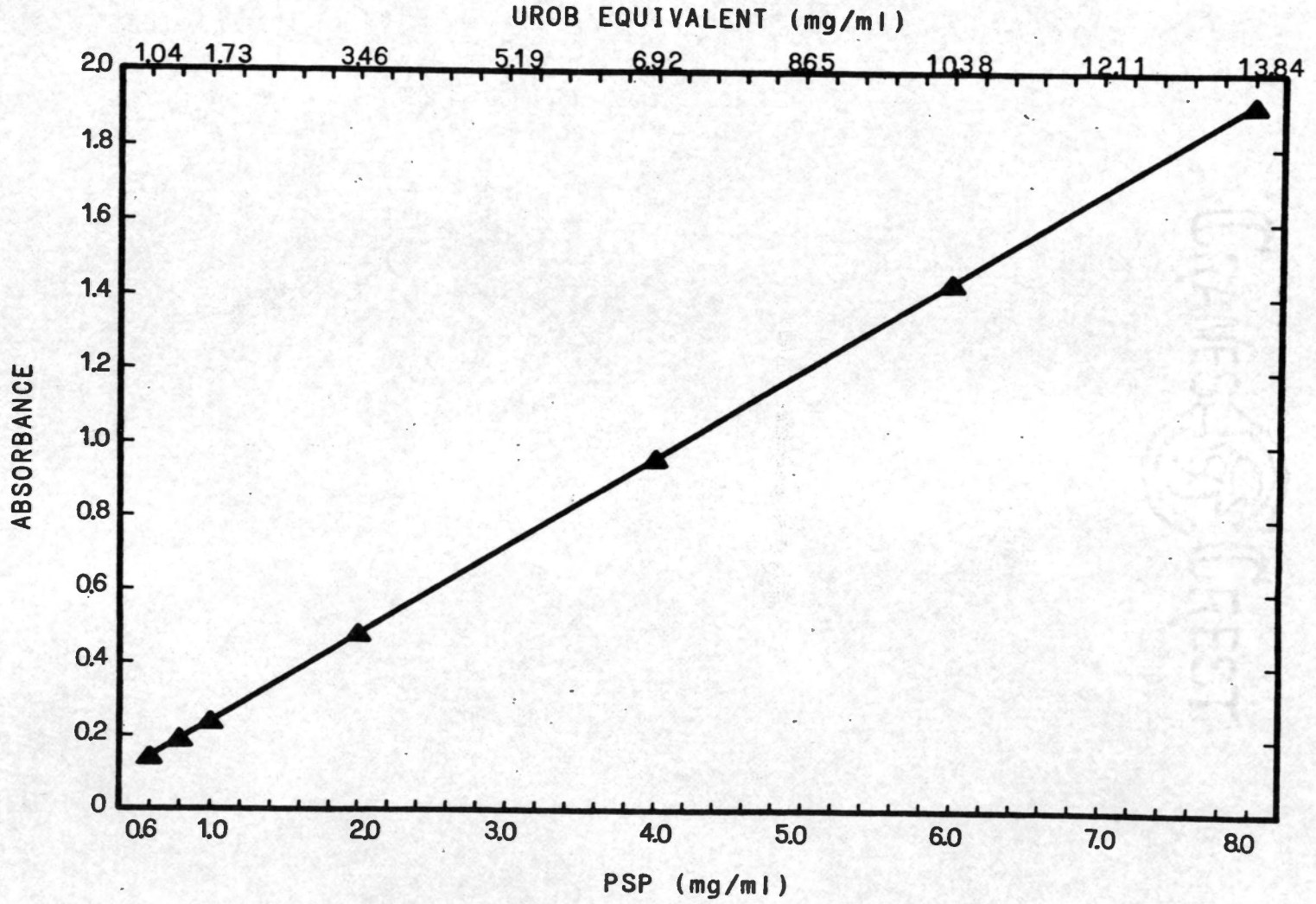


Figure 2. Standard curve derived for converting UROB absorbance to UROB (mg/ml).

V. STATISTICAL ANALYSIS

Regression analyses were conducted using the independent variables (Table 3) N, ADL, ADF, UROB, Animal Weight (ANWT) and Metabolic Animal Weight (MANWT). These variables were used to predict four dependent variables: (1) FDMO; (2) DMI; (3) DDM; and (4) Digestible Dry Matter Intake (DDMI).

Models were fitted to these dependent variables in an effort to determine which independent variables were useful in explaining the variation of the dependent variables. Stepwise and multiple regression procedures were employed according to the methods of Sokal and Rohlf (1969).

In calculating the stepwise regressions, all the default options of SAS (Barr et al., 1976) were used, so that an intercept term was included. The significance level for entry of a variable into the equation was 0.50, and the significance level for a variable to remain in the equation at each step was 0.10.

TABLE 3

VARIABLES FROM 1974 AND 1975 DIGESTIBILITY TRIALS
USED IN REGRESSION ANALYSES

VARIABLE	MEAN	S.D.	RANGE	
			LOW	HIGH
Animal weight (kg)	332.45	32.41	273.52	402.80
Metabolic Animal weight	63.84	4.68	55.19	73.79
DM Intake (kg/day)	5.41	1.14	3.83	7.76
Fecal DM output (kg/day)	1.91	0.26	1.39	2.63
Digestible DM (percent)	63.54	6.12	49.41	76.11
Fecal Nitrogen	11.21	1.47	9.03	14.15
Acid Detergent Fiber	46.87	5.16	36.07	56.22
Acid Detergent Lignin	12.32	2.24	8.65	16.91
Urobilinogen (mg/g feces)	50.92	9.85	35.30	74.41

CHAPTER V

RESULTS AND DISCUSSION

Results will be discussed according to the dependent variables in the following order: FDMO, DMI, DDM, and DDMI.

Fecal Dry Matter Output

Selected regression equations for predicting FDMO are shown in Table 4. ANWT was the first independent variable to be fitted in all the prediction equations constructed. This was done to adjust the other independent variables for variation in ANWT.

From Table 4, it is seen that ANWT was significant ($P < 0.03$) in the model, however variation in ANWT explained only 13.7 percent of the variation in FDMO. Several factors affect FDMO, including DM intake, DM digestibility, and ANWT. The influence of ANWT upon FDMO would more correctly be a response to the variation in DMI. The larger the animal, the greater the total DM intake, therefore a greater volume of FDMO would be expected from the larger animals. Due to this relationship, and the relatively small number of experimental animals used in this study, an expression of an ANWT-FDMO relationship may have been inhibited.

When UROB was added to the model containing ANWT, the coefficient of determination was increased to 0.349 ($P < 0.003$). No other single variable included in a model with ANWT explained as much of the variation in FDMO as did the variation in UROB. This is an indication of the value of this fecal constituent as a component in fecal indexes

TABLE 4

REGRESSION EQUATIONS FOR PREDICTING FDMO

b values									
INTERCEPT	ANWT	MANWT	UROB	NLUROB	ADF	ADL	N	R^2	S.D.
2.0197	0.0030							0.137	0.5398
2.8364	0.0038		-0.0274					0.349	0.4579
7.0087	0.0038			-1.4378				0.356	0.4736
1.0433	0.0034				0.0133			0.148	0.5445
4.4186	0.0032		-0.0318		-0.0198			0.368	0.4764
5.4706	0.0025		-0.0376			-0.0985		0.432	0.4514
1.6760	0.0028					0.0386	0.0343	0.147	0.5449
2.5274	0.0036		-0.0273				0.0205	0.357	0.4804
4.0588	0.0032		-0.0313		-0.0176		0.0231	0.370	0.4831
5.2088	0.0025		-0.0373			-0.0965	0.0257	0.436	0.4572
4.9801	0.0025		-0.0368		0.0040	-0.1009		0.436	0.4646
1.3062		0.0205						0.136	0.5402
1.9187		0.0261	-0.0275					0.349	0.4759
6.0880		0.0267		-1.4438				0.356	0.4736
0.2336		0.0238			0.0131			0.146	0.5450
3.6560		0.0221	-0.0320		-0.0200			0.368	0.4726
1.4104		0.0201				-0.0038		0.136	0.5483
4.8535		0.0176	-0.0376			-0.0985		0.432	0.4514
0.9848		0.0197					0.0391	0.146	0.5452
1.6297		0.0254	-0.0274				0.0348	0.357	0.4804
3.2888		0.0221	-0.0314		-0.0178		0.0208	0.371	0.4829
4.5971		0.0172	-0.0373			-0.0964	0.0235	0.436	0.4572
4.3689		0.0176	-0.0369		0.0038	-0.1006	0.0260	0.436	0.4644

designed to predict FDMO. FDMO decreased at the rate of 0.0274 percent per unit increase in UROB (Figure 3). This inverse response is the result of the dilution of UROB in the fecal excreta. UROB excretion into the small intestine is relatively constant, therefore when FDMO increases, the percentage UROB in the feces decreases. Figure 3 is in agreement with the results tabulated by Schwartz and Bracho (1972) which showed a similar negative UROB-FDMO relationship.

ANWT was replaced in the models (Table 4) by Metabolic Animal Weight (MANWT) to determine if animal weight expressed on a metabolic basis would explain more of the variation in FDMO. This approach did not increase the coefficients of determination.

When ADL was included in a model containing ANWT, the coefficient of determination was relatively low (0.147). However, the positive b value obtained for this equation indicated that FDMO increased at the rate of 0.0386 percent per unit increase in ADL. This equation is supported by the work of several researchers (Ellis et al., 1946; Forbes and Garrigus, 1948; Cook and Harris, 1951) who stated that as lignin in the diet increased, digestibility decreased. A decrease in digestibility caused by increasing amounts of ingested lignin would result in an increase in FDMO. From this, the positive b value can be correctly interpreted as an indicator of digestibility and consequently FDMO.

A stepwise regression procedure was employed as a means of determining which independent variables were the most useful in explaining the variation in FDMO (Table 5). The results of this procedure showed that the best (highest R^2) one variable model for

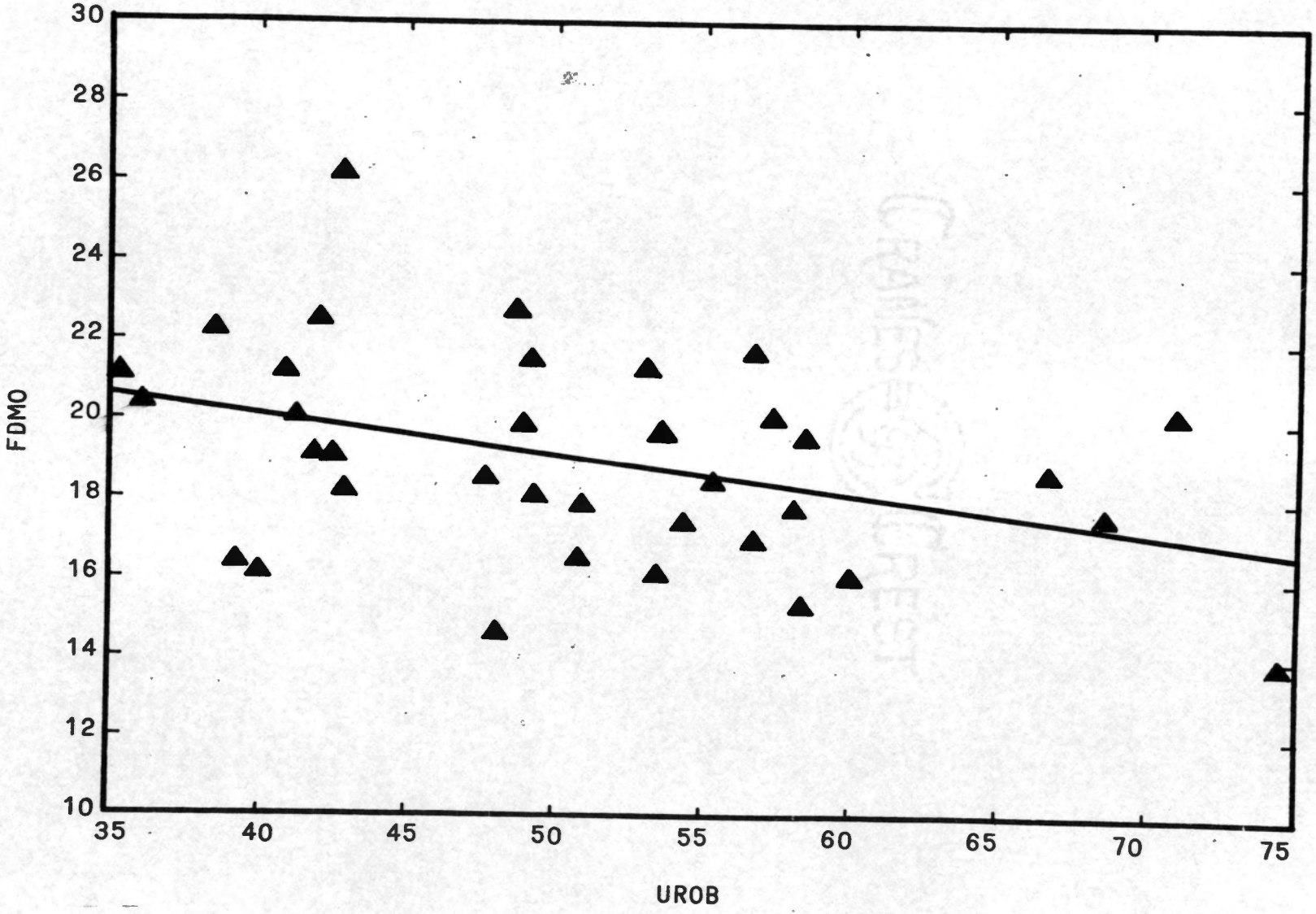


Figure 3. Relationship between FDMO (kg/day) and UROB (mg/g).

TABLE 5

STEPWISE REGRESSION EQUATIONS PREDICTING THE DEPENDENT VARIABLE FDMO

	b values							
INTERCEPT	UROB	ADL	ANWT	NLUROB	MANWT	N	ADL^2	R^2
5.3015	-0.0217							0.139
7.9296	-0.0390	-0.1417						0.358
5.4704	-0.0376	-0.0985	0.0025					0.432
11.0793		-0.0972	0.0026	-1.9484				0.437
10.4441		-0.0971		-1.9525	0.0184			0.437
10.1473		-0.0951		-1.9373	0.0181	0.0231		0.441
10.6385		-0.1728		-1.9381	0.0181	0.0201	0.0032	0.441
7.3636		-0.1789	- .0137	-1.9582	0.1135	0.0221	0.0034	0.442

explaining FDMO variation contained the independent variable UROB (R^2 = 0.139, $P < 0.003$).

ADL was the second variable to enter the stepwise procedure. This independent variable, when included in a model containing UROB, significantly ($P < 0.002$) helped in explaining the variation in FDMO by increasing the coefficient of determination to 0.358.

ANWT entered the procedure as the third independent variable ($P < 0.05$). A resulting R^2 improvement to 0.432 was achieved. This increase in R^2 showed that size of the animal (ANWT), when included in a model containing UROB and ADL, was beneficial in explaining the variation in FDMO.

Further additions of independent variables in the model did not significantly improve the predictability of FDMO.

Dry Matter Intake (DMI)

Selected regression equations predicting DMI are shown in Table 6. Neither ANWT nor MANWT ($P < 0.03$) alone, explained much of the variation in DMI (R^2 = 0.129). With a DMI range of 3.83 to 7.76 kg/day and a range in body weight from 273.5 to 402.8 kg, it would seem that more of the DMI variation would have been explained by the variation in ANWT. This lack of affect imposed by ANWT upon DMI in this study is not easily explained. Additional research will be necessary involving a larger experimental population before a valid conclusion can be made in regard to this relationship.

Addition of UROB to the model containing ANWT did not substantially increase the coefficient of determination (R^2 = 0.136, $P < 0.006$).

TABLE 6

REGRESSION EQUATIONS FOR PREDICTING DMI

INTERCEPT	b values ANWT	MANWT	UROB	NLUROB	ADF	ADL	N	R^2	S.D.
2.6607	0.0126							0.129	2.3757
3.3014	0.0133		-0.0215					0.136	2.4020
6.5923	0.0133			-1.1330				0.136	2.4016
11.4263	0.0081				-0.1197			0.175	2.3474
18.1249	0.0078		-0.0632		-0.1855			0.220	2.3178
16.1973	0.0038					-0.5721		0.327	2.1208
25.9552	0.0026		-0.1089			-0.8471		0.456	1.9358
-7.000	0.0092		-0.1089				1.0837	0.521	1.7891
-6.4495	0.0097		-0.0176				1.0809	0.525	1.8081
0.1653	0.0077		-0.0349		-0.0760		1.0213	0.538	1.8119
14.7002	0.0005		-0.0965			-0.7611	0.9927	0.781	1.2481
8.2437	0.0021		-0.0835		0.1136	-0.8844	1.0675	0.803	1.2030
-0.4225		0.0877						0.129	2.3760
0.0645		0.0922	-0.0218					0.136	2.4020
3.3991		0.0926		-1.1539				0.136	2.4016
9.4004		0.0578			-0.1199			0.175	2.3474
16.1977		0.0544	-0.0635		-0.1856			0.221	2.3170
15.3280		0.0259				-0.5727		0.326	2.1212
25.3058		0.0185	-0.1090			-0.8471		0.456	1.9358
-0.3331		0.0646					1.0847	0.522	1.7873
-8.9085		0.0683	-0.0180				1.0819	0.526	1.8059
-1.8353		0.0543	-0.0352		-0.0757		1.0221	0.539	1.8098
14.468]		0.0042	-0.0965			-0.7596	0.9924	0.781	1.2479
7.6532		0.0149	-0.0835		0.1137		1.0676	0.803	1.2025

Although there was a very slight negative relationship between DMI and UROB (b = -0.00063) as shown in Figure 4, the slope of the line was almost undetectable. Nevertheless, the tendancy toward an inverse relationship between DMI and UROB shown by the figure, would possibly be more pronounced in actual grazing trials if the following assumptions are valid: (a) as DMI increases, holding digestibility constant, UROB would decrease, (b) selective grazing increases digestibility, therefore, (c) selectivity would be reflected in a higher level of UROB due to the selection of more digestible plants and/or plant parts, which would (d) result in a decrease in FDMO and thereby (e) increase the percentage of UROB in the feces.

These assumptions are based upon results of selective grazing studies and interpretations of research dealing with UROB excretion into the feces. Before these relationships can be proven, more research is needed involving actual grazing animals. Nevertheless, there appears to be evidence in this study to support these assumptions.

In the stepwise procedure (Table 7), N was shown to be the best single variable for predicting DMI. N alone explained over 45 percent of the variation in DMI. The regression equation showed that DMI increased at the rate of 1.1535 percent per unit increase in N. This equation is supported by the work of Gallup and Briggs (1948), Blaxter and Mitchell (1948), Lancaster (1949a,b) and Homb and Breirem (1952) who concluded that N is closely associated with DMI. In addition, Milford (1957) found a highly significant regression of DMI on total N, but added that there are different relationships for different grasses. Langlands et al. (1963) added to Milford's (1957) conclusion

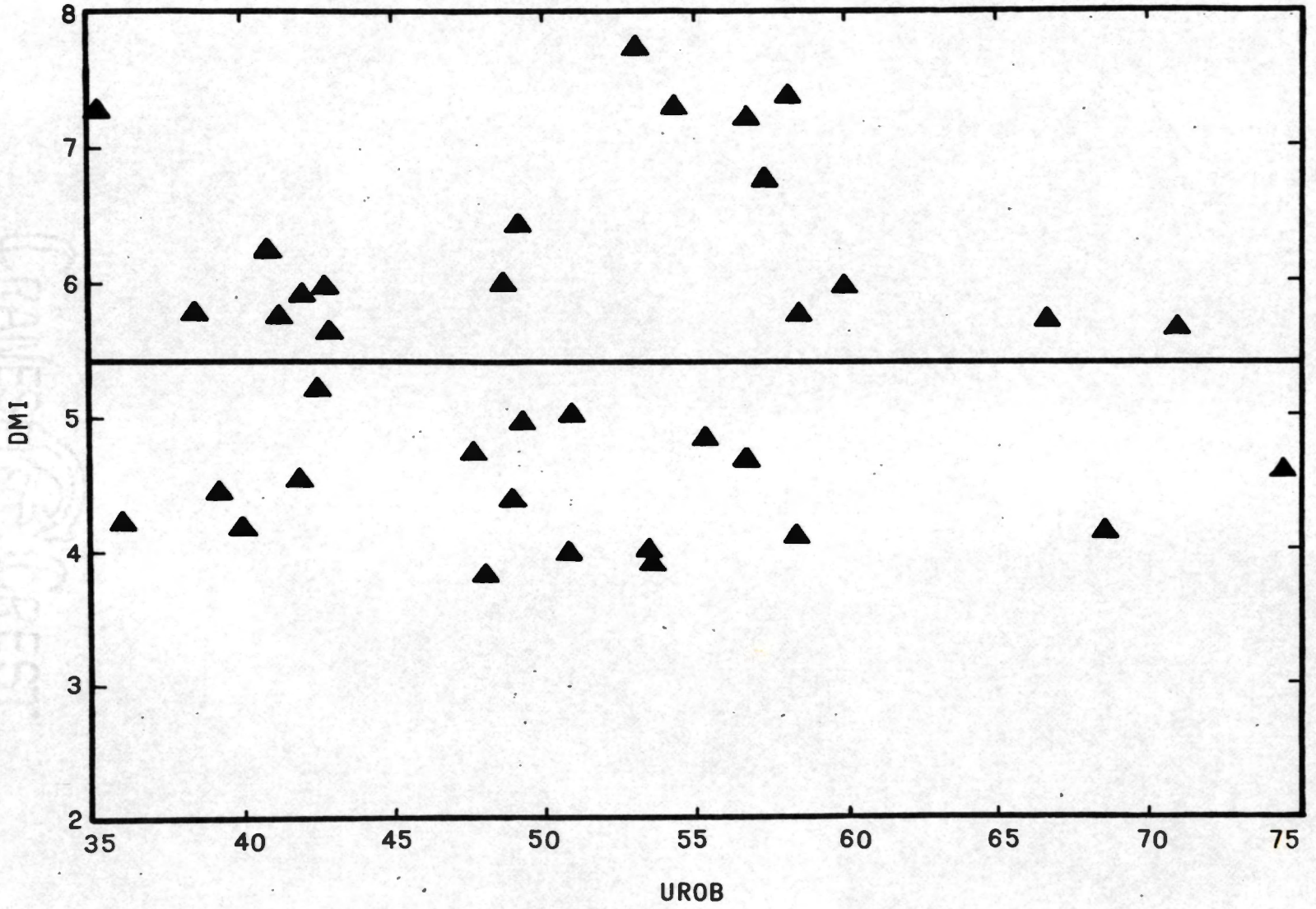


Figure 4. Relationship between DMI (kg/day) and UROB (mg/g).

TABLE 7

STEPWISE REGRESSION EQUATIONS PREDICTING THE DEPENDENT VARIABLE DMI

	values						
INTERCEPT	N	ADL	$UROB^2$	$UROB^3$	ADF	ADL^2	R^2
-1.0007	1.1535						0.453
6.8960	1.0383	-0.5371					0.679
12.6606	0.9926	-0.7668	-0.0009				0.782
8.1225	1.0724	-0.9216	-0.0008		0.1132		0.806
18.2516	1.0021	-2.6057		-0.0000	0.1138	0.0682	0.825

when he stated that it is necessary to use "local" regression equations derived from the same growth of herbage that is being studied. Langlands et al. (1963) felt that equations derived from one trial were not necessarily applicable to other trials in other locales because the N relationship would be different.

Milford (1957), examining the relationship between dry matter intake and fecal N, concluded that no general fecal N relationship could be derived for all grasses. His data suggested that forage species could fall into groups, the relationship for each group being different.

The regressions found in the present study are not implied to be transferable to other studies. Rather, any attempt at using these regression relationships to estimate intake under free grazing conditions would require previous knowledge of the species under consideration. It is clear that for a pasture consisting of two or more species in which each species has a different relationship between DMI and N, the use of these regressions would give inaccurate results. Instead, the equations found in this study show promise, that with proper application, N values of the feces can be valuable in predicting DMI.

ADL ($P < 0.0001$) explained a significant amount of the variation in DMI (R^2 increased from 0.453 to 0.679) when added to the model already containing N (Table 7). DMI decreased at the rate of 0.5371 percent per unit increase in ADL. Several researchers have concluded that lignin is not digested to a significant degree and largely recoverable in the feces (Ellis et al., 1946; Forbes et al., 1946; Swift et al., 1947; Forbes and Garrigus, 1948, and 1950a,b; Cook and

Harris, 1951). Forbes and Garrigus (1948) reported the average recovery of lignin in their digestion trials to be 102 ± 7 percent. Consequently, passage of ingested lignin into the feces justifies the use of fecal ADL as an indicator of diet ADL. From this it may be concluded that as fecal ADL increases, diet lignin has increased. Therefore, the negative response produced by the regression equation in this study is in agreement with the work of Deimum and Van Soest (1969) who reported that as lignin in the plant increased, DMI decreased.

Over 78 percent of the variation in DMI was explained by a model containing N, ADL, and UROB squared ($UROB^2$). Excluding ADF, which insignificantly increased the R^2, further improvements in the coefficient of determination were achieved only by performing mathematical operations upon those independent variables previously included in the prediction equation.

Digestibility of Dry Matter (DDM)

Selected prediction equations for DDM are presented in Table 8. ANWT alone had no real value ($P < 0.02$) in predicting digestibility of the ration ($R^2 = 0.048$). This is supported by numerous researchers who have examined digestibility in respect to animal age directly, and animal weight indirectly. Armsby and Fries (1911), Christensen and Simpson (1914), Schneider et al., (1953), Raymond et al., (1954) and Blaxter et al. (1966) used a very large age difference and a wide range in animal weights in their studies, and concluded that there was no significant difference in the ability to digest the same ration among their experimental animals.

TABLE 8

REGRESSION EQUATIONS FOR PREDICTING DDM

INTERCEPT	b values							R^2	S.D.
	ANWT	MANWT	UROB	NLUROB	ADF	ADL	N		
49.8429	0.0187							0.048	6.0623
44.2796	0.0133		0.1865					0.134	5.8687
15.7932	0.0128			9.8172				0.136	5.8590
79.2957	0.0042				-0.4023			0.134	5.8674
65.9965	0.0053		0.1254		-0.2717			0.164	5.8548
88.9231	-0.0069					-1.6516		0.324	5.1850
86.9893	-0.0066		0.0216			-1.5971		0.325	5.2621
27.9263	0.0109						2.4584	0.386	4.9407
21.8240	0.0053		0.1954				2.4892	0.480	4.6153
22.2969	0.0051		0.1941			-0.0054	2.4849	0.480	4.6891
60.6003	-0.0117		0.0507			-1.3955	2.3275	0.625	3.9851
38.8389	-0.0064		0.0948		0.3829	-1.8110	2.5796	0.667	3.8164
45.1817		0.1304						0.048	6.0609
41.0340		0.0926	0.1861					0.134	5.8688
12.7448		0.0886		9.7942				0.136	5.8592
78.0402		0.0306			-0.4012			0.134	5.8668
64.6367		0.0373	0.1252		-0.2716			0.164	5.8545
90.6211		-0.0478				-1.6521		0.324	5.1850
88.6287		-0.0463	0.0218			-1.5973		0.325	5.2620
24.9875		0.0781					2.4583	0.387	4.9376
20.3925		0.0377	0.1950				2.4888	0.481	4.6143
20.7856		0.0369	0.1940		-0.0042		2.4855	0.481	4.6881
63.2409		-0.0797	0.0511			-1.3924	2.3248	0.624	3.9875
40.2305		-0.0436	0.0951		0.3839	-1.8105	2.5788	0.667	3.8172

Variation in UROB was not significantly related ($P < 0.20$) to the variation in DDM ($R^2 = 0.134$). This would not be expected in grazing trials because an increase in DDM would decrease FDMO, thus increase UROB. However, in this study, the animals were fed a ration higher in digestibility than most pastures (Table 1, page 29). Consequently the low R^2 may have resulted from the confounding of DDM and DMI. The treatment rations ranged in mean DDM from 56.06 to 72.95 percent. This range in digestibility could have resulted in two different DMI responses and consequently FDMO and UROB would have been affected. As digestibility increased over 66.7 percent, DDM may have been negatively related, whereas those below 66.7 percent DDM may have been positively related to DMI (Conrad et al., 1964; Montgomery and Baumgardt, 1965).

The intake responses to ration digestibility may have indirectly affected the UROB excretion and therefore inhibited the explanation of DDM variation by UROB. However, a UROB-DDM relationship may exist as shown in Figure 5. This figure supports the dilution effect of UROB by FDMO. Such a relationship may be more pronounced in grazing studies where the digestibility of the diet would generally be lower than those used in these analyses.

ADL ($P < 0.008$), when added to the model containing ANWT, aided in explaining the variation in DDM (R^2 increased from 0.048 to 0.324). This is understandable if lignin in the diet is considered to be mostly excreted in the feces as discussed in the preceeding section.

The stepwise procedure (Table 9) showed N ($P < 0.0001$) to be the best single variable for predicting DDM ($R^2 = 0.370$). Raymond et al.

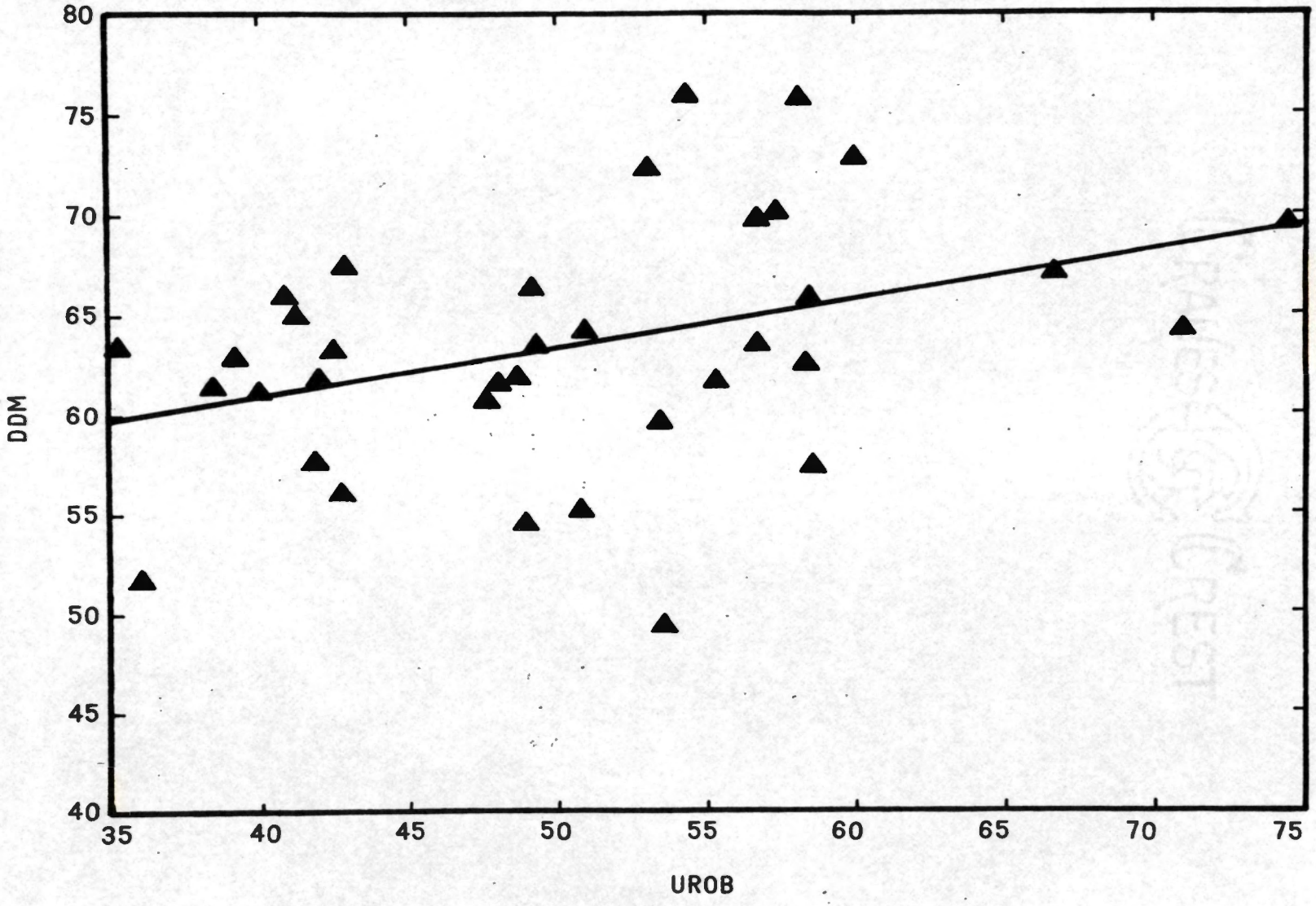


Figure 5. Relationship between DDM (percent digestible) and UROB (mg/g).

TABLE 9

STEPWISE REGRESSION EQUATIONS PREDICTING THE DEPENDENT VARIABLE DDM

	b values								
INTERCEPT	N	ADL	ADF	NLUROB	UROB	ADL^2	ANWT	$UROB^2$	R^2
35.0570	2.5402								0.370
54.7279	2.2558	-1.3380							0.605
42.4604	2.4891	-1.8905	0.3511						0.646
11.7409	2.5963	-1.7384	0.4341	6.0711					0.669
-128.3017	2.6556	-1.8822	0.5306	52.7104	-0.09003				0.690
-124.6575	2.5373	-4.7560	0.5260	57.8778	-1.0027	0.1168			0.698
-122.9370	2.5462	-4.8690	0.4943	60.3757	-1.0584	0.1176	-0.0082		0.704
-579.3951	2.5991	-5.1708	0.4687	249.8656	-8.3818	0.1289	-0.0106	0.0344	0.710

(1954) found a linear regression between digestibility and fecal nitrogen. Woolfolk (1950) stated that the relationship between percent N in the feces was quite satisfactory for predicting digestibility of the ration. Furthermore, Richards et al., (1958) stated that N of the feces was very useful in estimating digestibility of the diet. Kennedy et al. (1959) found fecal N to provide more reliable indexes than fecal pigments in predicting DM digestibility of the ration. The 2.5402 percent increase in DDM per unit increase in N in the present study indicates that there is a positive relationship between N and DDM. It is pointed out however, that interpretation of these results must be cautiously undertaken in light of the additions of soybean meal to the treatment rations. However, since each treatment duplicate was equally allocated the soybean meal, the relationships found should be valid.

The second independent variable to enter the stepwise procedure was ADL ($P < 0.0001$) which increased R^2 to 0.605. This equation showed that there was a decrease of 1.3380 percent in DDM per unit increase in ADL. Returning to the assumptions of lignin digestibility discussed earlier, the relationship between ADL and DDM is supported.

ADF ($P < 0.06$) was the next variable added in the stepwise procedure and resulted in a slight increase in R^2 to 0.646. Richards et al. (1958) found that crude fiber of both forages and feces to be highly correlated ($r = -0.839$) with digestible DM in all cases except in Sudan grass forage. However, Vera (1973) found that ADF when used in DDM prediction equations gave equations with large errors. Vera (1973) stated that the low correlation between ADF and DDM was unexpected and

that the correlation is improved if ADF is expressed on a DM basis free of ash, insoluble in acid detergent. The increase in R^2 produced by inclusion of ADF in the present prediction equations was not large enough to be of great importance in these analyses.

UROB entered the stepwise procedure as the fourth independent variable in the form of the Natural Log of UROB (NLUROB). NLUROB ($P < 0.15$) was not significant in the model and only increased the R^2 by 0.023 units ($R^2 = 0.669$). However, the b value indicated that with the other variables accounted for in the model, for each unit increase in DDM, there was a 6.0711 percent increase in NLUROB. The hypothesis that as DDM increases, the percentage of UROB in the feces increases, is supported by Figure 5. This relationship lies in the fact that as DDM increases, FDMO decreases causing the proportion of UROB in the feces to increase.

Digestible Dry Matter Intake (DDMI)

Table 10 contains selected prediction equations for DDMI. ANWT did not explain a significant amount of the variation in DDMI ($R^2 = 0.107$). ANWT was fitted first in the model so that any relationship between ANWT and DDMI could be seen. Extrapolation of these results to the grazing animal would indicate that animal weight and selectivity are not related. The hypothesis being that as an animal selectively grazes, the digestibility of the diet increases. On this premise, DDMI can be viewed as a measure of selective grazing. ANWT ($P < 0.01$), although a significant source of the variation in DDMI, did not explain enough of the DDMI variation to conclude that it has a definite effect

TABLE 10

REGRESSION EQUATIONS FOR PREDICTING DDMI

INTERCEPT	b values							R^2	S.D.
	ANWT	MANWT	UROB	NLUROB	ADF	ADL	N		
0.2065	0.0102							0.107	2.1373
-0.1146	0.0099		0.0108					0.109	2.1668
-1.8576	0.0098			0.5949				0.109	2.1664
10.2647	0.0053				-0.1374			0.183	2.0749
13.0490	0.0050		-0.0262	-0.0262	-0.1647			0.193	2.0943
14.0710	0.0011					-0.5859		0.369	1.8233
20.0959	0.0004		-0.0673			-0.7558		0.432	1.7574
-8.7417	0.0071		0.0144				1.0037	0.533	1.5695
-9.1900	0.0066		0.0002		-0.0621		1.0060	0.536	1.5871
-3.7857	0.0050		-0.0557			-0.6754	0.9573	0.547	1.5936
9.5773	-0.0016		-0.0433		0.1078	-0.7923	0.9277	0.791	1.0817
3.4518	-0.0001						0.9987	0.817	1.0310
-2.2958		0.0709						0.107	2.1371
-4.2154		0.0688	0.0105					0.109	2.1668
8.9529		0.0367			-0.1373			0.183	2.0746
11.7858		0.0353	-0.0265		-0.1647			0.193	2.0938
13.8326		0.0076				-0.5864		0.369	1.8234
19.9904		0.0030	-0.0673			-0.7558		0.432	1.7574
-10.5462		0.0495					1.0044	0.534	1.5677
-10.8776		0.0466	0.0140				1.0066	0.537	1.5857
-5.1132		0.0351	0.0000		-0.0617		0.9578	0.548	1.5922
9.8654		-0.0556	-0.0556			-0.6741	0.9272	0.791	1.0822
3.3899		-0.0001	-0.0432		0.1080	-0.7917	0.9986	0.817	1.0310

on DDMI and selective grazing. These results may be an artifact of the lack of ANWT affect on DMI (Table 6, page 45). ANWT did not explain the variation in DMI and in view of the formula used to calculate DDMI (DDMI = DMI X DDM) it is understandable that it likewise would not explain the variation in DDMI. Because of the confounding of DMI and DDMI in this study, more research would be appropriate concerning the effect of ANWT upon selectivity. Perhaps if the variation in DMI had been explained by ANWT variation, DDMI would have similarly been explained.

Neither UROB nor ADF, when included in a model with ANWT, accounted for much of the variation in DDMI (R^2 = 0.109 and 0.183, respectively). The b value indicated that for each unit decrease in ADF, there was a 0.1374 percent increase in DDMI. It is generally believed that as crude fiber in the diet increases, digestibility decreases (Schneider and Flatt, 1975). Schneider et al., (1953) examined various relationships of the forages and feces fiber in regard to digestibility and found that as the level of crude fiber increased, the digestibility of dry matter decreased. The crude fiber in the diet tends to exert a protective influence against the digestibility of all nutrients in the ration. Therefore, the level of crude fiber would be inversely related to the digestibility of the ration.

The stepwise procedure (Table 11) gave a better picture of which variables were the best for explaining the variation in DDMI. N ($P < 0.0001$) was the best single independent variable for predicting DDMI, explaining over 48 percent of the DDMI variation. These results

TABLE 11

STEPWISE REGRESSION EQUATIONS PREDICTING THE DEPENDENT VARIABLE DDMI

	b values						
INTERCEPT	N	ADL	$UROB^3$	ADF	ADL^2	$UROB^2$	R^2
- 4.1608	1.0562						0.483
3.4494	0.9463	-0.5176					0.748
6.336	0.9176	-0.6498	-0.0000				0.794
1.9140	0.9981	-0.8144	-0.0000	0.1156			0.827
12.1206	0.9308	-2.3309	-0.0000	0.1072	0.0618		0.844
8.8976	0.9423	-2.5373	-0.0000	0.1441	0.0692	0.0028	0.859

are similar to those found for DDM and are undoubtedly related since DDM would be carried over in the calculation of DDMI as indicated by the folmula given previously. It is shown that for each unit increase in N there is a 1.0562 percent unit increase in DDMI. Milford (1957) and Hutchinson (1958) also found a positive relationship between fecal N and digestible DM intake.

ADL was the second independent variable to be included in the stepwise procedure. When corrected for variations in N, ADL was a significant variable ($P < 0.0001$) in explaining the variation in DDMI (R^2 increased from 0.483 to 0.748). This large improvement in R^2 is a strong indication that ADL is a good predictor of DDMI. This further supports the conclusion of the earlier discussion involving DDM in regard to lignin in the feces. The 0.5859 percent unit increase in DDMI for each unit decrease in ADL (Table 10) supports the idea that as lignin in the ration (recoverable in the feces) increases, DDMI decreases.

UROB entered the stepwise procedure in the cubed form ($UROB^3$) increasing the R^2 to 0.794 ($P < 0.01$). Although not increasing the coefficient of determination by a large amount, its inclusion indicates that it is useful in predicting DDMI. The DDMI-FDMO-UROB relationship is similar to the DDM-UROB relationship discussed previously. As the digestibility of the DM intake increases, the amount of FDMO would decrease, resulting in higher UROB values.

CHAPTER VI

SUMMARY

From the data utilized in this study, it is concluded that no single fecal index may be adaptable to predicting all the independent variables of interest in evaluating grazed forages. Rather, several indexes may be necessary from which different dependent variables may be predicted.

Concerning the dependent variable FDMO, it appears that the best prediction equation includes the independent variables UROB, ADL, and ANWT. Using these independent variables, over 43 percent of the variation in FDMO can be explained.

It was found that 78 percent of the variation in DMI could be explained by a model containing the independent variables N, ADL, and $UROB^3$. This large amount of explained variation indicates the possible value of this index in predicting DMI of freely grazing animals.

This research showed that a model containing N, ADL, ADF, and NLUROB as the independent variables explained 67 percent of the variation in DDM.

DDMI was the most predictable of all the dependent variables studied in this research. Almost 83 percent of the variation in DDMI was explained by a model containing the independent variables N, ADL, ADF, and $UROB^3$.

The large amount of explained variation in the dependent variables considered in this study supports the belief that fecal

indexes can be a valuable tool for evaluating forages grazed by normal, unrestrained animals.

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