EFFECTS OF CONCENTRATE LEVEL, PARTICLE SIZE AND GRAIN PROCESSING ON THE DIGESTION OF FEEDSTUFFS IN MOBILE DACRON BAGS

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PREFACE

This thesis represents a portion of my work at Oklahoma State University. The work reported here is not an end in itself, but rather it created more questions than it answered. The feeding and care of animals was superbly supervised by two of the finest animal care technicians I have encountered, Ken Poling and Steve Welty.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

CHAPTER V

Table Page

CHAPTER VI

Table Page

CHAPTER VII

CHAPTER VIII

APPENDIX

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Although total dietary supply of energy and protein commonly are used as indicators of nutrient status of ruminants, site as well as extent of digestion can be important.

The post-ruminal supply of protein, amino acids or starch can limit production by ruminants. Based on this concept, as illustrated by the booklet "Nitrogen Usage in Ruminants" (NRC, 1985), the feed industry is currently searching for new values for more precise formulation of diets. Ruminal escape of protein plus its digestion in the intestines are of particular concern. Unfortunately, in vivo protein escape measurements are expensive and time consuming to determine and values are imprecise (Owens, 1986a). The mobile dacron bag (MDB) method may be a useful tool to examine site and extent of digestion of a wide array of feed components (Owens, 1986a).

For many years, researchers have measured the disappearance of nutrients from polyester bags suspended in the rumen, a technique variously and interchangeably called on in situ, or in sacco procedure. Using bags in the intestines, however, is a more recent development which was

devised to study protein nutrition (amino acid availability) with pigs (Sauer et al., 1983). Though some early workers constructed mobile nylon bags (MNB) to pass through the intestines, the high N content of nylon makes dacron preferable. Other Canadian researchers have employed the MDB technique in an attempt to determine the site of digestion in ruminants (Kirkpatrick and Kennelly, 1984). Small differences in the extent of small intestinal digestion or amino acid composition may explain why animal responses differ to various dietary sources which have similar bypass values. The MDB procedure permits researchers to measure on a more selective, individual basis the intestinal availability of undegraded dietary components of a wide number of feeds simultaneously. Besides its research potential, this tool should prove useful in the feed industry as a rapid and reliable quality control method over ingredients. For example, the MDB technique could be used to screen batches of feed rapidly to discrimate against batches with low protein digestibility.

Further technique development and testing certainly are needed. Our studies examined the MDB procedure to examine factors influencing results of MDB studies. In addition, we employed the MDB technique to study factors limiting extent of ruminal and postruminal digestion of specific feed components with special attention to effects of diet composition, particle size and grain processing.

Cereal grain processing can dramatically increase starch digestion in the rumen. Ruminal escape of both protein and starch from cereal grains can be altered by processing (Aguirre et al., 1984; Hibberd et al., 1985). The magnitude and even the direction of these changes as well as effects on small intestinal digestion are poorly defined. We used the MDB technique to determine the site and extent of digestion of protein and starch from several cereal grains processed by various commercial procedures.

Differences in particle size can explain some of the differences among grain processing methods. With soybean meal, Weakley (1983) noted that rate of protein ruminal digestion was lower for coarse than for fine soybean meal. How much of the differences in ruminal escape among various roughages, cereals and protein supplements can be attributed to particle size is uncertain. For in situ measurements, feeds are typically ground, but the influence of particle size on in situ disappearance of various constituents and on ruminal residence time remains to be determined.

The objective of this dissertation was to evaluate the MDB procedure and to employ this technique to test the impact of various dietary and feed factors on site and extent of digestion in ruminants.

CHAPTER II

REVIEW OF LITERATURE

The Mobile Dacron Bag (MDB) technique is relatively new procedure in ruminant nutrition research. It is a logical next step in the progression from in vivo to in vitro and in situ or in sacco procedures in an attempt to more precisely measure digestion in animals. This review will discuss factors altering results and verification of the MDB technique as well as the nutritive value of several processed grains and the effect of particle size in the disappearance of nutrients which can be studied using the MDB procedure.

Measurements of Digestibility in Animals

Animal production is the multiple of quality (balance and digestibility) and quantity (intake) of feedstuffs as they relate to the animal's requirements for maintenance and production. Techniques to measure in vivo and in vitro digestibilities have been described in a number of review publications (Van Soest, 1983; Church, 1987). Although in vivo estimates are the ideal benchmarks, limitations in time, space and feed and in variability of results have encouraged researchers to seek alternative methods to

appraise feeds, to study physiological limits to digestion and to test new drugs and concepts which can alter digestion.

The research in this dissertation deals with disappearance of nutrients from several feedstuffs placed in dacron bags and exposed to fermentation or digestion in the gastrointestinal tract of cattle. One of the objectives of this research was to determine the validity of digestibility estimates from MDB of various feedstuffs and different components of these feeds. Although in sacco and in vitro procedures are widely employed to estimate digestibility, technique validation and factors affecting MDB results have received limited attention.

Polyester Bags for "In Situ" Digestion

Ørskov and Mehrez (1975) and Mehrez and Ørskov (1977) used polyester fibers (dacron or nylon) bags in situ to measure ruminal disappearance of protein at different time intervals. Later, Ørskov et al. (1980) presented a full description of this "in sacco" procedure and listed its potential applications. Protein digestibility from in situ bags proved to be dependent on dietary conditions of the animal (Mehrez and Ørskov, 1977; Owens and Zinn, 1982). Disappearance of cottonseed meal was much greater in the rumen of cattle fed roughage than of those fed concentrate diets. Ørskov and Mehrez (1975) had found earlier that disappearance of dry matter from dried grass was greater

from bags incubated in rumens of lambs fed on whole barley than in lambs fed pelleted barley diets. Nevertheless, if the proper incubation time was chosen, dacron bags could quite closely approximate the amount of protein that escaped ruminal fermentation for a number of different protein sources (meat meal, casein, dehydrated alfalfa meal, cottonseed meal and soybean meal; Zinn and Owens, 1983).

The Mobile Dacron Bag Technique (MDB)

Because digestion trials are very time-consuming and expensive to conduct and results often are imprecise, more rapid, reliable and simple assays for ruminal and total tract digestibility have been sought. One assay method which may help is the MDB technique. With MDB, one can estimate in vivo disappearance of 4 feeds using 2 steers every 24 h. Full digestion trials for 4 feeds in 2 steers would require at least 8 weeks. The MDB procedure was developed for use in pigs. Much of the development initially, work involved comparison with in vivo measurements.

Swine Research. In 1978, Petry and Handlos used mobile nylon bags (MNB) in pigs to determine the digestible energy content of various feedstuffs. They inserted bags containing the feeds into the mouth and recovered them from feces. Results were not as good as expected, presumably due to a prolonged retention of bags in the stomach. Sauer et al. (1983) revived but modified this MNB procedure for use in protein nutrition studies to determine amino acid availabil-

ity from proteins in specific feedstuffs. They fitted their pigs with duodenal cannula, and inserted the bags into this cannula rather than orally. As this point is anterior to gastric digestion, they predigested bags and contents in an acid pepsin solution as a replacement.

Based on the experience and success of Sauer et al. (1983), other Canadian workers employed MNB with pigs (Cherian et al., 1985; de Lange et al., 1986). In Europe, MNB were used with swine by Graham et al. (1985) and in Australia by Taverner and Campbell (1985) to evaluate digestibilities of various protein sources and cereal grains.

In the first experiments in Alberta with the MNB of Sauer et al. (1983), estimates of protein digestibility were more variable than similar estimates from conventional digestibility studies. Later, researchers in Alberta studied the reasons for their large experimental variation. Cherian (1985) studied both predigestion factors (influence of length of time for predigestion with pepsin, pH of the pre-digestion solution, activity of pepsin), feed factors (degree of fineness, sample size) and mesh size of the bag. Results were compared to apparent protein digestibilities obtained in digestion trials. They concluded that a predigestion period of 2.5 to 4.0 h at pH of 2 with a pepsin activity of 377 IU/l, using .5 g samples in nylon bags resulted in values close to those obtained by in digestion trials with pigs (Cherian, 1985).

Sauer (1986) with MNB found that protein digestibility from canola meal in pigs ranged from 77 to 80%. This compares favorably with 79% from conventional digestion studies. Measuring the protein digestibility for 14 different of feedstuffs, Sauer et al. (1987) found that the apparent protein digestibilities from cereal grains were underestimated with the MNB although both methods ranked digestibility coefficients for feeds in the same order.

After successfully estimating protein disappearance with MNB Sauer et al. (1984) began to employ the technique to measure dry matter and energy digestibilities from feedstuffs by pigs. Close agreement with digestibilities determined with a marker (Dysprosium) demonstrated that MNB can be used to determine the digestibility of the energy from feedstuffs. In contrast, with these results, de Lange et al. (1986) concluded that MNB underestimated energy digestibilities of various cereal grains. Nevertheless, a high correlation between digestibilities values from MNB and conventional methods allowed one to quite accurately predict energy digestibility by regression.

Australian researchers used the MNB to estimate dry matter digestibilities by pigs for 39 different feedstuffs and compared results with digestibilities measured by total collection of feces. Again, digestibilities were underestimated ($P<0.001$) by MNB, but results, of these two procedures were closely and linearly related ($r^2= 0.89$; Taverner and Campbell, 1985).

Ruminant Research. Canadian researchers have used MNB recently to predict site of digestion in ruminants (Kirkpatrick and Kennelly, 1984; de Boer et al., 1986, 1987; Robinson et al., 1987). In Europe and Australia, degradation of protein in the rumen and in the total tract has been studied by Hvelplund (1985), Rooke (1985), Voigt et al. (1985) and Deacon et al. (1986).

A modification of the MNB procedure described by Sauer et al. (1983) was employed by Kirkpatrick and Kennelly (1984) to predict the digestibility of six diets for dairy cattle. Dry matter digestibilities were consistently underestimated by the MNB. This may have been due to a very high ratio of sample size to the bag surface area (75-100 mg/cm^2). This topic had received consideration in ruminal in situ studies by Van Hellen and Ellis (1977), Weakley et al. (1983), and Oldham (1986) though in only one report was the effect of surface area on disappearance from MDB examined (Kirkpatrick and Kennelly, 1984). They justified their underestimation of the MNB due to the ratio sample volume to the MNB surface area. One bottle neck of the MNB is the sample size because the sample has to be enough for laboratory analysis after the total tract digestion but small to reduce damage of the intestinal mucose or to block the intestinal tract of the animals under experimentation.

Hvelplund (1985) in Denmark compared post-ruminal digestion with MNB of eight protein sources with small intestinal digestion values determined from duodenal infusion

of similar protein sources. The correlation was good but not perfect (R^2 = 0.66, P = 0.03). Postruminal digestibilities of an additional thirteen protein sources were estimated with MNB. Protein disappearance values ranged from .11 to .97 though few values below .75 were observed.

In 1986, Oklahoma State University initiated a project to evaluate feedstuffs using MDB. Nalsen et al. (1987a, 1987b) reported results of the first trial examining total tract disappearance of ground samples of wheat grain, a wheat diet (60% with 40% sorghum silage), corn grain and a corn diet (60% with 40% sorghum silage). Total tract crude protein disappearance values were 97, 98, 92 and 87%, respectively. In a second trial, total tract disappearance of starch from wheat and corn particles of various sizes (<250, 250-500, 500-1000, 1000-2000 and >2000 microns of diameter) was measured. Respective values for starch disappearance from wheat vs corn for the various particle sizes were 96 vs 100, 96 vs 94, 97 vs 58, 83 vs 47, and 88 vs 33%. Starch in wheat was more extensively digested than starch from corn and was less depressed by a larger particle size.

Protein sources can be protected from microbial degradation in the rumen by heat and chemical treatments. Deacon et al. (1986) and Kennelly and de Boer (1986) examined the effects of heat (jet-sploding and extrusion) and formaldehyde treatment on ruminal and intestinal digestibility of canola and soybean meal using MNB. They concluded that the

in sacco procedure was an effective tool to separate ruminally degraded from undegraded protein and to measure intestinal disappearance of escape protein. Their results agreed with those of Voigt et al. (1985). The latter workers used MNB to measure crude protein digestibility of feedstuffs in the gastrointestinal tract of ruminants. Their random error for the method was 1.3% (absolute value) using just 2 animals with 2 bags passed through each. Intestinal (small plus large) disappearance of protein exceeded 90% for concentrates but fell to between 75 and 90% for forages.

For quality control of processed products, another concern is heat damage of the feedstuffs. Heat damage was examined by Robinson et al. (1987) using MNB. With dehydrated alfalfa, the MNB successfully evaluated the nutritive value and degree of damage.

Retrieving Mobile Nylon Bags from the Distal Ileum. Digestion in the total postruminal tract (small plus large intestine) has been the topic of all previous trials with MNB. Fermentation in the large intestine means that total postruminal disappearance will overestimate digestion in the small intestine. Uptake of glucose and amino acid from the small is much more valuable to ruminants than fermentation of these nutrients in the large intestine. So if bags could be retrieved from the terminal ileum, rather than from feces, small intestinal disappearance could be measured directly and small intestinal digestibility could be estimated more precisely. Hvelplund (1985) using cows equipped with

duodenal and ileal T cannulas retrieved 12 of 100 bags from an ileal cannula using a pair of tweezers. Comparison of disappearance of feed from these bags with those recovered from feces revealed, that fecal digestibilities considerably overestimated disappearance from the small intestine. Overestimates in N disappearance were 27% and 50% for soybean meal and rapeseed meal, respectively. Such large loss in the large intestine makes total tract or postruminal protein disappearance questionable as reliable indices of amino acid or glucose uptake by the small intestine.

The Alberta group working with pigs also has attempted to retrieve MNB from the distal ileum in order to determine ileal amino acid availabilities (Sauer and Ozimek, 1984). Their results were not satisfactory, presumably due to a low bag recovery rate; all of their reports present total tract, not small intestinal disappearance values (Sauer et al., 1983, 1984, 1987; Sauer, 1986; Sauer and Ozimek, 1984). As we had similarly low success is retrieving bags through a T-cannula, we attempted to surgically modify animals in order to develop a physiological model animal which permitted digesta to bypass the large intestine.

Ileo-Rectal Anastomosis. Although retrieval of bags from the distal ileum might be feasible in cattle equipped with a reentrant ileal cannula, success with a regular T cannula has been limited. Thus, we attempted novel approach we equipped two steers with an ileo-rectal anastomosis to by-pass the large intestine. Workers at the Animal and

Dairy Science Research Institute of South Africa (Pienaar, 1986) have similarly equipped pigs and sheep with ileo-rectal anastomoses in a program to study amino acid digestibility. In the USA, researchers at Purina Mills, St. Louis, MO also surgically equipped pigs with ileo-rectal anastomoses to study amino acid availability. Surgeries for Purina Mills were performed by Carey (1987). But neither of these groups have combined their procedure with the MDB technique.

Though ileostomies and colostomies are routine procedures in treating specific disorders in humans, the only report of an ileo-rectal anastomosis in animals I could locate was presented by Hennig et al. (1986) with pigs. They tested two types of surgical reconstruction: a side to side versus an end to side ileo-rectal anastomosis. In these operations, the ileum is severed or transected and fastened to the side of the rectum near the anus. When the side of the ileum was fastened to the side of the rectum, the ileum end dilated and perforated, and the pigs died. Pigs with end (ileum) to side (rectum) anastomoses survived. Results of biochemical and morphological adjustments were satisfactory and animals with ileo-rectal anastomosis were used to estimate pre-cecal nutrient digestibility (Hennig et al., 1986).

Surgical anastomoses have not been reported to date with beef or dairy cattle. Based on information provided by Pienaar (1986) and Carey (1987); S. Barron (1987) at Oklahoma State University performed an ileo-rectal anastomosis

on several dairy calves for the Animal Science Department of Oklahoma State University. Two such calves were used in several of the studies reported in this thesis.

Nutritive Value of Processed Grains

The rate, site, and extent of digestion of feedstuffs can be altered by ruminal conditions and feed processing (Owens and Goetsch, 1984). Heat generated during feed processing will gelatinize starch granules which in turn increases the rate and extent of digestion of starch in the total gastrointestinal tract, (Galyean et al., 1981 and Aguirre et al., 1984).

Owens et al. (1986b) and Theurer (1986) at a symposium on starch utilization by ruminants concluded that the principal function of grain processing is to increase the digestibility of starch from cereal grains. Rate and extent of digestion of starch varies with species of cereal grain. Processing generally is not needed with the small cereal grains (barley, oats, wheat) because starch digestion from these grains by ruminants is rapid and almost complete. In contrast, starch from milo grain and corn grain is tightly held in a protein matrix which resists microbial and enzymatic attack (Rooney, 1986; Owens and Hicks, 1987).

Commercial processing of grain substantially increases the extent of starch digestion in the rumen. Certain processing methods not only increase total tract digestibility, but also can alter the site of digestion and absorption of

protein and starch (Hibberd, 1982). Although ruminal escape of cereal protein can be affected by processing (Aguirre et al., 1984; Hibberd et al., 1985), the magnitude and even direction of effects of various processing methods on ruminal and small intestinal digestion of protein remain unclear.

To meet the protein requirements for cattle, both the ruminal N requirements for the microflora and the postruminal amino acid needs of the animal must be provided. Models to calculate supply of postruminal amino acids rely on estimates of ruminal escape and of intestinal digestion of escape protein from feedstuffs which generally include cereal grains a roughage source and a protein supplements. Because 50 to 90% of the dietary protein in beef cattle rations is provided by the cereal grain, escape values for cereal grains need special attention (Owens, 1986a). Escape values for protein from common grains are rare and the effects of grain processing on escape are poorly defined. The NRC (1985) provides only three estimates of undegradable protein: barley, 0.21; corn, 0.65; and sorghum grain, 0.52. The low protein content of cereal grains makes escape estimation by difference very difficult to measure accurately. In situ procedures have promise for precisely estimating escape values but provide no information on intestinal availability. More information on escape and postruminal availability as might be obtained with MDB will permit nutritionists to formulate more economical rations for high producing ruminants.

Effects of Particle Size in the Disappearance of Nutrients

Though enzyme limitations and residence time can limit extent of starch digestion in the small intestine of ruminants, particle size also appears important (Owens et al., 1986b). Galyean et al. (1981) separated corn with sieves into fraction with mean particle sizes of 6000, 3000, 1500 and 750 microns. Ruminal dry matter disappearance was very low from particles over 3000 microns in diameter, but rate of disappearance increased as particle was decreased to 1500 and to 750 microns. Ruminal starch disappearance paralleled dry matter disappearance. Kim and Owens (1985) also studied the site of digestion of corn starch particles in cannulated steers. They sieved a duodenal, ileal and fecal matter and calculated passage from chromium flow. The smaller the particle size, the greater the postruminal and total tract disappearance. Most starch particles over 2000 microns which passed through the duodenum were found in feces undigested. Particles from 1000 to 2000 microns appeared most desirable to provide intestinal starch as they had a postruminal digestibility of 94%. Because these results were calculated from disappearance of starch from particles at various sites, they are subject to a large sampling error. With MDB, effects of particle size of starch disappearance can be examined more directly.

Generally as particle size increases, extent of dry matter digestion in the rumen decreases. McLeod and Minson

(1969) demonstrated this relationship with grass hay. Lindberg (1981) found that a very coarse milling of silage, concentrate or oats reduced in sacco (ruminal) dry matter disappearance from these feeds.

The effect of particle size on the total tract disappearance of starch from MDB with wheat and corn particles was tested by Nalsen et al. (1987b). Decreasing the particle sizes from 2000 to < 250 microns increased the disappearance of starch from corn in MDB. Total tract disappearance of crude protein was much higher for wheat than corn as had been determined previously (Voigt et al., 1985; Nalsen et al., 1987a).

Effects of particle size on protein digestion in the rumen or postruminally are less certain. With soybean meal, Weakley et al. (1977) noted that rate of ruminal digestion of protein was much lower for coarse than for fine particles. However, subsequent in vivo studies, by Netemeyer et al. (1980) found that particle size had no effect on either ruminal escape or total tract protein digestibility. Presumably, this was because either ruminal retention time was greater for larger particles or that chewing removed the difference in particle size. Extent of ruminal escape differs considerably among and between roughages, cereals, and protein supplements (Lindberg, 1985). Because some of these differences may be an artifact of particle size, the proper particle size for dacron bag studies deserves attention.

Otherwise, in situ values will not accurately predict in vivo responses.

To improve the reproducibility of degradation measurements obtained with the in sacco procedures, sample preparation, incubation techniques, particle size of the samples should be standardized (Lindberg, 1985). To check the importance of various factors on the disappearance of protein from MNB in swine, Cherian (1985) conducted several experiments as discussed earlier. Decreasing the particle size of soybean meal from 2.0 to 0.5 mm increased its protein digestibility from 88 to 91%, presumably due to the greater surface area to mass, ratio which increased accessibility of protein to proteolitic enzymes (Cherian, 1985). Extent of fermentation in the large intestine also should be increased by fine grinding.

Validation of the Mobile Dacron Bag Procedure

New procedures need to be standardized before they can produce reliable results from widespread ruminant nutrition laboratories (Owens, 1987). Three research groups have employed MNB to date. At the University of Alberta, several experiments were conducted with pigs with duodenal cannulas, to examine the effects of length of time of pre-digestion with pepsin, pH of the pre-digestion solution, activity of the pepsin, degree of fineness of grinding, sample size and

mesh of the bag on the digestibility of protein (Cherian, 1985).

In Denmark, Hvelplund (1985) compared post-ruminal digestion in cows of proteins from MNB with small intestinal disappearance in sheep of several infused protein sources. Disappearance of nitrogen from MNB during passage through the small plus large intestines of cows was regressed against small intestinal digestibility of sheep. Finally, Rooke (1985) compared protein disappearance values obtained with MNB to true N digestibilities measured with rats. These two procedures ranked his three protein sources in the same order according to the following equation:

True N digestibility = - 0.89 + 2.01 MNB disappearance of N; (R^2 = 0.92).

Disappearance of other nutritive components from MNB has been compared with more conventional digestibility procedures by several workers (Sauer et al., 1983, 1984; Cherian, 1985; Taverner and Campbell, 1985; de Lange et al., 1986; Sauer, 1986). Values obtained with the conventional system generally have been higher than from the MNB. Only one of these comparisons has used ruminants. Kirkpatrick and Kennelly (1984) applied the MNB method of Sauer et al. (1983) to dairy cows. They found a tendency for MNB to underestimate apparent digestibility. Their results contrast with the report of Hvelplund (1985) who reported that protein disappearance from MNB overestimated disappearance of protein from the small intestine of sheep. Differences both

in the technique, in washing the bags after collecting in the feces, and in the baseline (small intestinal disappearance versus MNB passage through the small plus large intestine) are probably responsible for these differences in results. In 1987, de Boer et al., pointed out that Hvelplund (1985) was comparing MNB disappearance with true digestibility whereas other researchers have typically compared MNB values to apparent digestibility. If true, however, values are even more erroneous because true digestibility of protein always exceeds apparent digestibility and Hvelplund's (1985) MNB values were overestimates, not underestimates. Thus, site of comparison (small intestine vs total tract) is a more logical explanation for this discrepancy.

CHAPTER III

EFFECT OF CONCENTRATE LEVEL UPON DISAPPEARANCE OF DRY MATTER AND PROTEIN FROM MOBILE DACRON BAGS

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ABSTRACT

Four beef heifers (500 kg) fitted with ruminal and duodenal cannulas were fed diets (2% of body weight) with either a moderate or high level of concentrate (50 or 80%) in a crossover design. Disappearance of the dry matter and crude protein of four feedstuffs: dehydrated alfalfa hay, cottonseed meal, and the total diets 80:20 and 50:50 were determined using the mobile dacron bag (MDB) technique. All bags were incubated in the rumen for 15 h. The bags for total tract or disappearance were incubated in a pepsin-HCl solution before being placed in the duodenal cannula and recovered 10 to 30 h later in feces. Ruminal, postruminal, and total tract disappearance of DM and protein from MDB were calculated. Total tract disappearance of the dry matter from dacron bag averaged 54.8, 72.8, 72.3 and 66.4% for the four feedstuffs, respectively. Ruminal

and total tract disappearance of dry matter were greater ($P<.05$) with the 50% than the 80% concentrate diet whereas total tract disappearance of N from MDB was greater with the 80% than the 50% concentrate diet. Total tract disappearance of crude protein was: 78.4, 83.0, 67.6 and 57.2% for the four feedstuffs, respectively.

Total tract dry matter disappearance within feedstuff increased with postruminal residence time (h) following a cubic relationship (DMD,% = 36.1 + 0.2 T - 0.0008 T^2 - 0.00001 T^3;$r = 0.83$; $P<0.01$) while total tract crude protein disappearance was linearly related to residence time: (DCP % = 56.6 + 0.3 T ;$r = 0.89$; $P<0.01$). Results can be interpreted to indicate that in situ digestibility of a feed both in the rumen and in the postruminal tract will vary with diet. The mobile bag system appears to be an easy, rapid and reliable method to rank feedstuffs regarding site and extent of true digestion in ruminants.
(Key Words: Mobile Dacron Bag, Protein Digestion, Intestine Transit Time, Roughage, Rumen Fermentation, Protein Sources).

Introduction

The Mobile Nylon Bag (MNB) technique has been used successfully in protein nutrition studies with swine (Sauer et al., 1983) to determine amino acid availability from protein in specific feedstuffs. Canadian researchers also have tested this technique to predict site of digestion in

ruminants (Kirkpatrick and Kennelly, 1984; Cherian, 1985; de Boer et al., 1986, 1987; Kirkpatrick and Kennelly, 1987). In Denmark, Hvelplund (1985) compared post-ruminal digestion values from MNB with those estimated from duodenal infusion of specific protein sources. Postruminal digestibilities by the MNB ranged from .11 to .97 though few values fell below .75. Differences in small intestinal digestion may explain why animal responses differ to dietary protein sources which have similar ruminal escape values (Owens, 1986b). The MNB holds promise to appraise the intestinal availability of undegraded dietary protein though further development and testing are needed.

The influence of diet on ruminal degradation was studied initially by Ørskov and Mehrez (1975). They examined the rate of disappearance of dried grass incubated in the rumen of sheep receiving either whole or pelleted barley. Extent of dry matter degradation at various times of incubation was much greater from bags incubated in rumens of the sheep fed on whole barley. Later, Weakley (1983) demonstrated that as dietary roughage level increased, extent rate of protein degradation of vegetable protein sources increased in situ and in vivo. Barrio et al. (1986) analyzed the effects of dietary concentrate on in situ dry matter and nitrogen disappearance of different feedstuffs. Protein from soybean, cottonseed, and linseed meals was more extensively digested after 24 h of ruminal incubation in steers fed a high roughage diet (60%) than in

steers fed a low roughage diet (20%). For other feedstuffs, differences were small. This differences could be ascribed to either changes in the ruminal milieu (pH, osmolarity, microbial numbers or activity). The degree to which the microbial population in the rumen and its digestive capacities can be altered by dietary changes was examined by Jeraci et al. (1980) and by Strobel and Russell (1987). Ruminal digestion rates in cattle were generally reduced by feeding concentrates. Rates of cell wall digestion was depressed most. As much of the insoluble protein is associated with cell walls, reduced cell wall digestion should reduce protein digestion.

Loerch et al. (1983) measured in situ (in sacco) of a number of protein sources. They detected an interaction between composition of the dietary protein supplement and the incubated feedstuff. When soybean meal was included in the diet, the N disappearance at 12 h of in situ incubation appeared to be greater. They proposed that in situ disappearance of protein from various sources will vary with the source of protein in the diet, presumably, due to microbial adaptation to the source of protein. Their conclusion has not been verified by other research to date. If diet alters in situ disappearance drastically, reliable in situ studies will be more complex to conduct.

The objective of this experiment was to examine the effect of diet upon disappearance of dry matter and protein from feedstuffs in mobile dacron bags (MDB) in the rumen

and in the intestine of cattle. The diet contained either 50% or 80% concentrate and feedstuffs tested in MDB included cottonseed meal (CSM) and dehydrated alfalfa hay (ALF) and the two diets being fed.

Materials and Methods

Four beef heifers averaging 500 kg were fitted with 10 cm (internal diameter) rumen and duodenal cannulas and confined in metabolism crates for feces collection. They were used in a cross-over design with 2 - wk periods. Two basal rations were fed. These consisted of either 80% concentrate and 20% roughage or 50% concentrate and 50% roughage (Table 1). The roughage came from prairie hay or chopped alfalfa hay in the 80 and 50% concentrate diets, respectively. Added alfalfa hay increased the protein content of the diet. In each period, the first week was for diet adaptation and the remaining week was used for collecting digesta samples and measurements with MDB. The diet was fed at 2% of body weight (DM basis) in two equal feedings at 0800 and 1600 hours.

Site and Extent of Digestion

Chromic oxide was included in the diet at 0.2% of dietary dry matter as an indigestible marker. It was added in a mixture of cottonseed hulls, molasses and chromic oxide.

Duodenal and fecal samples were taken each day of the second week of each period immediately after feeding. The pH of digesta from the duodenum and the rectum was measured at the time it was collected. Duodenal samples (200 ml) were composited on a wet basis and refrigerated. Individual fecal samples were frozen.

Duodenal and fecal samples were dried at 55 C for 48 h and allowed to air equilibrate. These and feed samples were ground through a 2 mm screen. Feed, duodenal and fecal samples were analyzed for DM, ash, N (AOAC, 1975), acid detergent fiber (ADF; Goering and Van Soest, 1970), starch (MacRae and Armstrong, 1968) and chromium (Hill and Anderson, 1958). Ratios of chromium to feed constituent concentrations in feed, duodenal and fecal samples were used to calculate flow at various points and thereby, ruminal and total tract digestibilities of feed constituents. Nucleic acid-nitrogen (NA-N; Zinn and Owens, 1986) analyses were conducted on duodenal samples. Microbial nitrogen passage was calculated from NA-N content of samples and the NA-N to N ratio in bacterial cells. A microbial ash estimate of 20% (Smith, 1975) and the nitrogen content of isolated bacterial cells were used to estimate microbial or-

ganic matter (Goetsch and Owens, 1986). True ruminal OM digestion and true ruminal N digestion were calculated by subtracting the microbial contributions from respective OM and N flows at the duodenum. Digestion in the small plus large intestines was estimated as the difference between apparent digestion in the rumen and in the total tract.

Mobile Dacron Bag (MDB) Technique

Ruminal (R), postruminal (PR) and total tract (TT) disappearance of DM and crude protein (CP) of four feedstuffs were measured using MDB bags procedures. These feeds included: dehydrated alfalfa hay (ALF), cottonseed meal (CSM), diet A and diet B (Table 1). The MDB procedure previously described by Sauer et al. (1983) was followed except in certain aspects of bag construction and in that bags recovered from feces were thoroughly washed prior to analysis.

The bags, measuring 3.5 by 5.5 cm, were constructed of dacron cloth with a mean pore size measured microscopically of 60 to 70 microns. The dacron cloth was manufactured by Poly-Air, N. Erlanger, Blumgart & Co., Inc. 1450 Broadway, New York, NY 10018 and was described as 100% dacron Polyester. Approximately 2.5 g of dry feed which had been ground through a 2 mm screen was placed in each bag which then was sewn shut and the needle holes were glued closed. The ratio of sample to bag surface was 65 mg/cm^2, considerably higher than the 10 mg/cm^2 recommended by Van Hellen

and Ellis (1977), the 5 mg/cm^2 recommended by Weakley et al. (1983) and 10 to 15 mg/cm^2 recommended by Oldham (1986) for in situ disappearance. A large sample to surface ratio was employed so that an adequate amount of residual material would remain for analysis and yet the bags would not place undue stress on the insertion cannula or the small intestine. Transferring residue from larger ruminal bags to smaller intestinal bags as practiced by Hvelplund (1985) considerably complicates the MDB procedures. Bags were trimmed with rounded corners to reduce abrasion of the intestines. Bags containing all the feedstuffs were inserted into nylon stockings and suspended in the rumen for 15 h. On removal from the rumen, bags used to measure postruminal digestion were incubated in a pepsin-HCl solution [1g pepsin (570 Units per mg protein) per L of .1N HCl adjusted to pH 2] at 37.5 C for 3 hours with constant stirring as described by Kirkpatrick and Kennelly (1984).

After rumen and pepsin digestion, all bags were refrigerated at 4 C until they were inserted into the small intestine via the duodenal cannula at rate of one bag each 30 minutes. As bags were defecated with feces, they were collected on a screen covering the fecal pan. Feces were rinsed through the screen to recover each bag. Then, each bag was washed thoroughly by hand under cold running tap water until the wash water was colorless. Finally, bags were dried in a forced air oven at 90 C for 24 hours. Bags plus contents (feed residues) were weighed to determine dry

matter disappearance. For Kjeldahl nitrogen analysis, bags were digested intact. Blank bags containing a known weight of strips of Tygon tubing were used to correct for influx of non-adherent nitrogen.

Because washing should remove endogenous and a portion of the bacteria from the bags, estimates of disappearance by this procedure should more closely approximate true than apparent digestion of dry matter and crude protein as discussed by de Boer et al. (1987). Hence, disappearance would be expected to exceed apparent digestibility values due to washout of endogenous material from either the rumen or the intestines.

To examine the validity of disappearance values obtained, comparisons with true digestions of DM and N in the rumen and in the total tract seems logical, though the proper comparison for intestinal disappearance is not as obvious. In vivo, duodenal flow contains a mixture of feed residues and microbial matter (NRC, 1985). In contrast MDB contents should be primarily undigested feed residues. Thus intestinal disappearance from MDB cannot be directly compared with either apparent or true intestinal digestion of Nitrogen.

Bags removed after only ruminal disappearance were used to calculate extent of digestion at various sites; postruminal disappearance (% of diet) was calculated as the difference between the total tract minus the ruminal disappearance divided by the total tract an that result divided

by the initial weight of the feedstuff; postruminal disappearance (% of flow) was calculated as explained above but the final result was divided by the undegradable ruminal dry matter. Quadruplicate bags were used with all 4 feedstuffs and 4 animals across the 2 diets (two periods). T able 2 enumerates the disposition of the 272 bags used in this trial.

Statistical analysis. Results of the site and extent of digestion experiment were tested by analysis of variance using heifer, diet and period in the statistical model. When effects were significant different ($P<.05$), means were compared by a Duncan's multiple range test (Steel and Torrie, 1980).

Protein and dry matter disappearance in the rumen and the total tract from MDB were calculated using the mean value of all bags for each feed within each heifer and period. Postruminal disappearance was calculated by subtracting ruminal from total tract disappearance.

Extent of dry matter and protein degradation in the rumen, total tract and postruminal tract (both as percent of the diet and percent of flow) from MDB were tested by analysis of variance first for all feedstuffs and then separately for each feedstuff using period, animal and diet in the statistical model. Interactions between the diets fed and the same diets in bags (80 and 50% concentrate diets) were tested by analysis of variance using the mean for each feed within each animal in the statistical model. Total

tract and postruminal degradation were tested by including animal, diets, feedstuffs, and interactions among these factors of the model. When the effects were significant ($P<.05$) means were compared by a Duncan's multiple range test (Steel and Torrie, 1980).

Because residence time in the postruminal tract differed among bags, we tested for linear, quadratic, cubic and quartic effects of residence time of the mobile dacron bag on postruminal disappearance of the dry matter and crude protein by regression analysis (Steel and Torrie, 1980) with variation due to animal, period, diet and feedstuff removed.

Results and Discussion

In Vivo Digestibilities

The pH in the rumen and of feces tended to be lower with the higher concentrate diet though duodenal pH was higher ($P<.05$) with this diet (Table 3). Ruminal pH was not as low as expected for a high concentrate diet probably due to the low feed intake employed.

Differences between diets in dry matter and organic matter digestion were small (Table 3) though digestibilities of certain components differed ($P<.05$).

Postruminal N disappearance as a percent of flow averaged 63.6% and was not altered by diet. The absolute value for postruminal N digestion tended to be lowest with the 50% concentrate diet. These apparent small intestinal

digestion values agreed closely with the mean for steers cited by NRC (1976) of 68.2%.

Total tract apparent N digestion was higher ($P<.05$) with the 50% than with the 80% concentrate diet. Apparent N digestion is expected to be greater for higher protein diets. Expected N digestibilities for the two diets by the NRC (1978) equation [ND = (.9N% - .48)/N%] were 70.8 and 66.2%, respectively. These compare closely with the measured values of 74.3 and 66.4% from our trial. The 50% concentrate diet had a higher level of ADF than the 80% concentrate diet (Table 1). This, plus the change in the fiber source (alfalfa vs prairie hay) may be the reason for the lower ($P<.05$) total tract digestibility of ADF (36.5% vs 47.4%) with the 50% concentrate diet. Total tract ADF digestion for prairie hay and alfalfa hay were estimated by Rust (1983) to be 37.2 and 25.0% in 80% concentrate diets. Results are opposite from what one would expected with a single roughage source. For example, Poore et al. (1987), found that NDF digestion decreased when concentrate level was increased from 30 to 90% of the total diet. Between 36.0 and 58.7% of the dietary ADF disappeared in the rumen for 50% and 80% concentrate diets compared to 36.5 and 47.4% digested in the total tract. Greater ruminal than total tract ADF disappearance reflects sampling errors. Nevertheless, these values indicated that postruminal digestion of ADF was nil as a percent of flow as noted in most previous site of digestion studies.

Amounts of nitrogen leaving the abomasum, organic matter fermented, efficiency of microbial growth and escape of feed N (Table 4) were not significantly altered by roughage level. Zinn and Owens (1980) reported that an addition of dehydrated alfalfa hay to a diet altered the properties of the intestinal contents in a fashion similar to the changes we observed. In contrast to their trial in which ALF was added, we modified several diet components in our trial in addition to alfalfa to alter the roughage level.

Dry Matter Disappearance from
Mobile Dacron Bags

Ruminal dry matter disappearance at 15 h incubation differed ($P<.05$) among the four feed types as expected (Table 5). Disappearance was lower for the 80% concentrate diet (26.4%) than for other feeds. Reasons for the lower ruminal disappearance with this mixture are not apparent. A number of workers including de Boer et al. (1986) have noted differences among protein sources in ruminal disappearance. Estimates from Barrio et al. (1986) for CSM disappearance of DM was 38.9%.

Analyzed for each feedstuff individually, disappearance of DM in the rumen was not altered by composition of the diet being fed (Table 6). Nevertheless, ruminal disappearance of DM for each feed was greater with the moderate concentrate diet than with the high concentrate diet (Table 6). These results agree with those of Weakley et al.

(1983) and Barrio et al. (1986) who noted that disappearance of DM from certain feeds as soybean meal was greater with a high roughage diet. They suggested that fiber barriers may limit ruminal degradation. Our differences in DM disappearance due to diet are smaller but more consistent than others have reported.

Analysis across feedstuffs revealed that ruminal DM disappearance was highest with the 50% concentrate diet (33.0%) vs 80% concentrate (30.2%) (table 6); these results agree with those of Barrio et al. (1986) who found greater in situ dry matter disappearance across feeds with their 40% than their 80% concentrate diet.

These results conflict with in vivo measurement in which ruminal and total tract digestion of DM tended to be higher for the higher concentrate diet (Table 3). The reason for this discrepancy may be that for disappearance of DM from dacron bags incubated in the rumen, bags were incubated for a constant time of 15h. In vivo, retention times of various feed components will differ which thereby can alter extent of digestion.

The effect of diet on in situ disappearance was first demonstrated by Ørskov and Mehrez (1975) with dried grass suspended in the rumen of lambs fed either whole or pelleted barley. Disappearance of grass DM was greater with the whole barley diet. Barrio et al. (1986) found that the disappearance of the dry matter was significantly greater for the 40 than for the 80% concentrate diet for certain

feedstuffs including soybean, cottonseed and linseed meals. With other feedstuffs, no significant effect of diet on the disappearance of dry matter was detected.

No significant interaction (P>.6) was detected between feedstuff and diet fed in ruminal disappearance of the dry matter (Table 6).

Postruminal Disappearance of Dry Matter

Postruminal DM disappearance partially compensated for differences in ruminal disappearance with the 80% concentrate diet from the MDB being highest (45.9% of the diet and 74.6% of flow) among the feedstuffs (Table 5). Postruminal disappearance of DM from the dehydrated alfalfa was lowest (P<.05) both as a percent of the diet and a percent of flow.

In 1986, de Boer et al. reported values of intestinal availability of rumen undegradable dry matter for corn gluten meal, fish meal, meat and bone meal, soybean meal, canola meal, and alfalfa hay of 62.0, 38.0, 16.4, 7.2, 1.9 and 2.3%, respectively. Their low postruminal disappearance of alfalfa hay matches with our results.

Analyzed for each feedstuff individually, extent of postruminal disappearance of dry matter was not altered by composition of the diet being fed (Table 6). Disappearance tended to be higher with the roughage diet for three cases of the four feeds.

Average across feedstuffs, postruminal disappearance was not significantly (P>.05) altered by diet (Table 6) although a mean of 3.0% more of diet DM disappeared postruminally with the higher roughage diet. As a percentage of DM presented, disappearance averaged more with the higher roughage diet. This difference may be due to more desirable conditions in the intestines for enzimatic digestion or microbial fermentation. The information of Kirkpatrick and Kennelly (1987) did not separately report postruminal disappearance of the dry matter. But for the results they reported, disappearance of DM appeared to be greater with dairy cattle fed 19% protein diet than those fed a 16% protein diet.

No significant interactions (P>.6) was detected between diets and feedstuff for postruminal disappearance of dry matter. Postruminal differences in disappearance among and within feedstuffs was more variable than ruminal or total tract disappearance.

Total Tract Disappearance of Dry Matter

Significant differences among the feedstuffs (P<.05) were noted with lower disappearance of dry matter for the dehydrated alfalfa hay and the higher roughage diet than cottonseed meal or the 80% concentrate diet (Table 5). These generally match expected total tract digestibilities for these four feedstuffs. Total tract DM disappearance was almost 6 percent units higher for the 80 than the 50%

concentrate diet (72.3 vs 66.4%). Values are slightly below expected digestibilities of 78.6 and 69.6% (Table 1) those measured in this study (74.3 and 73.9%; Table 3). Another explanation could be that grinding the 50% concentrate diet for ruminal incubation increased its susceptibility to digestion more than chewing of the normal diet. Ruminal retention time was specified and uniform for feeds ingredients in the dacron bags. In the rumen, retention times vary with diet and particle factors. Either extended or selective ruminal retention of the less digestible components (coarse, fibrous particles) of the 50% concentrate diet would increase in vivo digestion of the less digestible diet, opposite from the effects we observed in situ. This leads to the suggestions that either particulate retention in the rumen was less with the 50 than the 80% concentrate diet or that the less digestible feed components of the 50% concentrate diet left the rumen most rapidly. The former suggestion is supported by the review by Owens and Goetsch (1984) from which one would predict mean in vivo particulate retention times in the rumen of 6.3 and 7.2 $\%h^{-1}$. The latter suggestion is supported by South Africa studies by Pienaar et al. (1980). Selective retention of only the potentially digestible components would relieve ruminal distension and permit feed intake to decrease at some sacrifice in digestibility. Selective retention, would be an ecological advantage for cattle grazing low digestibility forage.

A physical explanation of such segregation has been presented by Elliot (1980). As ruminal retention is primarily dependent on particle density, and particles being digested entrap CO_2 produced in minute bubbles, such particles are buoyed to the rumen surface and are retained longer than non-fermented particles. Such selective retention cannot be detected in most marker trials because all fractions of a feedstuff are tagged. Presence of selective retention and multiple pools of ruminal particles would invalidate most marker studies of single, disuniform feedstuffs except those which employ ruminal evacuation.

Zinn and Owens (1983) suggested that roughage level altered the flow of nutrients to the intestines. This should cause disappearance to be lower with the roughage diet, contrary to the results we obtained.

Analyzed within feedstuffs, disappearance of DM in the total digestive tract tended to be higher with the lower concentrate diet for all feeds though it was significantly higher ($P<.05$) only for the cottonseed meal (74.0 and 71.7%; Table 6). Canadian researchers (de Boer et al., 1987) found 70.3% and 73.7% total tract MNB dry matter disappearance for 50% concentrate diets which contained 11% and 17% of crude protein, respectively, though their diets contained 31.0% ADF, primarily from barley silage. With pigs, Petry and Handlos (1978) found that total tract disappearance of dry matter from MNB was not influenced by diet composition.

Averaged across feeds, the higher roughage diet increased ($P<.05$) DM disappearance in the total tract digestibility by a mean of 3.7 percentage units for a 5.8% increase (Table 6). Disappearance of DM from dacron bags containing the diets was higher for animals being fed the 50% concentrate diet than those being fed the 80% concentrate diet (Table 6). Disappearance from MDB matched in vivo digestibilities (Table 3) when most precisely feeds were passed through rumen and intestine of animals fed the diet being evaluated.

Jeraci et al. (1980) compared inocula from animals fed concentrate versus roughage diets. Extent of digestion for most feeds was greater with inocula from roughage-fed animals. This was similar to our results.

No significant interactions ($P>.6$) were detected between diets and feedstuff for total tract disappearance of the dry matter. Only one interaction was significant (Table 6) (animal * diet * feedstuff; $P<.03$). This appears that the animal differences are very important because other interactions (diet * feed and animal * feed) were not significant. Despite the potential animal effect, it seems desirable to use three or more animals in research of this type to reduce variation. This contrasts with the statement of Voigt et al. (1985) who concluded that the digestibility can be measured with enough accuracy with MNB using two animals. They found total tract digestibility could be estimated two MDB per sample so that the mean was

estimated with a standard deviation was 1.30%. This compares to a value of 1.36% for the four animals and two diets used in our trial.

Protein Disappearance from
Mobile Dacron Bags

Crude protein disappearance from MDB in the rumen was higher for alfalfa (70.5%) than for cotton seed meal and the 50% roughage diet (54.0 and 46.2%, respectively; Table 7). Ruminal escape protein from the cotton seed meal was moderate (46.0%) being quite close to the 50% of value for cottonseed meal reported for Zinn and Owens (1983) and somewhat higher than values of 34 to 38% reported by Goetsch and Owens (1985).

Apparent ruminal disappearance of protein were both considerably lower in vivo than in situ. True ruminal digestion of protein in vivo more closely matched in situ values. But effects of roughage level in vivo were reverse that noted in situ. Ruminal N digestibility differences would be expected as ruminal loss as ammonia is greater with higher protein/calorie ratios and protein content of the 50% and 80% concentrate diets were 15.6 and 12.6%, respectively.

Analyzed within each feedstuff, in situ disappearance of protein, was not altered by level of roughage in the diet (Table 8). Among the feedstuffs, disappearance was greater with the 50% than the 80% concentrate diet for only

one feedstuff--cottonseed meal. Weakley (1983) found that the level of roughage affects protein degradation from soybean meal in the rumen due to changes in either pH, retention time or microbial species. In his work, ruminal degradation of soybean meal placed in dacron bags was the greater with a 83% roughage level than 20% roughage. Similarly, Barrio et al. (1986) found greater loss of protein from soybean, cottonseed and linseed meals with a 60% roughage than a 20% roughage diet though other feedstuffs were not affected.

Ruminal disappearance of the crude protein averaged across feedstuffs show no general effect of diet. This contrast with DM disappearance for which the higher roughage diet increased DM disappearance from MDB. Reasons for this discrepancy are not clear though it implies that loss of protein does not parallel loss of DM from bags across diets. Within a diet and a feedstuff disappearances of the two were correlated in one previous study Barrio et al. (1985).

An interaction between diet and animal in ruminal disappearance of crude protein from MDB was noted ($P<.0001$), the behavior of one of the heifers was slightly different than the others during the first period of the trial.

Postruminal disappearance of the crude protein as a percent of the diet and a percentage of flow was higher ($P<.05$) with cotton seed meal (29.0% and 52%) than with other feeds tested (Table 7). Postruminal protein diges-

tion as a percent of flow was lowest for the 80% concentrate feed. Nalsen et al. (1987) measured ruminal protein disappearance of four feeds (wheat, wheat diet, corn and corn diet). His ruminal disappearance values (15 h) are high than ours, possibly due to the small particle size of his feeds. Effects of particle size on ruminal MDB disappearance of protein will be discussed in Chapter V.

Analyzed within feedstuffs, extent of postruminal disappearance (% of diet) from three of the feeds tended to be lower with the higher roughage diet (Table 8). As a percent of postruminal flow, these differences, except for the 50% roughage diet, disappeared. Hence, the main difference appears to be a compensatory effect for the differences in ruminal disappearance.

Averaged across feedstuffs tested, postruminal disappearance of crude protein from mobile bags were much greater (14.3 vs 11.7%) for the 80% than the 50% concentrate diet (Table 8). This effect is reduced when expressed as a percentage of flow.

Again, an interaction between animal, diet and feed in the bag was noted ($P<.0001$) for a postruminal N disappearance from MDB. No reason for this interaction is apparent.

Total tract disappearance of the protein supplements were considerably higher ($P<.01$) than for the two feeds and tended to be lower for the 50% than the 80% concentrate feed (Table 7). This is opposite from the in vivo results (Table 3).

Dacron bag values should be more precise estimates of disappearance of feed N than are in vivo digestibilities due to metabolic secretions of N into the guts. However, true digestibilities of feed protein, as predicted from the NRC (1976) equation or from mobile dacron bag studies (Hvelplund, 1985) near 90% should be. That is considerably greater than our values from this experiment for mixed diets.

Total tract disappearance of crude protein from MDB, averaged across feedstuffs was greater ($P<.05$) for the 80% concentrate than the 50% concentrate diet (72.9 vs 70.2%; Table 8). Again values for diets do not match apparent protein digestibilities measured in vivo (66.4 vs 74.3%). Among the feeds tested, a drop occurred in protein disappearance of the 50% concentrate diet when passed through the GI tract of animals fed the 50% concentrate.

Kirkpatrick and Kennelly (1984), however, reported that total tract disappearance of protein from six feedstuffs in MNB was greater ($P<.05$) when dairy cattle were fed a 19% protein diet than when were fed a 14% protein diet.

Two significant interactions among feedstuffs in the disappearance of crude protein were detected: an animal * diet ($P<.0001$) and an animal * diet * feedstuff effect ($P<.0001$). No feed by diet interaction was detected. This conflicts with the suggestion of Loerch et al. (1983) who reported an interaction between the protein supplement

being fed and that being incubated. Although we tested the total diet, not simply the protein supplement, we expected to detected an interaction if one exits. In the study of Loerch et al. (1983) direction of the interaction was inconsistent; hence responses probably should have been considered variable rather than reflecting adaptation to the protein source. Voigt et al. (1985) described a significant interaction animals * days ($P<.01$). In responses to roughage level, disappearance of crude protein changed differently from disappearance of dry matter (Tables 6 and 8).

Residence Time in the Intestines

Extent of postruminal digestion can be limited by enzymatic or microbial activities, access to the particles or by exposure time for either enzyme digestion in the small intestinal or microbial fermentation in the large intestine. To examine the relationship of intestinal residence to disappearance of dry matter and protein from MDB, retention times were subdivided and compared by analysis of regression. Animal and feedstuff effects were removed a lest square means were calculated (Table 9).

Though over half of the bags were defecated in less than 30 h, some bags remained over 60 h. This is considerably less than post-ruminal retention times of 20 h to 25 days for bags placed into the abomasums of steers (Snell et al., 1975). With residence times up to 40 h, DM disappearance tended to increase with residence time. Overall, a

cubic effect (r =.83, P<.01) was detected. The equation relating small intestinal disappearance of the dry matter to time (h of residence in the post-ruminal tract) was: DMD (%) = 36.1 + 0.2 T - 0.0008 T^2 - 0.00001T^3.

The disappearance of crude protein (% of the diet) from the MDB was more variable than DM (Table 9) and in the range of 50 to 59.5 hours the value was lower and significantly different to the others (P<.05). Small intestinal crude protein (% of the diet) disappearance (DCP) also was positively correlated (r =.89, P<.01) with residence time but quadratic and cubic relationships were not detected (P>.05). The regression equation for disappearance of the crude protein (%) was: DCP (%) = 5.66 + 0.3 T.

Voigt et al. (1985) in Berlin worked on retention time of the bags in the intestine. They found the mean retention time of the bags in his cows was 13.3 ± 1.9 h from the abomasum to the feces. This compares with 26.0 ± 2.6 h (n = 256) for all the feedstuffs and the diets in our experiment; the median residence time for our bags was 20.5 h. Passage time of bags from duodenum to defecation of sheep of two feedstuffs, soybean and rapeseed meals, was 14.2 h according to Hvelplund (1985). With dairy heifers, Kirkpatrick and Kennelly, (1987) found an average of residence time of bags in the intestine of was 15.8 h with a marked variation in passage time. They indicated that residence time did not appeared to affect the crude protein disap-

pearance. The low slope of our regression line supports their conclusion.

In conclusion, our results can be interpreted to indicate that digestibility of feedstuffs as measured with the mobile dacron bag technique can vary with diet of the test animal and post-ruminal residence time. This mobile bag system appears to be an easy, rapid and reliable method to rank feedstuffs in site and extent of true, not apparent digestion in ruminants.

TABLE 1. COMPOSITION OF THE EXPERIMENTAL DIETS

Diet	A	B
Concentrate: Roughage Ratio	80:20	50:50
Ingredient:	------% of DM------	
Corn dent, ground	66.2	36.2
Cottonseed hulls	12.0	12.0
Alfalfa hay, chopped	-	38.0
Prairie hay, chopped	8.0	-
Soybean meal	10.1	10.1
Molasses cane	2.0	2.0
Limestone	0.5	0.5
Dicalcium phosphate	0.5	0.5
Trace mineralized salt[a]	0.5	0.5
Chromic oxide	0.2	0.2
Composition:		
Dry matter	89.1	88.7
Crude protein	12.6	15.6
Starch	51.9	30.4
Acid detergent fiber	13.2	16.4
Ash	4.3	6.6
Digestibility[b]	78.6	69.6

[a]Trace mineral salt contained in %: 92 to 95 NaCl; 0.25 Mn; 0.20 Fe; 0.03 S; 0.033 Cu; 0.025 Co; 0.007 I; 0.005 Zn.

[b] Calculated from TDN values of components (NRC, 1984).

TABLE 2. DISPOSITION OF BAGS FOR MEASURING THE DRY MATTER AND CRUDE PROTEIN DISAPPEARANCE WITH MOBILE DACRON BAGS OF 4 FEEDSTUFFS

Procedure	Feedstuffs			
	ALF	CSM	80:20	50:50
Rumen incubation only	32	32	32	32
Rumen incubation plus pepsin-HCl plus small and large intestine (Total tract)	32	32	32	32
Blank bags				
Rumen	2	2	2	2
Total tract	2	2	2	2
Total	68	68	68	68

TABLE 3. EFFECTS OF TWO LEVELS OF CONCENTRATE ON SITE AND EXTENT OF DIGESTION IN BEEF HEIFERS

Item	Diets		
	80:20	50:50	SEM[c]
Digesta pH,			
Ruminal	6.5	6.6	0.10
Duodenal	2.8[a]	2.6[b]	0.10
Fecal	5.8[a]	6.3[b]	0.20
Ruminal digestion, % of diet,			
DM	52.9	51.8	1.3
OM(Apparent)	58.8	56.4	1.2
OM(True)	67.8	64.9	1.8
N(Apparent)	2.0[a]	32.2[b]	4.7
N(True)	52.4	64.6	2.5
ADF	58.7[a]	36.0[b]	2.5
Starch	78.8	82.8	3.7
Postruminal digestion, % of flow,			
DM	45.4	45.6	2.8
OM	42.7	44.0	3.0
ASH	69.5	59.9	2.8
N	65.2	62.1	1.6
Starch	69.1	89.4	3.5
Total tract digestion, % of diet,			
DM	74.3	73.9	0.9
OM	76.3	75.6	1.0
ASH	47.7[a]	56.8[b]	1.0
N	66.4[a]	74.3[b]	0.6
ADF	47.4[a]	36.5[b]	1.4
Starch	93.1	98.3	1.1

[ab] Means in a row with different superscripts differ statistically ($P<.05$).

[c] Standard error of the means.

TABLE 4. EFFECTS OF TWO LEVELS OF CONCENTRATE IN THE METABOLISM IN THE TRACT OF BEEF HEIFERS

Item	Diet 80:20	Diet 50:50	SEM[b]
N flow to the duodenum, g/day	146.2	145.9	9.48
Microbial N flow to the duodenum, g/day	74.3	69.7	5.56
Microbial OM flow to the duodenum, g/day	729.3	683.3	54.51
Apparent ruminal OM disappearance, %	58.8	56.4	1.21
OM Truly Fermented, kg	5.5	5.2	0.15
Microbial efficiency, g MN/kg OMF	13.7	13.3	0.74
Escape of feed N, %	47.6	35.4	2.52
Ruminal digestion of feed N, %	52.4	64.6	2.52

[a] Means in a row with different superscripts differ statistically ($P<.05$).

[b] Standard error of the mean.

TABLE 5. DISAPPEARANCE OF THE DRY MATTER OF THE DIFFERENT FEEDSTUFS BY THE MOBILE DACRON BAG TECHNIQUE

Feedstuff	Ruminal 15h % of diet	Postruminal % of diet	Postruminal % of flow	Total tract % of diet
	----------% Disappearance----------			
ALF	32.5[a]	22.3[c]	31.6[c]	54.8[c]
CSM	34.2[a]	38.7[b]	63.9[a]	72.8[a]
80:20	26.4[b]	45.9[a]	74.6[a]	72.3[a]
50:50	33.3[a]	33.2[b]	45.0[b]	66.4[b]
SEM[e]	0.95	1.65	4.30	1.36

[abcd] Means in a column with different superscripts differ statistically ($P<.05$).

[e] Standard error of the means.

TABLE 6. DISAPPEARANCE OF DRY MATTER WITH TWO DIETS OF FOUR FEEDSTUFFS BY THE MOBILE DACRON BAG TECHNIQUE

	Ruminal 15h % of diet			Postruminal % of diet			Postruminal % of flow			Total Tract % of diet		
	Diet			Diet			Diet			Diet		
	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]
Item	% Disappearance											
ALF	30.1	34.9	2.4	22.0	22.6	1.7	20.0	23.1	1.9	52.1	57.5	1.6
CSM	33.6	34.7	0.6	38.1	39.3	0.9	60.0	67.8	3.2	71.7[a]	74.0[b]	1.0
80:20	25.7	27.2	1.8	44.4	47.4	2.4	66.4	82.8	8.0	70.1	74.6	2.0
50:50	31.6	35.0	1.0	33.5	32.8	1.0	43.7	46.2	3.9	65.1	67.8	2.0
Means	30.2	33.0	(8.9%)	34.5	35.5	(3.0%)	47.5	55.0	(15.7%)	64.8	68.5	(5.8%)
SEM[d]	1.5			1.5			4.3			1.6		

[ab] Means within the same site of digestion in a different column with different superscripts differ statistically (P<.05).

[c] Standard error of the mean for diet effect within the feedstuff.

[d] Standard error of the mean for feedstuffs.

Interaction in the total tract DM of animal * diet * feed (P<.03).

TABLE 7. DISAPPEARANCE OF THE CRUDE PROTEIN OF THE DIFFERENT FEEDSTUFS BY THE MOBILE DACRON BAG TECHNIQUE

Feedstuff	Ruminal 15h % of diet	Postruminal % of diet	Postruminal % of flow	Total tract % of diet
	----------% Disappearance----------			
ALF	70.5[a]	7.9[a]	29.6[b]	78.4[a]
CSM	54.0[b]	29.0[b]	52.0[a]	83.0[a]
80:20	63.3[a]	4.3[a]	7.9[c]	67.6[b]
50:50	46.2[b]	11.0[b]	15.7[b]	57.2[b]
SEM[e]	2.680	3.710	6.300	2.230

[abcd] Means in a column with different superscripts differ statistically ($P<.05$).

[e] Standard error of the means.

TABLE 8. DISAPPEARANCE OF CRUDE PROTEIN WITH TWO DIETS OF FOUR FEEDSTUFFS BY THE MOBILE DACRON BAG TECHNIQUE[12]

	Ruminal 15h % of diet			Postruminal % of diet			Postruminal % of flow			Total Tract % of diet		
	Diet			Diet			Diet			Diet		
	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]
Item	-----------------------------------% Disappearance-----------------------------------											
ALF	70.6	70.5	1.6	8.0	7.7	2.8	7.6	8.5	2.9	78.6	78.2	1.7
CSM	53.0	55.0	0.6	30.1	27.8	1.5	51.6	52.4	1.6	83.1	82.8	1.2
80:20	64.6	62.0	3.2	3.8	4.8	1.7	6.1	9.7	1.9	68.4	66.8	2.7
50:50	46.2	46.2	4.7	15.3	6.6	9.1	22.4	9.0	15.3	61.5	52.8	4.9
Means	58.6	58.4	(-0.3%)	14.3	11.7	(-18%)	21.9	19.9	(-9.2)	72.9	70.2	(-3.8)
SEM[d]	2.5			3.8			5.4			2.6		

[ab] Means within the same site of digestion in a different column with different superscripts differ statistically (P<.05).

[c] Standard error of the mean for diet effect within the feedstuff.

[d] Standard error of the mean for feedstuffs.

[1] Ruminal interaction animal * diet (P<.0001).

[2] Postruminal N disappearance interaction of animal * diet * feed (P<.0001).

TABLE 9. EFFECT OF INTESTINAL RESIDENCE TIME ON THE DISAPPEARANCE OF THE DM AND N USING THE MOBILE DACRON BAG TECHNIQUE

Residence time (Hours)	N	Disappearance			
		DM (%)	SEM[d]	N[f] (%)	SEM[e]
10 - 19.5	109	35.3[abc]	0.61	9.0[ab]	0.75
20 - 29.5	88	34.4[bc]	0.58	14.6[a]	0.70
30 - 39.5	23	39.8[a]	2.01	8.7[ab]	2.43
40 - 49.5	22	37.5[ab]	2.05	12.0[a]	2.48
50 - 59.5	5	37.9[ab]	1.90	7.1[b]	2.29
> 60.0	9	32.1[c]	2.39	11.9[a]	2.89

[abc] Means in a column with different superscripts differ statistically ($P<.05$).

[d] Standard error of the means of the disappearance of DM.

[e] Standard error of the means of the disappearance of CP.

[f] % of diet.

CHAPTER IV

EFFECT OF DIET ON POSTRUMINAL DISAPPEARANCE OF DRY MATTER AND PROTEIN FROM FEEDSTUFFS IN MOBILE DACRON BAGS.

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ABSTRACT

Four beef heifers (500 kg) fitted with ruminal and duodenal cannulas were fed diets (2% of body weight) with either a moderate or high level of concentrate (50 vs 80%) in a crossover design. Disappearance of dry matter and crude protein of four feedstuffs (cottonseed meal, dehydrated alfalfa hay, and the two mixed diets) were determined using the mobile dacron bag (MDB) technique developed for ruminants. To detect effects of diet on postruminal disappearance, half of the bags were transferred from the rumen of heifers fed one diet into the duodenum of heifers fed the other diet. Postruminal disappearance of dry matter was 6.2 higher ($P<.05$) with the 50% than for the 80% concentrate diet. Postruminal disappearance of crude protein tended to be higher (1 to 22%) with the 50% than with the 80% concentrate diet. Differences are presumably due

to an alteration in times or rates of fermentation in the large intestine.

(Key Words: Mobile Dacron Bag, Protein Digestion, Protein Sources, Roughage, Feed Evaluation, Intestine Transit Time.)

Introduction

No experiments to date have studied the effect of diet on the postruminal digestion independently from ruminal digestion. Several researches who have measured site of digestion (Weakley, 1983; Goetsch et al., 1985) have commented that post-ruminal digestion can compensate partially if ruminal digestion is reduced. Such findings, however, are confounded with ruminal differences and, because supply of nutrients flowing to the duodenum may influence extent of digestion, cannot be considered as unbiased measurements. As information on factors limiting postruminal digestion is sparse, it is helpful to review factors which can limit ruminal digestion. These include microbial factors, feed factors and residence time.

Mehrez (1976) examined the effect of supplemental urea on ruminal fermentation in sheep. He reported that rate of protein degradation in situ and extent of fermentation of high and low protein barley, maize and high and low protein grass were increased by adding urea to the diet. This is presumably an effect on microbial activity in the rumen. Nutrient supply and fermentation conditions for fermenta-

tion in the large intestine also could alter digestion in the hindgut (Hoover 1978; NRC, 1985).

Weakley et al. (1983) observed that ruminal escape of soybean meal protein from ruminal degradation was 48% greater when steers were fed a high concentrate diet than when they were fed a 50% roughage diet. In 1986, Barrio et al. reported that ruminal disappearance of nitrogen from soybean meal, cottonseed meal and linseed oil meal was more extensive with steers fed an higher (60%) roughage diet than when fed a lower (20%) roughage diet. Again, changes in conditions for microbial conditions for fermentation were considered to be responsible.

The European Association of Animal Production (EAAP, 1987) is attempting to standardize the artificial fibre bag procedure to use in evaluation of feedstuffs for protein escape from the rumen. Their primary concerns are: bag specification (dimensions, pore size), sample preparation, animal number and type, the feeding protocol, number of replications, the washing procedures and the method of data presentation. They recommended limit feeding a 50% roughage diet be fed to animals used for ruminal incubation of bags. Though such a diet will give more uniformity results, findings may not be applicable with other diets.

One evaluation of the effect of diet on total tract digestion from MNB was conducted with pigs by Taverner and Campbell (1985). They fed two diets differing in crude fiber content (5.9 and 4.6%). Digestibilities for dry mat-

ter were 75.0 to 77.6%, respectively. Feedstuff disappearance from MNB was affected (P<.001) by fiber content of the basal diet, being consistently lower with the higher fiber diet.

As dietary fiber content is one of the most variable diet components which alters ruminal digestion, we examined its effect on postruminal and total tract disappearance of different feedstuffs from MDB. To test postruminal disappearance independently, we transferred bags among animals fed different diets.

Materials and Methods

Animals, diets and methods were similar to those described in Chapter III. Disappearance of dry matter (DM) and crude protein (CP) of four feedstuffs was measured using MDB procedures. These four feeds included: dehydrated alfalfa hay pellets (ALF), cotton seed meal (CSM), diet A (80% concentrate) and diet B (50% concentrate). The latter two diets also were used for feeding animals in this experiment.

Ruminal disappearance values were determined in the heifers as previously presented (Chapter III). Following removal from the rumen and pepsin digestion, all bags were refrigerated at 4 C (maximum of 8 h) until they were inserted into the small intestine via the duodenal cannula at rate of one bag each 30 minutes.

The effect of diet on postruminal disappearance (% of diet and % of flow) of dry matter and protein digestion was studied by transferring bags between heifers eating the two different diets. Thus, bags fermented in the rumen of the two heifers fed the 50% concentrate diet were inserted into either its own duodenum or into the duodenum of the two other heifers fed the 80% concentrate diet. In a similar fashion, postruminal digestion of bags ruminally fermented in the two heifers fed the 80% concentrate diet was measured in the two heifers fed both the 50% and the 80% concentrate diet.

As they were defecated, the dacron bags collected on a screen with 0.5 by 0.5 cm openings covering the fecal pan. Feces were rinsed through this screen; each bag was collected and thoroughly washed manually under cold running tap water until the wash water was colorless. Finally, the bags were dried in a forced air oven at 90 C for 24 hours. Bags plus residue contents were weighed to determine dry matter disappearance and were digested intact for Kjeldahl nitrogen analysis. Blank bags containing a known weight of Tygon tubing strips were used to correct for influx of non-adherent nitrogen. Table 1 lists the disposition of the 272 bags used for disappearance measurements in this study.

Statistical Analysis of DM and CP Disappearance of the Bags

Protein and dry matter disappearance in the rumen and

the total tract were calculated using mean values from the four quadruplicate bags of each feed within each animal and period.

Extent of dry matter and protein disappearance in the postruminal tract (both as a percentage of the diet and a percentage of duodenal flow) and in the total digestive tract was tested by analysis of variance of a crossover design using feedstuff, period, animal, and diet fed for ruminal digestion and diet fed for postruminal digestion in the statistical model. Interactions of diets and the two feedstuffs fed (80% and 50% concentrate only) in the bags were tested by analysis of variance using period, animal, diet, feedstuff and the interaction of diet with feedstuff as classes in the statistical model. To test the effects of total tract and post-ruminal environment on the disappearance of DM or CP of the feedstuffs an additional analysis of variance was used by including diet for ruminal incubation, diet, feedstuff, diet for post-ruminal digestion and all interactions among these factors in the model. When effects were significant ($P<.05$), means were compared by a Duncan's multiple range test (Steel and Torrie, 1980).

Results and Discussion

Flow diagrams presenting results are found in Figures 1 and 2. Ruminal DM disappearances has been discussed previously (Chapter III).

Postruminal Disappearance of Dry Matter

As expected, postruminal disappearance of dry matter differed among the four feedstuffs (Table 2). Among the feeds, the highest post-ruminal disappearance was for the 80% concentrate diet (46.8%) whereas the lowest was for the dehydrated alfalfa pellets (22.4%). Dry matter disappearance values for the other two feedstuffs were 37.2% for cotton seed meal and 34.0% for the 50% concentrate diet.

Analyzed across feedstuffs, postruminal disappearance, as a percentage of the diet, was greater ($P<.05$) with the moderate concentrate diet by 6.2% (Table 3). This difference is quite similar to the 3.0% greater postruminal disappearance value with the moderate concentrate diet in the previous experiment where bags were not transferred between animals fed different diets (Chapter III).

Increased post-ruminal DM disappearance also might be attributed to improved conditions for microbial fermentation in the large intestine. Fecal pH and, thereby, presumably large intestinal pH with the higher fiber diet remained closer to the pH range for rapid fermentation (6.3 vs 5.8; Chapter III). If rate and extent of DM fermentation is greater at a high pH, as discussed by Strobel and Russell (1987), rate of post-ruminal DM fermentation should be increased by a diet yields a higher pH in the large intestine. This hypothesis needs to be checked by measuring postruminal DM digestion in steers equipped with an ileo-rectal anastomosis (Chapter V). If this effect of added

fiber is due to changes in the large intestinal fermentation or residence time, postruminal diet should have no effect on postruminal DM disappearance in animals equipped to bypass the large intestine.

Total Tract Disappearance of Dry Matter

Total tract disappearance of dry matter differed among the four feedstuffs (Table 2). Among the feeds, the highest total tract disappearance was for the 80% concentrate diet (73.2%) whereas the lowest was for the dehydrated alfalfa pellets (54.8%). Dry matter disappearance values for the other two feedstuffs were 72.8% for cotton seed meal and 67.3% for the 50% concentrate diet.

Canadian researchers (de Boer et al., 1987) found total tract MNB dry matter disappearance for two different diets (50% concentrate) with 11% and 17% of crude protein were 70.3 and 73.7%.

In 1978, Petry and Handlos fed several diets to pigs and measured digestibility of DM using MNB. They found no effect of diet on disappearance of DM from bags. In contrast, Taverner and Campbell (1985) measured disappearance from MNB in pigs fed basal diets containing 4.6 and 5.9% crude fiber. Disappearance of dry matter was greater for pigs fed the lower fiber diet.

Analyzed (see Appendix Tables 1 to 8) for individual feedstuffs, diets of animals used for postruminal measurement had a significant effect on total tract disappearance

of DM although the feed by postruminal diet interaction was not detected for total tract disappearance (P>0.63). This is illustrated in Table 5. No interaction between ruminal and postruminal diet on DM disappearance in the total tract was detected P = 0.64. For total tract disappearance of DM, an animal by diet by feedstuff interaction (P<.03) was observed. The interaction is presumably caused by greater increase in total tract digestion of the two diets than the two pure feeds. No explanation for this interaction is apparent.

Protein Disappearance from MDB

Postruminal disappearance of crude protein also differed among the four feedstuffs (Table 4) with values for CSM being highest in all cases. Postruminal disappearance of protein, as a percent of the diet as a percent of supply was not altered (P>.05) by diet being fed (Table 6). Trends in crude protein disappearance were the opposite from those for DM, with disappearance being 22.5% greater with the 50% concentrate diet (Table 7). These trends were the opposite in direction to the -9.2 higher postruminal (% of flow) disappearance of crude protein with the higher concentrate diet noted in heifers in which bags were not transferred (Chapter III). Analyzed within feedstuff, postruminal diet had no effect on postruminal disappearance of protein (Table 7).

Total tract disappearance of crude protein also differed among the four feedstuffs (Table 4) with values for low concentrate diet being lowest. Again, total tract disappearance of protein was not altered (P =.80) by diet being fed (Table 6). Trends in crude protein disappearance were the opposite from those for DM, with disappearance being 0.7% greater with the 50% concentrate diet (Table 7). These trends were the opposite in direction to the -4% higher total tract disappearance of crude protein with the higher concentrate diet noted in heifers in which bags were not transferred (Chapter III).

No interaction between ruminal and postruminal diet on protein disappearance was detected (P =.83 for total tract crude protein disappearance). Although a feedstuff by postruminal diet interaction was apparent (P < 0.001 for total tract disappearance of crude protein).

Crude protein disappearance of 6 diets was measured using mobile nylon bags in dairy cattle fed a 50% concentrate diet by Kirkpatrick and Kennelly (1987). They fed two levels of dietary protein, 14% and 19%. No effect of diet on total tract disappearance of crude protein was detected. Our two diets provided 12.6% and 15.6% crude protein and our results would support the suggestion of Kirkpatrick and Kennelly (1987) that dietary protein level had no significant effect on total tract protein disappearance from MDB.

Interactions between diet in the bag and diet fed at the two sites was examined using only the 50 and 80% concentrate diets individually. No interaction between diet fed to animals versus diet of animal used for post-ruminal measurement was noted (Table 6). Certainly, microbial adaptation to specific substrates has been demonstrated for soluble nutrients, and pH can alter extent of fiber digestion in the rumen. But lack of interaction between postruminal diet and feed in the MDB indicates that adaptation to diet for compensatory postruminal digestion in this trial was minor. Loerch et al. (1983) indicated that feeding a protein source can alter its extent of ruminal degradation. Their observation is not supported by results of our study. No evidence of either ruminal (Chapter III) or post-ruminal adaptation to the diet being fed was detected.

Reasons for the higher post-ruminal DM (% of diet) and a trend toward the higher crude protein disappearance with the moderate concentrate diet are not clear. One might speculate that added fiber could dilute digestive enzymes and thereby reduce digestion. Alternatively, added fiber might be expected speed passage through the small intestine; though the results of Goetsch and Owens, 1986 noted that particle passage rate was slower with the forage diets (.258) than with the concentrate diets (.156 units/h). Both of these actions of added fiber should decrease DM disappearance, not increase it as was observed.

Interactions between animal and diet, animal and feedstuff and feedstuff and diet fed to animals used for postruminal disappearance measurement were detected. Reasons for these interactions are not apparent. Disappearance of protein in the total tract was more drastically altered by animal to animal variation and diet effects. This indicates that these two factor interaction need closer control when estimating protein than when estimating DM disappearance from feedstuffs.

Appendix Tables 1 to 8 illustrate the relative amounts of variation which can be attributed to animal, diet and feedstuff for the ruminal, postruminal (% of diet and % of flow) and total disappearance of the dry matter and crude protein. The difference among feedstuffs was responsible for most of the variation. For dry matter disappearance, animal and diet used for ruminal incubation were relatively important though the postruminal diet and a diet by feed interaction was no detected as discussed earlier.

Residence Time in the Intestines

Postruminal residence time of the MDB in this study was greater with the 50% than the 80% concentrate (27.6 h vs 24.6 h) which could explain the effect of diet. As residence time in the small intestine may be reduced by higher fiber levels (Owens et al., 1986), the longer residence time is presumably in the large intestine. In humans, Ehle and Robertson (1983) have observed that added

fiber decreased residence time in the large intestine, opposite in pigs can expand to handle larger quantities of fiber (Cannon, 1985) but factors controlling volume and retention time of the hindgut remain to be defined (Hoover, 1978). Though Faichney et al. (1983) have suggested that with low quality forages passage through the hindgut may limit total tract rate of passage in adult sheep, with other diets or with cattle, the rumen rather than the hindgut is considered to be the bottleneck. Nevertheless if hindgut residence time were increased, extent of fermentation in the hindgut should increase.

In conclusion, results of this study can be indicated that the differences in total tract or postruminal disappearance of the two diets fed to the animals are due to an alteration in times or rates of fermentation in the large intestine.

TABLE 1. DISPOSITION OF BAGS FOR MEASURING THE DRY MATTER AND CRUDE PROTEIN DISAPPEARANCE WITH MOBILE DACRON BAGS OF 4 FEEDSTUFFS

Procedure	Feedstuffs			
	ALF	CSM	80:20	50:50
Rumen incubation only	32	32	32	32
Total tract-transfer postruminal disappearance	32	32	32	32
Blank bags	4	4	4	4
Total	68	68	68	68

TABLE 2. DISAPPEARANCE (%) OF THE DRY MATTER OF THE DIFFERENT FEEDSTUFS FROM MOBILE DACRON BAGS

Feedstuff	Postruminal % of diet	Postruminal % of flow	Total Tract % of diet
	--------% Disappearance---------		
ALF	22.4[d]	35.3[c]	54.8[c]
CSM	37.2[b]	63.0[a]	72.8[a]
80 % Concentrate	46.8[a]	76.8[a]	73.2[a]
50 % Concentrate	34.0[c]	48.4[b]	67.3[b]
SEM[e]	1.54	2.52	1.82

[abcd] Means in a column with different superscripts differ statistically ($P<.05$).

[e] Standard error of the means.

TABLE 3. DISAPPEARANCE OF DRY MATTER WITH TWO DIETS OF FOUR FEEDSTUFFS BY THE MOBILE DACRON BAG TECHNIQUE

	Ruminal 15h % of diet			Postruminal % of diet			Postruminal % of flow			Total Tract % of diet		
	Diet			Diet			Diet			Diet		
	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]
Item	% Disappearance											
ALF	30.1	34.9	2.4	20.2	24.6	1.7	31.9	38.9	1.9	55.1	54.7	1.6
CSM	33.6	34.7	0.6	39.1	37.9	0.9	67.4	56.9	3.2	73.8	71.5	1.0
80:20	25.7	27.2	1.8	46.3	49.0	1.6	83.9	73.9	8.0	73.5	74.7	1.6
50:50	31.6	35.0	1.0	33.4	36.4	1.5	53.5	50.2	2.2	68.4	68.0	1.7
Means	30.2	33.0	(8.9%)	34.7	37.0	(6.2%)	59.2	55.0	(-7.1%)	67.7	67.2	(-0.7%)
SEM[d]	2.0			1.5			2.5			1.8		

[ab] Means within the same site of digestion in a different column with different superscripts differ statistically ($P<.05$).

[c] Standard error of the mean for diet effect within the feedstuff.

[d] Standard error of the mean for feedstuffs.

TABLE 4. DISAPPEARANCE (%) OF THE CRUDE PROTEIN OF THE DIFFERENT FEEDSTUFS FROM MOBILE DACRON BAGS

Feedstuff	Postruminal % of diet	Postruminal % of flow	Total tract % of diet
	--------% Disappearance----------		
ALF	8.3[c]	27.4[b]	78.8[b]
CSM	28.7[a]	50.8[a]	82.7[a]
80 % Concentrate	3.9[d]	13.3[c]	67.2[c]
50 % Concentrate	13.4[b]	20.0[b]	59.7[d]
SEM[e]	4.17	7.15	2.98

[abcd] Means in a column with different superscripts differ statistically (P<.05).

[e] Standard error of the means.

TABLE 5. TOTAL TRACT DISAPPEARANCE (%) OF THE DRY MATTER FROM MOBILE DACRON BAGS FOR HEIFERS FED 50 AND 80% CONCENTRATE DIETS

	Diet	
Feedstuff	50 %	80 %
	% Disappearance	
ALF	78.9	78.9
CSM	83.8	81.5
80 % Concentrate	66.2	68.1
50 % Concentrate	57.2	62.1

TABLE 6. SIGNIFICANCE OF INTERACTION BETWEEN DIET AND DIET IN THE MOBILE DACRON BAG FOR DRY MATTER AND CRUDE PROTEIN DISAPPEARANCE AT SEVERAL SITES

Component	Ruminal % of diet	Postruminal % of diet	Postruminal % of flow	Total tract % of diet
		Pr > F		
DM	0.8583	0.9010	0.6745	0.6766
CP	0.8294	0.9286	0.8391	0.8030

TABLE 7. DISAPPEARANCE OF CRUDE PROTEIN WITH TWO DIETS OF FOUR FEEDSTUFFS BY THE MOBILE DACRON BAG TECHNIQUE

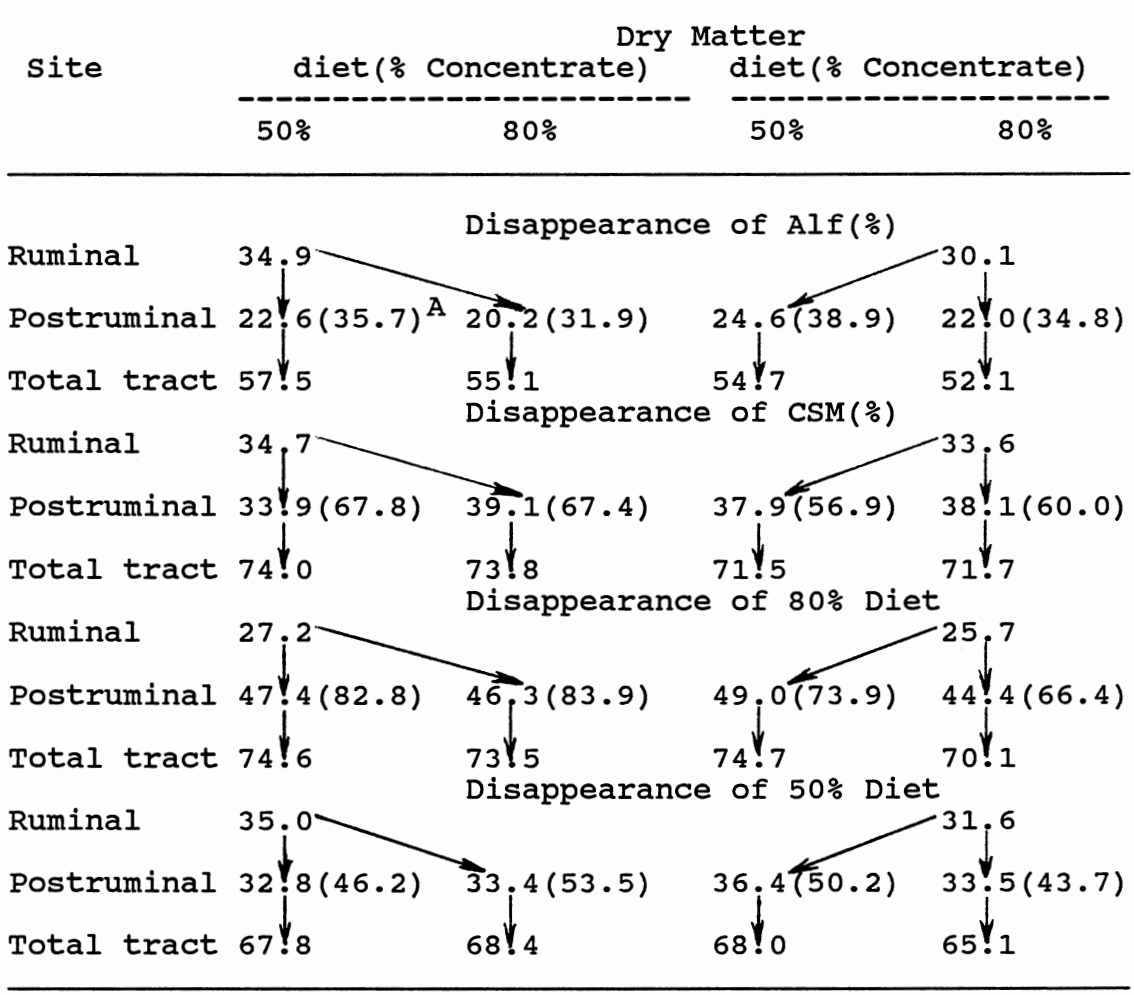
	Ruminal 15h % of diet			Postruminal % of diet			Postruminal % of flow			Total Tract % of diet		
	Diet			Diet			Diet			Diet		
	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]	80:20	50:50	SEM[c]
Item	-----------------------------------% Disappearance-----------------------------------											
ALF	70.6	70.5	2.4	8.6	8.9	1.7	24.6	30.3	1.9	79.1	79.5	1.6
CSM	53.0	55.0	0.6	24.9	31.9	0.9	45.4	53.9	3.2	79.9	84.9	1.0
80:20	64.6	62.0	1.6	5.8	1.1	2.3	10.6	26.7	8.0	67.8	65.7	1.7
50:50	46.2	46.2	1.5	16.6	15.3	2.2	24.4	24.2	4.7	62.8	61.5	1.6
Means	58.6	58.4	(-0.3%)	14.0	14.3	(0.02%)	26.2	33.8	(22.5%)	72.4	72.9	(0.7%)
SEM[d]	2.5			4.7			7.1			3.0		

[ab] Means within the same site of digestion in a different column with different superscripts differ statistically ($P<.05$).

[c] Standard error of the mean for diet effect within the feedstuff.

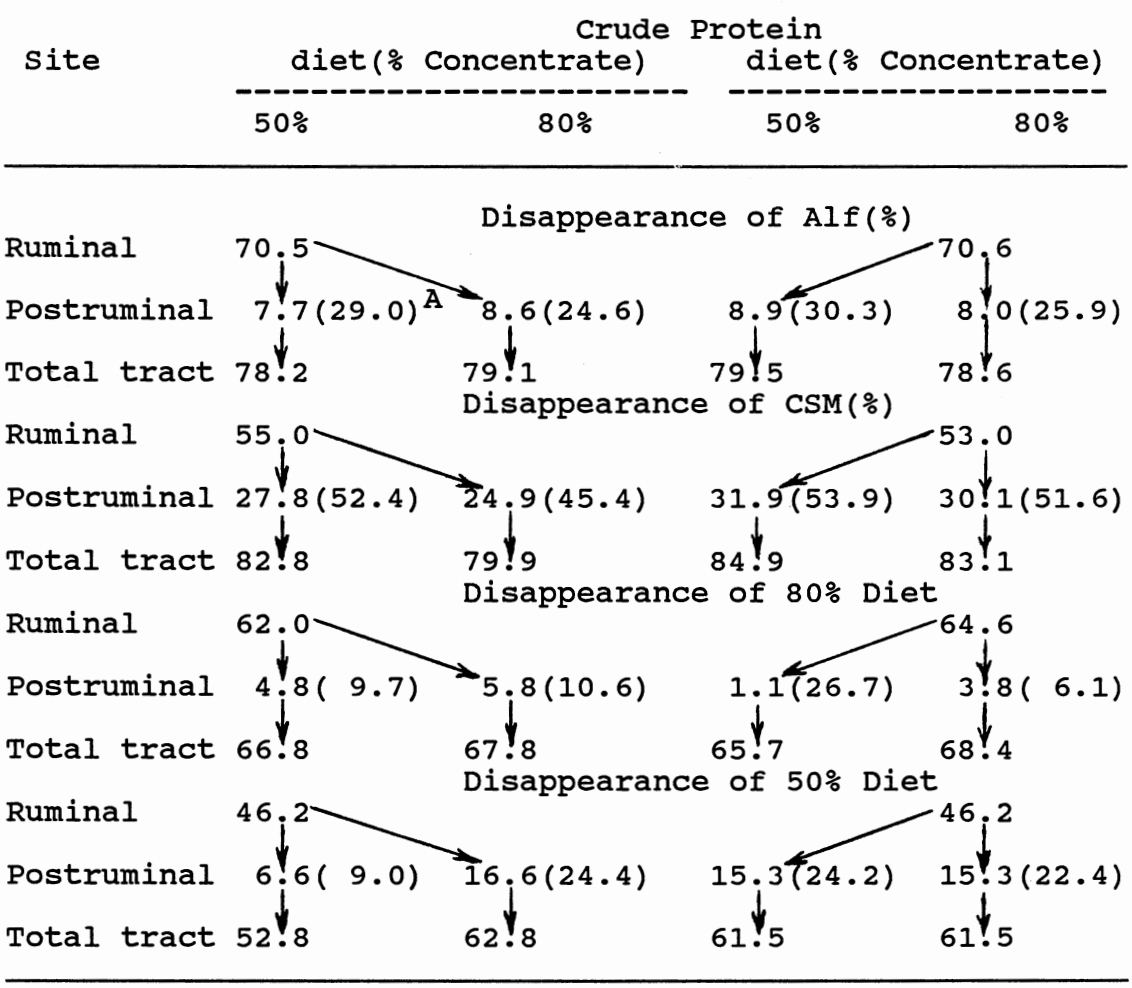
[d] Standard error of the mean for feedstuffs.

FIGURE 1. FLOW DIAGRAM FOR RUMINAL, POSTRUMINAL AND TOTAL TRACT DRY MATTER DISAPPEARANCE FROM MOBILE DACRON BAGS



A Postruminal disappearance (% of flow).

FIGURE 2. FLOW DIAGRAM FOR RUMINAL, POSTRUMINAL AND TOTAL TRACT CRUDE PROTEIN DISAPPEARANCE FROM MOBILE DACRON BAGS



A Postruminal disappearance (% of flow).

CHAPTER V

PROCESSED GRAINS: DRY MATTER, CRUDE PROTEIN AND STARCH DISAPPEARANCE FROM MOBILE DACRON BAGS.

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ABSTRACT

Site and extent of disappearance of dry matter, crude protein and starch from 14 different combinations of grains and processing (corn, milo, oats, wheat, barley processed by various commercial methods) were measured using two dairy calves (150 kg) equipped with duodenal T cannulas and an ileo-rectal anastomosis. An 80% concentrate diet at 2.5% of body weight was fed. Ruminal disappearance after 15 h of incubation was measured. For postruminal digestion, this was followed by 3 h of pepsin-HCl digestion and insertion of fermented bags into the duodenal cannula with recovery on defecation. Rolling whole corn or oats increased disappearance of dry matter, starch and protein in the rumen and in the small intestine. Compared with rolled grain, steam flaked grain had greater disappearance of DM and protein in the rumen for corn and milo grains but

not for wheat and barley. Ruminal starch disappearance was increased by flaking each of the grains. Ensiling rolled corn, especially at 35% moisture, increased ruminal and intestinal disappearance of dry matter, starch and crude protein. Generally, processing increased both ruminal and intestinal disappearance. Supply of intestinally disappearing starch and protein ranged from 3 to 78 and 0 to 100%, respectively of that provided in the diet. As corn and milo were more extensively processed, intestinal supply of digestible starch tended to increase. Changes in site of digestion with processing of wheat, barley and oats were minor.

(Key Words: Mobile Dacron Bag, Protein Digestion, Starch Digestion, Grains, Processing, Intestine Bypass.)

Introduction

Despite extensive research on the requirements of protein for beef cattle (NRC, 1984, 1985), certain basic information needed to balance diets to meet postruminal protein requirements of the ruminant more precisely is not available. Models which calculate requirements rely on estimates of ruminal escape of ruminal and intestinal digestibility values, and reliable estimates of ruminal bypass and intestinal digestion to date are limited primarily to protein supplements. In most diets, however, cereal grains and forage supply 50 to 90% of the total dietary protein. Hence, escape values for protein for commercially

processed cereal grains are needed to match with the information on protein supplements to correctly balance diets. Such information will improve our ability to formulate lower cost rations, and potentially could increase production levels for high producing ruminants.

Escape values for protein from the common grains are rare. In the NRC (1985) publication only three estimates of undegradable protein are provided: barley (0.21), corn (0.65) and sorghum grain (0.52).

Commercial grain processing procedures, commonly used to increase total tract digestion of starch, also can alter ruminal escape of cereal grain protein (Aguirre et al., 1984; Hibberd et al., 1985). The magnitude and even the direction of change varies with the method of processing. Effects of various methods of processing of cereals on ruminal escape and small intestinal digestion of protein need to be tested.

The mobile dacron bag (MDB) technique has been used in pigs to measure digestibilities of various protein sources and cereals (Sauer et al., 1983; Cherian et al., 1985; Graham et al., 1985; Taverner and Campbell, 1985; de Lange et al., 1986) and in ruminants to test ruminal and post-ruminal degradation of different protein sources (Hvelplund, 1985; Rooke, 1985; Voigt et al., 1985; Deacon et al., 1986; Kennelly and de Boer, 1986; de Boer et al., 1987; Kirkpatrick and Kennelly, 1984; Nalsen et al., 1987; Robinson et al., 1987). Although these reports provide information

on disappearance of DM and of crude protein, no information on the effect of grain processing on disappearance from mobile dacron bags has been published to date.

Though enzyme limitations and residence time can limit extent of starch digestion in the small intestine of ruminants, particle size also may limit digestion (Owens et al., 1986b). Galyean (1984) listed several variables which can alter in situ (rumen) digestion. These included diet of the donor animal and storage, grinding and processing of samples. To determine extent of digestion, a 48 h in vitro incubation period was recommended. Considering that ruminal retention time more likely is only 15 to 24 h, Kirkpatrick and Kennelly (1984) recommended that ruminal in situ measurements should last for 15 h. Hence, we employed 15 h incubations in our study.

The objective of this experiment was to determine the impact of grain source and grain processing on the extent of disappearance of dry matter, protein and starch in the rumen and the small intestine of calves. Calves were equipped with an ileo-rectal anastomosis to reduce extent of fermentation in the large intestine. Thereby, estimates of postruminal disappearance should more closely represent digestion in the small intestine alone.

Materials and Methods

Two Holstein calves (150 kg) vaccinated against IBR,

PI_3, and four-way blackleg and dewormed with Ivermectin were fitted with duodenal cannulas. They also were equipped with an anastomosis between the distal ileum and the distal rectum so that digesta from the small intestine would bypass the large intestine and flow directly from the small intestine to the rectum for defecation. The calves were housed in individual pens with a flooring of iron screen and hardware cloth (Sherman wire 1300 E. Pacific St., Sherman, TX 75090) which had small holes (5 by 1 cm) to simplify collection of defecated mobile dacron bags. Calves were fed a concentrate diet composed of 20.1% dry rolled corn, 20% of chopped prairie hay, 19.8% dry rolled sorghum, 13.8% soybean meal, 12.6% dehydrated alfalfa hay crumbles, 5.9% molasses, 5.9% dry ground wheat, 0.9% dicalcium phosphate and 1% salt plus a trace mineral supplement (94.5% sodium chloride, 3.2% of magnesium oxide, 1.8% potassium chloride, 0.25% manganous oxide, 0.2% iron oxide, 0.033% copper oxide, 0.007% calcium iodate, 0.005% zinc oxide, 0.005% cobalt carbonate). The salt plus trace mineral supplement was mixed with the diet at each feeding. Animals were adjusted to their diets for a minimum of 14 days prior to any measurements and were fed a total of 2.5% of body weight (dry matter basis, DM) split into two equal feedings at 0800 and 1600 hours. Feed samples were collected during the trial for analysis of dry matter, crude protein and starch. Calves had free access to fresh water.

Mobile Dacron Bag (MDB) Technique

Site of disappearance of dry matter, protein and starch from MDB was measured at two different sites. These were A) ruminal disappearance which consisted of incubation in the rumen for a period of 15 h and B) total tract disappearance which consisted of ruminal incubation A), pepsin-HCl digestion and insertion into the duodenum and recovery from feces. Extent of digestion in the small intestine was calculated as total tract minus ruminal disappearance. To calculate this as a percent of input or duodenal flow, this was divided by ruminal escape.

Bags. The dacron bags, measuring 3.5 by 5.5 cm, were constructed of dacron cloth with a mesh size of 60 to 70 microns. Approximately 2.0 g of dry feedstuff was placed in each bag after which each bag was sewn shut and the sewing holes were glued closed with Duco cement (Devcon Corporation., Wood Dale, IL 60191). Corners of bags were rounded to reduce abrasion of the intestine (Sauer et al., 1983).

Feeds. Fourteen different unground feedstuffs were employed (dry feed). They consisted of corn in the dry rolled, whole shelled, steam flaked and high moisture forms, with the latter at both 25 and 35% moisture levels; milo in the rolled and steam flaked forms; wheat in the

rolled and steam flaked forms; barley in the rolled and steam rolled forms; oats in the whole and rolled forms; and the diet as fed to the calves. Table 1 presents the chemical composition and the geometric diameter of these feedstuffs. The same batch of grain was processed to form each of the processed forms by a number of feedlots and feed companies (B. Doran, personal communication).

Incubation. Twelve bags containing each of the 14 feedstuffs were inserted into nylon stockings and incubated for 15 h in the rumen of a 400 kg cannulated beef steer fed the diet described ration above. Upon removal from the rumen, four bags of each feedstuff were washed thoroughly, dried and analyzed. The other eight bags of each feed were incubated for 3 h at 37.5 C as described by Kirkpatrick and Kennelly (1984) with constant stirring in a pepsin-HCl solution [1g pepsin (570 units per mg protein) per liter of .1N HCl] adjusted to pH 2. After pepsin digestion, each bag was refrigerated at 4 C until it could be inserted into the small intestine via the doudenal cannula. Bags were inserted into the duodenal T cannula at a rate of one bag each 30 min. As they were defecated, the dacron bags collected on the hardware cloth. Each bag then was washed by hand under cold running tap water until the wash water was colorless. This procedure was identical to that used for bags retrieved from the rumen. All washed bags were dried in a forced air oven at 90 C for 24 h.

Feed samples and bags containing residues were subjected to dry matter, Kjeldahl nitrogen (AOAC 1975) and starch (MacRae and Armstrong, 1968) analyses. Blank bags containing a known weight of sliced Tygon tubing were used to correct for influx of non-adherent nitrogen and starch. Bags plus residue contents were weighed and digested intact for Kjeldahl nitrogen analysis and crude protein was calculated as 6.25 times N.

Because washing should remove endogenous secretions and a portion of the bacteria in the bags, disappearance estimates by this procedure should more closely approximate true than apparent digestibility of dry matter and crude protein and would be expected to exceed apparent digestion values (de Boer et al., 1987).

Surgical Procedure

For the ileo-rectal anastomosis, surgery under general anesthetic was performed via the right flank with the calf in left lateral recumbency. The ileum was clamped and transected as close as considered feasible (approximately 10 cm) to the ileo-cecal junction. The distal stump of the ileum was closed with a double row of inverting sutures. The clamped free end of the transected ileum was then moved posteriorly and an "end to side" ileo-rectal anastomosis was formed on the right lateral aspect of the rectum approximately 20 cm from the anus. The abdominal wall access incision was then sutured (Barron, 1987).

For duodenal cannulation, a separate incision was made through the right abdominal wall just posterior to the last costochondral junction. The pylorus and proximal duodenum were exteriorized and a plastisol T cannula was sutured in place in the duodenum approximately 10 cm from the pylorus. The duodenum, with the fixed and stoppered cannula, was pushed into the abdominal cavity and the cannula stem was exteriorized through a small incision between the 12th and 13th ribs. The flank access incision was then sutured (Barron, 1987).

Statistical Analysis

Extent of dry matter, crude protein and starch disappearance in the rumen, total tract and in the small intestine were tested by analysis of variance using calf and feed in the statistical model. When differences were significant ($P<.05$), means were compared by a Duncan's multiple range test. To see the specific effect of the method of processing the grains and the grain sources for all the nutritive components (DM, protein and starch) a total of 72 contrasts (Steel and Torrie, 1980) were run: whole vs rolled, corn vs oats and their interaction; steam flaked vs rolled, all the grains, and their interaction; high moisture vs rolled corn and high moisture (35%) vs high moisture corn (25%) (Tables 7-15).

Results and Discussion

Dry matter disappearance at various sites of the digestive tract 14 feeds are presented in Table 2. Processing drastically altered ruminal, total tract and small intestinal disappearance of dry matter. Ruminal, total tract and postruminal DM disappearance of whole corn and whole oats were very low; digestion at each site was increased ($P<.05$) by dry rolling. Compared to rolling of the grain, steam flaking increased ($P<.05$) ruminal digestibility for corn and milo but not for wheat or barley (Steam rolling for barley). Steam flaking also increased ($P<.05$) intestinal disappearance, both as a percent of the DM presented and as a percent of diet, as well as total tract disappearance of DM from corn, milo and wheat but not for barley (which was steam rolled). Ensiling rolled corn to form the high moisture form tended to increase ruminal, intestinal and total tract disappearance of DM. Though ruminal and total tract disappearance were changed less by fermentation than by steam flaking, amounts of dietary DM disappearing in the small intestine from these two processing methods were virtually identical.

Significant differences between methods of processing grains were found for whole vs rolled grains, steam flaked vs rolled grains, high moisture vs dry rolled corn and 25% vs 35% high moisture corn in the total tract, ruminal and intestinal disappearance of DM ($P<0.01$; Tables 7-9).

Galyean et al. (1981) reported values of ruminal dry matter disappearance of high moisture corn (24% moisture) of 25.1, 25.0, 26.4 and 29.6% at 2, 4, 6 and 8 hours of rumen incubation, respectively; those values agree closely with ours (19.4%) at 15 h. Lack of a continued increase reflects that digestion must have been completed in a very short time. Hibberd et al. (1985) measured 18 h in vitro DM disappearance of dry rolled sorghum grain. Although they found significant differences among three varieties, their in vitro digestion value for hetero-yellow (49.5) for exceeds both our ruminal and total tract disappearance values for rolled milo (17 and 46%, respectively).

Nalsen et al. (1987a) measured ruminal and total tract disappearance of DM from finely ground wheat and corn with the MDB. His values for both ruminal (56 and 38%) and total tract (90 and 60%) disappearance were greater than from our two rolled (46 and 19%; 80 and 46%) grains though the ranking of these two grains was similar.

Total tract dry matter disappearance in pigs of ground barley from MNB passed through the small plus large intestines ranged from 81 to 85% (Sauer et al., 1984). This also exceeded our total tract disappearance in anastomosed steers of 68%, possibly due to fermentation in the large intestine of the pigs. Fine grinding, as discussed in Chapter VI, also would increased extent of disappearance in the rumen and the small intestine.

Starch Digestion

Rolling of whole corn or oats increased (P<.05) extent of starch disappearance in the rumen, small intestine and total tract (Table 3). Compared to rolling the grain, steam processing increased (P<.05) starch disappearance from the rumen, small intestine and total tract for four grains tested (corn, milo, wheat, barley). Extent of starch digestion in the rumen was not changed by ensiling of rolled corn at 25% moisture, but with ensiling at 35% moisture, extents of ruminal and total tract starch disappearance were increased (P<.05) and intestinal disappearance tended to increase. Ruminal starch disappearance at 15 h ranked these 13 combinations of grains and processing methods corn as: 1) steam flaked corn, 2) steam flaked wheat, 3) steam flaked milo, 4) rolled oats, 5) rolled wheat, 6) steam rolled barley and 7) high moisture corn (35%). Though reasonably similar to the ranking of these grains in acidosis potential (Britton, 1986). Ruminal, starch disappearance of high moisture grain was considerably below the value expected. Perhaps a digestion time shorter than 15 h would more accurately predict acidosis potential or perhaps the higher moisture feeds increase the probability of acidosis by reducing salivation.

Significant differences between methods of processing grains were found for steam flaked vs rolled in the total tract and ruminal disappearance of starch (P<0.001); with respect to the total tract disappearance of starch all the

contrasts were significant different ($P<0.05$); no significant differences were found in the intestinal disappearance of the methods of processing the grains (Tables 10-12).

The quantity of starch disappearing in the small intestine (% of supply) within each grain increased as particle size was reduced (Tables 3 and 1, respectively) except for steam flaking of corn, because the flakes are flat not spherical in shape as the other grains are. With that feed, geometric mean diameter probably underestimates surface area due to the shape of the flake. But across feedstuffs, particle size was poorly related to intestinal starch digestion which was quite low for rolled oats (2096 um) and for several forms of corn (2329-4758 um) and rolled milo (1482 um), intermediate for barley (2418-2644 um) and flaked milo (1333 um) and highest for wheat (1865-2245 um) and processed corn (2430-3388 um; Tables 1 and 3). This ranking probably reflects differences in starch structure or accessibility among these grains. This ranking quite closely parallels expected rate of gain of feedlot cattle fed these grains.

Only one comparison for starch disappearance from mobile bags is available. Graham et al. (1985) found with ground samples that disappearance of starch in the total tract of pigs for barley was 99.9%. This is considerably greater than our values for barley grain which may be due to particle size as discussed in Chapter VI. One additional factor to consider is that our estimates of intesti-

nal and total tract disappearance in this study do not include disappearance from the large intestine whereas all the literature reports to date have permitted MDB to pass through the large intestine after leaving the small intestine.

Protein Digestion

Rolling of whole corn or oats greatly increased ($P<.05$) protein disappearance from MDB, both in the rumen (38 vs 4%; 52 vs 2%) and the total tract (60 vs 4%; 61 vs 3%; Table 4). Rolling of either corn or oats, also increased ($P<.05$) both the fractional and the total disappearance of protein from the small intestine in a manner similar to effects of rolling on dry matter and starch. Fractional disappearance of crude protein was very low compared to the theoretical true protein digestibility of most protein sources (90%; NRC 1976) or the mean intestinal digestibility of escape protein of 66% (NRC, 1985). Only with thoroughly processed grains were these values attained. Except for wheat and 25% moisture corn, more extensive processing and smaller particle size increased fractional protein disappearance in the small intestine. This is the first time that processing effects on postruminal protein disappearance has been reported. Differences suggest that previous estimates of true total digestibility by regression are subject to error (Owens, 1986b) and that in vivo intestinal disappearance values for

cereal grains maybe overestimated due to underestimation digestibility of microbial protein in the intestine.

Significant differences between methods of processing grains were found for whole vs rolled grains, steam flaked vs rolled, high moisture vs dry rolled corn and 25% vs 35% high moisture corn in the total tract disappearance of crude protein ($P<0.05$), steam flaked vs rolled grains were different in ruminal and intestinal disappearance of crude protein ($P<0.02$; Tables 13-15).

As compared with dry rolled grain, steam flaking greatly increased ruminal, intestinal (as a fraction of supply) and total tract disappearance of protein from corn and milo grains. With these grains, total tract disappearance was virtually complete. The amount of protein reaching the small intestine from the grain, however, was reduced by flaking. This contrasts with the suggestion of Hinman et al. (1974) that flaking denatures milo protein and increases ruminal escape. They attributed an increased post-ruminal protein flow to the grain alone and may not have accounted fully for increased microbial protein synthesis associated with flaking of milo. Flaking or steam rolling of wheat and barley had little impact compared to dry rolling on site or extent of protein disappearance from these grains. This parallels effects of processing on dry matter but not on starch with these grains. No explanation for this differential response among grains is apparent.

Published information on the effects of grain source and grain processing on site and extent of digestion is limited. The total tract apparent digestibility of nitrogen from whole and dry rolled corn was reported by Aguirre et al (1984) to be 69.4 and 70.9% respectively, compared with MDB losses of 4 and 60% for these corn forms in our anastomosed steers (Table 4) due presumably to differences in particle size and animal preparation. Hvelplund (1985) indicated that recovery of the MDB from the ileum gave 6% lower estimates for protein digestion than recovery of bags from the feces.

Four ground cereals (rye, triticale, wheat and barley) were tested in pigs using MDB by Graham et al. (1985). Crude protein disappearance from all four grains exceeded 94%. Only steam flaked corn and milo attained degradations that high in our trial, but again, grinding extent will alter protein exposure for digestion.

In situ N disappearance of rolled oats reported by Barrio et al. (1986) at 24 h was 78% compared with our value at 15 h of 51.5% (Table 4). Factors such as surface area, bag pore size and washing will affect the loss of particles and thereby of protein from dacron bags.

Processing to increase the surface area of the cereal for contact with the ruminal microbes and enzymes presumably is responsible for the extensive disappearance of crude protein from steam flaked corn and milo. The higher the amount of corneus endosperm (corn, milo) the greater

the response in protein disappearance to starch gelatinization and fine grinding.

Nalsen et al. (1987a) measured disappearance of wheat or corn from MDB in heifers. Ruminal disappearance of protein was 65.5% and 33.3% (vs 56 and 38% in our study) for these two grains, respectively. Total tract disappearance values for protein from ground wheat and ground corn noted by Nalsen et al. (1987a) were 96.9 and 91.5%, considerably higher than values of 90 and 60% for our rolled grains. Again this difference may be due to differences in particle sizes or to large intestinal fermentation as discussed by Hvelplund (1985). Another difference is that Nalsen et al (1987) adapted their heifers to the same grain that they tested with the MDB. In Chapter III, we could detect no interaction between diet fed and disappearance of the dry matter of the diet from MDB even though Loerch et al. (1983) reported an interaction of the N disappearance of protein sources and the dietary protein supplement. Though desirable, it does not seem practical to adapt each receptor animal to each feedstuff being studied in situ. Lindberg (1985) and J. Oldham (personal communication) proposed that an ideal diet for measuring in situ protein disappearance should contain 50% hay (10-12% crude protein) and 50% of a concentrate mix (17-18% crude protein), it appears that diet allow the most rapid development of a considerable variety of typical rumen microorganisms. Although their standard concentrate mix contains a wide variety of

feeds (barley, oats, dried sugar-beet pulp, and soybean meal), they seemed more concerned about standardizing ruminal conditions than about any potential effect of diet on in situ disappearance.

Rumen escape of protein was markedly altered by processing (Table 5). It ranged from under 5% of fed protein for steam flaked corn and steam flaked milo to over 40% of grain protein for dry rolled corn, wheat and steam rolled barley and over 90% for whole grains.

These protein escape values are much more variable and poorly match in vivo escape means (NRC, 1985) for corn (.65), sorghum grain (.52) and barley (.21). Values of escape of protein from the NRC (1985) and from Verite et al. (1979) are different at the results of Table 5. These do support the concept which our results would advance, namely that processing can markedly alter escape values. In vivo measurements must contend with extensive dilution of feed protein with microbial protein. In situ estimates, however, ignore possible differences in ruminal retention time of these feeds and in particle size changes which occur during mastication, rumination and dentrition.

Escape alone is an incomplete index of protein value as intestinal disappearance ranged from 0 to 100%. Moderate processing have the maximum supply of intestinally digestible protein.

In addition to feed protein, one must consider the supply of microbial protein potentially synthesized in the

rumen. Based on an assumption that 160 g of microbial crude protein (or 128 g of true protein of which equals 84 g digestible true protein) are formed per kg of dry matter fermented in the rumen, and that disappearance from MDB approximates ruminal fermentation, one can calculate total flow of total N and digestible true protein to the small intestine from these data as presented in Table 6. Differences among processed grains in expected total protein flow are surprisingly small. Because particle size limited ruminal disappearance of both DM and protein from MDB from unprocessed grains, the fraction of microbial protein varied.

Among the processed grains, flow of total protein to the small intestine calculates to range from .58 to 1.03 of that fed. The value tended to decrease as processing was more extensive. Intestinally digested protein, as a percent of crude protein fed among then processed grains ranged from 20 to 62%, increasing as corn was processed but decreasing as wheat was processed. The fraction of digested protein which was of microbial origin (% of microbial flow) calculated to range from 43 to 100%, for the processed grains being considerably greater for processed corn and milo than from wheat and barley. This should change amino acid composition and biological value of the intestinal protein.

In summary, in situ procedures previously have predicted ruminal protein escape quite well (Zinn and Owens,

1983). As in vivo measurement of protein escape from cereal grains is difficult and imprecise due to the low protein content of grains and extensive but variable degress of microbial dilution, some technique to predict escape is needed. The MDB technique is a reasonably simple method to estimate both ruminal protein escape as well as intestinal digestion of rumen escape protein. Greater verification of the technique is warranted, especially regarding the appropriate ruminal incubation time and the appropriate particle size of MDB contents.

TABLE 1. CHEMICAL COMPOSITION OF COMMERCIALLY PROCESSED CEREALS GRAINS

Feedstuff	Percent			Geometric diameter	
	Dry matter	Crude protein	Starch	um[a]	SD[b]
Corn					
whole shelled	88.8	9.9	49.5	4758	±1006
dry rolled	88.4	10.3	65.3	2785	± 823
25% moisture	75.0	12.0	53.7	2329	± 716
35% moisture	65.0	14.0	82.1	2430	± 737
steam flaked	83.8	10.5	53.5	3388	±1007
Milo					
rolled	87.0	10.8	55.3	1482	± 472
steam flaked	85.0	11.4	50.7	1333	± 444
Oats					
whole	91.8	13.3	29.0	2438	± 655
rolled	91.5	12.0	31.7	2096	± 639
Wheat					
rolled	89.7	15.7	42.7	1865	± 576
steam flaked	88.1	15.9	44.9	2245	± 682
Barley					
rolled	88.7	14.1	39.2	2418	± 722
steam rolled	88.2	15.0	40.6	2644	± 780
Diet	91.4	19.0	36.5	1922	± 624

[a] Mean (um = Microns).
[b] Standard Deviation.

TABLE 2. EFFECT OF PROCESSING IN THE DRY MATTER DISAPPEARANCE OF GRAINS FROM MOBILE DACRON BAGS

Feedstuff	Mobile bag disappearance site: Ruminal (15H)	Total tract	Small intestine by difference	% of supply
	% Disappearance			
Corn				
whole shelled	3.8^{g}	4.8^{g}	1.0^{g}	1.0
dry rolled	19.1^{ef}	46.1^{e}	27.0^{e}	33.4
25% moisture	19.4^{ef}	56.7^{d}	37.3^{cd}	46.3
35% moisture	27.2^{cd}	66.3^{c}	39.1^{cd}	53.7
steam flaked	49.1^{a}	86.5^{a}	37.4^{cd}	73.5
Milo				
rolled	16.9^{ef}	45.9^{e}	29.0^{e}	34.9
steam flaked	33.8^{bc}	82.0^{ab}	48.2^{b}	74.3
Oats				
whole	0.1^{g}	4.1^{g}	4.0^{g}	4.0
rolled	15.2^{f}	30.4^{f}	15.2^{f}	17.9
Wheat				
rolled	46.1^{a}	79.9^{b}	33.8^{ed}	62.7
steam flaked	27.3^{cd}	86.5^{a}	59.2^{a}	81.4
Barley				
rolled	23.3^{ed}	67.9^{c}	44.6^{bc}	58.1
steam rolled	22.7^{ed}	68.3^{c}	45.6^{bc}	59.0
Diet	35.0^{b}	68.4^{c}	33.4^{de}	51.4
SE[h]	2.00	2.00	3.00	

^{abcdefg} Means in a column with different superscripts differ statistically ($P<.05$).

[h] Standard Error of the Means.

TABLE 3. EFFECT OF PROCESSING IN THE STARCH DISAPPEARANCE OF GRAINS FROM MOBILE DACRON BAGS

Feedstuff	Mobile bag disappearance site			
	Ruminal (15H)	Total tract	Small intestine difference	% of supply
	% Disappearance			
Corn				
Whole shelled	0.0^{h}	7.4^{g}	7.4^{b}	7.4
Dry rolled	9.4^{f}	20.4^{f}	11.0^{b}	12.1
25% Moisture	4.0^{h}	11.6^{g}	7.6^{b}	7.9
35% Moisture	21.7^{e}	56.3^{a}	34.6^{b}	44.2
Steam flaked	49.4^{b}	82.5^{a}	33.1^{b}	65.4
Milo				
rolled	3.5^{h}	15.0^{f}	11.5^{b}	11.9
steam flaked	36.5^{d}	56.9^{b}	20.4^{b}	32.1
Oats				
whole	18.8^{f}	21.2^{f}	2.4^{c}	3.0
rolled	26.4^{e}	31.6^{e}	5.2^{c}	7.1
Wheat				
rolled	24.7^{e}	56.6^{b}	31.9^{b}	42.4
steam flaked	44.3^{c}	87.6^{a}	43.3^{a}	77.7
Barley				
rolled	17.1^{f}	38.8^{d}	21.7^{b}	26.2
steam rolled	21.8^{e}	47.4^{c}	25.6^{b}	32.7
Diet	82.6^{a}	95.0^{a}	12.4^{b}	71.3
SEM[1]	8.5	4.6	10.5	

^{abc} Means in a column with different superscripts differ statistically ($P<.05$).

[i] Standard Error of the Means.

TABLE 4. EFFECT OF PROCESSING IN THE CRUDE PROTEIN DISAPPEARANCE OF GRAINS FROM MOBILE DACRON BAGS

Feedstuff	Mobile bag disappearance site			
	Ruminal (15h)	Total tract	Small intestine by difference	% of supply
	% Disappearance			
Corn				
whole shell	3.5^{f}	3.8^{g}	0.3^{e}	0.3
dry rolled	38.3^{d}	60.0^{ef}	21.7^{bcd}	35.1
25% moisture	72.2^{b}	74.4^{c}	2.2^{e}	7.9
35% moisture	90.4^{a}	100.0^{a}	9.6^{de}	100.0
steam flaked	99.0^{a}	100.0^{a}	1.0^{e}	100.0
Milo				
rolled	60.4^{c}	75.8^{c}	15.4^{cde}	38.9
steam flaked	97.9^{a}	100.0^{a}	2.1^{e}	100.0
Oats				
whole	2.5^{f}	2.6^{g}	0.1^{e}	0.1
rolled	51.5^{c}	61.3^{ef}	9.8^{ed}	20.2
Wheat				
rolled	55.6^{c}	89.8^{b}	34.2^{ab}	77.0
steam flaked	55.6^{c}	76.8^{c}	21.2^{bcd}	47.7
Barley				
rolled	57.2^{c}	72.8^{cd}	15.6^{cde}	36.4
steam rolled	52.6^{c}	77.9^{c}	25.3^{bc}	53.4
Diet	22.4^{e}	68.5^{cde}	46.1^{a}	59.4
SEM[i]	3.2	3.1	4.6	

^{abc} Means in a column with different superscripts differ statistically ($P<.05$).

[i] Standard Error of the Means.

TABLE 5. EFFECT OF PROCESSING IN THE PROTEIN BYPASS OF GRAINS USING MOBILE DACRON BAGS

	Escape % of protein	Intestinal digestion %of escape	Escape intestinally digested protein % of protein
Corn			
whole shelled	96.5	0.3	0.3
dry rolled	61.7	35.1	21.7
25% moisture	27.8	7.9	2.2
35% moisture	9.6	100.0	9.6
steam flaked	1.0	100.0	1.0
Milo			
rolled	39.6	38.9	15.4
steam flaked	2.1	100.0	2.1
Oats			
whole	97.5	0.1	0.1
rolled	48.5	20.2	9.8
Wheat			
rolled	44.4	77.0	34.2
steam flaked	44.4	47.7	21.2
Barley			
rolled	42.8	36.4	15.6
steam rolled	47.4	53.4	25.3
Diet	77.6	59.4	46.1

TABLE 6. EXPECTED FLOWS OF TOTAL AND DIGESTIBLE PROTEIN TO THE SMALL INTESTINE PER KILOGRAM OF GRAIN DM CONSUMED

	Flow total protein				Flow digestible protein				
	Microbial[a]	Grain[b]	Total	% of fed	Microbial[c]	Grain	Total	% of fed	% of microbial flow
Corn									
whole shelled	6	84	90	102	3	3	6	7	50
dry rolled	30	56	94	103	16	20	3	40	44
25% Moisture	31	25	56	62	16	2	18	20	89
35% Moisture	44	9	53	58	23	9	32	35	72
steam flaked	79	0	79	90	42	0	42	48	100
Milo									
rolled	27	37	64	68	14	14	28	30	50
steam flaked	54	2	56	58	29	2	31	32	94
Oats									
whole	0	119	119	98	0	1	1	1	0
rolled	24	53	97	88	13	11	24	22	54
Wheat									
rolled	74	63	137	97	39	48	87	62	45
steam flaked	44	62	106	76	23	30	53	38	43
Barley									
rolled	37	54	91	73	20	19	39	31	51
steam rolled	36	63	99	75	19	33	52	39	37

[a] Calculated as ruminal dry matter disappearance times 160g microbial cells per kg digested in the rumen.

[b] Ruminal escape times protein content of feed DM (g/kg).

[c] Microbial crude protein flow times .8 g true protein/g crude protein times .66g digested protein/g true protein.

TABLE 7. ANALYSIS OF VARIANCE FOR CONTRASTS FOR RUMINAL DISAPPEARANCE OF DRY MATTER

Contrast	DF	Contrast SS	MS	F	Pr > F
Whole vs rolled grains (1)	1	0.19394	0.19394	47.97	0.0001
Corn vs oats (2)	1	0.23943	0.23943	59.23	0.0001
Interaction 1*2	1	0.00145	0.00145	0.36	0.5500
Steam flaked vs rolled (3)	1	0.31250	0.31250	77.30	0.0001
Grains (4)	2	0.36555	0.18277	45.21	0.0001
Interaction 3*4	2	0.19977	0.09988	24.71	0.0001
High moisture vs rolled	1	0.20953	0.20953	51.83	0.0001
Between high moisture corn	1	0.14163	0.14163	35.04	0.0001

TABLE 8. ANALYSIS OF VARIANCE FOR CONTRASTS FOR TOTAL TRACT DISAPPEARANCE OF DRY MATTER

Contrast	DF	Contrast SS	MS	F	Pr > F
Whole vs rolled grains (1)	1	0.77882	0.77882	358.36	0.0001
Corn vs oats (2)	1	1.74836	1.74836	804.48	0.0001
Interaction 1*2	1	0.01917	0.01917	8.82	0.0037
Steam flaked vs rolled (3)	1	1.13231	1.13231	521.02	0.0001
Grains (4)	2	1.41841	0.70920	326.33	0.0001
Interaction 3*4	2	0.60756	0.30378	139.78	0.0001
High moisture vs rolled	1	0.73941	0.73941	340.23	0.0001
Between high moisture corn	1	0.01737	0.01737	8.00	0.0057

TABLE 9. ANALYSIS OF VARIANCE FOR CONTRASTS FOR INTESTINAL DISAPPEARANCE OF DRY MATTER

Contrast	DF	Contrast SS	MS	F	Pr > F
Whole vs rolled grains (1)	1	0.19547	0.19547	33.56	0.0001
Corn vs oats (2)	1	0.69378	0.69378	119.10	0.0001
Interaction 1*2	1	0.01007	0.01007	1.73	0.1916
Steam flaked vs rolled (3)	1	0.25510	0.25510	43.79	0.0001
Grains (4)	2	0.54264	0.27132	46.58	0.0001
Interaction 3*4	2	0.30160	0.15080	25.89	0.0001
High moisture vs rolled	1	0.16171	0.16171	27.76	0.0001
Between high moisture corn	1	0.25823	0.25823	44.33	0.0001

TABLE 10. ANALYSIS OF VARIANCE FOR CONTRASTS FOR RUMINAL DISAPPEARANCE OF STARCH

Contrast	DF	Contrast SS	MS	F	Pr > F
Whole vs rolled grains (1)	1	143.99045	143.99045	1.00	0.3354
Corn vs oats (2)	1	641.89445	641.89445	4.44	0.0537
Interaction 1*2	1	1.56645	1.56645	0.01	0.9186
Steam flaked vs rolled (3)	1	2863.81203	2863.81203	19.79	0.0006
Grains (4)	2	432.97380	216.48690	1.50	0.2577
Interaction 3*4	2	217.76046	108.88023	0.75	0.4893
High moisture vs rolled	1	16.24013	16.24013	0.11	0.7426
Between high moisture corn	1	316.12840	316.12840	2.18	0.1615

TABLE 11. ANALYSIS OF VARIANCE FOR CONTRASTS FOR TOTAL TRACT DISAPPEARANCE OF STARCH

Contrast	DF	Contrast SS	MS	F	Pr > F
Whole vs rolled grains (1)	1	274.01405	274.01405	6.48	0.0233
Corn vs oats (2)	1	312.50000	312.50000	7.39	0.0166
Interaction 1*2	1	3.15005	3.15005	0.07	0.7889
Steam flaked vs rolled (3)	1	6075.45000	6075.45000	143.65	0.0001
Grains (4)	2	2634.32261	1317.16130	31.14	0.0001
Interaction 3*4	2	499.59361	249.79680	5.91	0.0138
High moisture vs rolled	1	246.43203	246.43203	5.83	0.0301
Between high moisture corn	1	1998.09000	1998.09000	47.24	0.0001

TABLE 12. ANALYSIS OF VARIANCE FOR CONTRASTS FOR INTESTINAL DISAPPEARANCE OF STARCH

Contrast	DF	Contrast SS	MS	F	Pr > F
Whole vs rolled grains (1)	1	20.73680	20.73680	0.09	0.7645
Corn vs oats (2)	1	58.64445	58.64445	0.26	0.6155
Interaction 1*2	1	0.27380	0.27380	0.00	0.9725
Steam flaked vs rolled (3)	1	596.85307	596.85307	2.69	0.1235
Grains (4)	2	998.15351	499.07675	2.25	0.1426
Interaction 3*4	2	98.60685	49.30342	0.22	0.8038
High moisture vs rolled	1	136.14803	136.14803	0.61	0.4469
Between high moisture corn	1	724.68640	724.68640	3.26	0.0925

TABLE 13. ANALYSIS OF VARIANCE FOR CONTRASTS FOR RUMINAL DISAPPEARANCE OF CRUDE PROTEIN

Contrast	DF	Contrast SS	MS	F	Pr > F
Whole vs rolled grains (1)	1	1290.28950	1290.28950	32.10	0.0001
Corn vs oats (2)	1	2894.86503	2894.86503	72.03	0.0001
Interaction 1*2	1	1535.02146	1535.02146	38.19	0.0001
Steam flaked vs rolled (3)	1	2195.88105	2195.88105	54.64	0.0001
Grains (4)	2	2487.42228	1243.71114	30.94	0.0001
Interaction 3*4	2	1231.80952	615.90473	15.32	0.0001
High moisture vs rolled	1	62.63907	62.63907	1.56	0.2190
Between high moisture corn	1	0.00333	0.00333	0.00	0.9928

TABLE 14. ANALYSIS OF VARIANCE FOR CONTRASTS FOR TOTAL TRACT DISAPPEARANCE OF CRUDE PROTEIN

Contrast	DF	Contrast SS	MS	F	Pr > F
Whole vs rolled grains (1)	1	1017.52706	1017.52706	25.58	0.0001
Corn vs oats (2)	1	3688.60660	3688.60660	92.74	0.0001
Interaction 1*2	1	274.45886	274.45886	6.90	0.0121
Steam flaked vs rolled (3)	1	599.25523	599.25523	15.07	0.0004
Grains (4)	2	1483.74486	741.87243	18.65	0.0001
Interaction 3*4	2	860.61594	430.30797	10.82	0.0002
High moisture vs rolled	1	150.51644	150.51644	3.78	0.0586
Between high moisture corn	1	340.22404	340.22404	8.55	0.0056

TABLE 15. ANALYSIS OF VARIANCE FOR CONTRASTS FOR INTESTINAL DISAPPEARANCE OF CRUDE PROTEIN

Contrast	DF	Contrast SS	MS	F	Pr > F
Whole vs rolled grains (1)	1	16.17567	16.17567	0.19	0.6638
Corn vs oats (2)	1	48.02420	48.02420	0.57	0.4549
Interaction 1*2	1	511.32741	511.32741	6.06	0.0181
Steam flaked vs rolled (3)	1	500.88851	500.88851	5.94	0.0193
Grains (4)	2	1179.49660	589.74830	6.99	0.0024
Interaction 3*4	2	121.86999	60.93499	0.72	0.4918
High moisture vs rolled	1	407.35346	407.35346	4.83	0.0337
Between high moisture corn	1	338.09750	338.09750	4.01	0.0520

CHAPTER VI

INFLUENCE OF PARTICLE SIZE OF DRY ROLLED CORN ON RUMINAL AND POSTRUMINAL DISAPPEARANCE USING THE MOBILE DACRON BAGS TECHNIQUE

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ABSTRACT

Two dairy calves (150 kg) equipped with duodenal T cannulas and ileo-rectal anastomoses were fed (2.5% of body weight) an 80% concentrate diet. Site and extent of disappearance of dry matter, crude protein and starch from dry rolled corn sieved to obtain particle sizes of <125, 125-250, 250-500, 500-1000 and 1000-2000 microns were measured using mobile dacron bags (MDB) procedures. Disappearance was measured : A) after 15 h of rumen incubation, B) after A plus 3 h pepsin - HCl digestion, insertion in the duodenal cannula and recovery from feces and C) after passage through the intestines only. Total tract, ruminal and small intestinal disappearance of dry matter, starch and crude protein all were greater ($P<.05$) for smaller particle sizes. Particles greater than 500 microns diameter lost no

starch during postruminal passage; their extent of intestinal disappearance of dry matter and protein was less than half that of smaller (<250 microns) particles. Prefermentation of larger particles in the rumen tended to increase intestinal disappearance of dry matter and starch but to decrease intestinal disappearance of protein. Total tract dry matter disappearance was positively correlated ($r = .90$, $P<.05$) with intestinal residence time. To optimize supply of intestinally digested starch and protein, the optimal sizes for corn particles were 125 to 250 microns and 250 to 1000 microns, respectively. Results can be interpreted to indicate that particle size can alter the site and extent of disappearance of dry rolled corn.
(Key Words: Mobile Dacron Bag, Protein Digestion, Starch Digestion, Cereals, Intestinal Transit Time, Intestine Bypass.)

Introduction

The mobile dacron bag (MDB) technique has been used to evaluate digestibilities of various protein sources and cereal grains with pigs (Sauer et al., 1983; Cherian et al., 1985; Graham et al., 1985; Taverner and Campbell, 1985; de Lange et al., 1986) and to test ruminal degradation of different protein sources (Hvelplund et al., 1985; Rooke, 1985; Voigt et al., 1985; Deacon et al., 1986; Kennelly and de Boer, 1986; de Boer et al., 1987; Kirkpatrick and Kennelly, 1987; Nalsen et al., 1987; Robinson

et al., 1987). Many of these reports provide information on disappearance of DM, and of crude protein, but no information on starch disappearance has been published.

Though enzyme limitations and residence time can limit extent of starch digestion in the small intestine of ruminants, particle size also appears important (Owens et al., 1986b). It is well known that several variables alter in vitro starch disappearance. These include diet of the donor animal, retention time and grinding or processing of the feed samples. To determine extent of digestion, a 48 h in vitro incubation period has been used most in ruminant nutrition laboratories to predict extent of digestion. Considering that ruminal retention time usually is only 15 to 24 h, a shorter time than 48 h seems more appropriate for grains (Kirkpatrick and Kennelly, 1984). They used 15 h for ruminal (in situ) measurements.

Weakley et al. (1983) used dacron bags to study dry matter and nitrogen disappearance of soybean meal and distillers grain samples. Disappearance was greater from samples which were pulverized. Galyean et al. (1981) separated corn with sieves into fractions with mean particle sizes of 6000, 3000, 1500 and 750 microns. Extent of 24 h in situ ruminal disappearance of DM from grain particle sizes above 1500 microns was very low; extent of disappearance increased as particle decreased. Ruminal starch disappearance paralleled dry matter disappearance. Kim and Owens (1985) studied the effect of particle size on corn

digestion in cannulated steers. Duodenal, ileal and fecal matter were sieved. The smaller particle sizes disappeared more extensively during postruminal digestion. As particle size increased, total tract digestibility decreased. Most starch particles over 2000 microns which passed from rumen were recovered in feces undigested. To maximize ruminal escape and postruminal disappearance, particle sizes between 1000 to 2000 microns appeared most desirable. Postruminal disappearance of starch from such particles was 94%. Because these results were calculated from disappearance of starch from particles retrieved from various sites, they are subject to large sampling errors. Changes in particle size, also, cannot be quantitated readily by digesta sampling alone. The mobile dacron bag procedure obviates these errors and permits one to examine the effect of particle size on extent of digestion more directly.

The objective of this experiment was to determine the influence of particle size of dry rolled corn on extent of in situ disappearance of dry matter, protein and starch. Disappearance in the rumen, in the rumen plus small intestine and in the small intestine alone was measured. Fermentation in the large intestine was avoided by equipping calves with an ileo-rectal anastomosis. The effects of residence time in the small intestine upon disappearance also was estimated.

Materials and Methods

Two Holstein calves (150 kg) vaccinated against IBR, PI_3, and four-way blackleg and dewormed with Ivermectin were fitted with duodenal cannulas. They also were equipped with an anastomosis between the distal ileum and the distal rectum to bypass the large intestine and flow directly from the small intestine to the rectum for defecation as described in Chapter V. These calves were housed in single pens with a flooring of iron screen and hardware cloth with openings 5 cm by 1 cm (Sherman wire 1300 E. Pacific St., Sherman, TX 75090) to simplify collection of defecated mobile dacron bags. Calves were fed a diet composed of 20.1% dry rolled corn, 20% chopped prairie hay, 19.8% dry rolled sorghum grain, 13.8% soybean meal, 12.6% dehydrated alfalfa hay crumbles, 5.9% molasses, 5.9% dry ground wheat, 0.9% dicalcium phosphate, and 1% salt plus mineral supplement (94.5% sodium chloride, 3.2% of magnesium oxide, 1.8% potassium chloride, 0.25% manganous oxide, 0.2% iron oxide, 0.033% copper oxide, 0.007% calcium iodate, 0.005% zinc oxide and 0.005% cobalt carbonate). Potassium and magnesium were added because fecal loss of these minerals was elevated by the large intestinal bypass. The salt plus trace mineral supplement was added to the diet at feeding. Animals were adjusted to the diets for a period of 7 days and were fed at 2.5% of body weight [dry matter (DM) basis] in two equal feedings at 0800 and 1600 hours. Feed samples were collected during the trial for

analysis for dry matter, crude protein and starch. The Calves had free access to fresh water.

Mobile Dacron Bag (MDB) Technique

Site of disappearance of dry matter, protein and starch from the mobile dacron bags was measured by three different methods. For ruminal disappearance, bags were incubated in the rumen for 15 h. For total tract disappearance, bags removed from the rumen were soaked in a pepsin-HCl digestion mixture for 3 h, placed into the duodenum and recovered from feces. For small intestinal disappearance, bags neither pre-incubated in the rumen nor pre-digested with pepsin-HCl, were placed into the duodenum and recovered from feces.

The dacron bags, measuring 3.5 by 5.5 cm, were constructed of dacron cloth with a mesh size of 60 to 70 microns. Approximately 2.0 g of dry rolled corn of the specified particle size was placed in each bag after which each bag was sewn closed and the sewing holes were glued shut. To reduces abrasion of the intestine, the bags were trimmed with rounded corners as described by Sauer et al. (1983). To attain various particle sizes, dry rolled corn was sieved through a series of screens to obtain particle sizes with five different diameters: <125, 125-250, 250-500, 500-1000 and 1000-2000 microns (um). Eighteen bags containing each particle size were inserted into nylon

stockings, soaked in water at 39 C for 3 minutes and incubated for 15 h in the rumen of a 400 kg cannulated beef steer fed the diet described above. Upon removal from the rumen, six bags of each size were washed thoroughly, dried and analyzed. The other twelve bags were incubated in a pepsin-HCl solution [1g pepsin (570 units per mg protein) per liter of .1N HCl adjusted to pH 2] at 38 C for 3 h with constant stirring as described by Kirkpatrick and Kennelly (1984). After pepsin digestion, each bag was refrigerated at 4 C until it could be inserted into the small intestine via the doudenal cannula. Bags were inserted at a rate of one bag each 30 min. As bags were defecated, they collected on the hardware cloth. Each bag collected was washed by hand under cold running tap water until the wash water was colorless using a procedure identical to that described by Weakley et al. (1983). All washed bags were dried in a forced air oven at 90 C for 24 h. Feed samples and bags containing residues were subjected to dry matter, Kjeldahl nitrogen (AOAC, 1975) and starch (MacRae and Armstrong, 1968) analysis procedures. Blank bags containing a known weight of sliced Tygon tubing were used to correct for influx of non-adherent nitrogen and starch. Bags plus residual contents were weighed and were digested intact for Kjeldahl nitrogen analysis.

Because washing should remove endogenous secretions and a portion of the bacteria in the bags, results of this procedure should more closely approximate true than appar-

ent disappearance of dry matter and crude protein and may exceed apparent digestion values (de Boer et al., 1987). For starch (total alpha glucosan), apparent and true digestibilities should be equal.

Statistical Analysis

Extents of dry matter, crude protein and starch disappearance in the rumen, total tract and postruminally, both as a percent of the diet and a percent of duodenal flow, were tested by analysis of variance using animal and particle size in the statistical model. When differences due to particle were significant ($P<.05$), means were compared by a Duncan's multiple range test (Steel and Torrie, 1980).

The effect of intestinal residence time on the disappearance of dry matter, crude protein and starch from MDB was tested for linear, quadratic, cubic and quartic effects of time using regression analysis (Steel and Torrie, 1980) with animal and particle size included as class variables.

Results and Discussion

Dry Matter Disappearance

Disappearance of the dry matter from MDB in the rumen, total tract and in the small intestine, both of predigested (calculated by difference) and samples not predigested is presented in Table 1. Extent of DM disappearance in the rumen increased linearly as particle size was reduced

(Figure 1). This closely matches the ruminal disappearance reports of by McLeod and Minson (1969), Teeter et al. (1980), Galyean et al. (1981), and Kim and Owens (1985). As some particles inevitably are lost through pores in the bags, and such loss should be greater for smaller sized particles, these results are subject to some bias though loss should be minimal except for very small particles as the bag pore size was 60 to 70 um.

Small intestinal disappearance of DM, both from undigested and pre-digested particles, was inversely related to particle size. This confirms the suggestion of Kim and Owens (1985). Disappearance of DM in cases was higher for predigested than undigested particles. Less extensive digestion of the pre-digested particles does not support the concept that the smaller, more digestible particles are preferentially attacked in the rumen leaving only the more resistant material to flow to the intestine. Instead, the results can be interpreted to suggest that ruminal or abomasal digestion increased susceptibility to intestinal digestion, perhaps by removing some barrier(s) to digestion.

The unsieved dry rolled corn had a geometric mean diameter of 2282 ± 700 um, the largest particle size. It also had the lowest DM disappearance (Table 1) both in rumen and in the total tract (18.5 and 45.5%, respectively).

Starch Disappearance

Ruminal and postruminal disappearance of starch from corn grain sieved to various size particles is presented in Table 2. Starch from smaller particle sizes disappeared to a much greater degree not only in the rumen, but also in the small intestine and total tract. This is presumably due to an increase in the amount of surface area exposed for microbial or enzymatic attack.

As with DM, small intestinal digestion of starch from corn tended to be increased by fermentation in the rumen or in pepsin. Whether this is due to greater wetting, to fermentation or to pepsin attack is unknown. Hvelplund (1985) indicated that if the pepsin solution has a high pH, postruminal protein digestion from MDB will be reduced.

Feedlots using dry rolled grain would never grind feedstuffs as finely as described in this paper, but with steam flaking, exposure of starch should approach that of small particles used in this study. The particle sizes of this study should be compared not to grain as it is fed, but instead to grain particles as they reaches the sites of fermentation or digestion following mastication, rumination and dentrition. The low disappearance values for ruminal and postruminal disappearance of large particles indicate that extent of digestion is extremely low for unchewed particles. This concept is supported, by the observation that much of the fecal starch from steers fed whole or rolled

corn diets is found in large particles (Teeter et al., 1980).

Values for intestinal disappearance of starch (Table 2), between 0.5% and 39.2%, are unduly low when compared with estimates of 67% postruminal disappearance of starch from ground corn (Owens et al., 1986b), 82% for intestinal digestion of starch from corn (Theurer, 1986) and 95% intestinal degradation of starch (Spicer et al., 1986). Reasons for this discrepancy are not apparent though our values are for grain which bypassed fermentation of the large intestine whereas these in vivo values would include loss from both the small and large intestine.

The results of Table 2 support the concept that particle size can limit the extent of postruminal digestion of starch as suggested previously by Teeter et al. (1979), Teeter et al. (1980) and Rust (1983). Nevertheless, with processed grains, other factors also might become limiting (Ørskov, 1986; Theurer, 1986).

If the energy value of starch digested in the small intestine exceeds that of starch fermented in the rumen (Owens et al., 1986b; Brethour, 1987), grain processing to a specific size might be employed to shift the degradation from the rumen to the small intestine. Assuming relative metabolizable energy values of .8 and 1.0 for ruminal and small intestinal digestion, one can calculate values for grain of the various sizes as presented in Table 2. According to these calculations, particles between 125 and

250 microns should supply the greatest amount of metabolizable energy. Particles under 125 microns were not as fully fermented in the rumen, or the total tract whereas larger particles were not extensively attacked in either the rumen or the small intestine and, thereby, had lower total tract disappearance. Reasons for the low ruminal disappearance of starch from the smallest particles are not apparent. Feeding corn whole to increase the intestinal supply will be counterproductive if total tract digestibility is sacrificed. The particle size which limits access of intestinal enzymes appears to be greater than the size which limits microbial access as might be expected. Microbial enzymes in the rumen are primarily found closely associated with microbial cell surfaces whereas amylase in the intestine should enter all spaces that fluid can penetrate.

Protein Disappearance

Disappearance of the protein in the rumen and postruminally changed quadratically with particle size, but generally was greater for smaller particles. These trends agree with the report of Weakley et al. (1977) which noted that rate of in situ digestion in the rumen of soybean meal was much lower for coarse than for fine soybean meal. Yet, rate of ruminal digestion may be not related to its extent. Netemeyer et al. (1980) found that particle size of soybean meal had little effect on ruminal protein escape or total tract protein digestibility, presumably due to longer rumi-

nal retention of the larger particles of soybean meal.

Extent of ruminal escape tended to be greater for larger particles and tended to be decreased by pre-fermentation in the rumen. However, the impact of particle size on intestinal protein digestion, though detectable, was less dramatic than its effect on starch digestion. As for starch, intestinal disappearance of protein was very low compared with an expected true digestibilities (90%; NRC, 1976) and an intestinal N disappearance average across feedstuffs of 68% (NRC, 1985). Except for spurious values for 125 to 250 um particles, intestinal digestion of pre-fermented and unfermented grain increased as particle size decreased indicating that particle size can limit extent of protein digestion in the small intestine. Despite this decrease, supply of intestinally digested protein tended to be greater for larger (250 - 1000 um) than for smaller corn particles. One potential benefit from feeding corn in a larger particle size is that it may increase ruminal escape of postruminally digested protein.

Small Intestinal Transit Time

A second factor which could limit digestion in the small intestine is time for digestion. The residence time of dacron bags filled with sieved dry rolled corn in steers with an ileal-rectal anastomosis varied from less than 10 h to 29.5 h.(Table 4). The general mean for all the treatments between insertion into the duodenum and

collection in the feces was 14.7 ± 2.0 h (n=72) thought the mode was only 12 h.

The transit time of liquid and particles through the small intestine of steers was measured by Owens et al. (1986a) by recording the time of ileal appearance of a duodenally dosed dye. They reporting a mean residence time of 4.5 h for steers fed various diets. Hvelplund (1985) using dacron bags in sheep reported that the passage time of bags between a duodenal T cannula and a ileal T cannula was 5.3 h. Retention times for bags in our trial longer than 5 h must reflect an abnormally slow intestinal transit or retention of bags in the terminal rectum. Only 6% of our bags had residence times under 5.5 h.

With the exception of the two bags retained over 25 h, dry matter, starch and crude protein disappearance increased as residence time was extended (Table 4). The magnitude of these regressions ($P<.05$) indicate that increasing small intestinal residence time should increase extent of energy, starch or protein digestion. Such a change by reducing dietary fiber levels (Owens et al., 1986a) or suppressing intestinal passage rate with drugs or hormones. Voigt et al. (1985) noted with nylon bags that disappearance of dry matter increased with retention time in the small plus large intestine but he did not observe a similar effect for crude protein.

Regression analyses for small intestinal disappearance of dry matter, crude protein and starch vs residence time

(T) in hours of the MDB in the small intestine gave the following regression equations :

Small intestinal DM disappearance

DM disappearance (%) = $12.0 + 14.8T - 2.0T^2$;

($r=.90$, $P<.05$).

Small intestinal crude protein disappearance

CP disappearance (%) =

$104.1 - 21.2\ T - 2.7\ T^2 - 0.1\ T^3 - 0.002\ T^4$;

($r=.91$, $P<.001$).

Small intestine starch disappearance

Starch disappearance (%) =

$-550.2 - 150.2\ T - 14.8\ T^2 - 0.6\ T^3 - 0.009\ T^4$.

($r=.88$, $P<.05$).

As noted in Tables 2 and 3, disappearance from unsieved grain was generally lower than any of the sieved fractions. To determine how the values for unsieved grain check against the value one would expect based on its component particle sizes, fractions in each size were multiplied by disappearance for each size and summed. Percentages of each particle size were 0.30, 1.40, 3.30, 11.00, 43.30 and 41.00 for fractions < 125, 125 to 250, 250 to 500, 500 to 1000, 1000 to 2000 and > 2000 microns. It was assumed that the small fraction of particles larger than 2000 microns in diameter had a degradation rate half that of the 1000-2000 um particles. The results observed and values predicted from component particles matched quite well (Table 5). The poorest predictions were for intestinal and total tract

disappearance which were starch underestimated. Apparently, presence of a variety of particle sizes did not markedly alter disappearance of components from MDB.

To optimize extent of digestion at some specific site, one can employ estimates determined in this study. Particle sizes which maximize specific factors for corn from MDB are presented in Table 6. For dry matter, disappearance in the rumen was numerically highest for particles between 125 and 250 um whereas postruminal and total tract disappearance were greatest for smaller (<125 um particles. Postruminal supply of DM was greatest with particles between 1000 and 2000 um, but total supply of postruminally digested DM was equally high for all particles under 500 um.

Extent of ruminal, postruminal and total tract digestion of starch was highest for particles in the 125 to 250 um size range. Even though postruminal supply was greatest for 500 to 1000 um particles, the highest postruminal supply of digestible starch was from 125 to 250 um particle sizes. For protein; ruminal, postruminal and total tract disappearance were highest for the smallest particles (<125 um), but the calculated supply of postruminally digested protein was greater for larger particles (250-1000 um). Thus, some sacrifice in ruminal and total tract digestion is necessary to shift site of digestion to the intestines.

These differences can be extrapolated to various feeding conditions. If energy digestible limits performance,

the smaller particle sizes of corn would be ideal providing acidosis is avoided. If postruminal digestion of starch is energetically more efficient than ruminal digestion particles up to 250 um are useful, and if postruminal protein supply limits intake or performance, particles up to 1000 microns are desirable.

In conclusion, reducing particle size increases extent of digestion in rumen, and total tract of dry matter, crude protein and starch. Particle size also limits extent of digestion of material flowing through the small intestine. Our results can be interpreted to indicate that particle size can limit extent of digestion of dry rolled corn both in the rumen and in the small intestine and that some sacrifice in digestibility is needed to shift nutrient digestion postruminally.

TABLE 1. EFFECT OF PARTICLE SIZE ON THE DRY MATTER DISAPPEARANCE OF DRY ROLLED CORN FROM MOBILE DACRON BAGS

Treatment	Mobile Dacron Bag Disappearance Site				
	Ruminal (15H)	Total tract	Small intestine		
			% of diet	% of duodenal supply	Not prefermented
Particle Diameter	% Disappearance				
2000-1000 um	20.0[d]	55.2[d]	35.2	44.0	22.7[e]
1000-500 um	37.0[c]	74.3[c]	37.3	59.2	49.5[c]
500-250 um	39.6[bc]	85.0[b]	45.4	75.1	66.8[b]
250-125 um	53.5[a]	90.6[a]	37.1	79.8	85.5[a]
<125 um	44.9[b]	92.6[a]	47.7	86.6	87.9[a]
Not sieved[f]	18.5[d]	45.5[e]	27.0	33.1	33.4[d]
S.E.[g]	2.1	1.5	2.7	2.7	2.8

[abcde] Means in a column with different superscripts differ (P<.05).

[f] Geometric mean diameter measured to be 2282 ± 700 um.

[g] Standard Error of the Means.

TABLE 2. EFFECT OF PARTICLE SIZE ON STARCH DISAPPEARANCE OF DRY ROLLED CORN FROM MOBILE DACRON BAGS

Treatment	Mobile Dacron Bag Disappearance Site					
	Ruminal (15H)	Total tract	Small intestine			
			% of diet	% of duodenal supply	Not prefermented	Value[d]
Particle diameter	% Disappearance					
2000-1000 um	5.0[b]	0.4[c]	0	0	0.5[b]	4.0
1000-500 um	3.6[b]	1.3[c]	0	0	2.2[b]	2.9
500-250 um	6.7[b]	43.8[ab]	37.1	39.8	10.0[b]	42.5
250-125 um	27.1[a]	73.3[a]	46.2	63.4	10.0[b]	67.9
<125 um	21.7[a]	55.7[a]	34.0	43.4	39.2[a]	51.4
Not sieved[e]	7.4[b]	16.3[b]	8.9	9.6	5.8[b]	14.8
S.E.[f]	3.8	9.5	-	-	3.9	-

[abc] Means in a column with different superscripts differ (P<.05).

[d] Value = % rumen disappearance * .8 + SI (by difference).

[e] Geometric mean diameter measured to be 2282 ± 700 um.

TABLE 3. EFFECT OF PARTICLE SIZE ON THE CRUDE PROTEIN DISAPPEARANCE OF DRY ROLLED CORN FROM MOBILE DACRON BAGS

Treatment	Mobile Dacron Bag Disappearance Site				
	Ruminal (15H)	Total tract	Small intestine		
			% of diet	% of duodenal supply	Not prefermented
Particle diameter			% Disappearance		
2000-1000 um	53.0[b]	72.3[b]	19.3	41.1	64.2[b]
1000-500 um	24.3[d]	62.1[c]	37.8	49.9	36.4[d]
500-250 um	34.5[cd]	72.4[b]	37.9	57.9	43.3[cd]
250-125 um	72.4[a]	75.3[b]	2.9	10.5	91.0[a]
<125 um	82.9[a]	99.5[a]	16.6	97.1	100.0[a]
Not seived[e]	39.8[bc]	59.7[d]	19.9	33.1	56.4[bc]
S.E.[f]	3.3	3.2			4.6

[abcd] Means in a column with different superscripts differ (P<.05).

[e] Geometric mean diameter = 2282 ± 700 um.

[f] Standard Error of the Means.

TABLE 4. EFFECT OF RESIDENCE TIME ON INTESTINAL DISAPPEARANCE OF THE DRY MATTER (DM), STARCH AND CRUDE PROTEIN (CP) FROM CORN GRAIN USING THE MOBILE DACRON BAG TECHNIQUE

Residence time (hours)	N	DM (%)	SEM[c]	Starch (%)	SEM[d]	Protein (%)	SEM[e]
< 10	4	46.0[b]	4.07	9.89[b]	9.1	6.5[b]	2.0
10.0 - 14.5	38	45.6[b]	0.88	8.97[b]	4.4	15.2[b]	5.3
15.0 - 19.5	24	48.6[b]	1.45	30.92[b]	4.4	45.0[a]	2.5
20.0 - 24.5	4	80.1[a]	0.55	68.04[a]	16.5	71.5[a]	7.4
25.0 - 29.5	2	48.0[b]	0.00	8.65[b]	16.3	17.1[b]	6.6

[ab] Means in the column with different superscripts differ ($P<.05$).

[cde] Standard Error of the Means of DM, starch and CP, respectively.

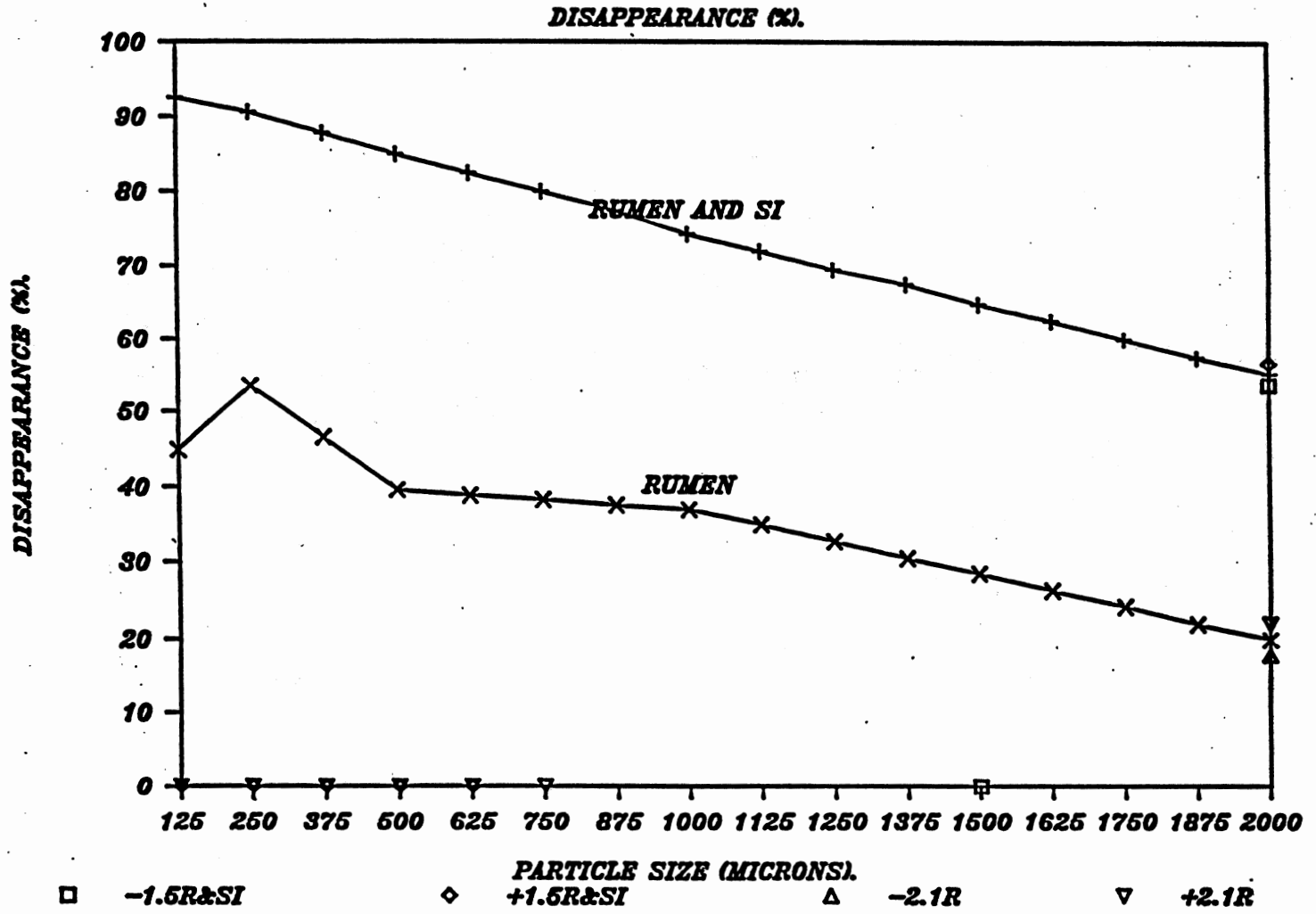
TABLE 5. COMPARISON OF OBSERVED VS EXPECTED RUMINAL, TOTAL TRACT AND POSTRUMINAL DISAPPEARANCE OF NOT SIEVED CORN

Component	Ruminal Disappearance		Total Tract		Small Intestine	
	Observed	Expected	Observed	Expected	Observed	Expected
	% Disappearance					
Dry matter	18.5	18.9	45.5	47.4	33.4	23.4
Starch	7.4	4.2	16.3	3.1	5.8	1.1
Protein	39.8	38.6	59.7	56.4	56.4	47.7

TABLE 6. INFLUENCE OF PARTICLE SIZES ON RUMINAL, POSTRUMINAL AND TOTAL TRACT DISAPPEARANCE OF DRY ROLLED CORN

Component	Total Tract Disappearance	Ruminal Disappearance	Post-Ruminal Disappearance	Post-Ruminal Supply	Supply of Post-Ruminally Digested Component
Dry matter	<125	125-250	<125	1000-2000	<500
Starch	125-250	125-250	125-250	500-1000	125-250
Protein	<125	<125	<125	500-1000	250-1000

EFFECT PARTICLE SIZE IN DRY MATTER.



CHAPTER VII

VALIDATION OF THE MOBILE DACRON BAGS PROCEDURE

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ABSTRACT

Two dairy calves (150 kg) equipped with duodenal T cannulae and ileo-rectal anastomoses were fed with an 80% concentrate diet at 2.5% of body weight. Particles greater than 250 um were isolated from the duodenum and placed in mobile dacron bags (MDB). Disappearance of DM, protein and starch from bags was compared with intestinal digestion in the intestine of particles over 250 um. Small intestinal digestion of the dry matter, starch and crude protein from particles > 250 um was no significantly greater than disappearance from dacron bags although values tended to be 10 to 15% lower for particles in the bags. Comparison of MDB with in vivo digestion values for an 80% concentrate diet and grains commercially processed also revealed underestimation by MDB. This is probably due to differences in particle size, retention time or presence of a dacron barrier which can inhibit microbial attack and enzyme entry.
(Key Words: Mobile Dacron Bag, Protein Digestibility,

Starch Digestion, Intestine Bypass, Digestibility, Total Digestible Nutrients.)

Introduction

Ideally, new methods are extensively verified and standardized shortly after they are devised. Unfortunately many common procedures in animal science research including techniques for amino acids and fiber analyses never have been standardized despite their widespread acceptance and use (Owens, 1987). Even in situ ruminal disappearance, though widely correlated with various solubility estimates, has been compared to in vivo measurements in only one study (Zinn and Owens, 1983).

Study of digestion of encapsulated food passing through the digestive tract should not be considered a new procedure. It was used in the 1700's (de Reauner, 1756; and Spallanzani, 1782) in animals and humans.

Workers at Texas A&M inserted nylon bags into the abomasum of steers (Snell and Lichtenwalner, 1975; and Snell et al., 1975). Bags were recovered up to 25 days later in feces. At Oklahoma State University, post-ruminal digestion of cotton cloth strips was examined in 1978 (A.B. Johnson, personal communication) but recovery of strips was incomplete. Sauer et al. (1983) renewed research with the Mobile Nylon Bag (MNB) technique using it for evaluating feedstuffs with pigs. The MNB has been used for ruminants over the past 5 years in laboratories in Denmark

(Hvelplund, 1985), in Canada (Cherian, 1985) and in England (Rooke et al, 1985) though only three experiments have attempted to validate it. Lack of gross differences between MDB disappearance and in vivo digestibility values has led to the tacit acceptance of this technique.

Verification methods employed in the past have certain limitations. Hvelplund (1985) duodenally infused protein sources and compared the increase in ileal N flow to the amount of residual N remaining in fecally recovered mobile dacron bags containing the same protein source across 12 protein sources. Bags were recovered from feces by washing the feces in a sieve basket. Comparing ileally undigested protein with fecal residues in bags means that extent of digestion could differ. Indeed, pre-ileal disappearances was less than pre-rectal loss (Hvelplund 1985). Infused protein contained small as well as large particle sizes. Although fine particles would contribute to ileal N flow, they may not be fully retained by the nylon cloth (22 micron pore size) of the mobile bags. In our studies, protein from corn particles was less extensively digested from larger than smaller particles (Chapter VI), so particle size may have altered their results.

Cherian (1985) compared protein digestibilities from mobile bags with apparent digestion coefficients for swine. Recovered bags were patted dry and not washed. This should leave a portion, albeit variable, of endogenous material inside. So again, particle size and presence of endogenous

secretions complicates interpretation of his results. Finally, Rooke et al. (1985) compared protein digestibilities from mobile bags with true protein digestibilities from rat studies. Crude protein digestibilities may differ among species. Compared with non ruminants, ruminants produce pancreatic secretions much richer in pancreatic ribonuclease and weaker in amylase.

Several factors are of concern when extrapolating from disappearance from dacron bags to in vivo digestibilities whether the site of comparison is the rumen or the intestine. First, particles, microbes and fluids containing solutes can enter through pores in the cloth of the bag and cause disappearance to be underestimated. If attached to particles, these are difficult to remove. Thorough washing appears to remove most of the microbial matter as measured by nucleic acids (Weakley, 1983) though residual amounts of ^{15}N (Varvikko et al, 1985) and DAP (Olubobokum et al, 1987) can be large. Proteolytic enzymes in the intestine should not attach to particles as tenaciously as ruminal bacteria. One of our objectives was to measure the quantity of DM diffusing into mobile dacron bags which is not remove by "patting dry" but can be removed by thorough washing.

The second concern is disappearance of non-digested material from the bag. Particles with diameters less than the pore size of the bag and soluble compounds can sift or diffuse out of the bag even though they are not digested. In this case, disappearance will overestimate digestibil-

ity. This loss complicates interpretation of any particle size - digestibility relationship. Yet, if smaller particles are more extensively digested in the rumen than larger particles, this error should be proportionately quite small.

Because duodenal infusion experiments as conducted by Hvelplund (1985) are complex to conduct and interpret, and our primary concern was whether presence of dacron cloth around particles would reduce their digestibility. Hence we sieved duodenal digesta and placed it in dacron bags to measure disappearance of DM, protein and starch during transit through the small intestine. These values were compared with disappearance of DM, protein and starch from particles of a similar size between duodenum and ileum. We assumed that particle size was not altered during passage through the small intestine. This is supported by similar sizes of abomasal and fecal particles (Poppi et al., 1985) Though ruminal digestion can either decrease or increase particle size (Kennedy, 1984), changes in particle size in the small intestine should be minimal.

The objectives of this experiment were: to compare the disappearance of dry matter, protein and starch of a particulate fraction of duodenal fluid from mobile dacron bags versus in vivo during small intestinal transit in calves equipped with an ileo-rectal anastomosis; to quantitate diffusion of DM into mobile dacron bags; and to compare estimates of dacron bag disappearance of various feed-

stuffs with those determined in vivo both in our studies and from tables of digestibility figures.

Materials and Methods

Trial 1

Animal, housing, feeding and surgical procedures were described elsewhere (Chapter V).

Mobile Dacron Bag (MDB) Technique

Five hundred ml of duodenal chyme were collected from each animal and sieved through a 250 um screen. The filtrate was discarded and the residues were rinsed with cold running tap water. This particulate fraction (particles bigger than 250 um) was divided in two portions. One was kept for chemical composition analysis and the second was packed wet into mobile dacron bags. Disappearance of dry matter, protein and starch from these bags was measured by inserting them into the duodenum and recovering them as they were defecated. For comparison, coefficients of digestibility of particulate material greater than 250 um were calculated by collecting, sieving, and rinsing particles larger than 250 um from fecal samples. The quantities of particle passing the duodenum and appearing in feces were calculated from chromium content of non-sieved duodenal and fecal digesta.

Approximately 2.0 g of wet duodenal particulate material was placed into each bag after which each bag was tied

shut with a polyester thread. Bag construction, insertion and recovery was described elsewhere (Chapter V). Twelve replicate bags (3 per day for 2 days for each of two animals) containing particles bigger than 250 um were analyzed for DM recovery. Half were used each for crude protein [Kjeldahl nitrogen (AOAC, 1975)] and half for starch (MacRae and Armstrong, 1968) analysis. Blank bags containing a known weight of sliced Tygon tubing were used to measure and correct for influx of non-adherent DM, nitrogen and starch. Bags plus residual contents were weighed and digested intact for Kjeldahl nitrogen analysis.

Statistical Analysis. Extents of dry matter, crude protein and starch disappearance in the intestines versus MDB from particles > 250um were tested by analysis of variance using calf, day and estimation method (dacron bag vs in vivo) in the statistical model. When differences were significant ($P<.05$), means were compared by a t test (Steel and Torrie, 1980).

Trial 2

Animal, housing and feeding procedures are described elsewhere (Chapter III).

Mobile Dacron Bag (MDB) Technique

Slices of tygon tubing (two grams) were packed into mobile dacron bags. Bags were inserted into the duodenum and recovered after passing through the small plus large

intestine of 500 kg heifers them as defecated. For comparison, two treatments were used: A) in four heifers, sets of two bags each with tygon tubing were passed through the intestines for a total of eight defecated bags. These collected on the screen covering the fecal pan. Each of the bags was thoroughly washed by hand under cold running tap water until the water was colorless. A second set of eight bags similarly packed with Tygon tubing were inserted and collected as defecated. Instead of the washing these bags, they were simply patted dry with a paper towel. All bags were dried in a forced air oven at 90 C for 24 h and weighed.

Statistical Analysis. Differences in dry matter, recovery between washed bags and patted dry bags were tested by analysis of variance using heifer and treatment in the statistical model. When differences were significant ($P<.05$), means were compared by a t test (Steel and Torrie, 1980).

Trial 3

Animal, housing, feeding, MDB and statistical procedures were described elsewhere (Chapter III).

Results and Discussion

Trial 1

Coefficients of digestibility for particles greater than 250 um in diameter by the mobile dacron bag procedure vs in vivo are presented in Table 1.

Mean values for disappearance of DM, protein and starch from particles in dacron bags were consistently not significantly lower (11%, 16% and 10%) than in vivo values. Lower disappearance of DM and crude protein values with MDB procedures than in vivo agrees with results of studies of MNB using pigs (Sauer et al., 1983, 1984; Cherian, 1985; de Lange et al., 1986; Sauer, 1986) and with the cattle (Kirkpatrick and Kennelly, 1984). Using a dynamic model, Ewing and Johnson (1987) suggested that rate of ruminal starch digestion by the nylon bag method must be underestimated.

That smaller particles are more completely digested than larger particles is apparent from Table 1. This supports the findings with MDB containing corn particles of various sizes (Chapter VI).

The technique originally designed and used by the Canadian researchers purportedly gave estimates of apparent digestibility because they wiped the bags from feces with tissue. Graham et al. (1985) working with pigs altered this original technique and extensively washed the bags on recovery with cold water to reduce contamination of the

undigested residue with fecal material. Hence, the more recent disappearance values should more closely approximate true digestibilities (Rooke, 1985; de Boer et al, 1987). Their values for organic matter and crude protein degradation from MNB, however, were higher than conventionally measured apparent digestibilities (Graham et al, 1985). No explanation for this increase in disappearance is apparent.

Hvelplund (1985) washed defecated bags in his protein digestion comparison with the small intestinal digestion of ruminal fermented or non-fermented duodenally infused into lambs. Though related, disappearance from bags recovered in feces exceeded true digestibility of protein infused into the small intestine by an average of almost 12 percentage units. Disappearance was less for bags recovered at the ileum than those recovered from feces. This can partly explain the difference.

Rooke (1985) also washed the bags and he collected in the fecal material and contrasted duodenal to fecal disappearance of N to true N digestibility coefficients determined with rats. True digestibilities were 20% higher for rats than MNB disappearance for fish meal, rapeseed meal and meat and bone meal though results from these two procedures were related ($r^2 = 0.92$, $P<0.05$). Washing of the particles before placing them in the mobile dacron bags and after recovery of the bags from feces should most closely approximate true digestibility. Hence, the data Rooke (1985) should be a reliable comparison to validate the MDB.

In his study disappearance from bags under-estimated true digestibility of the crude protein by about 20%, not markedly different from the 16% reduction we observed.

Reasons for in vivo digestion to exceed MDB loss of N are not certain. Perhaps fluid flux through the pores is inhibited so that rate of digestion is reduced. Also, intestinal enzymes are secreted into the intestines both by the pancreas and by the intestinal mucosa. For mucosal enzymes, close proximity of the substrate should speed digestion. Therefore, presence of a dacron mesh barrier may reduce rate of particle digestion. If rate of passage of bags through the digestive tract were greater than for digesta, or if indigestible particles were preferentially retained in the gastro-intestinal tract, one also would expect in vivo digestibility to exceed disappearance from dacron bags. Hvelplund (1985) suggested that the residence time of bags in the small intestine is not shorter but, indeed, may be longer than for particulate matter. Comparative in vivo intestinal residence times from work by O'Connor et al. (1984) seems very short (2.7 h) compared to more recent estimates from steers of about 4.5 h (Owens et al., 1986a). Retention times in the post-ruminal tract of calves with an ileo-rectal anastomoses seems longer than would be expected, also. So slow passage of bags can not explain the tendency for digestibilities to be underesti-mated by MDB procedures.

Finally, disuniform passage of particles and fluids, though possible in the large intestine is doubtful in the small intestine based on results of Owens et al. (1986b). Entry and adherence of microbes is another possibility. But in our comparison of bagged vs non-bagged particles among of microbial adherence should be similar, not greater for bagged particles as the results imply.

Sauer et al. (1987) summarized several potential problems of the mobile dacron bags. First, the bag does not allow intimate interaction between the feedstuff and the intestinal tract. Secondly, microbial matter may enter and adhere to feedstuff and bag material. Third, soluble components are assumed to digested, and finally, may inhibit mixing of feed with the hydrolytic enzymes. In addition Owens (1987) noted that rate of passage may be disuniform, with retention of certain fractions for more extensive digestion in the rumen, in the abomasum and in the cecum. Graham et al. (1985) suggested that the major source of error with mobile dacron bags is particle loss from the bags, which, in turns varies with pore size of the bag, and the particle size of the feedstuffs being studied and extent of washing.

Site of bag recovery also is an important variable. Disappearance of nitrogen was higher from bags recovered in the feces than for the bags recovered at the ileum in a study by Hvelplund (1985). Though his validation study compared disappearance of infused protein from the small

intestine with total post-ruminal disappearance from bags, sites of bag and digesta recoveries in our trial were identical.

Trial 2

System of cleaning the mobile dacron bags after recovery (thorough washing vs patting dry) considerably altered the small intestinal disappearance of DM (Table 2). More ($P<.05$) dry matter remained inside the bags patted dry with tissue as compared with the thoroughly washed bags (+0.027g vs -0.001g). With half gram samples of 50% digestibility, this would cause true disappearance to be underestimated by over 10% assuming no appropriate blanks were run. Residual variation was much greater with patted than washed bags. This indicated that statistical precision is improved by washing recovered bags.

The procedure described by Sauer et al. (1983) for evaluating various protein supplements and determining the availability of amino acids in the intestine of pigs was recommended that recovered bags should be cleaned only with a tissue to remove adhering feces in order to obtain estimates of apparent digestibility. His group always compares their estimates to apparent digestibility values obtained with external markers or by total fecal collection. Ruminant nutritionists in Alberta, Canada used the same patting dry method proposed by Sauer et al. (1983), in the first report with dairy cattle (Kirkpatrick and Kennelly, 1984)

but they thoroughly washed bags retrieved from the rumen. The endogenous and microbial contamination of the tiny bags from the rumen was not considered. Such contamination probably quantitatively less important in Swine than in ruminants. Hvelplund (1985) and Rooke (1985) modified the technique of Sauer et al. (1983). After recovering of MNB in the feces, bags were manually washed thoroughly with tap, cold water. In Canada, de Boer et al. (1987) stated that with washing of MNB with results should more closely relate to true digestibility than to apparent digestibility. Ours is the first approach to compare washing with blotting. The results can be interpreted to suggest that contamination with fecal material can result in underestimates of true disappearance of DM by over 10% and will considerably increase the variability in recovery of dry matter.

Trial 3

One means to check validity of the MDB is to compare values for disappearance of dry matter and crude protein against values measured in vivo (Chapter III). Table 3 summarizes information of the site and extent of digestion of the high concentrate diet (80%). Values can be compared only to apparent digestibilities in the total tract for the feed, apparent postruminal digestion of feed plus microbial matter (for which RNA analysis indicates that 80% of DM and

8.16% of N is of microbial origin) and either apparent or true diet digestibility in the rumen.

Another set of digestibility comparisons can be drawn from the results of Chapter V.

Values for disappearance of DM and crude protein from MDB matched total tract apparent digestibilities reasonably well though both were slightly low. Because true always exceeds apparent digestibility, MDB disappearance must be underestimating true digestibilities of both components.

Ruminal disappearance of DM overestimated true digestion and underestimated apparent digestion. In contrast, crude protein disappearance in the rumen was reasonably similar to true crude protein digestion there but considerably above apparent ruminal digestion. If bag washing adequately removes microbial matter from the bags, MDB results should provide estimates of true, not apparent ruminal digestion. Particle size and differential passage rates are probably responsible. Particles in the diet would be reduced in size by chewing during eating and rumination and by digestion and dentrition. In MDB, particle size reduction would be minimal, so the greater extent of ruminal digestion may be due to particle size reduction, as observed in Chapter VI. If true, however, protein digestion should similarly be increased even though the two may not be exactly parallel (Chapter VI). Selective ruminal retention of the potentially digestible DM could be involved, too. In bags, all particles were held for a 15 h

digestion period. In the rumen, if indigestible particles escape more rapidly than digestible particles, retention time may be extended and extent of ruminal digestion will be increased as a result. Such a phenomenon cannot be simulated by the MDB technique.

Post-ruminal digestion of dry matter entering the duodenum was overestimated for DM and underestimated for crude protein. As digesta contains microbial DM and crude protein, the discrepancy between postruminal disappearance and digestion is not surprising. However, the estimate of postruminal protein disappearance is far below the estimates made in vivo (NRC,1985).

In fact that MDB disappearance generally underestimated in vivo true digestion agrees with results from studies using pigs (Sauer et al., 1983, 1984; Cherian, 1985; de Lange et al., 1986; Sauer, 1986) and one study with dairy cattle (Kirkpatrick and Kennelly, 1984). Differences in particle size, in particle retention as well as presence of a dacron cloth barrier around the feedstuffs are probably responsible; none of these limitations are not readily surmounted to improve accuracy of prediction by MDB procedures.

Digestibility values, which are quite similar to TDN values, for feed grains processed in various manners (NRC, 1984) can be compared with the total tract disappearance values from MDB for the processed grains of Chapter V (Table 4). Again, TDN values were underestimated by MDB

disappearance. In addition, an effect of particle size is apparent with very low MDB disappearance values for the whole shelled corn and whole oats (4.8 and 4.1%) compared with TDN values for these two grains (90 and 77%). The smaller the particle size, the closer the agreement between MDB disappearance and TDN values. This indicates that mastication and rumination are extremely important for both ruminal and total tract digestion as discussed in Chapter V.

The kind and size of the grains appeared to interact with processing with milo and corn rolling alone produced MDB disappearance values only 55 and 58%, with barley and wheat MDB disappearances were 81 and 91% of TDN values, respectively. Values for processed materials (steam flaked milo and corn) were reasonably close to TDN values, presumably due to their small particle size. Greater study of retention time is needed to correlate with rates of digestion of various particle sizes in order to mathematically model ruminal digestion and escape of starch and protein from cereal grains processed by various methods.

Some additional comparisons can be drawn regarding site and extent of starch digestion from several grains from MDB (Chapter V) versus values summarized by Owens et al. (1986). These are presented in Table 5. Comparisons again reveal a closer similarity between MDB starch disappearance and in vivo starch digestions for grain processed that reduced the particle size. Again, the factor which

appears to limit attack of microbial population and enzimatic digestion is particle size.

Additional factors deserving attention are effects of the mass to surface area ratio of the MDB (we used 65 mg/cm^2) and the pore size of the bag probably inhibited protozoal and prohibited fungal entry. These factors were discussed in Chapter II. Unfortunately, on must strike a balance between analytical needs (large residue desired), labor (transfer from large to small bags is time consuming) and accuracy of simulating the feedstuff (small pore size desired to prevent washout) against the optimal conditions for ruminal and postruminal digestion from bags (small mass/volume, small post-ruminal bags, large pore size to enhance flushing). The ideal may be infeasible.

Our results may be interpreted to indicate that disappearance from MDB both in the rumen and postruminally will underestimate true digestion of the dry matter, crude protein, and starch. The difference appears greater for feeds with larger particles. In general, protein digestion was more accurately predicted by MDB than DM or starch digestion probably because it is less affected by particle size. If in vivo chewing and retention times could be simulated in MDB studies, predictability MDB results would be improved.

TABLE 1. DISAPPEARANCE OF THE NUTRITIVE COMPONENTS OF A DAIRY RATION OF MOBILE DACRON BAGS VS IN VIVO COEFFICIENTS OF DIGESTIBILITY

Component	Dacron Bags Particle >250 um.	SEM[b]	In vivo digestibility of particles >250 um.	SEM[b]	In vivo digestibility of all material	SEM[b]
	---------- % Disappearance----------					
Dry matter	72.8	4.4	81.8	2.5	84.5	4.00
Crude protein	23.9	3.3	28.4	2.3	34.0	3.00
Starch	61.9	5.4	68.9	2.1	89.2	2.00

[a] Means in a row with different superscripts differ (P<.05).

[b] Standard Error of the Means.

TABLE 2. CHANGES IN THE WEIGHT OF MOBILE DACRON BAGS UNDER TWO SYSTEMS OF CLEANING

	Treatment			
	Washed	SEM[c]	Patted dry	SEM[c]
Initial weight, g	2.441	0.0046	2.440	0.0070
Final weight, g	2.440	0.0048	2.467	0.0082
Weight change, g	-0.001[a]	0.0005	0.027[b]	0.0020

[ab] Means in a row with different superscripts differ ($P<.05$).

[c] Standard Error of the Means.

TABLE 3. DISAPPEARANCE OF THE NUTRITIVE COMPONENTS OF A 80% CONCENTRATE DIET FROM MOBILE DACRON BAGS VS IN VIVO COEFFICIENTS OF DIGESTIBILITY

Component	Dacron bags	SEM[a]	In vivo true digestibility	SEM[a]	In vivo apparent digestibility	SEM[a]
	----------% Disappearance----------					
Total tract						
Dry matter	70.1	1.6	-	-	74.3	1.0
Crude protein	68.4	2.6	-	-	66.4	0.6
Ruminal						
Dry matter	25.7	1.5	67.8	1.8	58.8	1.2
Crude protein	64.6	2.5	52.4	2.5	2.0	4.7
Post ruminal (% of flow)						
Dry matter	66.4	4.3	-	-	45.4	2.8
Crude protein	6.1	5.4	-	-	65.2	4.7

[a] Standard Error of the Means.

TABLE 4. COMPARISON OF THE TOTAL TRACT DRY MATTER DISAPPEARANCE OF GRAINS FROM MOBILE DACRON BAGS VS TDN[a]

Feedstuffs	Percent TDN[a]	Total Tract
	% Disappearance	
Corn		
Whole shelled	90.0	4.8
Dry rolled	80.0	46.1
25% Moisture	92.0	56.7
35% Moisture	93.0	66.3
Steam flaked	95.0	86.5
Milo		
Rolled	84.0	45.9
Steam flaked	92.0	82.0
Oats		
Whole	77.0	4.1
Wheat		
Rolled	88.0	79.9
Barley		
Rolled	84.0	67.9

[a] From NRC (1984).

TABLE 5. COMPARISON OF THE TOTAL TRACT STARCH DISAPPEARANCE OF GRAINS FROM MOBILE DACRON BAGS VS STARCH DIGESTIBILITY COEFICIENTS[a]

Feedstuff	Total Tract Starch Digestion		Ruminal Starch Digestion		Small Intestine Starch Digestion	
	In Vivo[a]	MDB	In vivo[a]	MDB	In vivo[a]	MDB
Corn						
Whole shelled	91.7	7.4	58.9	0.0	17.0	7.4
Dry rolled	93.2	20.4	71.8	9.4	16.1	11.0
35% moisture	94.6	56.3	86.0	21.7	5.5	34.6
Steam flaked	97.8	82.5	82.8	49.4	15.6	33.1
Milo						
Rolled	86.4	15.0	67.8	3.5	13.4	11.5

[a] From Owens et al. (1986).

CHAPTER VIII

EFFECTS OF UREA AND LINSEED OIL ON RUMINAL PROTOZOA NUMBERS AND NUTRIENT DIGESTION BY STEERS

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ABSTRACT

Seven steers (350 kg) equipped with ruminal and duodenal cannulas were limit fed (1% of body weight) a high concentrate diet. Urea or linseed oil were added at 1% or 5% of the diet in two crossover trials. On day 9 to 13 of each period, ruminal protozoa were counted and digestion of dry matter, fiber and N were estimated. Mean protozoal counts were reduced by 35% ($P<.05$) and 42% ($P<.01$) with urea and linseed oil, respectively. Dry matter digestion in the total tract was reduced by approximately 11% ($P<.01$) when the animals received linseed oil. Digestion of N in the total tract was reduced by 8% (LSO). Total tract dry matter and N digestibilities were related ($r = .61$, $P<.05$ and $r = .67$, $P<.01$) to the ruminal protozoa numbers with linseed oil. Total tract fiber digestion was reduced by

34% by these antiprotozoal compounds. Efficiency of microbial growth, ruminal escape of dietary protein and total flow of N to the duodenum were not significantly altered by urea or linseed oil. Reduced digestibilities of dry matter, fiber and N with these antiprotozoal compounds may decrease rate of particle size reduction in the rumen and thereby reduce feed intake of roughages-based diets.
(Key words: Rumen Protozoa, Antiprotozoal Agents, Digestion, Protein Digestibility, Urea, Linseed Oil.)

Introduction

Numbers of protozoa in the rumen vary with dietary conditions. Protozoa numbers are generally inversely related to bacterial numbers within the rumen, but numbers can fluctuate with time after a meal.

Presence or absence of protozoa in the rumen can alter microbial efficiency. Feeding a chemical to remove protozoa from the rumen (defaunation) of sheep increased efficiency of microbial growth by 130% (Demeyer and Van Nevel, 1979). Because protozoa remain sequestered within the rumen and seldom leave, they contribute only limited amounts of protein to the postruminal supply (Firkins et al., 1987). But whether the increases in microbial efficiency observed with defaunation are due simply to inefficient growth of protozoa or to other changes which accompany defaunation (e.g. decreased proteolysis and methane formation) remains to be determined.

Presence of protozoa can alter nutrient requirements. One of the benefits from simultaneous feeding of soluble sugar with urea probably is due to maintenance of a higher protozoal population in the rumen even though protozoa do not use ammonia as a source of N.

Feeding anti-protozoal drugs with certain practical diets has proven useful. In three studies with lambs fed low-protein diets, removal of protozoa increased growth rate of lambs by 18, 40 and 54% (Demeyer et al., 1982). Though many antiprotozoal drugs are toxic to animals at low concentrations (Lovelock et al., 1982), certain common non-toxic compounds (eg. urea and linseed oil) have antiprotozoal activity and need to be tested for their efficacy under practical feeding conditions. Though of value with low protein, high energy diets, defaunation may not be desirable with high fiber diets. Removal of protozoa reduced in vitro digestion of grasses by a mean of 52% in a study by Amos and Akin (1978).

The objectives of this research were to determine the effects of daily feeding of urea and linseed oil on ruminal protozoa numbers and on nutrient digestion. Of particular interest was the influence of these two compounds on digestibility of fiber.

Materials and Methods

Seven dairy steers averaging 350 kg with ruminal and

duodenal T-type cannulae were used in a two trials, each with a crossover design with two 2-week periods. The first week of each period was for adaptation to the diet (table 1) and the second week of each period was used to collect samples. In the first trial, steers received two treatments: a) 5% linseed oil (LSO) and b) control. In the second experiment the treatments were: a) 1% added urea (U) and b) control. Supplements (LSO and U) were added and mixed into the diet at feeding time. Steers were maintained in 2.8 x 3.1 m pens with water available at all times. Feed supply was limited to 1% of body weight (dry matter basis, DM) in two equal feedings (0800 and 1600 hours).

Chromic oxide was included in the diet at .2% of dietary dry matter as an indigestible marker by adding 10 g of a mixture of 64% of wheat flour and 16% of chromic oxide to each meal. Feed samples were collected during the trial.

Ruminal, duodenal and fecal samples were taken on days 3, 5 and 7 of the second week of each period immediately after feeding; this sampling time was chosen based on a preliminary diurnal sampling study in which a dramatic increase in the protozoa counts was noted after feeding ([F = Feeding]; figure 1). The pH of digesta from duodenum and rectum was measured at the time of collection. Duodenal samples (200 ml), were composited on a wet basis and refrigerated. Individual fecal samples were frozen. Ruminal

samples (200 ml) were extracted from the mid-ventral region of the rumen and the pH was measured immediately. These samples were strained through four layers of cheesecloth, acidified by adding 1% of 1ml of 20% (V/V) H_2SO_4 and refrigerated.

Before acidification, a one ml sample was withdrawn with a broad bore pipette and mixed with 9 ml of a .85% saline solution containing 10% (V/V) formalin to fix the protozoa. Protozoa in duplicate samples were counted with a hemocytometer chamber at a magnification of 450 X (Doran, 1985) on each of the 3 sampling days.

Duodenal and fecal samples were dried at 55 C for 48 h and then allowed to air equilibrate. These and feed samples were ground through a 2 mm screen. Feed, duodenal and fecal samples were analyzed for DM, ash, N, NH_3-H (AOAC, 1975), acid detergent fiber (ADF; Goering and Van Soest, 1970), starch (MacRae and Armstrong, 1968) and chromium (Hill and Anderson, 1958). Rations of chromium to feed constituent concentrations in feed, duodenal and fecal samples were used to calculate ruminal and total tract digestibilities of feed constituents. Apparent dry matter digestion in the rumen was calculated as DM intake minus duodenal DM divided by DM intake. Digestion in the small intestines was estimated as the difference between calculated apparent digestion in the rumen and apparent digestion in the total tract.

In the experiment with urea, 2 liters of rumen fluid were withdrawn on day 28 to obtain isolated bacterial cells (IBC). Rumen fluid was strained through four layers of cheesecloth and bacteria were separated by differential centrifugation (Merchen and Satter, 1983). The IBC were dried at 55 C and crushed to a fineness suitable for mixing. Nucleic acid-N (NA-N; Zinn and Owens, 1986) analyses were conducted on IBC and on duodenal samples. Microbial N passage was calculated from NA-N content of duodenal samples and the NA-N to ratio in IBC. A microbial ash estimate of 20% (Smith, 1975) and the N content of IBC were used to estimate microbial organic matter flow (Goetsch and Owens, 1986). True ruminal digestion was calculated by subtracting the microbial contribution to duodenal organic matter (OM) from total duodenal OM flow.

Data were tested by analysis of variance using steer, treatment and period in the statistical mode. Simple correlations also were calculated between various measurements. A multivariate analysis of variance was done and finally, partial correlation coefficients from the matrix associated with the error effect were calculated between various measurements (Stell and Torrie, 1980).

Results and Discussion

Both LSO and urea depressed the numbers of protozoa in ruminal fluid after feeding (Table 2). Mean protozoa counts were reduced by 42% ($P<.01$) and 35% ($P<.05$) with LSO

and urea, respectively. Addition of LSO to the diet in the previous studies also has reduced rumen protozoal numbers (Purser and Moir, 1966; Czerkawski, 1973; Knight et al., 1978; Demeyer and Van Nevel, 1979; Demeyer, 1981). The toxicity of LSO fatty acids toward protozoa has been studied by Ikwuegbu and Sutton (1982) and Czerkawski and Clapperton (1983). In general, the numbers of bacteria in the rumen tend to increase when the protozoa population is decreased, presumably to fill the niche vacated by the protozoa.

Ruminal NH_3-N differences were small. The slight increase in ruminal NH_3-N with 1% added urea was not significant and was much smaller than expected. The slight decrease ruminal ammonia concentration with LSO (13.3 vs 12.0 mg/dl) is similar to defaunation results of Ikwuegbu and Sutton (1982) and may be due to reduced proteolysis by protozoa. A similar effect also could be responsible for failure of added urea to greatly increase ruminal ammonia concentrations.

Except for ruminal pH with LSO, both compounds tended to reduce digesta pH at all points in the gastro-intestinal tract. This may be attributed to an increased bacterial acid production in the rumen in the absence of protozoa. Protozoa engulf starch and reduce rate of ruminal fermentation which helps avoid rapid fermentation and thereby holds pH higher (Purser and Moir, 1966).

Total tract DM digestion tended to be lower with both treatments, being reduced by 11% when animals received LSO ($P<.01$). In an extensive review by Demeyer (1981), defaunation consistently reduced apparent digestibility of OM. Knight et al. (1978) and Demeyer (1981) suggested that defaunation shifted a larger proportion of digestion of dry matter to the large intestine. In this experiment, 58 and 70% of the total DM digested in the animals treated occurred postruminally for LSO and U, respectively; these values were not altered by treatment.

Extent of digestion of nitrogen in the total tract was reduced by 8% with LSO. Ikwuegbu and Sutton (1982) previously reported that apparent digestibility of nitrogen decreased from 77% to 68% with daily dosing of sheep with 40 ml of LSO.

Total tract ADF digestion tended to be reduced with both LSO and U (Table 2). Studies with defaunated ruminants (Knight et al., 1978; Amos and Akin, 1978; Demeyer, 1981) all indicate that total tract fiber digestibility is decreased by the absence of protozoa. A regression analysis with the LSO trial detected a positive correlation between ruminal protozoa numbers and total tract digestibilities of both dry matter and N ($r = 0.61$, $P<.05$ and $r = 0.67$, $P<.01$). The partial correlation coefficients from the matrix associated with the error effect detected in the LSO trial a positive relation between protozoa numbers and

total tract digestibilities of dry matter and Nitrogen ($r = 0.50$, $P<.08$ and $r = 0.64$, $P<.02$).

Added LSO and urea did not significantly alter the amount of N leaving the abomasum, ruminal escape of feed nitrogen or efficiency of microbial growth (table 3). These results do not in agree with findings of Knight et al. (1978), Demeyer and Van Nevel (1979) or Demeyer (1981) who have reported that defaunation increased net microbial synthesis. In our study, LSO and urea were fed at 5% and 1% of the diet respectively, and did not completely defaunate the rumen at these levels. Thus, effects of these compounds on microbial efficiency was not as great as others have reported.

The trend toward reduced digestibilities of DM, ADF and N with LSO and U supplementation may reflect a reduced rate of particle size reduction in the rumen. If true, this could reduce intake of roughage-based diets. It seems desirable to continue defaunation research using feed supplements like LSO and U which do not cause toxicity problems as some antiprotozoal drugs do (Lovelock et al., 1982). Higher levels and another compounds need testing. Czerkawski and Clapperton (1983) suggested that considerable amounts of LSO could be fed without causing adverse effects on ruminants. Another important advantage of LSO and urea as antiprotozoal compounds is that they can be fed continuously. These materials also provide nutrients in contrast to various antiprotozoal drugs and chemicals.

TABLE 1. COMPOSITION OF EXPERIMENTAL DIET

ITEM	PERCENTAGE
Ingredient, % of DM	
Cracked corn	63.5
Dehy alfalfa hay	6.0
Cotton seed hulls	14.0
Soybean meal	10.0
Molasses, cane	5.0
Salt and minerals	1.5

TABLE 2. EFFECTS OF LINSEED OIL AND UREA ON PROTOZOA NUMBERS AND NUTRIENT DIGESTION IN DAIRY STEERS FED HIGH CONCENTRATE DIET

ITEM	LSO			UREA		
	Control	5%	SE	Control	1%	SE
Ruminal protozoa numbers ($x10^4$/ml)	5.3[A]	3.1[B]	1.9	4.8[a]	3.1[b]	2.0
Ruminal NH_3-N, mg/dl	13.3	12.0	0.8	14.5	14.9	0.4
Digesta pH						
Rumen	6.6	6.7	0.1	6.6	6.5	0.1
Duodenal	3.0	2.9	0.2	3.0	2.9	0.1
Feces	6.6	6.5	0.2	6.6	6.5	0.1
Total tract digestion, %						
DM	73.8[a]	66.8[b]	1.2	71.8	71.6	2.3
N	55.6	51.4	1.4	32.2[a]	50.6[b]	4.9
ADF	26.7	17.7	3.3	13.6	9.0	0.1
Ruminal digestion, % of total						
DM	20.2	18.8	0.1	23.0	22.0	1.2
OM	31.2	28.1	0.1	16.0	15.5	1.1
ADF	95.1	95.0	0.1	94.3	93.7	0.1
Intestinal tract digestion, % of total						
DM	65.5	58.0	2.7	72.7	69.7	1.9
N	69.2	65.0	1.5	74.6	72.6	2.4
ADF	10.3	11.1	0.1	33.4	30.8	0.1

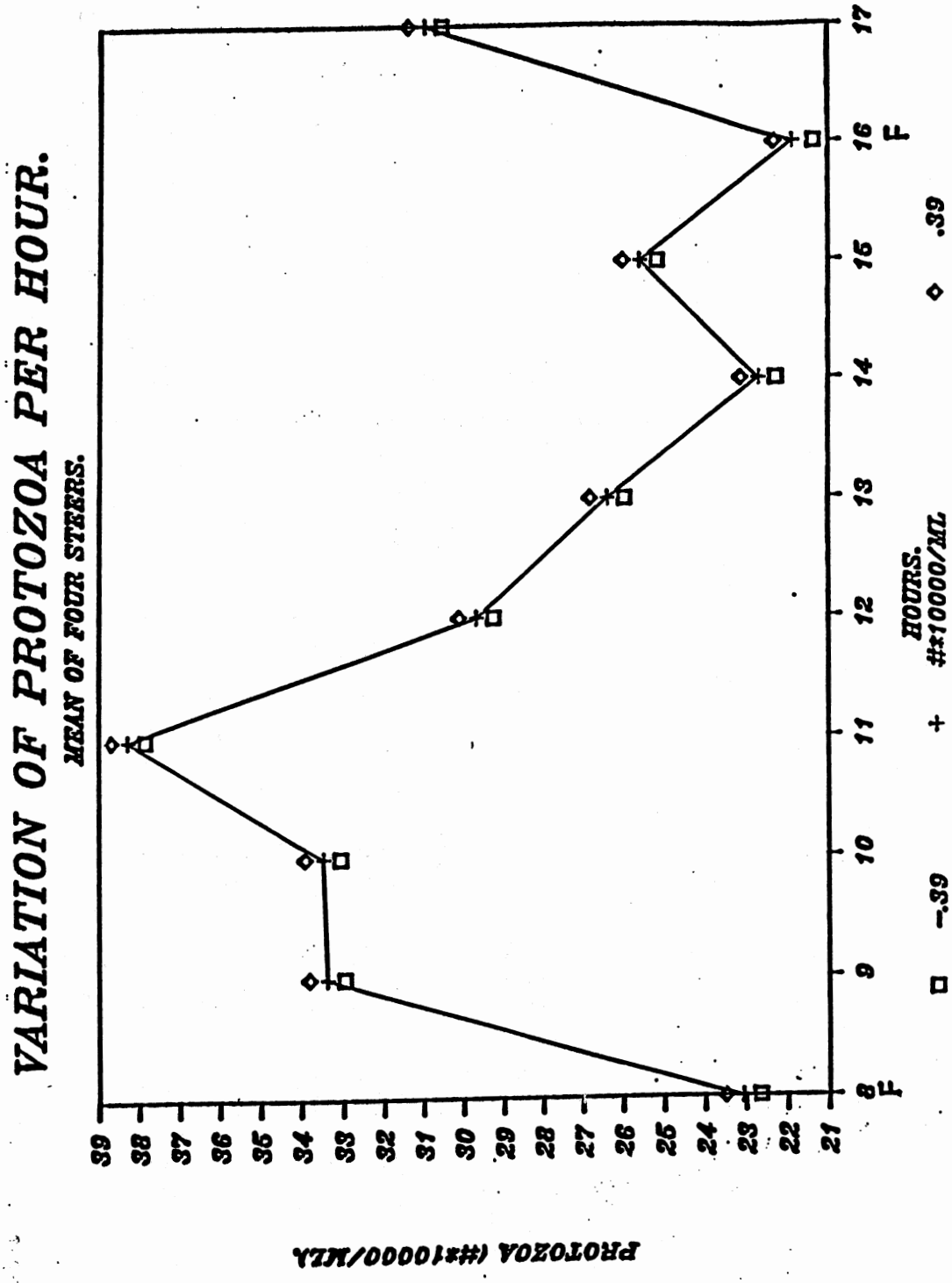
[ab]Means in a row within an experiment with different superscripts differ statistically ($P<.05$).

[AB]Means in a row within an experiment with different superscripts differ statistically ($P<.01$).

TABLE 3. EFFECTS OF TWO ANTIPROTOZOAL COMPOUNDS IN THE NITROGEN METABOLISM IN THE DIGESTIVE TRACT OF DAIRY STEERS

ITEM	LSO		UREA	
	Control	5%	Control	1%
N flow to the duodenum, g/day	66.6	63.4	95.0	82.9
Escape of feed N, %	45.0	42.0	56.4	42.5
Microbial efficiency, g MN/kg OMF *	59.2	48.6	34.3	37.2

*Grams of microbial N/kg OM fermented in the rumen.


VARIATION OF PROTOZOA PER HOUR.
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□ -.39
+ #*10000/ML
◇ .39

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APPENDIX

LISTING OF ANALYSIS OF VARIANCE

TABLE 1. OVERALL ANALYSIS OF VARIANCE FOR TOTAL TRACT DRY MATTER DISAPPEARANCE FROM MOBILE DACRON BAGS

Source of variation	DF	SS	MS	Pr>F
Between animals (A)	3	122.8	40.9	0.1627
Between diets (D)	1	145.7	145.7	0.0140*
Between feedstuff(F)	3	10982.2	3660.7	0.0001**
Interaction A*D	3	287.6	95.9	0.0081**
Interaction D*F	3	203.4	67.8	0.0382*
Interaction A*F	9	91.4	10.2	0.9183
Interaction A*D*F	9	456.7	50.7	0.0280*
Postruminal Diet(P)	1	129.6	129.6	0.0204*
Interaction D*P	1	5.1	5.1	0.6434
Interaction A*P	3	184.6	61.5	0.0537
Interaction F*P	3	41.2	13.7	0.6283
Interaction A*D*P	3	161.6	53.9	0.0814

* Significantly different at 5%.

** Significantly different at 1%.

TABLE 2. OVERALL ANALYSIS OF VARIANCE FOR TOTAL TRACT CRUDE PROTEIN DISAPPEARANCE FROM MOBILE DACRON BAGS

Source of variation	DF	SS	MS	Pr>F
Between animals (A)	3	468.4	156.1	0.0012**
Between diets (D)	1	268.3	268.3	0.0024**
Between feedstuff(F)	3	27003.9	9001.3	0.0001**
Interaction A*D	3	554.5	184.8	0.0003**
Interaction D*F	3	216.5	72.2	0.0579
Interaction A*F	9	524.6	58.3	0.0361*
Interaction A*D*F	9	763.9	84.9	0.0024**
Postruminal diet(P)	1	109.5	109.5	0.0511
Interaction D*P	1	1.4	1.4	0.827
Interaction A*P	3	1003.0	334.3	0.0001**
Interaction F*P	3	642.2	214.1	0.0001**
Interaction A*D*P	3	88.7	29.6	0.3757

* Significantly different at 5%.

** Significantly different at 1%.

TABLE 3. OVERALL ANALYSIS OF VARIANCE FOR POSTRUMINAL DRY MATTER DISAPPEARANCE FROM MOBILE DACRON BAGS

Source of variation	DF	SS	MS	Pr>F
Between animals (A)	3	78.4	26.1	0.3192
Between diets (D)	1	534.7	534.7	0.0001**
Between feedstuff(F)	3	7070.5	2356.8	0.0001**
Interaction A*D	3	427.6	142.6	0.0005**
Interaction A*F	9	667.0	74.1	0.0014**
Interaction A*D*F	9	676.9	75.2	0.0012**

* Significantly different at 5%.

** Significantly different at 1%.

TABLE 4. OVERALL ANALYSIS OF VARIANCE FOR POSTRUMINAL DISAPPEARANCE DRY MATTER (PERCENT OF FLOW) FROM MOBILE DACRON BAGS

Source of variation	DF	SS	MS	Pr>F
Between animals (A)	3	665.0	221.7	0.0487
Between diets (D)	1	1624.7	1624.7	0.0001**
Between feedstuff(F)	3	55739.9	18579.9	0.0001**
Interaction A*D	3	733.4	244.4	0.0339*
Interaction A*F	9	3150.5	350.1	0.0001**
Interaction A*D*F	9	5129.9	570.0	0.0001**

* Significantly different at 5%.

** Significantly different at 1%.

TABLE 5. OVERALL ANALYSIS OF VARIANCE FOR POSTRUMINAL DISAPPEARANCE CRUDE PROTEIN (% OF DIET) FROM MOBILE DACRON BAGS

Source	DF	SS	MS	F	Pr > F
Between animal (A)	3	462.759753	154.253251	3.61	0.0175
Between diet (D)	1	2.589134	2.589134	0.06	0.8062
Interaction A*D	3	2154.265179	718.088393	16.83	0.0001
Between feedstuff (F)	3	24699.096893	8233.032298	192.90	0.0001
Interaction A*F	9	872.281769	96.920197	2.27	0.0273
Interaction A*D*F	9	2068.927399	229.880822	5.39	0.0001

TABLE 6. OVERALL ANALYSIS OF VARIANCE FOR POSTRUMINAL CRUDE PROTEIN (% OF FLOW) FROM MOBILE DACRON BAGS

Source	DF	SS	MS	F	Pr > F
Between animal (A)	3	3275.455610	1091.818537	11.82	0.0001
Between diet (D)	1	1243.138791	1243.138791	13.46	0.0005
Interaction A*D	3	8506.559890	2835.519963	30.71	0.0001
Between feedstuff (F)	3	73923.460258	24641.153419	266.86	0.0001
Interaction A*F	9	3181.840934	353.537882	3.83	0.0006
Interaction A*D*F	9	9423.281773	785.273481	8.50	0.0001

TABLE 7. OVERALL ANALYSIS OF VARIANCE FOR RUMINAL DISAPPEARANCE DRY MATTER FROM MOBILE DACRON BAGS

Source	DF	SS	MS	F	Pr > F
Between animals (A)	3	269.35730	89.78577	17.23	0.0001
Between diets (D)	1	189.04413	189.04413	36.29	0.0001
Interaction A*D	3	459.42142	153.14047	29.39	0.0001
Between feedstuffs (F)	3	1246.69859	415.56620	79.76	0.0001
Interaction A*F	9	167.30965	18.58996	3.57	0.0008
Interaction A*D*F	9	249.37279	27.70809	5.32	0.0001
Interaction D*F	3	57.21118	19.07039	3.66	0.0155

TABLE 8. OVERALL ANALYSIS OF VARIANCE FOR RUMINAL DISAPPEARANCE OF CRUDE PROTEIN

Source	DF	SS	MS	F	Pr > F
Between animals (A)	3	336.45932	112.15311	9.24	0.0001
Between diets (D)	1	2.28980	2.28980	0.19	0.6650
Interaction A*D	3	281.17090	93.72363	7.72	0.0001
Between feedstuff (F)	3	11849.55700	3949.85233	325.35	0.0001
Interaction A*F	9	710.12312	78.90257	6.50	0.0001
Interaction A*D*F	9	850.69880	94.52209	7.79	0.0001

VITA

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