AN EVALUATION OF THE NYLON BAG TECHNIQUE FOR ESTIMATING RUMEN UTILIZATION OF CONCENTRATES

by

Wayne C. Figroid

A Thesis Submitted to the Faculty of the

DEPARTMENT OF ANIMAL SCIENCE

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1967

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Wayne C. Feigeid

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

William H. Hale
WILLIAM H. HALE
Professor of Animal Science

Jan 6, 1967
Date

ACKNOWLEDGMENTS

The author wishes to express his gratitude to his major professor, Dr. W. H. Hale, for his suggestions, criticisms and guidance throughout the course of the experiments and writing of this thesis. Sincere appreciation goes to Dr. Donald Ray for his generous assistance with the statistical portion of the problem.

Thanks also go to Robert Nunez for his help in handling the animals throughout the experiments. An additional expression of thanks is due to Dr. Clark B. Theurer and the staff of the Animal Science Department for the assistance provided whenever needed.

TABLE OF CONTENTS

TABLE OF CONTENTS--Continued

LIST OF TABLES

LIST OF TABLES--Continued

LIST OF TABLES--Continued

LIST OF TABLES--Continued

LIST OF TABLES--Continued

LIST OF FIGURES

ABSTRACT

The nylon bag technique was studied to evaluate several variables affecting utilization of grains from nylon bags. Incubations at 2, 4, 6, 8 and 24 hours showed eight-hours of incubation to be indicative of 24-hour dry matter disappearance. Samples of 2, 4, 6, 8 or 10 grams of 20 mesh milo incubated in four by seven inch bags for eight hours showed no differences in per cent dry matter disappearance. Flaked milo and barley samples of 6, 10, 14 and 18 grams incubated in two by seven, three by seven and four by seven inch bags indicated that both bag size and sample size significantly influenced the rate of dry matter disappearance. Four inch wide bags and 10 gram samples were selected for use. Significant differences were observed in grain utilization between five cloth types and mesh sizes of 9-20, 20-28 and 28-35 for milo and barley samples. The nylon bag technique ranked grains and grain processes in the same order as did digestion trials. Generally, treatment means differed between steers, days and rations. However, the ranking of each treatment was the same when comparing combinations of steers, rations and days.

INTRODUCTION

In recent years many new grain varieties and hybrids have been developed in the United States. This fact, coupled with new processing techniques and variations within each technique, offer the livestock industry a great number of grain products. Consequently, some rapid method of evaluating these products is desirable.

The use of digestion or feedlot trials for feedstuff evaluations is somewhat limited from the standpoint of time and expense. Chemical analyses of a feedstuff yields essential information conerning its usefulness, but the final evaluation must be based on its nutritive value to the animal. As a result, considerable attention has been given to screening techniques to evaluate the utilization of feedstuffs. Screening techniques usually utilize small samples and laboratory procedures, thus permitting a rapid and inexpensive evaluation of a large number of feedstuffs.

Annison and Lewis (1) report that as much as 85 per cent of the digestible dry matter may disappear from the rumen of steers. Therefore, any estimate of digestion in the rumen of a fistulated steer should offer a good indication of the overall utilization of the feedstuff by the animal.

The *in vivo* nylon bag technique with rumen fistulated steers appears to offer a quick, easy method of estimating utilization, yet it has received only limited use in evaluating concentrates. This technique has a valid basis. Fistulation apparently has no effect upon digestion according to Hayes, Little and Mitchell (17). Van Dyne (35) working with forages obtained excellent relationships between digestion trials and the nylon bag technique.

The work reported in this thesis was undertaken to evaluate several variables of the nylon bag technique in measuring grain utilization.

REVIEW OF LITERATURE

In recent years a number of workers have investigated means of obtaining an estimate of forage feeding values by micro-methods. Many researchers have studied the use of nylon, dacron and silk bags *in vivo* techniques.

Cotton threads were suspended in the rumen of cows by Balch and Johnson (4) and the rate of digestion was observed. Quin, Van Det Wath and Myburg (32) suspended silk bags in the rumen of fistulated sheep and observed the rate of disappearance of feeds. McAnally (29) studied the digestion of oat and wheat straw by placing samples in silk bags and incubating them in the rumens of fistulated sheep.

Cellulose digestibility of alfalfa, brome grass and timothy hay were compared by Hopson, Johnson and Dehority (20) using a conventional digestion trial and the dacron bag technique. The results showed that 36 and 42 hour incubated samples had a significant correlation to the digestibility coefficients of cellulose as determined in a conventional digestion trial. Gallinger and Kercher (12) found a significant correlation ($P \leqslant .05$) for organic matter disappearance from nylon bags and organic matter digestibility of native hays by steers; whereas with wethers the correlation between the two methods approached significance ($P > .05$). The correlation for dry matter disappearance from nylon bags and dry matter digestibility of native hays by steers and wethers approached significance ($P > .05$).

In a study reported by Kercher, Gallinger and Eikenberry (26), the nylon bag technique ranked alfalfa, oat, native and crested wheatgrass hays for dry matter and cellulose digestibilities in the same order as did the total collection method. The correlation between the nylon bag technique and total collection for organic matter disappearance was significant ($P \leq .05$) for steers and approached significance ($P > .05$) with wethers; whereas the dry matter correlations approached significance ($P > .05$) with both steers and wethers.

Lusk, Browning and Miles (28), in a conventional digestion trial, found that the cellulose digestion coefficient for coastal Bermuda was 63.7 per cent. It was not significantly different from the coefficient of 61.0 per cent for 72 hour incubations in nylon bags. Cellulose digestibility of alfalfa hay was 56.5 per cent as determined by a conventional digestion trial, which was not significantly different from the digestion coefficient of 55.2 per cent determined by the nylon bag technique. A correlation of +0.83 was obtained when the 48 hour legume and 72 hour grass hay digestion coefficients were compared with results of conventional digestion trials.

The procedures used in the nylon bag technique varies from worker to worker. Van Keuren and Heinemann (36) placed 10 grams of forage, ground through a 20 mesh screen, in nylon bags, which were then suspended in the rumen of a fistulated animal. The nylon bags measured two by four inches and were made of 72 thread count woven nylon cloth, double sewn with a smooth interior and no exposed edges. The bags, identified by plastic tags, were secured to an acrylic plastic stick weighted at one end with lead. The plastic sticks were secured to the fistula plug with nylon fishing line. This restricted the bags to the same area of the rumen, which they felt was related to the low variation observed. The nylon bags remained in the rumen for a predetermined length of time. Upon removal, the bags were cleaned of adhering ingesta and oven dried for 48 hours at 65 degrees centigrade. The bags were removed from the sticks and the material in the bags was crushed by hand, then the bags were dried at the same temperature for an additional 24 hours. The bags were weighed and dry matter digestibility was determined by weight difference.

Van Dyne (35) used similar methods in his studies. His nylon bags were two by four inches in size and made from parachute cloth with 120 threads per inch. Two to 10 grams of the forage sample and two glass marbles, used to aid mixing, were placed in each bag. The bags were affixed in a manner similar to those of Van Keuren and Heinemann (36). The bags were identified with plastic ear tags or labeled directly with ink. After removal from the animals, the bags were soaked in a 75 per cent ethyl alcohol solution and rinsed in water as a group. Various rinsing procedures described later were used. The bags were oven dried and weighed, then placed in a centrifuge tube and cellulose was determined on the bags and samples by the procedure of Crampton and Maynard (7).

Lusk, Browning and Miles (28) ground forages through a 10 mesh screen and placed three gram samples into two by four inch nylon bags. The bags were made of parachute material 80 by 130 threads per inch with thread spaces of 6 by 11 microns. The sample bags were weighted with washers and nylon cords were used to attach the bags to the fistula cap. Upon removal, the bags were placed in 75 per cent ethanol to stop microbial activity.

The effect of fistulation upon the digestibility of hay and grains by cattle has been studied by several workers. Haynes et al (18) compared digestibilities of various hay to grain ratios comparing 500 lb. fistulated steers and mature milking cows. Steers exhibited significantly ($P \leqslant .01$) reduced crude fiber and ether extract digestibilities. They concluded fistulation depressed digestibility; however, the comparison of cows and 500 pound steers has obvious limitations. Drori and Loosli (8) paired six steers and introduced a rumen fistula in one of each pair. No significant differences were noted between the digestion coefficients of intact and fistulated steers.

Apparent digestion coefficients for dry matter, energy, crude protein, ether extract, crude fiber and nitrogen free extract were studied by Hayes, Little and Mitchell (17) with fistulated steers and their intact twins. Fistulation did not significantly affect apparent digestion coefficients.

Miles (31) obtained a significant increase in digestion in the ventral area as compared to the dorsal area of the rumen. This concurs with work of Balch and Johnson (4), who found that cotton was digested much faster in the ventral than in the dorsal area of the rumen. Balch and Johnson (4) also observed that the rate of breakdown of the dry matter of hay suspended in the rumen in silk bags was about three times faster in the ventral than dorsal sac of the rumen.

Miles (31) washed silk sacks containing feed with water and failed to force out particles of material from within the sacks. Lambert and Jacobson (27) placed nylon bags containing cellulose in plastic bags filled with autoclaved rumen contents. The contents were kneaded for a considerable length of time with no appreciable loss of cellulose from the nylon bags. This suggested that some enzymatic change occurs in the cellulose prior to its' disappearance from the bags.

Hopson et al (20) used fistulated wether lambs to study differences in rates of cellulose digestion for alfalfa, brome grass, and first and second cutting timothy hays incubated in dacron bags. They found that digestion of cellulose in alfalfa hay could be detected ealier than with the grass hays. Maximum digestibility of alfalfa occurred 6 to 12 hours ahead of the grass hays, which indicated a more rapid rate of digestion for alfalfa. In a second trial, the effects of feeding a forage to the fistulated steer different from the forages in the dacron bags were investigated. The cellulose digestion of the four test forages in the dacron bags was significantly greater in the steers fed the alfalfa hay ration than with the steers fed the grass hays.

Elliston (10) found alfalfa hay and barley straw in nylon bags were digested to a greater extent ($P \leqslant .01$) when incubated in a fistulated steer fed alfalfa hay as compared to a fistulated steer fed barley straw. Gallinger and Kercher (12) observed that when fistulated steers were fed chopped alfalfa hay, significantly ($P \leqslant .05$) more dry and organic matter of native hays disappeared from nylon bags than when the fistulated steers were fed a mixture of rolled barley and chopped alfalfa hay.

A possible explanation for this is offered by the work of Johnson, el Shazly and Dehority (23). Using filter paper in nylon bags, cellulose digestion rates were inhibited when ratios of 1:2 or higher of corn:hay were fed. Further experiments showed that the inhibition was due primarily to a competition for nutrients, the most important one being urea nitrogen.

Hale et al (14) fed fistulated steers alfalfa hay or rations containing 60 per cent of either barley or milo. The rate of dry matter disappearance of milo and barley incubated in the milo fed steer was reduced compared with the rate of disappearance in the steers on either the alfalfa or barley rations. However, due to the design of the experiment, rations and steers were confounded since only one steer was fed each ration.

Van Dyne (35) found that cellulose digestion increased with increasing time periods of fermentation up to 60 to 72 hours. Per cent cellulose digestion was directly related to fermentation time. Van Keuren and Heinemann (36) found that dry matter digestibilities of various forages converged at greater time periods of incubation.

Elliston (10) noted increasing cellulose and dry matter disappearance of forages from nylon bags for each succeeding 24 hour period through 96 hours. He also checked the disappearance of solka floc from nylon bags for incubations between 8 and 72 hours. Dry matter losses showed a definite increase with increasing time, from 3.4 to 56.7 per cent, respectively.

Cadena (5) found the per cent disappearance of ground milo and barley dry matter from nylon bags, when incubated in an alfalfa fed steer, increased with increasing incubation times. Incubation times of 2 to 24 hours showed 35.7 to 87.0 and 55.8 to 86.6 per cent dry matter disappearance, respectively, for milo and barley. He noted that the difference between grains was not as great at 24 hours as it was for the two, four and six hour periods.

Per cent cellulose digestion was inversely related to sample sizes of 2 to 10 grams in work reported by Van Dyne (35). Van Keuren and Heinemann (36) showed an effect of forage sample size on dry matter digestibility for shorter incubation periods. Generally, a larger sample size resulted in a lower dry matter digestibility. The effect decreased as the length of time in the rumen increased and was not significant for 72 to 96 hour incubations. According to Erwin and Elliston (11) as the amount of feed (milo, barley, barley straw, and alfalfa hay) was increased from 10 to 24 grams, digestibility was linearly decreased.

The influence of fineness of grind (20, 40 and 60 mesh) showed very little effect on the per cent dry matter digestibility of alfalfa hay in work reported by Van Keuren and Heinemann (36). Elliston (10) found that fineness of grind of alfalfa or barley straw using either a 5, 10 or 20 millimeter diameter screen accounted for the smallest amount of variation in the analysis of variance. Erwin and Elliston (11) reported fineness of grind of milo, barley, barley straw and alfalfa had less effect on digestibility as time of incubation was increased. The results also indicated that the position of the bags on a chain or between chains within a steer did not influence dry matter disappearance.

The ability of several animals to digest cellulose and dry matter in nylon bags has been studied. However, these studies of the variation between animals offer conflicting results. Elliston(10)found the between animal variation was not significant ($P \geqslant .01$) in forage dry matter studies with steers. Van Dyne (35) found the variation between animals of the same class (cattle and sheep) for forage dry matter disappearance was significant at the 5 per cent level between cattle and the 1 per cent level between sheep. The difference between sheep and cattle was significant ($P \leqslant .01$) when grazed upon the range, but no differences were noted when they were fed oat hay.

The method of washing the bags after incubation has a significant effect upon the amount of dry matter disappearance. Van Keuren and Heinemann (36) studied eight soaking treatments on alfalfa. These involved pre- and post-incubation-soaking in water with and without agitation for 24 hours and only long enough to wet the sample. The post-incubation-soaking treatments increased the amount of dry matter disappearance, but coefficients of variation were not affected by any of the treatments. Thorough versus light washing of the bag and its contents after incubation was studied by Van Dyne (35). There was more than a 10 per cent difference between the estimates of forage dry matter digestion by the two washing procedures, which was significant ($P \leqslant .01$). Further washing of the bags indicated that essentially all of the soluble material had been removed from the bags by the thorough washing method. Erwin and Elliston (11) washed the bags and their contents until the wash water remained clear. Elliston (10) studied the disappearance of solka floc from nylon bags incubated in a water bath as a water control check. He found only 2.6 per cent of the dry matter disappeared in 72 hours.

Since the cereal grains contain approximately 70 per cent starch, the digestibility of this fraction in the rumen becomes very important. The amount of starch that passes from the rumen to the abomasum was studied by Weller and Gray (37). They found that increasing the amount of starch fed from 3 to 150 grams produced an increase of one to eight grams of starch passing through the abomasum.

Karr, Little and Mitchell (25) fed balanced rations containing 20, 40, 60 or 80 per cent corn to steers. Average daily starch intake, passage of starch to the abomasum and recovery of starch from the feces, respectively, were as follows: 20 per cent corn - 977 grams, 357 grams and 16 grams; 40 per cent corn - 1976 grams, 511 grams and 19 grams; 60 per cent corn - 2401 grams, 1062 grams and 41 grams;

80 per cent corn - 2641 grams, 841 grams and 42 grams. Apparent digestion of starch was 98 to 99 per cent for all rations. The proportionate shares of digestion occuring anterior and posterior to the rumen were not consistently affected by the different rations. However, the absolute amount of starch passing out of the rumen increased at higher levels of starch intake. These conflicting reports may be due to the different levels of starch fed or the form in which it was fed, which was isolated starch as compared to starch from whole corn. However, these reports concur in that most of the starch fed is digested in the rumen.

Archibald, Owen and Barnes (3) studied the digestibility of dry matter, protein, n-free extract, ether extract, energy, cellulose, lignin, and pentosans for alfalfa and timothy hays. Two procedures involving fistulated cows were used: the conventional digestion trial and the dacron bag technique. The digestion coefficients were remarkably uniform for the the individual forages for most of the fractions determined, regardless of the procedure used. This was especially true of the values for energy, fiber, cellulose and pentosans.

They did find a higher protein digestibility by the dacron bag technique. This was probably due to metabolic nitrogen in the feces. The dacron bag technique yielded lower values for fiber, cellulose and pentosans. This was considered to be due to the lack of rumination of the material in the bags. They also noted higher values for ether extract for the dacron bag technique.

Cadena (5) studied water soluble losses by placing control bags of dry rolled and ground milo and barley in water and removing them from the water at the same time interval that the bags were removed from the rumen. He corrected the rumen incubated bags for water soluble losses assuming that this material was digestible, but does not represent microbial digestion in the nylon bag. The water losses were constant in relation to time with no increase in losses after the first two hour period for a two, four, six and eight hour study. It must be noted that both Elliston (10) and Cadena (5) failed to state if they had subjected the water control treatments to the same washing procedure as the rumen incubated treatments. If they did not, the validity of these water soluble losses must be questioned.

Cadena et al (6) placed dry rolled milo and steam rolled barley in nylon bags and incubated them in a fistulated steer fed alfalfa hay. The rate of dry matter disappearance of barley was three times greater than for milo suspended in the rumen for seven hours. Differences in the rate of disappearance favored barley through the 48 hour incubation. Fecal samples of feedlot heifers on these feeds showed fecal starch was consistently higher for heifers on the milo ration than those on the barley ration. Average fecal starch values were 25.1 per cent for the milo ration and 7.7 per cent for the barley ration. In a feedlot trial Hale et al (16) found a steam rolled barley ration resulted in higher and more efficient gains than an equivalent dry rolled milo ration. These trials suggest, as do the nylon bag results, a more complete utilization of the starch of barley than milo.

Cadena (5) conducted a digestion trial with steers fed 56 per cent dry rolled milo or 56 per cent steam rolled barley rations. Protein and gross energy digestibilities were significantly higher for the barley than for the milo ration. As the only variable of the rations was milo and barley, the digestion trial suggested that the digestibility of barley was higher than milo. A study of these grains with nylon bags incubated in a steer fed alfalfa hay showed the dry matter disappearance for barley was 50 per cent greater than for milo.

Hale et al (13) studied the rate of dry matter disappearance of dry rolled and medium ground milo and barley from nylon bags incubated in a steer fed alfalfa hay. The rate of dry matter disappearance from dry rolled barley was 60 per cent greater than for dry rolled milo and suggests a more rapid and greater total digestion of barley than milo. A digestion trial by Hale et al (14) found the dry matter digestibility of dry rolled milo and barley to be 63.4 and 78.0 per cent, respectively, which confirmed the nylon bag results.

Milo and barley, dry rolled or ground through a 20 mesh screen, were compared for rate of dry matter disappearance from nylon bags by Cadena (5). Grinding the milo increased the rate of dry matter disappearance by 14.1 per cent over dry rolling. He attributed this increase to the smaller particles being able to pass through the bag and to the greater surface area presented for microbial attack. This observation suggests that ground milo would be superior to dry rolled milo in a fattening ration. However, Hubbert et al (21) reported ground milo depressed gains compared to dry rolled milo in a 52 per cent milo ration fed in a feedlot trial. They suggest considerable fine materials were present in ground milo rations, which steers apparently found unpalatable. This agrees with results from the Kansas Station (24) which showed feed intake was reduced with finely ground grain. It must be noted that Mehen (30) in a total collection digestion trial found digestion coefficients of dry rolled and fine ground milo to be 68.85 and 70.69 per cent, respectively. However, these differences were not statistically different. The results of the digestion trial are not in agreement with those in the nylon bag study in respect to dry rolled and fine ground milo.

These facts indicate that the nylon bag technique does have merit in estimating utilization of feedstuffs; however, there has been little research conducted with concentrates. Many variables remain unexplained and the results of any nylon bag study must be carefully evaluated.

EXPERIMENTAL PROCEDURE

Experimental Animals and Rations

Rumen fistulas were established in six Hereford steers according to the method reported by Dougherty (9). Fistula plugs constructed of two heavy pieces of rubber and connected by an inflatable neck were used to close the fistulas. The steers were maintained on constant feed intake in individual, partially shaded concrete-floored pens, with free access to water and salt. During the trials the steers received three rations. Two steers each received ground alfalfa hay, steam processed milo or steam processed barley rations. Some difficulty was experienced in keeping the grain fed steers on feed. Consequently the grain levels of the rations were lowered. The formulation of the grain rations for the various experiments is given in Tables 1 and 2. The cattle were allowed three weeks on constant feed intake prior to the nylon bag studies.

Nylon Bag Technique

The adoption of the nylon bag technique for the purpose of feed evaluation required a bag that would be large enough to hold a sufficient quantity of feed for analysis. The bag had to be constructed of material

Table 1. Grain rations for fistulated steers. Trials 1-8.

Ingredients	Pounds	
	Milo Ration	
Ground alfalfa	5.00	
Cottonseed hulls	15.00	
Steam processed milo	68.25	
Cottonseed pellets	4.50	68.25% grain
Molasses	5.00	
Dicalcium phosphate	0.50	
Urea	0.60	
Salt	0.50	
Ground limestone	0.60	
Trace minerals*	0.05	
	100.00	
Vitamin A-10-P	10 gm.	
	Barley Ration	
Ground alfalfa	5.00	
Cottonseed hulls	10.00	
Steam processed barley	74.85	
Cottonseed pellets	3.00	74.85% grain
Molasses	5.00	
Dicalcium phosphate	0.50	
Urea	0.50	
Salt	2.50	
Ground limestone	0.60	
Trace minerals*	0.05	
	100.00	
Vitamin A-10-P	10 gm.	

*Manganese, 12.20%; Iodine, 0.38%; Cobalt, 0.26%; Iron, 9.60%; Copper, 0.73%; Zinc, 0.67%; Calcium, 9.15%.

Table 2. Grain rations for fistulated steers. Trials 9-12.

Ingredients	Pounds	
	Milo Ration	
Ground alfalfa	15.00	
Cottonseed hulls	15.00	
Steam processed milo	56.50	56.50% grain
Cottonseed pellets	6.00	
Molasses	6.00	
Dicalcium phosphate	0.50	
Ground limestone	0.50	
Salt	0.50	
	100.00	
Vitamin A-10-P	10 gm.	
	Barley Ration	
Ground alfalfa	15.00	
Cottonseed hulls	15.00	
Steam processed barley	57.50	
Cottonseed pellets	5.00	57.50% grain
Molasses	6.00	
Dicalcium phosphate	0.50	
Ground limestone	0.50	
Salt	0.50	
	100.00	
Vitamin A-10-P	10 gm.	

with a weave sufficiently loose to permit the entrance of rumen fluid and egress of the gases produced, yet fine enough to prevent loss of the feed material. In all, six different cloth samples of synthetic fibers were tested.

Bags four by seven inches double sewn on all edges with nylon thread were constructed. Identification of the bags was by numbered plastic ear tags or Coors Porclein Ink. The weight of each nylon bag was recorded and 10 grams (except as noted) of air dry feed was then placed in each bag.

A five hundred gram weight was attached to each end of a 15 inch chain, passing link size number 00. The closed bags were attached to the weighted chains, one to each link, with nylon fishing line. The bags were closed three and one-half inches from the bottom with nylon fish line. All samples were incubated in the anterior dorsal sac of the rumen of a fistulated steer for eight hours (except as noted). Following incubation, the bags still attached to the chain, were group rinsed thoroughly in tap water until the water remained clear and were then removed from the chains and dried in a forced air oven at 100 degrees C. for 24 hours and weighed. The dry matter disappearance from the bags during incubation was used as the criteria of digestion.

Incubation periods were from the tenth through the seventeenth hours after feeding for hay-fed steers and from the third through tenth hours for grain-fed steers (except as noted). All new bags were "conditioned" by incubating them in the rumen of a fistulated steer for 72 hours prior to use.

Grain particle sizes are given as Tyler screen scale equivalents. Weights were recorded to the nearest 0.1 gm. for trials 1-4 and to the nearest 0.01 gm. for trials 5-12. Bags were made from the six different nylon cloths and designated A, B, C, D, E and F. All statistical analyses were conducted according to Steel and Torrie (34).

Trial 1

This trial was composed of two one way classification studies utilizing bag E. Three replications were incubated for 2, 4, 6, 8 and 24 hour periods. In part A, dry rolled milo and three steam processed milo treatments, poor rolled, regular rolled and flat rolled, were compared. In part B, dry rolled milo was compared with pressure cooked flaked, pressure cooked flaked and ground, steam processed flaked, and steam processed flaked and ground milo. When ground, the moist flakes were processed through a vegetable chopper. The moist heat treated grains were added to the nylon bags without drying. Dry matter determinations were made from the same batch of processed feed.

The same alfalfa hay fed steer was used for both experiments, which were conducted on different days. The bags were placed in the rumen between the third and fifth hours after feeding and the steer was fed once during each 24 hour incubation interval.

This trial was conducted to study the effect of length of incubation time and to compare digestibility in the nylon bag of various grain treatments with feedlot performance and conventional digestibility values. The effect of grinding (surface area) on the grain treatments was also observed.

Trial 2

Dry rolled milo was placed in nylon bags A, B, C, D and E and incubated for eight hours in a steer fed alfalfa hay.

The purpose of this trial was to study the effect of cloth type on the rate of dry matter disappearance. A minimum of 18 replications was used.

Trial 3

A series of one-way classification studies were conducted to check the performance of bags C, D and E when they contained several milo treatments. The treatments studied, with a minimum of eight replications, were steam processed milo flakes larger than 9 mesh in size, 9-20 mesh ground milo, 20-28 mesh ground milo and 28-35 mesh ground milo. All the bags were incubated in alfalfa hay fed steers. Each study was conducted on a different day.

The 9-20 mesh ground milo represents grain particles that went through a nine mesh screen and were caught on a twenty mesh screen. The 20-28 and 28-35 mesh grain fractions were determined in a similar manner.

Trial 4

A three by three factorial design was used to study three bags, C, D and E, and three particle sizes of ground milo. The grain treatments were 9-20, 20-28, and 28-35 mesh ground milo.

In the experimental design, position of the chains in the rumen was confounded with bag type in that one chain was used for each bag type. All bags were incubated at the same time in an alfalfa hay fed steer. Six replications were used.

Trial 5

Milo and barley were ground in a Wiley Mill through a two mm. screen and the percentage by weight of the various particle sizes was determined. The particle sizes were 9-20, 20-28, 28-35 and greater than 35 mesh. Proximate analyses of the first three particle sizes and the whole grain of milo and barley were analyzed according to AOAC (2).

Trial 6

The effect of atmospheric moisture on the weight of bags C, D, E and F was determined. The empty bags were heated for three hours in a vacuum oven at 100 degrees centigrade, cooled in a desicator and weighed. They were then exposed to the air over night, reweighed and the difference in weight was called moisture uptake by the bags. Three replications were used.

Trial 7

A four by three factorial experiment was conducted to study steer, chain, bag and grain particle size effects. Bags C, D, E and F were compared containing 9-20, 20-28 and 28-35 mesh ground milo across two alfalfa hay fed steers. Six chains were used with each chain having one replication of all twelve treatment combinations. There were three replications within each steer or six replications of each grain treatment.

Trial 8A

Two one way classification studies were conducted with steam processed milo and barley flakes with the four bags, C, D, E and F. Steam processed milo flakes were incubated in a milo fed steer and steam processed barley flakes were incubated in a barley fed steer during the same time period. There were nine replications of each bag within each grain. Each chain contained three replications of each grain by bag treatment combination.

Trial 8B

Two four by three factorial experiments were used to study three particle sizes of milo and barley in bags C, D, E and F. The particle sizes were 9-20, 20-28 and 28-35 mesh. Milo and barley were incubated respectively in milo and barley fed steers. Each chain contained one replication of each treatment combination.

Trial 9

A water control study was conducted to determine the amount of dry matter leaching that occurs independently of any digestion. Three particle sizes plus whole ground grain of both milo and barley were studied. The particle sizes were 9-20, 20-28 and 28-35 mesh and whole grain ground through a two mm. mesh screen in the Wiley Mill. All samples were incubated in an agitated water bath for eight hours at 39 degrees centigrade. The bags were then subjected to the same washing and drying procedure as previously outlined.

Trial 10A

The effect of sample size upon dry matter disappearance from bag F was studied using milo ground through a 20 mesh screen. Samples of 2, 4, 6, 8 and 10 grams were incubated for three and six hour periods in a milo fed steer. Simultaneous water controls were conducted for each time period. However, these water controls were simply removed from the water and not washed as previously described. The water control values were subtracted from the *in vivo* values to obtain net values.

Trial 10B

Two three by four factorial experiments, one with steam processed milo flakes and the other with steam processed barley flakes, were conducted with steers fed milo and barley, respectively. The purpose was to determine the effect of sample size and bag size on dry matter disappearance. The bags used were constructed in accordance with the previously outlined manner except that they were of two, three and four inch widths. These bags with sample sizes of 6, 10, 14 and 18 grams of steam processed milo and barley flakes were studied with three replications.

Trial 11

Two steers on each ration, milo and barley, were utilized to determine if there was a steer difference when using the nylon bag technique. Steam processed flaked and dry rolled milo and barley were incubated, respectively, in milo and barley fed steers during the same time period. Four replications were used.

Trial 12

The effect of the steer's ration on the nylon bag technique was examined in this trial. Six steers, two on each ration, alfalfa hay, milo and barley, were utilized. The milo and barley treatments used were dry rolled, steam processed poor rolled and steam processed excellent flake. Three replications of each treatment were incubated in all steers at the same time.

RESULTS AND DISCUSSION

Trial 1A

The results of these one way classification studies (Tables 3 and 4) showed that there was an effect of incubation time upon dry matter disappearance from nylon bags. The longer the incubation time, the greater the dry matter disappearance. Van Dyne (35) noted the same trend for cellulose digestion and Elliston (10) for dry matter disappearance.

In part A the four milo treatments studied were: dry rolled and three steam processed treatments, poor rolled, regular rolled and flat rolled. As indicated in Table 3, the treatments were ranked in the following order on dry matter disappearance (lowest to highest): dry rolled, steam processed poor rolled, steam processed regular rolled and steam processed flat rolled. The nylon bag technique ranked the grain treatments in the same order as digestion trials conducted by Husted (22) and Mehen (30).

Trial 1B

Part B of this trial compared five milo treatments. These were: dry rolled, pressure cooked flaked, pressure cooked flaked and ground, steam processed flaked and steam processed flaked and ground. The

Table 3. The effect of grain processing treatment and time on dry matter disappearance, per cent. Trial 1A.

Hrs. in Rumen	Dry Rolled	S. P.[a] Poor Rolled	S. P. Regular Rolled	S. P. Flat Rolled
2	16.7[b]	21.1	43.8	55.4
4	13.1	21.3	40.5	45.5
6	20.6	24.1	48.1	56.4
8	25.1	28.7	51.0	63.1
24	52.4	48.5	73.2	85.2

a. Steam processed.
b. Mean value of three observations.

Table 4. The effect of grinding (surface area) and time on dry matter disappearance, per cent. Trial 1B.

Hrs. in Rumen	Dry Rolled	P. C.[a] Flaked	P. C. Flaked and Ground	S. P.[b] Flaked	S. P. Flaked and Ground
2	12.5[c]	41.3	42.4	44.1	46.0
4	15.5	42.4	43.1	45.8	49.0
6	14.8	43.1	48.8	45.1	49.3
8	21.9	44.0	49.1	50.7	49.3
24	28.9	76.6	81.4	78.3	77.8

a. Pressure cooked.
b. Steam processed.
c. Mean value of three observations.

ground grains were processed in a moist condition through a vegetable chopper. The results in Table 4 indicate little effect of surface area (due to grinding) for either moist heat treatment. The moist heat treatment apparently altered the starch portion of the grain. As in Trial 1A, a definite effect of length of incubation time was noted. The three major treatments--dry rolled, pressure cooked flaked and steam processed flaked milo--were ranked in a similar manner as they were in a digestion trial by Mehen (30). Little difference was noted between the rate of dry matter disappearance for the moist heat treatments while that from the dry rolled treatment was definitely less.

Trial 2

The results of a one-way classification study in five nylon bag types, A, B, C, D and E, and dry rolled milo are presented in Table 5. The results indicated that bags A and B had very low rates of dry

Table 5. The effect of cloth type on dry matter disappearance, per cent. Trial 2.

Bag	A	B	C	D	E
Dry Matter Disappearance	11.8[a]	9.0	19.1	18.2	28.2

a. Mean value of a minimum of eighteen observations.

matter disappearance while bag E exhibited the highest rate. Bags A and B were discarded from further trials because they exhibited rates of dry matter disappearance so low as to make detection of treatment differences difficult. These results show a large effect of cloth type upon the rate of dry matter disappearance. Readily observable characteristics (threat count, weight, etc.) could not be correlated to rates of dry matter disappearance from the various cloth types.

Trial 3

The results of four one way classification studies conducted with bags C, D and E and four milo treatments are presented in Table 6. The grain treatments were steam processed milo flakes larger than

Table 6. The effect of bag type and grain treatment on dry matter disappearance, per cent.[a] Trial 3.

	Bag		
Grain Treatment	C	D	E
S.P. milo flakes	25.4	28.2	45.7
9-20 mesh milo	19.9	22.8	25.6
20-28 mesh milo	13.3	17.6	21.2
28-35 mesh milo	19.7	21.3	24.7

a. Each value represents a mean of at least eight observations.

9 mesh, 9-20 mesh ground milo, 20-28 mesh ground milo and 28-35 mesh ground milo. The data in Table 6 shows a definite difference in the rate of dry matter disappearance of these grain treatments for the three bags. It can be noted that each of the bags ranked the four milo treatments in identical order.,

The coefficients of variation for these treatment combinations presented in Table 7 showed no advantage of one bag over the other in this respect. It should be noted that in general the flakes showed the highest coefficients of variation while the more closely defined particle sizes exhibited less variation.

Table 7. Coefficients of variation for bag, grain treatment combinations. Trial 3.

Grain Treatment	Bag		
	C	D	E
S.P. milo flakes	14.8[a]	6.3	18.1
9-20 mesh milo	7.9	12.5	11.0
20-28 mesh milo	10.0	7.6	3.1
28-35 mesh milo	5.6	4.0	5.3

a. Mean value of at least eight observations.

A homogeneity of variance test was conducted to study the feasibility of statistically combining the separate studies in Trial 3.

The results showed that the variation in these studies was not statistically different and, therefore, a pooled analysis of variance could be run. To maintain a 95 per cent level of significance with a 90 per cent assurance of detecting a difference of 5 per cent, 8.2 replications were needed.

Trial 4

The results of Trial 4 are presented in Table 8. In this three by three factorial experiment bag type was confounded with chains (position in rumen) since one chain was used for each bag type. Assuming no position effect, the analysis of variance, Table 8, showed significant ($P \leqslant .01$) bag and particle size differences in addition to a significant ($P \leqslant .05$) bag by particle size interaction.

Even though confounding did occur, the treatment combinations were ranked in the same order as they were in Trial 7 using two steers when chain by bag confounding did not occur. Apparently there was little effect due to confounding. There was, as in Trial 3 (Table 6), a large bag and particle size difference. It can also be noted in Table 9 that each bag ranked the three grain treatments in identical order as was the case in Trial 3. Each bag type performs in a similar manner with the major difference between them being the mean values for the treatment combinations.

Table 8. Analysis of variance of Trial 4.

Source	df	Sum of Squares	Mean Squares	F Test
Reps	5	23.55	4.71	ns
Treatment	8	4585.03	573.13	56.41**
Bags	2	940.90	470.45	46.30**
Prt Size	2	3511.78	1755.89	172.82**
Bag X Prt Size	4	132.35	33.09	3.27*
Error	40	406.21	10.16	

**P ⩽ .01
*P ⩽ .05.

Table 9. Mean dry matter disappearance values in per cent for bag and particle size treatment combinations. Trial 4.

Bag	9-20 mesh	20-28 mesh	28-35 mesh
C	11.9	18.8	28.2
D	15.9	25.6	34.8
E	19.5	26.9	43.2

The size of experiment necessary to fulfill a 95 per cent level of significance with a 90 per cent assurance of detecting a difference of 5 per cent required 8.83 replications. The size of experiment necessary to fulfill a 90 per cent level of significance with a 90 per cent assurance of detecting a difference of 7.5 per cent was only 3.18 replications.

Trial 5

In an effort to define grain treatments, various particle sizes of milo and barley were studied to see if one particle size might have similar dry matter "digestibility" patterns to the whole ground or rolled grain. The different particle sizes represented readily definable and easily repeatable grain treatments with which to study several variables of the nylon bag technique.

The proportion of the various particle sizes from the whole grains is listed in Table 10.

The proximate analyses for three particle sizes and whole ground milo and barley are enumerated in Table 11. These data indicate that there is no particle size of either milo or barley which is comparable to the whole grain. This suggests that a specific particle size fraction should not be used to evaluate the whole grains by the nylon bag technique. Even though no one particle size will accurately represent

Table 10. Proportions of particle sizes of whole milo and barley ground through a two mm. mesh screen. Trial 5.

Tyler Screen Scale Equivalent Designation	Gms.	Per Cent (by weight)
	Milo	
9-20	42.7	15.27
20-28	128.8	46.05
28-35	38.8	13.87
35	69.4	24.81
	Barley	
9-20	77.6	23.37
20-28	157.7	47.50
28-35	43.5	13.10
35	53.2	16.02

the whole grains, it appears that for barley, particle size of 20-28 mesh most accurately represents the whole grain. There was no significant difference as tested by Duncan's Multiple Range Test ($P > .05$), between this particle size and the whole barley grain for any of the fractions of the proximate analysis studied.

Milo with a particle size of 20-28 mesh most accurately represented the whole grain; however, there were significant differences ($P \leq .05$) in ether extract and protein between this particle size and the whole grain.

Table 11. Proximate analysis for various particle sizes of milo and barley. All values on a dry matter basis.[a] Trial 5.

	Barley				Milo			
	Whole Ground	9-20 Mesh	20-28 Mesh	28-35 Mesh	Whole Ground	9-20 Mesh	20-28 Mesh	28-35 Mesh
N.F.E. %	79.10[b]	82.02[b]	82.32[b]	76.11[c]	81.25[b]	84.86[c]	82.58[b]	73.50[d]
Prot. %	10.32[b]	10.44[b]	10.34[b]	9.64[c]	11.22[b]	11.76[c]	12.28[d]	12.59[c,d]
C.F. %	5.21	2.76	3.06	8.29	1.41[b]	0.49[b]	1.24[b]	3.23[c]
E.E. %	2.54	2.78	2.02	2.60	4.52[b]	2.18[c]	2.42[c]	7.50[d]
Ash %	2.84[b]	2.00[c]	2.65[b]	3.40[d]	1.63[b]	0.74[c]	1.38[b]	3.18[d]

a. Each value mean of two observations.

b, c, d. Means within grains on the same line bearing different superscripts are significantly different ($P \leqslant .05$).

Trial 6

The amount of moisture adsorbed from the atmosphere by bags C, D, E and F is reported in Table 12.

Table 12. Moisture uptake by bags. Trial 6.

	C	D	E	F
Wt. in gm. moisture uptake	0.0959	0.0505	0.0485	0.0185

Bag C adsorbed significantly more moisture than the other three bags. Bag C adsorbed nearly one tenth gram of moisture. In some studies this level of moisture adsorption could affect the amount of dry matter disappearance by 5 per cent. These data showed that for all practical purposes, it was not necessary to handle bags D, E and F in a manner to prevent exposure to the air and subsequent absorption of atmospheric moisture. To place all the bags on an equal weight basis, both before and after incubation, the bags in subsequent trials were handled in a manner described in the Experimental Procedure of Trial 6.

Moisture uptake by bags containing samples was not studied. However, it can be theorized that the amount of moisture uptake by such bags would be even greater. The moisture uptake would certainly be variable because of the different amounts of sample remaining in each bag after incubation in a steer.

Trial 7

The results of this trial are shown in Table 13. The analysis of variance for this trial is shown in Table 40. Steers were significantly ($P \leqslant .01$) different in their ability to "digest" dry matter. Chains were not significant ($P \geqslant .05$), although they approached significance. Bags, particle size and the bags by particle size interaction were also significant ($P \leqslant .01$). The steer by bag and steer by bag by particle size interactions were not significant ($P \geqslant .05$); however, the steer by particle size interaction was significant ($P \leqslant .01$).

These data indicated that different bags can allow "digestion" of dry matter of certain particle sizes faster than other particle sizes and that fistulated steers, even though on the same ration, differ in their ability to influence the rate of dry matter disappearance.

As Figure 1 shows, bag F distributed the dry matter "digestion" of the three particle size means across a wider range and in a more linear fashion. The per cent dry matter disappearance for the three means were separated approximately equidistant.

Table 14 indicates that bag F had the lowest coefficients of variation across the three particle sizes.

The two steers ranked the treatment combinations (bags by particle sizes) in the same order except as noted in Table 13. Steers 1 and 2 ranked particle size 1 with bags E and F in a different order.

Table 13. The effect of steers, bags and particle size on the ranking of dry matter disappearance, per cent. Trial 7.

Steer I[a]	C1	D1	F1	C2	E1	D2	E2	C3	F2	D3	E3	F3
	10.6	13.4	16.0	16.6	18.5	23.0	25.8	27.9	30.2	32.7	42.2	43.6
Steer II	C1	D1	E1	C2	F1	D2	E2	C3	F2	D3	E3	F3
	9.6	11.5	15.4	16.3	17.3	18.9	21.9	22.1	26.7	26.7	35.0	39.4

a. The upper case letters refer to bag designation and the Arabic numerals refer to particle size as follows: 1, 9-20 mesh; 2, 20-28 mesh; 3, 28-35 mesh.

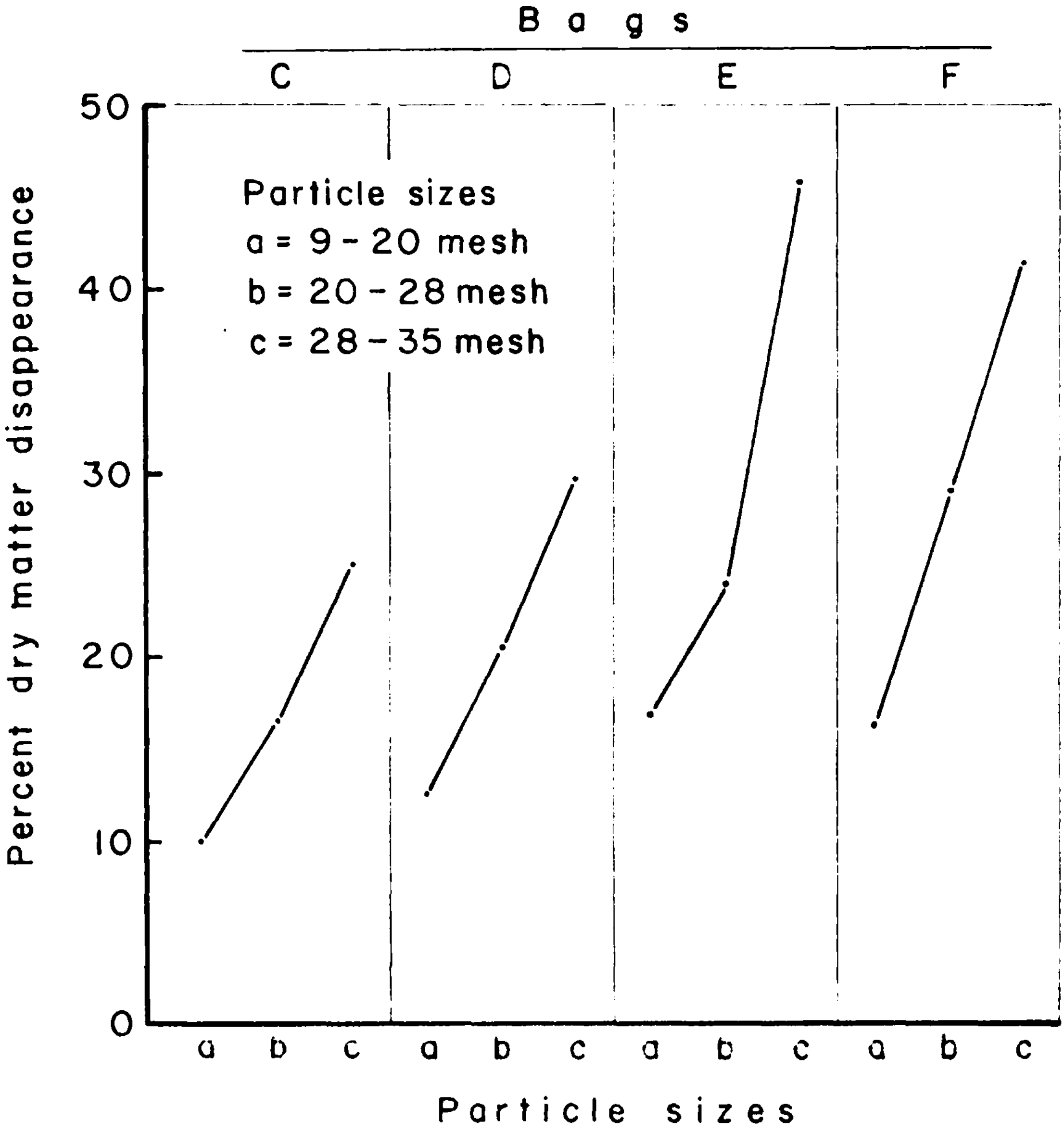

Figure 1: Percent dry matter disappearance of various milo particle sizes in different bags.

Table 14. Coefficients of variation of bag, particle size combinations. Trial 7.

Bags	Mesh		
	9-20 mesh milo	20-28 mesh milo	28-35 mesh milo
C	12.17[a]	11.00	16.15
D	27.45	17.22	15.30
E	14.52	12.25	9.44
F	9.38	9.84	6.17

a. Mean value of six replications.

Trial 8A

The results of Trial 8A are presented in Tables 15 and 16. Separate analyses of variance were run on the milo and barley portions of the study because steers and rations were confounded. That is, each of the two steers were fed a different ration.

Analysis of variance (Table 41) for the milo treatments found that chains, bags and chain by bag interaction were significant ($P \leqslant .01$). Analysis of variance for barley treatments found chains significantly ($P \leqslant .05$) different. Bags were significant ($P \leqslant .01$) while the chain by bag interaction was not significant ($P \geqslant .05$).

Table 15. Mean values in per cent dry matter disappearance for steam processed milo and barley incubated respectively in milo and barley fed steers. Trial 8A.

	Bags			
	C	D	E	F
S.P. Milo flakes	12.0[a]	16.0	24.4	25.5
S.P. Barley flakes	17.8	22.9	29.1	33.0

a. Mean value of nine observations.

Table 16 shows that the coefficients of variation were not lowest for bag F.

Table 16. Coefficients of variation for milo and barley flakes incubated in various bags. Trial 8A.

	Bags			
	C	D	E	F
S.P. Milo flakes	25.83	7.85	4.64	6.05
S.P. Barley flakes	17.40	15.33	18.20	16.19

Consideration must be given to the fact that the confounding of steer rations, grain samples and steers may invalidate these coefficients of variation and absolute dry matter disappearance values. However, all bags within a grain specie were randomly allotted to each steer which should subject the bags to a random location within a steer. Thus coefficients and absolute values within a grain or steer should be directly comparable while comparisons between steers or grains are not necessarily comparable. However, these data indicated that barley is more digestible than milo which is in agreement with Saba (33).

Trial 8B

Analysis of variance (Table 43) of the milo portion of the study showed a significant ($P \leqslant .01$) difference between bags, chains and particle size with all interactions being non-significant ($P \geqslant .05$), Table 43.

Coefficients of variation for the milo study are presented in Table 17. It can be noted that the variation was smallest for all particle sizes in bag F.

Analysis of variance for the barley portion of the study showed chains and bags significantly different ($P \leqslant .01$). Particle size, chain by bag, chain by particle size, and bag by particle size interactions were significant ($P \leqslant .05$). This indicated that there was a difference in the rate of dry matter "digestion" in nylon bags in various locations of the rumen due to the fact that chains occupied different spaces in the rumen. Bags and particle sizes and particle sizes within bags resulted in different rates of digestion dependent upon location in the rumen.

Table 17. Comparison of coefficients of variation for three particle sizes of milo in various bags incubated in a milo fed steer. Trial 8B.

	Mesh		
Bags	9-20	20-28	28-35
C	6.72	17.73	6.05
D	4.41	9.36	5.86
E	4.54	7.20	8.25
F	3.47	4.17	2.18

The dry matter disappearance values are presented in Table 18.

Coefficients of variation for the barley study are presented in Table 19. Bag F showed the lowest overall variation for the three particle sizes.

It was noted that the 28-35 mesh barley was "digested" at a slower rate than 20-28 mesh barley (Figure 2). This was in contrast to the milo portion of this study which showed increasing rates of dry matter disappearance for smaller particle sizes of grain. This was attributed to the barley becoming sticky in texture and forming a lump of material within the bag. Hoflund, Quinn and Clark (19) encountered a similar problem with cellulose clumping. This probably reduced the rate of

Table 18. Mean values in per cent dry matter disappearance of three particle sizes of milo and barley incubated respectively in milo and barley fed steers. Trial 8B.

	Particle Size					
	Milo			Barley		
Bags	9-20	20-28	28-35	9-20	20-28	28-35
C	11.5	15.5	26.5	31.4	33.5	28.7
D	14.0	19.7	28.2	35.2	47.4	34.1
E	16.3	21.7	32.7	59.8	67.5	64.2
F	16.1	21.4	32.8	66.7	71.8	68.1

Table 19. Comparison of coefficients of variation for three particle sizes of milo in various bags incubated in a milo feed steer. Trial 8B.

	Mesh		
Bag	9-20	20-28	28-35
C	12.34	18.98	21.66
D	8.50	14.95	9.54
E	11.04	8.87	6.86
F	6.07	5.04	5.77

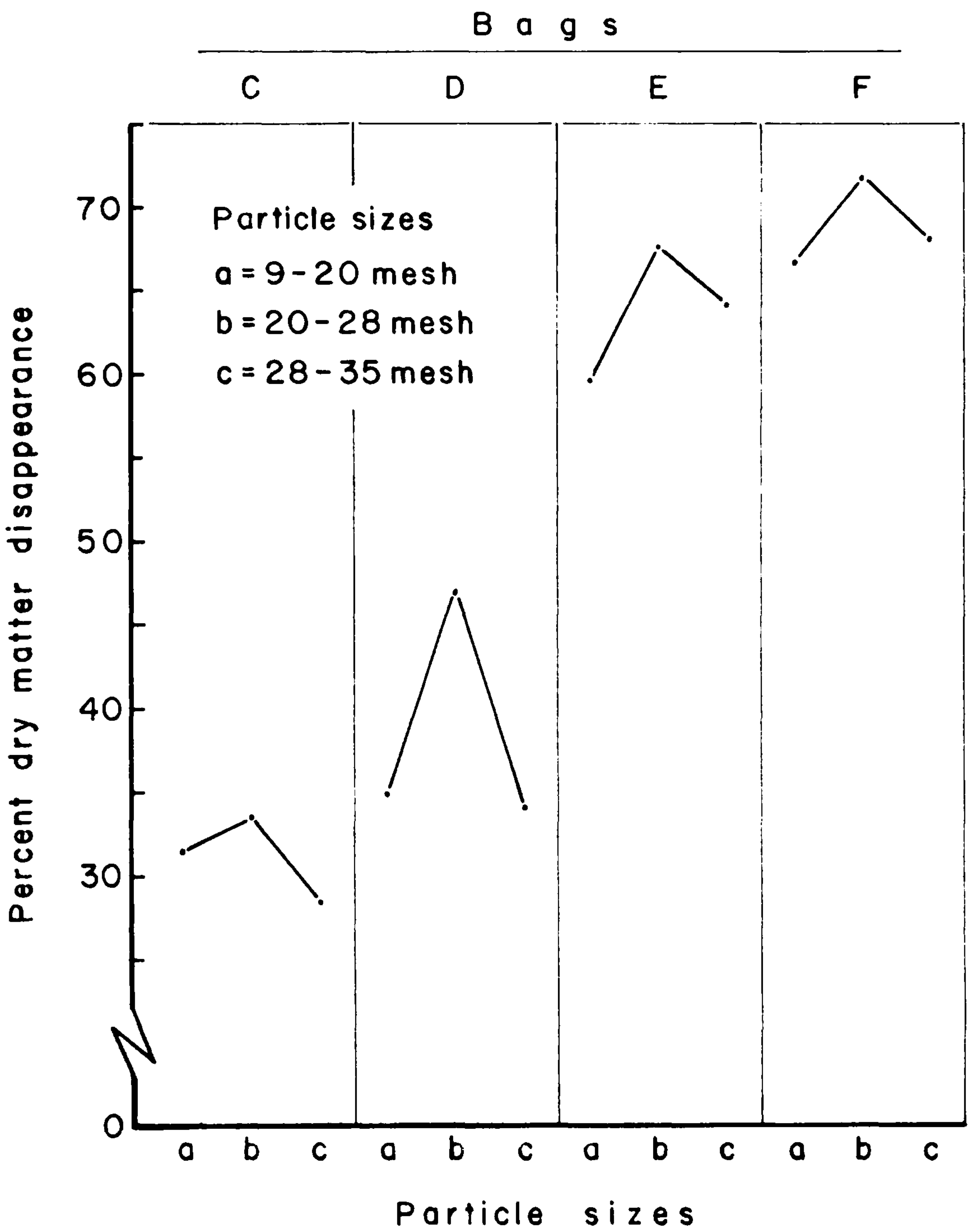

Figure 2: Percent dry matter disappearance of various barley particle sizes in different bags.

exchange of rumen fluid between the particles of grain and thus reduced the rate of "digestion."

Trial 9

The results of the water control studies with milo are presented in Tables 20 and 21.

Analysis of variance of the milo data (Table 45) found a significant ($P \leqslant .01$) difference in dry matter disappearance between bags, particle sizes and the particle size by bag interaction. It can be noted from the data in Table 20 that the dry matter disappearance of the various particle sizes of milo closely followed the *in vivo* trends of the same particle sizes (Trial 8B). A comparison of the coefficients of variation (Table 21) of the four milo treatments in the four bag types indicated, as with *in vivo* studies, that bag F was superior in reducing variation. However, bag F did not have the lowest variation with the whole ground grain.

The water control data for the barley treatments are presented in Table 22. Analysis of variance of the barley data (Table 46) found a significant ($P \leqslant .01$) difference between bags and particle sizes. The bag by particle size interaction was not significant ($P \geqslant .05$).

The dry matter disappearance values for the barley treatments did not follow those of the barley *in vivo* dry matter disappearance trends (Trial 8B), nor the *in vivo* or *in vitro* milo trends.

Table 20. Milo water control dry matter disappearance values in per cent. Trial 9.

	Mesh			
Bags	Whole Ground	9-20 Mesh	20-28 Mesh	28-35 Mesh
C	14.4	6.0	12.7	20.4
D	13.2	7.1	13.4	24.2
E	21.1	7.3	14.7	25.8
F	20.7	7.0	14.4	25.2

Table 21. Comparison of coefficients of variation for various bags and particle sizes of milo incubated in a water bath. Trial 9.

	Mesh			
Bags	Whole Ground	9-20 Mesh	20-28 Mesh	28-35 Mesh
C	18.56	9.20	4.19	8.77
D	14.38	3.94	6.24	4.84
E	2.32	5.36	3.06	1.08
F	5.81	0.96	2.49	0.28

Table 22. Barley water control dry matter disappearance values in per cent. Trial 9.

	Mesh			
Bag	Whole Ground	9-20 Mesh	20-28 Mesh	28-35 Mesh
C	19.6	15.1	18.9	23.2
D	23.9	15.7	21.0	24.7
E	24.5	15.6	20.5	24.0
F	25.7	17.8	21.2	23.7

Dry matter disappearance of the 28-35 mesh particle size was not suppressed as in the *in vivo* study. In view of the fact that the same grain treatment may react differently in a water control (*in vitro*) and *in vivo* situation sheds some doubt on the validity of subtracting water control values from the *in vivo* nylon bag results.

Evaluation of the coefficients of variation for the four bag types and four barley particle sizes did not indicate, as did the *in vivo* study, a definite advantage for bag F. These data are presented in Table 23.

Table 23. Comparison of coefficients of variation for various bags and particle sizes of barley incubated in a water bath. Trial 9.

	Mesh			
Bag	Whole Ground	9-20 Mesh	20-28 Mesh	28-35 Mesh
C	13.11	7.21	8.05	2.71
D	7.67	9.03	12.49	3.61
E	5.54	4.56	6.60	16.60
F	2.22	8.32	4.21	7.77

Trial 10A

The results of Trial 10A are presented in Table 24. There was no statistical difference ($P \gg .05$) in dry matter disappearance for either the three or six hour incubation periods nor between sample sizes within a time period. The water control values were quite low when compared to previous water control values. This was undoubtedly due to the method in which the bags were handled. They were not washed as previously described but merely removed from the water. It appeared that a great deal of dry matter was lost with the washing procedure in Trial 9. When comparing the results of the water control values of this study with the results of Trial 9, it appeared that the washing technique had a marked effect upon the amount of dry matter disappearance.

Table 24. Effect of sample size and length of incubation of in vivo and water control nylon bags upon dry matter disappearance in per cent. Trial 10A.

	Sample Size, Gms.				
	2	4	6	8	10
Three hours:					
In vivo	23.6[a]	23.2	23.9	23.6	23.0
Water control	3.9	5.5	5.3	5.2	4.5
Net	19.7	17.7	18.6	18.4	18.5
Six hours:					
In vivo	27.5	26.9	26.2	25.3	25.5
Water control	7.2	6.1	7.2	4.3	4.8
Net	20.3	20.8	19.0	21.0	20.7

a. Mean value of two observations.

Trial 10B

The mean dry matter disappearance values are listed in Table 25. Analysis of variance (Table 47) of the milo portion of the study found chains not significantly different ($P \geqslant .05$). Bags were significantly different ($P \leqslant .01$) and sample size difference was significant ($P \leqslant .05$). However, none of the interactions were significant ($P \leqslant .05$).

Analysis of variance (Table 48) of the barley portion of the study showed chains, bags, and sample size significantly different ($P \leqslant .01$).

Table 25. Per cent dry matter disappearance of 6, 10, 14 and 18 gram samples of flaked grain in two, three and four-inch wide bags.[a] Trial 10B.

Bag Width, in.	Sample Size, gms.	S.P. Barley Flakes	S.P. Milo Flakes
	6	43.4	48.7
	10	33.9	43.3
2	14	23.6	33.7
	18	20.5	24.5
	6	43.1	54.3
	10	44.4	43.5
3	14	34.7	45.7
	18	33.9	43.6
	6	51.0	55.7
	10	47.8	53.0
4	14	41.0	52.6
	18	43.2	49.1

a. Mean value of three observations.

The interactions of chains by bags, and chains by sample size were not significant ($P > .05$). The bag by sample size interaction was significant ($P \leq .05$).

Comparison of the two studies listed in Table 25 showed a definite effect of bag size on the various sample sizes. The narrower the bag the greater the effect upon dry matter disappearance. In the narrower bags the "digestion" of the larger samples was greatly reduced. With the four inch wide bag containing milo, sample size had less effect on "digestion" than it did in the barley study. The 14 and 18 gram sample sizes were large enough to considerably lower dry matter disappearance in even the four inch wide bag. The 10 gram sample size was chosen for subsequent use because in the four inch wide bag little depression of "digestion," over the 6 gram sample size occurred. The study in Trial 10A showed even less effect. The slight depression in dry matter disappearance was considered negligible in view of the greater accuracy in handling and weighing the 10 gram sample. The four inch wide bag was chosen for use because it had less effect upon sample size than did the other sized bags. The four inch bag width had been previously used and this trial validated its use. Sample volume could also possibly affect dry matter disappearance. Such volume affects would probably be less in the four inch wide bag than in the narrower bags. The four inch wide bag is a convenient size with which to work when filling, emptying and washing.

Trial 11

The results of Trial 11 presented in Table 26 showed that the two steers on each ration performed in a similar manner. The steer within grains variation accounted for only 1.72 per cent of the variation in the analysis of variance (Table 27). While the difference between steers on the same ration was statistically significant, the absolute differences were small.

Table 26. Effect of steer differences on grain dry matter disappearance, per cent. Trial 11.

	Steer	Treatment	
		Flaked	Dry Rolled
S.P. Milo flakes	1	34.0	22.9
	2	37.0	24.0
S.P. Barley flakes	3	28.3	52.1
	4	32.3	57.2

Analysis of variance showed grains, steers within grains, processes within grains, and the steers by processes within grains were significant ($P \leqslant .01$).

Table 27. Analysis of variance of per cent dry matter disappearance of dry rolled and steam processed flaked milo and barley incubated respectively in milo and barley fed steers. Trial 11.

Source of Variation	df	Mean Squares
Grains	1	1350.05**
Steers within grains	2	50.62**
Process within grains	2	1480.73**
Steer X process within grains	2	52.95**
Error	24	6.59

** ($P \leqslant .01$)

Trial 12

The results of Trial 12 are presented in Table 28. The analysis of variance (Table 49) showed steer rations to be significantly different ($P \leqslant .01$). This is attributed to the hay ration and its critical carbohydrate level. Steers within rations were significant ($P \leqslant .05$). This contributed the least to the variation in the analysis. Grains within steers and processes within grains were significant ($P \leqslant .01$). Apparently it makes little difference what rations the steers are fed in evaluating the relative differences between grains and grain processes.

Table 28. Effect of ration, grain and grain processing on dry matter disappearance from nylon bags, per cent. Trial 12.

Steer[b]	Milo[a] DR	PR	SF	Barley[a] DR	PR	SF	Steer Ave.
1	23.9	17.2	33.2	51.7	32.3	64.4	37.1
3	22.4	20.9	43.8	64.7	43.3	63.5	43.1
Average:		26.9			53.4		40.1
5	20.9	18.3	37.6	65.1	36.4	63.2	40.2
6	23.7	16.5	32.6	68.4	35.9	62.6	40.0
Average:		24.9			55.3		40.1
2	31.9	26.3	36.2	68.2	46.7	70.9	46.7
4	27.3	23.2	31.3	68.9	45.4	65.8	44.2
Average:		29.9			61.0		45.4

a. DR, dry rolled; PR, steam processed poor rolled; SF, steam processed flat flaked.

b. Steers 1 and 3 were fed milo; 5 and 6, barley; 2 and 4, hay.

The results of this trial are in agreement with Hale et al (15) that the digestibility of milo dry matter by steers was improved by steam processing and flaking as compared to dry rolled milo. The nylon bag trials indicate that disappearance of dry rolled and steam processed barley were similar, which agrees with digestion studies for barley similarly processed (Mehen 30). A difference was observed in dry matter disappearance between milo and barley which agrees with Mehen (30).

Observations on Repeatability

The mean dry matter disappearance of several milo particle sizes in various bags that were incubated for an eight hour period in the same alfalfa hay fed steer on different days were compared. It was noted that the treatment combinations (particle sizes and bags) were ranked in the same order with only one exception, between C2 and E1, as is indicated in Table 29.

Table 29. Comparison of per cent dry matter disappearance for combinations of several milo particle sizes and various bags incubated in the same alfalfa hay fed steer on different days.

Trial	C1[a]	D1	C2	E1	D2	E2	C3	D3	E3
4	11.9	15.9	18.8	19.5	25.6	26.9	28.2	34.8	43.2
7	9.6	11.5	16.3	15.4	18.9	21.9	22.1	26.7	35.0

a. The upper case letters refer to bag designation and the Arabic numerals refer to particle size as follows: 1, 9-20 mesh; 2, 20-28 mesh; 3, 28-35 mesh.

These data indicated that the nylon bag technique does indeed have repeatability over time.

The ability of two different alfalfa hay fed steers to "digest" the same treatment combinations (as in Table 29) in an eight hour period on the same day were compared. The two steers ranked the treatments in identical order as Table 30 shows. These data clearly showed

Table 30. Comparison of per cent dry matter disappearance for combinations of several milo particle sizes and various bags incubated in two alfalfa hay fed steers on the same day.

Trial	C1[a]	D1	C2	E1	D2	E2	C3	D3	E3
4	9.6	11.5	16.3	15.4	18.9	21.9	22.1	26.8	35.0
4	10.6	14.0	16.6	18.5	23.0	25.8	28.0	32.7	42.2

a. The upper case letters refer to bag designation and the Arabic numerals refer to particle size as follows: 1, 9-20 mesh; 2, 20-28 mesh; 3, 28-35 mesh.

that the mean values of dry matter disappearance may differ from animal to animal. However, both animals had the ability to "digest" a proportional amount of each treatment combination so as to rank their per cent dry matter disappearances in an identical manner.

Table 31 shows the ranking of the same treatment combinations as in Table 29 by different alfalfa hay fed steers on different days. The treatment combinations were ranked in an identical manner. It appeared that even though a statistical difference was noted between

Table 31. Comparison of per cent dry matter disappearance for combinations of several milo particle sizes and various bags incubated in two alfalfa hay fed steers on different days.

Trial	C1[a]	D1	C2	E1	D2	E2	C3	D3	E3
4	11.9	15.9	18.8	19.5	25.6	26.9	28.2	34.8	43.2
4	10.6	14.0	16.6	18.5	23.0	25.8	28.0	32.7	42.2

a. The upper case letters refer to bag designation and the Arabic numerals refer to particle size as follows: 1, 9-20 mesh; 2, 20-28 mesh; 3, 28-35 mesh.

steers and their ability to "digest" equivalent amounts of dry matter as in Trial 12 that these steers had the ability to rank treatment combinations in the same order with only minor exceptions.

Table 32 gives a comparison between milo flakes in various bags and steers fed a milo ration or an alfalfa hay ration. Both steers had the ability to rank these flake-bag treatment combinations in the same order. It was evident that the relationship between the various treatment combinations within a trial are valid. The comparison between steers on different rations and in different trials has little meaning as far as mean "digestion" values are concerned.

Comparing the same treatments in Table 33 (bags and particle sizes) it was determined that the same steer when on different rations

Table 32. Comparison of combinations of milo flakes and various bags incubated in alfalfa hay and milo fed steers on different days on per cent dry matter digestibility.

Steer Ration	Trial	Bag C	Bag D	Bag E
Hay	3	25.4	28.2	45.7
Milo	8	46.2	51.9	71.8

Table 33. Comparison of the ranking of treatment combinations (various bags and particle sizes) incubated in the same steer on different rations on per cent dry matter disappearance.

Ration	Trial	C1[a]	D1	C2	E1	D2	E2	C3	D3	E3
Hay	7	10.6	14.0	16.6	18.5	23.0	25.8	28.0	32.7	42.2
Milo	8	11.5	14.0	15.5	16.3	19.7	21.7	26.5	28.2	32.7

a. The upper case letters refer to bag designation and the Arabic numerals refer to particle size as follows: 1, 9-20; 20-28 mesh; 3, 28-35 mesh.

(alfalfa hay and milo) had the ability to rank these treatment combinations in exactly the same order on both rations.

These data and those in Table 32 indicate that it does not matter particularly what ration the fistulated steer is fed when various milo treatments are incubated to determine dry matter disappearance. The fistulated steer can rank the treatments in the same order on either ration.

GENERAL DISCUSSION

The results of several nylon bag studies have indicated that the nylon bag technique may have some validity in estimating digestibility and utilization of feedstuffs. However, in general, this work has been conducted with forages in reference to cellulose digestion. Hale et al (13), Cadena (5), and Erwin and Elliston (11) are the only previous workers who have studied the nylon bag technique as a measure of concentrate utilization. For the most part their studies attempted to relate a specific nylon bag study to a concurrent digestion or fattening trial.

The work reported in this thesis, with concentrates, was undertaken to evaluate several variables associated with the nylon bag technique. This was an effort to make the nylon bag technique more useful and, threfore, give it wider application. Dry matter disappearance on specific grains or grain processes in the nylon bag was compared with previous digestion and fattening trials.

Previous workers and this work have shown that the nylon bag technique appears to be reliable when comparing treatments within a grain species or between species of quite widely differing digestibilities. Although the bulk of this work was conducted with milo, some barley studies were also conducted. It was noted that these two grains behaved in a somewhat different manner under identical conditions. While the technique appears to be very valid for milo, there were some problems associated with the barley studies. Whether or not this was due to physical properties of the grain is not known. However, it was observed that the barley samples clumped together in the bags. It, therefore, appears that more work should be conducted to evaluate the nylon bag technique with many other grains and concentrates.

An important observation made from these studies was the ability of the various trials to rank the grain treatments in the same order on dry matter disappearance. When comparing a number of treatment combinations (bag types and grain treatments) within a grain, over various steers, rations and days, it was noted that the treatments were nearly always placed in the same order. Thus, the relative dry matter disappearance for these various treatment combinations were nearly always the same. This excellent repeatability, obtained with only three replications, indicates that the nylon bag technique should be a very useful screening tool to study the nutritional value of concentrates.

There are, however, many variables which affect absolute values of dry matter disappearance when grains are incubated in nylon bags in the rumens of fistulated steers. The results can vary widely between steers on the same ration, between rations of the steers, length of the sample incubation period, between chain units in the same steer, sample size, bag size, cloth type of bag, and grain preparation. Many interactions between the factors are also present.

Eight hour incubation periods of the nylon bags were chosen because of convenience and the eight hour incubations showed maximum reliable results. The relationship between milo and barley with the nylon bag technique was as expected and correlated well with digestion and growth trials reported in the literature.

SUMMARY

Nylon bag studies were conducted to evaluate such variables as particle size, sample size, cloth types, hours in rumen, grains, rations of fistulated steers and steer differences.

Ten grams of grain were added to each bag and dry matter disappearance from the nylon bags was determined. Incubations of 2, 4, 6, 8 and 24 hours found eight hour incubations showed maximum reliable differences between treatments. Incubations began three and ten hours after feeding, respectively, for grain and hay fed steers. After incubation the bags were group washed in water until the water remained clear.

Significant differences were observed between two steers, four cloth types and three particle sizes of milo in a factorial experiment. Complete proximate analyses were determined for 9-20, 20-28 and 28-35 mesh milo and barley. None of these three particle sizes were comparable to the whole grains, which suggests that a specific particle size fraction should not be used to evaluate whole grains. Moisture absorption by the various bags (cloth types) ranged from 0.02 to 0.1 grams, indicating this could be an important factor.

A difference in the behavior of grains was observed. Milo and barley flakes incubated, respectively, in milo and barley fed steers

produced a significant within grain difference between chains and bags, and a significant interaction with milo. Three particle sizes of milo and barley incubated in a water bath, at 39 degrees C., showed dry matter losses of 5 to 25 per cent and 15 to 25 per cent, respectively, for milo and barley.

Sample sizes of 2, 4, 6, 8 and 10 grams of 20 mesh ground milo incubated for three and six hour periods in four inch wide bags showed no differences. Samples of 6, 10, 14 and 18 grams of flaked milo and barley incubated in 2, 3 and 4 inch wide bags found bag size and sample size had a significant effect. The use of 4 inch wide bags and 10 gram samples was adapted.

Steers within grains (milo and barley) were significantly different; however, this accounted for only 1.72 per cent of the variation in the analysis of variance. The steers within grains ranked the grain processes in the same order. Hay and grain rations were significantly different, which was attributed to the hay ration and its critical carbohydrate level.

In general, means of treatment combinations (cloths and particle sizes) differed between steers, days and rations. However, the ranking of each treatment was the same when comparing the same hay-fed steer on different days, different hay-fed steers on the same day, different hay-fed steers on different days and hay-fed steers vs. milo-fed steers on different days.

The effect of particle size studied with pressure cooked flaked, pressure cooked flaked and then ground, steam processed flaked, and steam processed flaked and then ground milo showed almost no difference. Grinding flaked milo had no effect indicating the factor affecting disappearance from nylon bags was the processing technique and not surface area.

Elliston (10), Cadena (5) and this work have successfully ranked various milo and barley treatments in the same order as digestion trials. It appears that the value of the nylon bag technique lies in its ability to rank various treatments; however, any specific numerical value obtained has limited use.

APPENDIX

Explanation of Abbreviations

d. f.	degrees of freedom
Prt. Size	particle size

Table 34. Analysis of variance of per cent dry matter disappearance of milo flakes from nylon bags C, D and E incubated in an alfalfa hay fed steer. Trial 3.

Source of Variation	d.f.	Mean Squares
Among Bags	2	944.91**
Within Bags	23	16.18

** (P ⩽ .01)

Table 35. Analysis of variance of per cent dry matter disappearance of 9-20 mesh milo from nylon bags C, D and E incubated in an alfalfa hay fed steer. Trial 3.

Source of Variation	d.f.	Mean Squares
Among Bags	2	157.40**
Within Bags	53	6.27

**(P ⩽ .01)

Table 36. Analysis of variance of per cent dry matter disappearance of 20-28 mesh milo from nylon bags C, D and E incubated in an alfalfa hay fed steer. Trial 3.

Source of Variation	d.f.	Mean Squares
Among Bags	2	131.99**
Within Bags	23	1.55

**(P ⩽ .01)

Table 37. Analysis of variance of per cent dry matter disappearance of 28-35 mesh milo from nylon bags C, D and E incubated in an alfalfa hay fed steer. Trial 3.

Source of Variation	d.f.	Mean Squares
Among Bags	2	58.64**
Within Bags	23	1.14

**(P ⩽ .01)

Table 38. Analysis of variance of per cent dry matter disappearance of 9-20, 20-28 and 28-35 mesh milo in nylon bags C, D and E incubated in an alfalfa hay fed steer. Trial 4.

Source of Variation	d.f.	Mean Squares
Replication	5	4.71
Treatment	8	573.13**
Bags	2	470.45**
Prt. Size	2	1755.89**
Bags x Prt. Size	4	33.09*
Error	40	10.16

*(P ⩽ .05)
**(P ⩽ .01)

Table 39. Analysis of variance of weight of moisture uptake by dry nylon bags C, D, E and F exposed to the air. Trial 6.

Source of Variation	d.f.	Mean Squares
Among Bags	3	0.3062**
Within Bags	8	0.0008

**(P ⩽ .01)

Table 40. Analysis of variance of per cent dry matter disappearance of 9-20, 20-28 and 28-35 mesh milo in nylon bags C, D, E and F, incubated in two alfalfa hay fed steers. Trial 7.

Source of Variation	d.f.	Mean Squares
Steers	1	199.64**
Chains	4	10.50
Bags	3	500.04**
Prt. Size	2	2335.84**
Bag x Prt. Size	6	35.65**
Steer x Bags	3	6.63
Steer x Prt. Size	2	32.54*
Steer x Prt. Size x Bags	6	2.57

*(P ⩽ .05)
**(P ⩽ .01)

Table 41. Analysis of variance of per cent dry matter disappearance of milo flakes in nylon bags C, D, E and F incubated in a milo fed steer. Trial 8A.

Source of Variation	d.f.	Mean Squares
Chains	2	11.67**
Bags	3	382.77**
Chain x Bag	6	8.20**
Within Bags	24	2.05

**($P \leqslant .01$)

Table 42. Analysis of variance of per cent dry matter disappearance of barley flakes in nylon bags C, D, E and F incubated in a barley fed steer. Trial 8A.

Source of Variation	d.f.	Mean Squares
Chains	2	70.00*
Bags	3	406.81**
Chain x Bag	6	12.90
Within Bags	24	17.16

*(P ⩽ .05)
**(P ⩽ .01)

Table 43. Analysis of variance of per cent dry matter disappearance of 9-20, 20-28 and 28-35 mesh milo in nylon bags C, D, E and F incubated in a milo fed steer. Trial 8B.

Source of Variation	d.f.	Mean Squares
Chains	2	9.07**
Bags	3	65.36**
Prt. Size	2	750.78**
Chain x Bag	6	2.22
Chain x Prt. Size	4	3.04
Bags x Prt. Size	6	1.99
Chains x Bag x Prt. Size	12	1.16

**(P ⩽ .01)

Table 44. Analysis of variance of per cent dry matter disappearance of 9-20, 20-28 and 28-35 mesh barley in nylon bags C, D, E and F incubated in a barley fed steer. Trial 8B.

Source of Variation	d.f.	Mean Squares
Chains	2	211.70**
Bags	3	3066.99**
Prt. Size	2	170.48*
Chain x Bag	6	16.58*
Chain x Prt. Size	4	24.36*
Bag x Prt. Size	6	24.43*
Chains x Bags x Prt. Size	12	5.08

*($P \leqslant .05$)
**($P \leqslant .01$)

Table 45. Analysis of variance of per cent dry matter disappearance of whole ground, 9-20, 20-28, and 28-35 mesh milo in nylon bags C, D, E and F incubated in a water control bath. Trial 9.

Source of Variation	d.f.	Mean Squares
Bags	3	40.96**
Prt. Size	3	607.27**
Bag x Prt. Size	9	10.23**
Within Bags	32	1.09

**($P \leqslant .01$)

Table 46. Analysis of variance of per cent dry matter disappearance of whole ground, 9-20, 20-28 and 28-35 mesh barley in nylon bags C, D, E and F incubated in a water control bath. Trial 9.

Source of Variation	d.f.	Mean Squares
Bags	3	17.92
Prt. Size	3	156.99
Bag x Prt. Size	9	3.97**
Within Bags	32	3.14

**($P \leqslant .01$)

Table 47. Analysis of variance of per cent dry matter disappearance of 6, 10, 14 and 18 gram samples of milo flakes in two, three and four inch wide bags of nylon cloth type F incubated in a milo fed steer. Trial 10B.

Source of Variation	d.f.	Mean Squares
Chains	2	71.70
Bags	2	690.84**
Sample Size	3	298.80*
Chains x Bags	4	4.15
Chains x Sample Size	6	41.04
Bags x Sample Size	6	72.58
Chains x Bags x Sample Size	12	27.24

*(P ⩽ .05)
**(P ⩽ .01)

Table 48. Analysis of variance of per cent dry matter disappearance of 6, 10, 14 and 18 gram samples of barley flakes in two, three and four inch wide bags of nylon cloth type F incubated in a barley fed steer. Trial 10B.

Source of Variation	d.f.	Mean Squares
Chains	2	456.58**
Bags	2	717.21**
Sample Size	3	390.39**
Chains x Bags	4	9.19
Chains x Sample Size	6	36.09
Bags x Sample Size	6	42.03*
Chains x Bags x Sample Size	12	12.10

*($P \leqslant .05$)
**($P \leqslant .01$)

Table 49. Analysis of variance of the effect of ration, grain and grain processing on dry matter disappearance from nylon bags. Trial 12.

Source of Variation	d.f.	Mean Squares
Ration	2	339.04**
Steers within ration	3	127.03*
Grains within steers	6	3872.87**
Process within grain	24	408.82**
Error	72	28.17

*(P ⩽ .05)
**(P ⩽ .01)

LITERATURE CITED

1. Annison, E. F., and Dyfed Lewis. 1959. Metabolism in the rumen. John Wiley and Sons, New York, New York.

2. A. O. A. C. 1955. Official Methods of Analysis (8th ed.) Association of Official Agricultural Chemists. Washington, D. C.

3. Archibald, H. Fenner, D. F. Owen, Jr., and H. D. Barnes. 1961. Measurement of the nutritive value of alfalfa and timothy hay by varied techniques. J. Dairy Sci. 44:2232.

4. Balch, C. C., and V. W. Johnson. 1950. Factors affecting the utilization of food by dairy cows. Brit. J. Nutrition. 4:389.

5. Cadena, Melchor Cadena. 1963. Digestibility of dry rolled milo and steam rolled barley by fattening steers and in vitro utilization of various starches by rumen microorganisms. Unpublished M. S. thesis. University of Arizona.

6. Cadena, Melchor, W. H. Hale, Farris Hubbert, Jr., and Bruce Taylor. 1962. Digestibility of dry-rolled milo and steam-rolled barley by fattening steers. Arizona Cattle Feeder's Day Report. University of Arizona, Tucson, Arizona.

7. Crampton, E. W., and L. A. Maynard. 1938. The relation of cellulose and lignin content to the nutritive value of animal feeds. J. Nutrition. 15:383.

8. Dorori, K., and J. K. Loosli. 1959. Influence of fistulation on the digestibility of feed by steers. J. Animal Sci. 18:206.

9. Dougherty, R. W. 1955. Permanent stomach and intestinal fistulas in ruminants; some modifications and simplifications. Cornell Vet. 45:331.

10. Elliston, Nolie G. 1961. Comparative methods for the evaluation of feeds for ruminants. Unpublished M. S. thesis. University of Arizona.

11. Erwin, E. S., and N. G. Elliston. 1959. Rapid method of determining digestibility of concentrates and roughages in cattle. J. Animal Sci. 18:1518 (Abstract).

12. Gallinger, D. D., and C. J. Kercher. 1964. An in vivo method for determining forage digestibility. J. Animal Sci. 23:604 (Abstract).

13. Hale, W. H., W. J. Saba, Farris Hubbert, Jr. and Bruce Taylor. 1963. Utilization of milo and barley grains by steers fattened in drylot. Arizona Cattle Feeder's Day Report. University of Arizona, Tucson, Arizona.

14. Hale, W. H., W. J. Saba, James Kiernat and Bruce Taylor. 1964. Digestion of milo and barley by cattle. Arizona Cattle Feeder's Day Report. University of Arizona, Tucson, Arizona.

15. Hale, W. H., Bruce Taylor, W. J. Saba, Luis Cuitun and Brent Theurer. 1965. Effect of steam processing milo and barley on digestion and performance by steers. J. Animal Sci. 24:883 (Abstract).

16. Hale, W. H., Bruce Taylor, W. J. Saba, E. K. Keating and Kenneth Palmer. 1964. The value of three varieties of milo compared with steam-rolled barley in a high-grain fattening ration. Arizona Cattle Feeder's Day Report. University of Arizona, Tucson, Arizona.

17. Hayes, B. W., C. O. Little and G. E. Mitchell, Jr. 1964. Influence of ruminal, abomasal and intestinal fistulation on digestion in steers. J. Animal Sci. 23:764.

18. Haynes, E. H., R. F. Davis, R. G. Warner and J. K. Loosli. 1955. The digestion coefficients of feeds containing various ratios of hay to grain by fistulated steers and milking cows. J. Animal Sci. 14:1206. (Abstract).

19. Hoflund, S., J. I. Quinn and R. Clark. 1948. Studies on the alimentary tract of Merino sheep in South Africa:xv. The influence of different factors on the rate of cellulose digestion (a) in the rumen (b) in ruminal ingesta as studied in vitro. Ondestepoort Journ. Vet., Sci. and Animal Ind. 23(2):395.

20. Hopson, Jr D., Ronald Johnson and Burk A. Dehority. 1961. Use of the dacron bag technique as an estimate of the rate of in vivo cellulose digestion in the evaluation of forages. J. Animal Sci. 20:960 (Abstract).

21. Hubbert, F., Jr., B. Taylor, W. H. Hale, W. C. Carey and E. B. Stanley. 1962. Barley vs. milo in Arizona feedlot rations. Arizona Cattle Feeder's Day Report. University of Arizona, Tucson, Arizona.

22. Husted, W. T. 1966. The effect of various processing methods on milo digestibility by steers. Unpublished M.S. Thesis. University of Arizona, Tucson, Arizona.

23. Johnson, Ronald R., K. el Shazly and B. A. Dehority. 1960. Effect of starch on the digestion of cellulose in vitro and in vivo by rumen microorganisms. J. Animal Sci. 19:1268 (Abstract).

24. Kansas State University of Agriculture and Applied Science. 1962-63. Beef cattle investigations, a progress report. Bull. No. 45.

25. Karr, M. R., C. O. Little and G. E. Mitchell, Jr. 1965. Ruminal and post-ruminal digestion of starch by steers. J. Animal Sci. 24:890 (Abstract).

26. Kercher, C. J., D. D. Gallinger and H. D. Eikenberry. 1964. Nylon bag technique for measuring forage value. J. Animal Sci. 23:880 (Abstract).

27. Lambert, M. R. and N. L. Jacobson. 1958. The effect of chlortetracycline feeding on cellulose digestion in the bovine rumen. J. Animal Sci. 17:656.

28. Lusk. J. W., C. B. Browning and J. T. Miles. 1962. Small sample in vivo cellulose digestion procedure for forage evaluation. J. Dairy Sci. 45:69.

29. McAnally, R. A. 1942. Digestion of straw by the ruminant. Biochem. J. 36:392.

30. Mehen, S. M. 1966. Effect of steam processing on the digestibility of barley and steam processing, pressure cooking and fine grinding on the digestibility of milo by steers. Unpublished M.S. Thesis. University of Arizona, Tucson, Arizona.

31. Miles, J. T. 1951. Rumen digestion of certain crude fiber constituents. Unpublished Ph. D. Dissertation. University of Wisconsin.

32. Quin, J. I., J. G. Van Det Wath, and S. Myburg. 1938. Studies on the alimentary tract of Merino sheep in South Africa. IV. Description of experimental technique. Onderstepoort J. Vet. Sci. Animal Ind., 11:341.

33. Saba, W. J. 1964. Digestibility of milo and barley by beef cattle. Unpublished M.S. Thesis. University of Arizona, Tucson, Arizona.

34. Steel, Robert G. D. and James H. Torrie. 1960. Principles and prodedures of statistics. McGraw-Hill, New York.

35. Van Dyne, George M. 1962. Micro-methods for nutritive evaluation of range forages. J. Range Mgmt. 15:303.

36. Van Keuren, R. W., and W. W. Heinemann. 1962. Study of a nylon bag technique for <u>in vivo</u> estimation of forage digestibility. J. Animal Sci. 21:340.

37. Weller, R. A. and F. V. Gray. 1954. The passage of starch through the stomach of the sheep. J. Experimental Bio. 13:40.