IN VITRO DIGESTION OF MILO AND BARLEY STARCH BY RUMEN MICROORGANISMS

by

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INTRODUCTION

Sorghum grains are among the major cereal grains of the world. In areas other than the United States, a large proportion of the sorghum grains is consumed directly by the human population. In the United States practically all of the sorghum grains produced are used as livestock feeds.

During the last ten to twelve years sorghum grain production has increased by more than 300 percent. The major part of the increase has occurred in the southwestern part of the United States. The reason for this increase in the Southwest is that sorghum is highly drought resistant and the grain varieties are very suitable to irrigation as the water requirements are considerably lower than would be for corn in order to obtain similar grain yields. Furthermore, the sorghums are very adaptable to the high temperatures of the Southwest.

Commercial cattle feeding has increased by more than 600 percent in the Southwest during the last 14 years. Commercially fed cattle have a high requirement for grain. In the Southwest the grains used are milo and barley. As the barley acreage in the United States has remained fairly constant for many years, there has been an increasing amount of sorghum grain used for fattening cattle in the Southwest.

As the percentage of sorghum grains increased in relation to barley, the feeders were of the opinion that performance of fattening cattle on high sorghum rations was not as satisfactory as equivalent barley rations. These observations were contrary to the normally

accepted feeding values for sorghum grains and barley.

A series of feed lot trials initiated in 1960, at the University of Arizona, supported the view of the cattle feeders and further suggested that the nutritional value of the two grains should be completely re-evaluated based on current ration formulation and nutritional information. The University of Arizona trials suggested that digestion of the starch and protein of the sorghum grains were the limiting factors in the lower nutritional value of the grain.

Evaluation of the many various grain species or varieties within a specie by fattening trials or digestion trials becomes prohibitive from the standpoint of cost, time, and physical facilities. A laboratory method which will permit a rapid screening of grains would be highly desirable. One approach to this problem is the artificial rumen technique as several samples can be tested in one day's study.

It is generally accepted that the major portion of digestion in the ruminant occurs in the rumen. The results of the artificial rumen technique must be eventually validated with digestion or feeding trials. If the results between the two methods are in good agreement, only the more desirable samples would be further tested in feed lot trials.

The purpose of the studies reported in this thesis was to develop a procedure with the artificial rumen to evaluate the utilization of starch from milo and barley.

The preliminary studies were concerned with the finding of the optimum conditions for the artificial rumen technique in order to study

the utilization of starch from grains by rumen microorganisms.

The other areas studied were as follows:

1. Comparative study of the starch utilization of milos and barleys of various origins and treatments.
2. Effect of an artificial saliva and urea on the starch utilization from milo and barley.
3. Effect of feed grade enzymes on the digestibility of the starch of milo and barley as affected by artificial saliva and urea.

REVIEW OF LITERATURE

Morrison (27), in his well-known book, Feeds and Feeding, lists milo with digestion coefficients of 78 percent for crude protein, 91 percent nitrogen-free extract, and 79.4 percent for total digestible nutrients. For barley the corresponding values are 79 percent, 92 percent, and 77.7 percent, respectively. The small difference in total digestible nutrients between the two grains can be accounted for by the fact that barley has 6.2 percent crude fiber and milo has only 3.3 percent.

Schneider (36), in his compilation, Feeds of the World, selected digestion trials that could be critically evaluated, and further separated digestibility values of both milo and barley for various species of livestock. With cattle he gives the following digestion coefficients for milo: Crude protein 57 percent, nitrogen-free extract 83 percent, and total digestible nutrients 80 percent. The equivalent values for barley are 76 percent, 92 percent, and 84 percent, respectively. Observation of the above reveals a discrepancy between the two sets of values, especially with nitrogen-free extract and protein. Since nitrogen-free extract constitutes the major portion of both grains, the true digestibility of the fraction will be a major influence in the overall energy value of the grain.

The Pfizer feed formulator (30) gives a metabolizable energy value for milo of 1110 Cal/lb. and 800 Cal/lb. for barley. Titus (41), for poultry, reports a somewhat higher metabolizable energy value for

both milo and barley, listing 1552 Cal/lb. for milo and 947 Cal/lb. for barley. On the basis of metabolizable energy values, higher performance and better feed efficiency would be expected from the rations containing a high level of milo as compared to a similar barley ration.

Hale (21), of the University of Arizona Experiment Station, reported that barley rations increased gains by 8 percent and improved feed efficiency by 11 percent over comparable milo rations. Apparently the difference in gain between the two grains becomes higher as the grain level increased in the rations. When the grain levels were below 60 percent of the ration, the improvement in gain was 4 percent, whereas at levels of more than 60 percent the improvement reached a maximum of 14 percent. It is worth mentioning at this time that when protein was raised in the milo diets from 10.5 to 13.5 percent, the performance increased. A similar protein raise in the barley rations appeared to reduce performance.

Digestibility trials with milo and barley (56 percent milo or barley) rations conducted by Cadena (15) indicated that the barley rations were more digestible than the milo rations. The crude protein and gross energy digestion coefficient of the barley rations were significantly ($P<0.05$) greater than for the milo rations.

Cadena (15) employing the <u>in vivo</u> nylon bag technique showed a greater disappearance of barley dry matter than for milo dry matter for the first eight hours in the rumen. As time progressed the dry matter disappearance between the two grains became similar and by 24 hours were equal. This suggests that the rate of digestion of milo is slower than for barley. With tests made in the artificial rumen, barley starch

digestion was five times greater than that of milo during the first three hours of fermentation. At nine hours of fermentation the difference was only two and one-half times as great.

Sandstedt et al. (35), using amylolytic enzymes in vitro, showed that there was a wide variation in the digestibility of raw starches of various origins. In general, there appeared to be no direct relationship between digestibility and amylose content of the grains, as a 61 percent amylose corn was not as resistant to digestion as potato starch with 22 percent amylose. The resistance to starch digestion appeared to be a function of the particular amylose in question.

Ackerson (1), working with one day old chicks, demonstrated no noticeable trace of starch granules in the feces, when the diet consisted of normal ground corn. The diet of high amylose type ground corn resulted in the detection of many intact starch granules in the feces. Borchers (6), working with rats, found that high amylose corn starch had a digestibility of 66 percent to 77 percent, whereas normal corn starch was 95 percent digested.

The above findings suggest that the new hybrid milos may be high in amylose type of starch which is more resistant to the action of rumen microorganisms than is barley or the older varieties of milo. Burroughs et al. (12), testing cellulose digestion in good and poor quality roughages, found that a complex mineral solution did not improve the digestion of the good quality roughage, but the cellulose digestion of the poor quality roughage was improved by the addition of a mineral mixture.

Burroughs et al. (13), studying cellulose digestion as affected by other feeds, found that a complex mineral solution plus nitrogen improved the digestibility of ground corn, ground oats, and wheat bran over the control which was supplemented only with nitrogen.

Warner (42), compared the composition of mineral solutions with the composition of saliva and rumen liquor in sheep and discussed the effects of modifying a conventional saliva-type mixture to approach more closely the composition of the ruminal liquid.

McNaught, Owen, and Smith (25) demonstrated, in vitro, the requirements of rumen bacteria for iron, copper, cobalt, etc. The criteria used in these studies was the bacterial growth as related to the decrease of non-protein nitrogen in the fermentation medium. These findings were later confirmed by Little, Cheng, and Burroughs (23).

Bryant, Robinson, and Chu (7), working with Bacteroides succinogenes, determined that calcium, phosphorus, magnesium, sodium, potassium, and ammonium ions are essential for the normal growth of this organism. Barnett (3) lists calcium, magnesium, phosphorus, sodium, potassium, sulphur, cobalt, copper, iron, molybdenum, manganese, zinc, and chlorine as being the minerals normally involved in the various phases of the fermentation in the rumen.

Burroughs, Gerlaugh, and Bethke (11) found that when certain amounts of the ash of alfalfa hay were added to a high corn cob ration, the digestibility of the fiber was increased, concluding, therefore, that alfalfa ash and certain complex mineral mixtures definitely increased the ability of the rumen microorganisms to digest cellulose, as well as roughage dry matter, under laboratory conditions. Swift

et al. (38) corroborated Burroughs (11) findings by obtaining an increase of 10 percent fiber digestibility on a 40 percent corn cob ration supplemented with alfalfa ash. Tillman, Sirny, and MacVicar (40), working with a 60 percent cottonseed hulls ration, demonstrated that a complex mineral mixture based on the mineral composition of alfalfa ash gave as good results as the alfalfa ash itself.

McDougall (24) suggested that salivary phosphate may have a function in addition to that of a buffer of maintaining the bacterial population. This view is supported by Bryant and Burkey (8).

Pearson and Smith (29), studying the in vitro utilization of urea by rumen microorganisms, found that the urea splitting power of rumen liquor was not appreciably affected by bubbling CO_2 through the flask, but the addition of starch had a slight accelerating effect. Wegner et al. (43), working with an in vitro system, found that adding urea to a sample of rumen liquid did not provide a satisfactory in vitro system for his studies, for the mixture became acid quite rapidly.

Mills et al. (26), working in vitro, found that urea fed with hay alone was slowly hydrolized. He suggested this as being indicative of low microbial activity. The addition of starch accelerated digestion and protein synthesis.

Burroughs et al. (9) used an artificial rumen system to assess the effects of various sources of energy upon the utilization of urea. His results confirm Mills's (26) observations that the available energy is of major importance in urea utilization involving a fermentative system.

Bell, Gallup, and Whitehair (5) used yellow corn, dehydrated sweet potatoes, cracked milo, and rolled barley to determine the value of urea as a source of nitrogen and found that the addition of urea had little effect on the apparent digestibility of nutrients, other than protein, which was increased in all rations.

Burroughs et al. (10), using an artificial rumen, added the substrate (filter paper) to flasks containing $(NH_4)_2SO_4$ or urea, with and without a salt mixture patterned after saliva, and found that the complex salt mixture contributed to cellulose digestion.

Bell, Gallup, and Whitehair (4), working in feeding trials, assessed the value of urea in rations containing corn, barley, and milo. They used steers of about 550 pounds weight in metabolism tests to compare barley and milo with corn, and the effectiveness of urea supplementation in each ration. The apparent digestibility of the nitrogen-free extract for the steers receiving barley was 67.84 percent; however, when 45 grams of urea were added to the ration, the apparent digestibility of the nitrogen-free extract was 66.97 percent. The steers receiving milo gave a nitrogen-free extract an apparent digestibility of 66.30 percent. When 45 grams of urea were added to this ration, the apparent digestibility was 67.58 percent.

Conflicting evidence is obtained from a review of the literature on the use of enzymes in ruminant nutrition. Burroughs et al. (14) studying the value of a dried enzyme mixture of a bacterial origin with both amylolytic and proteolytic properties (Agrozyme) found that the addition of .015 lb. per animal increased liveweight gains by 7 percent and decreased feed required per unit of gain by 6 percent;

however, little or no difference was noted in feed consumption, dressing percentage, and federal carcass grade. Dry matter, organic matter, protein, and cellulose digestibility were not influenced by the enzyme supplementation.

Grainger and Stroud (19), working with ewes, found that a multiple enzyme preparation consisting of gumase, amylase and protease, and the three separate enzymes improved apparent dry matter, crude fiber and crude protein digestibility, and also improved nitrogen balance.

Kercher (22) found no significant differences in the average daily gain, live grade, and dressing percentage of steers fed 4 grams of Zymo-pabst enzyme per day, but the steers fed the additive had a significantly higher dressing percentage than the controls. Nelson and Catron (28) conducted 8 experiments with 582 steers to compare four enzymes: Agrozyme, Zymo-pabst, Rhozyme B-6+H-39, and Takamine and their relationship to Diethylstilbestrol (DES) in fattening rations and found that all enzymes resulted in improved gains and feed conversion when fed with DES, but only one, Agrozyme, improved gains without DES. Further experiments by the same researchers demonstrated that extra protein had no effect on enzyme response in this test. Smith and Kish (37), adding Zymo-pabst, Agrozyme, and Dawe-Nzyme to low moisture corn rations for finishing steers, obtained a weight gain improvement ranging from 4.3 to 11.3 percent and a grain feed efficiency improvement ranging from 2.9 to 9.8 percent. No differences in live or carcass grade were reported.

Dyer and Templeton (18), working with 27 steers, tested Rhozyme F-3C and Rhozyme F-4D and found no significant differences in average daily gain, but control animals required from 5 to 8 percent more feed per pound of gain than those receiving the enzymes. Clark, Dyer, and Templeton (16), studying the effects of three bacterial enzyme preparations (Rhozyme F-3C, Rhozyme F-4D and Argrozyme) on the performance of feed lot steers fed chiefly on barley, observed that all enzymes tended to increase the average daily gain of all treated groups but found no difference in feed required per pound of gain.

Ralston, Church, and Oldfield (31), using the nylon bag technique, tested Pectinase, XC 597, HT Protease, and amylase and found no differences on crude fiber, cellulose, or ether extract digestibility due to the treatment. Theurer, Woods, and Burroughs (39) obtained no improvement in feed consumption, rate of gain, or feed conversion by the supplementation of lamb rations with Agrozyme, a bacterial preparation, or Ficin, a proteolytic plant enzyme. In vitro studies by the same workers, using an artificial rumen, indicated that most of the proteolytic activity of these enzyme preparations was lost in the first two or three hours of fermentation. Rea and Ross (32) also reported no apparent response from enzymes added to rations of lambs.

MATERIALS

The rumen fluid used in these studies was obtained from fistulated steers fed alfalfa hay. The rumen fluid was collected in the early morning which was approximately 14 hours after the previous evening feeding. It was strained through a double layer of cheesecloth and placed in a previously warmed thermos jug in order to protect the microorganisms of the rumen fluid from a sudden change in temperature. In the laboratory the rumen fluid was centrifuged at the speed of 500 rpm for three minutes to spin down small particles of hay and other undesirable materials. The supernatant layer was used as the inoculum.

One hundred milligrams of the sample to be digested were placed in a 50 ml culture tube, and 25 ml of the centrifuged rumen fluid was added. The tubes were then placed in the water bath at 39°C. and CO_2 was bubbled into each tube in order to produce anaerobic conditions and to stir the sample.

Our preliminary investigations indicated the proper amount of centrifuged rumen fluid for each tube to be 25 ml. Similarly, the preliminary trials for centrifugation rate of the rumen fluid showed that 500 rpm for three minutes gave the best results. The results of the preliminary trials will be discussed in a following section.

The fermentation process took place in a water bath maintained at a constant temperature of 39°C. by a thermostat. A stirrer was used in order to minimize any variation in the temperature of the water in various locations in the bath.

After a five-hour incubation period, the tubes were removed from the water bath and 20 ml of a mixture of 25 percent butanol and 75 percent ethanol was added, and the tubes were centrifuged at 2000 rpm for 10 minutes. The supernatant liquid was poured off and 40 ml of the butanol-ethanol mixture were added to the sample and centrifuged for a second time. The purpose of the butanol-ethanol mixture was to precipitate the starch.

The starch determination of the samples was made by a modification of the method of Dubois et al. (17), as described below:

The culture tubes were emptied into 250 ml Erlenmeyer flasks, using water and a policeman to make sure that no particle remained in the culture tubes. Fifteen ml of 10 M KOH were then added in order to gelatinize the starch, and two drops of a one percent solution of Phenolphtalein in ethyl alcohol were added to be used later as an end point in the neutralization of the sample. The indicator was added at this point rather than before the neutralization because of its alcohol content. The samples were then boiled for 15 minutes to remove, by volatinization, the butanol-ethanol mixture, since both of these chemicals give a positive reaction for starch.

After the samples were allowed to cool, they were brought to a volume of 100 ml and filtered through a number 515 filter paper. A final dilution was made with demineralized water so that each ml would contain about .05 mg/ml of starch.

To a 10 ml Erlenmeyer flask, 1 ml of the diluted sample was pipetted and 1 ml of a 1/10 dilution of an 80 percent stock solution

of aqueous phenol and 5 ml of concentrated sulfuric acid from a buret were also added. The sulfuric acid was added rapidly but carefully.

Three blanks were prepared in the same manner, using 1 ml of water in place of the sample. The blank giving the greatest transmission in the Bausch & Lomb Spectronic 20 colorimeter was used. This was done in order to minimize the possibility of contamination. The samples were allowed to cool for at least fifteen minutes and then transferred to 3/4" Spectronic tubes. The wave length of the Spectronic 20 was set at 490 mu and the blank at 100 percent transmission.

The percent transmission of all the samples were read and recorded. Since this test is extremely sensitive, all samples were set up in triplicate and the two closest readings on the Spectronic 20 were averaged. Amylopectin was used as a standard and treated as described for the unknown samples alone.

The percent transmission readings of the starch samples and standard were converted to optical densities and applied in the following formula:

$$\text{Percent starch in the sample} = \frac{\text{Optical density of the sample}}{\text{Optical density of the standard}}$$

The composition of the artificial saliva used in the artificial rumen studies is listed in Table 1 and is a modification of the artificial saliva of McDougall (24) adapted to our laboratory conditions. The artificial saliva was added at the rate of 2 ml per tube in the studies which required the addition of the artificial saliva.

Table 1. Composition of the artificial saliva[a]

NaH_2PO_4	6.00	gr.
$NaHCO_3$	6.00	gr.
KCl	0.875	gr.
NaCl	0.875	gr.
$MgSO_4$	0.250	gr.
$CaCl_2$	0.0875	gr.
H_2O	100	ml.

a. The artificial saliva is a modification of McDougall's (24) artificial saliva formula.

When added, a three percent solution of urea in demineralized water was used at the rate of 1 ml per tube.

The enzymes studied were furnished by the Rohm & Haas Company and added at the rate of 1 percent of the grain substrate. The enzymes were from pilot production and developed for possible use in the feed trade.

RESULTS AND DISCUSSION

Only one reference was found that related to the type of study undertaken in this thesis. Salsbury et al. (34) measured starch digestion in the artificial rumen but used a method of starch determination that did not lend itself to routine use. A considerable portion of the research effort was spent in modifying the method of Dubois et al. (17) so that it would be applicable to the artificial rumen study and be satisfactory for routine determinations. The method developed has been described on the procedure section of this thesis.

The results presented in Tables 2 to 5 represent preliminary observations in order to establish criteria to be used for the remaining studies. The preliminary studies were not necessarily conducted in the order they are presented in the four tables; however, the results became the basis of operation for the main objectives of the study.

Table 2 presents the effect of grain level and volume of rumen fluid in the digestion flask. It was necessary to add a grain level which would permit sufficient starch for a determination following the digestion period. The results in Table 2 suggest that under the conditions of fermentation in this study, both volume of rumen fluid and absolute grain level in the artificial rumen are important. With both milo and barley, it appeared that starch digestion was maximum when 100 mg of starch and 25 ml of centrifuged rumen fluid were used.

Table 2. The effect of substrate level and artificial rumen volume on starch utilization from milo and barley.

Percent of starch utilized[a]

		MILO			BARLEY		
Sample	mg. Grain	100	100	50	100	100	50
Number	ml. Rumen Fluid	25	15	15	25	15	15
1		12.77	10.41	7.85	19.71	12.87	15.14
2		15.37	11.21	10.15	24.17	17.38	19.09

a. Rumen fluid centrifuged at 1500 rpm. for three minutes and incubated for five hours.

During the course of the preliminary work, it appeared that the centrifugation speed suggested by most workers could result in a large number of microorganisms being spun out. Strained rumen liquor was spun for three minutes at speeds ranging from 250 to 1500 rpm and utilized to digest milo and barley samples for five hours, after which a determination of the starch utilized by the microorganisms was made. Table 3 suggests that milo and barley starch utilization was lowest at 1000 and 1500 rpm; whereas at 250 rpm the determinations seemed to be affected by small feed particles remaining in the rumen liquid. 500 rpm appeared to give the optimum results and, therefore, was adopted for the remaining part of the research work.

The effect of the length of time of digestion in the artificial rumen upon starch utilization is given in Tables 4 and 5. Four trials were conducted with each milo and barley and determinations made at 2,

Table 3. The effect of centrifugation rate of strained rumen fluid on starch utilization from milo and barley.[a]

Percent starch digestion

	Centrifuge speed[b]			
	250 rpm	500 rpm	1000 rpm	1500 rpm
Milo[c]	9.85	13.00	10.54	10.54
Barley[c]	30.08	30.08	19.72	23.87

a. Samples digested for five hours.

b. All samples were spun for three minutes.

c. 100 mgs substrate added in 25 ml volume.

Table 4. The effect of the length of time of digestion in the artificial rumen upon starch utilization from milo.

Percent of starch digested[a]

Trial number	Digestion time - hours			
	2	4	6	24
1[b]	8.46	6.00	19.65	
2[b]	4.09	7.99	10.41	
3[c]	6.20	19.14	19.00	50.16
4[c]	15.38	23.66	30.65	45.45

a. Rumen fluid centrifuged at 1500 rpm for three minutes.

b. Starch determined by the method of Dubois et al. (17)

c. Starch determined by the method of Folin-Wo (2).

Table 5. The effect of the length of time of digestion in the artificial rumen upon starch utilization from barley

Percent of starch digestion[a]

Trial number	Digestion time - hours			
	2	4	6	24
1[b]	8.34	10.91	27.90	
2[b]	2.75	12.77	23.97	
3[c]	14.70	26.04	28.60	65.70
4[c]	22.87	26.56	43.35	59.49

a. Rumen fluid centrifuged at 1500 rpm for three minutes. 100 mg of substrate in 25 ml volume.

b. Starch determined by the method of Dubois et al. (17).

c. Starch determined by the method of Folin-Wo (2).

4, 6, and 24 hours of digestion. The rate of starch utilization for the grains was very rapid for the first 4 to 6 hours, and considerably slower for the 6 to 24-hour period. It was also noticed at 24 hours of digestion that milo and barley approached similar utilization. Subsequently, 5 hours of digestion time was selected because of convenience in the use of the laboratory facilities and other problems related to a 24-hour digestion. It is believed that a rapid rate of digestion in the rumen is desirable as this results in a more rapid passage of feedstuffs, thus permitting a greater intake of food by the animal. If the 24 hours of digestion were used as the end point, it would be difficult to detect small differences between the grains.

A comparative study of Iowa milo, Arizona milo, popped Arizona milo, and ensiled Arizona milo is presented in Table 6. The popped

Table 6. Starch digestion from Iowa milo, Arizona milo, popped Arizona milo, and ensiled Arizona milo.

Trial number	Iowa milo	Arizona milo	Popped Arizona milo[a]	Ensiled Arizona milo[b]
1				
% starch	78.17	77.14	78.00	83.92
% utilized	11.51	15.11	17.37	10.55
2				
% starch	77.15	79.24	76.86	81.73
% utilized	10.41	12.86	19.59	12.37

a. Popped in a pan using dry heat.

b. Ensiled in an airtight container for 21 days at 30% moisture at room temperature.

Arizona milo was utilized more completely than the Arizona milo, without any treatment. Conflicting evidence was obtained by Salsbury et al. (34), who found no appreciable difference in the digestion of untreated and dry heat treated corn starch during the first six hours of digestion; however, in the second half of the twelve-hour fermentation period, the dry heat treated corn starch showed slightly more rapid digestion than the untreated corn starch. The heat treatment

of the milo in this study was probably more drastic than that of Salsbury et al. (34).

Ensiling Arizona milo did not appear to increase starch utilization when digested for five hours in the artificial rumen. This finding was contrary to those of Saba (33) who found in nylon bag studies that ensiling at 30 percent moisture for 21 days improved the dry matter digestibility of milo. The in vitro fermentation appeared normal and the low starch utilization values may be related to starch determination with this type of product. As noted in Table 6, the starch composition value was considerably higher for the ensiled milo than for the untreated milo. The Arizona milo showed higher starch utilization than did the Iowa milo, which was grown under different temperature and moisture conditions.

The comparative studies on the starch utilization from California, Texas, Arizona, and Hegari milos presented in Table 7 suggest that the Arizona milo starch is better utilized than that of Texas, California, and Hegari milos. There seems to be very little difference in the starch utilization from the California and Texas milos, but there was 69.5 percent increase in the starch utilization from the Arizona milo as compared to Hegari, and 44.4 percent increase in starch utilization of the Arizona milo as compared to California milo. The increase in starch utilization of Texas milo was 41.6 percent greater than from the Hegari milo. The reason for this is not known, but it had been suspected that the starch of the open-pollinated varieties would be more digestible than the starch of the hybrid varieties. In fact, in

Table 7. Comparative starch utilization from California, Texas, Arizona, and Hegari milos.

Trial number	California milo	Texas milo	Arizona milo	Hegari milo[a]
1				
% starch	78.86	79.47	79.47	78.51
% utilized	17.18	19.61	23.32	15.13
2				
% starch	80.17	83.81	83.41	82.39
% utilized	14.08	20.45	26.84	15.14
3				
% starch	80.25	81.40	79.11	80.81
% utilized	16.39	17.57	18.68	10.30
Av. % utilized	15.88	19.21	22.94	13.53

a. Hegari is an open-pollinated milo variety grown locally in Arizona.

a recent feeding trial reported by Hale et al. (20), it appeared that Hegari resulted in improved performance and feed efficiency when compared to an Arizona and a Texas hybrid.

Table 8 gives the results which compare an Arizona barley with a barley variety grown in the Sacramento Valley of California. Starch utilization appeared to be similar with both varieties.

Table 8. Comparative starch utilization from the Arizona and California barleys.

Trial number	Arizona barley	California barley
1		
% starch[a]	74.36	71.09
% utilized	42.53	41.43
2		
% starch[a]	74.36	71.09
% utilized	38.46	42.83

a. The percent starch for barley was determined only once for both trials.

Table 9 illustrates a comparative study of the starch utilization from the Arizona barley and the Arizona, California, and Hegari

Table 9. Comparative starch utilization from the Arizona barley and the Arizona, California, and Hegari milos.

	Arizona barley	Arizona milo	California milo	Hegari milo
% starch	71.61	83.22	84.35	82.09
% utilized	28.15	25.00	21.20	21.80

milos. The starch from the Arizona barley was found to be utilized to a greater extent than the starch from the Arizona milo. This result is in keeping with Cadena (15) who found barley dry matter to be more digestible than that of milo. In this study, the starch utilization from the Arizona barley was lower than for previous artificial rumen runs. The reason for this is unknown. No appreciable difference was observed between the California and Hegari milos starch utilization, but in another study (Table 7), California milo outperformed Hegari milo. This variation can probably be attributed to the differences between the artificial rumen runs.

There are several sources of information on mineral mixtures related to artificial rumen systems. McDougall (24) suggested an artificial saliva to be used in this type of study, and the late Dr. Farris Hubbert, Jr. of the University of Arizona modified McDougall's artificial saliva to make it more adequate to our laboratory conditions. The effect of this modified artificial saliva (Table 1) on the starch utilization from milo and barley by rumen microorganisms was tested, using Arizona milo and Arizona barley as shown in Table 10. Barley starch

Table 10. The effect of artificial saliva on the utilization of starch of Arizona milo and barley.[a]

	Barley alone	Barley + saliva	Milo alone	Milo + saliva
% starch	71.47	71.47	80.87	80.87
% utilized	44.78	51.36	25.00	31.32

a. The artificial saliva of Table 9 was used at the rate of 2 ml per tube.

utilization was improved 14.7 percent by the addition of the artificial saliva; likewise, milo starch utilization was increased 25.3 percent by the addition of the same amount of artificial saliva. This finding is in agreement with *in vivo* studies of Burroughs et al. (11,12) and *in vitro* studies of Bryant, Robinson, and Chu (7) and McNaught, Owen, and Smith (25).

With the purpose of investigating the effect of urea on the starch utilization from milo and barley, a 3 percent solution of urea was prepared and used at the rate of 1 ml per tube. Table 11 presents the results of six trials on the effect of artificial saliva and urea on the utilization of Arizona milo. The artificial saliva proved to be beneficial to the milo starch utilization by rumen microorganisms by improving its average utilization by 85.0 percent. The urea addition improved starch utilization by 23.9 percent. Previous digestion trials by Saba (33) indicated that milo protein was poorly digested, and it appeared logical that a small addition of non-protein nitrogen might supply a readily available source of nitrogen for the rumen microorganisms, thus permitting more rapid digestion of the grain starch. The addition of both artificial saliva and urea to the samples produced an average increase of 63.4 percent, which was less than with the artificial saliva alone.

The effect of the artificial saliva and urea on the starch utilization of Arizona barley in the artificial rumen is presented in Table 12. It was found that the addition of artificial saliva produced an average increase of 49.2 percent over the control, and the addition of urea resulted in only 9.5 percent improvement as compared with the

Table 11. The effect of artificial saliva and urea on the utilization of Arizona milo.[a]

Trial number	Milo	Milo + saliva	Milo + urea	Milo + saliva + urea
1				
% starch	78.64	78.64	78.64	78.64
% utilized	17.49	47.95	23.87	46.31
2				
% starch	82.09	82.09	82.09	82.09
% utilized	37.22	50.54	33.79	48.99
3				
% starch	78.05	78.05	78.05	78.05
% utilized	37.75	49.44	39.44	44.63
4				
% starch	79.83	79.83	79.83	79.83
% utilized	17.48	29.90	17.48	12.11
5				
% starch	79.78	79.78	79.78	79.78
% utilized	21.80	39.10	31.83	42.55
6				
% starch	82.87	82.87	82.87	82.87
% utilized	13.52	51.82	33.64	42.83
Average				
% starch	80.21	80.21	80.21	80.21
% utilized	24.21	44.79	30.00	39.57

a. A 3% solution of urea was used at the rate of 1 ml per tube.

Table 12. The effect of artificial saliva and urea on the utilization of the starch of the Arizona barley

Trial number	Barley	Barley + saliva	Barley + urea	Barley + saliva + urea
1				
% starch	69.17	69.17	69.17	69.17
% utilized	35.82	60.15	41.24	61.51
2				
% starch	73.47	73.47	73.47	73.47
% utilized	34.49	50.92	39.35	50.36
3				
% starch	72.71	72.71	72.71	72.71
% utilized	28.07	35.67	27.18	36.05
Average				
% starch	71.78	71.78	71.78	71.78
% utilized	32.79	48.91	35.92	49.35

control. When both artificial saliva and urea were added, the improvement was 50.5 percent, thus confirming the results obtained with milo. Furthermore, the difference between the saliva treated group and the samples containing urea was 36.2 percent, and the difference between the saliva treated samples and the samples containing both artificial saliva and urea was only 0.9 percent. These data suggest that the

effect of urea was very small as compared with the effect of the artificial saliva. The agreement of Tables 11 and 12 seems to indicate that the artificial saliva has a definitely beneficial effect on the starch utilization from milo and barley, and that the addition of urea causes very little improvement in the utilization of starch from either milo or barley.

The results presented above clearly demonstrate the effectiveness of the artificial saliva in improving starch utilization from milo and barley in an in vitro system with rumen microorganisms. The reason for the artificial saliva effect is not known. As the mineral solution added has a strong buffering action, the effect observed may have been one of pH control in the artificial rumen fermentations. No pH measurements were made following digestion with and without artificial saliva. In general, studies have shown that a buffering solution need not be used when mildly centrifuged rumen fluid is used in short-term studies, such as the one described above. The possibility exists that certain of the mineral ions in the artificial saliva were necessary nutrients for the rumen microorganisms or necessary for certain enzyme activity related to starch hydrolysis and glucose utilization by the rumen microorganisms.

Several commercial enzymes furnished by the Rohm & Haas Co. of Philadelphia were tested for their ability to hydrolyze starch from the Arizona milo and barley. According to the specifications of the manufacturers, the enzymes were used at the rate of 1 percent by weight of the substrate. It is necessary to point out that the enzymes used are characterized primarily by their ability to hydrolyze protein rather than starch except Rhozyme J-25 and Rhozyme A-4 which are, in addition,

good sources of diastase activity; however, according to the manufacturers, all enzymes contained some amylase activity. The commercial name and the enzymatic activity of the enzymes are given in Table 13.

In performing the artificial rumen trials, it was necessary to make only a few runs at a time due to the small capacity of our equipment and the time involved in the determinations. Unfortunately, the results so obtained cannot be directly compared one with another but must be evaluated separately. The reason for this is the variability of the artificial rumen inoculum from one trial to another.

The first two enzymes to be evaluated were Rhozyme J-25 and Rhozyme H-39 and the results reported in Table 14. Neither of the enzymes appeared to improve starch utilization from milo, but when milo plus enzymes plus artificial saliva were digested there was a considerable improvement over the control for both enzymes. It is probable that this improvement was due to the artificial saliva rather than to the enzyme addition. With the samples containing milo plus enzymes plus urea, a decrease in the starch utilization was noticed. The reason for this is unknown.

The following trial was set up with Rhozyme W-15, Diastase 70, Rhozyme P-11, Rhozyme A-4, Rhozyme S, and Rhozyme 33. Table 15 presents the results obtained using milo grain. The Rhozyme W-15 and the Diastase 70 produced a slight improvement in starch utilization by rumen microorganisms over the control, but this increase was not large enough to consider of importance. The Rhozymes P-11, A-4, S, and 33 did not improve the starch utilization. On the contrary, the Rhozymes S and 33 appeared to decrease the starch utilization. The Rhozyme J-25

Table 13. Relative activity of the selective enzyme preparations furnished by the Rohm and Haas Co.

Enzyme	Casein solubilization units[a]	Dextrinizing units - SKB[b]
Rhozyme P-11	10,000	150
Rhozyme A-4	2,000	3,500
Rhozyme J-25	2,250	8,000
Rhozyme B-6	6,000	50
Rhozyme W-15	3,000	25
Protease 30	5,000	75
Protease 41	7,500	30
Rhozyme T-22	No information available	
Rhozyme 33	No information available	
Diastase 70	No information available	
Rhozyme S	No information available	
Rhozyme H-39	No information available	

a. An enzyme is said to have 1,000 casein solubilizing units if 200 mg of it produces 69.4 mg soluble nitrogen, or if it solubilizes 750 mg casein after a reaction period of 1 hour at 40°C and pH 8.0.

b. Number of grams of beta-amylase treated starch dextrinized by 1 gram of enzyme in 1 hour at 30°C and pH 4.7.

Table 14. The effect of the selective enzyme preparations on the utilization of the milo starch as affected by artificial saliva and urea additions.

Enzyme name	Control	Enzyme only	Enzyme + saliva	Enzyme + urea	Enzyme + saliva + urea
Rhozyme J-25					
% starch	81.13	81.13	81.13	81.13	81.13
% utilization	30.08	28.12	33.76	26.15	31.96
Rhozyme H-39					
% starch	81.13	81.13	81.13	81.13	81.13
% utilization	30.08	21.77	34.10	23.72	37.47

Table 15. The effect of the selective enzyme preparations on the utilization of the milo starch, with and without artificial saliva and urea additions.

Enzyme	Control	Enzyme only	Enzyme + saliva	Enzyme + urea	Enzyme + saliva + urea
Rhozyme W-15					
% starch	81.76	81.76	81.76	81.76	81.76
% utilized	11.71	15.23	23.97	12.83	19.70
Diastase 70					
% starch	81.76	81.76	81.76	81.76	81.76
% utilized	11.71	15.23	33.65	15.23	26.02
Rhozyme P-11					
% starch	81.76	81.76	81.76	81.76	81.76
% utilized	11.71	12.83	19.69	17.57	23.97
Rhozyme A-4					
% starch	81.76	81.76	81.76	81.76	81.76
% utilized	11.71	10.40	29.92	15.23	29.92
Rhozyme S					
% starch	81.76	81.76	81.76	81.76	81.76
% utilized	11.71	7.99	17.47	10.40	21.94
Rhozyme 33					
% starch	81.76	81.76	81.76	81.76	81.76
% utilized	11.71	7.99	37.36	29.90	17.47

and Rhozyme A-4 studied did not seem to produce the effect reported by the manufacturers of hydrolyzing starches. The sample containing milo plus enzymes, milo plus urea and a combination of the two appeared to follow the same pattern mentioned in the discussion of Table 14. This was, again, reason to suspect that the apparent improvement in starch utilization from this series of studies was due to the artificial saliva and not the enzymes.

In a separate trial, Protease 41, Rhozyme T-22, Protease 30, and Rhozyme B-6 were tested using milo as the substrate. The results of this test appear in Table 16. None of the enzymes tested appeared to improve starch utilization by the rumen microorganisms, but here, again, the beneficial effect of the artificial saliva was demonstrated. The addition of urea, in conjunction with the enzymes, added little or nothing to improvement in the digestibility of milo starch.

An adaptation of our artificial rumen set-up allowed the author to be able to test all twelve enzymes at the same time. The starch determinations were made progressively; four at a time. The remainder of the digested samples were stored in a freezer. This adaptation made it possible to compare all the samples one with another and determine with more precision which enzymes are beneficial and those that show no activity with barley as the substrate.

Table 17 gives the results of the study with barley. Only four enzymes showed an improvement of the starch utilization from barley over the control. The Rhozyme T-22 improved the starch utilization by 78.9 percent, while the Rhozyme A-4, which has high diastase activity, improved the starch utilization from barley by 24.7 percent. The

Table 16. The effect of the selective enzyme preparations on the utilization of milo starch, with and without artificial saliva and urea additions.

Enzyme	Control	Enzyme only	Enzyme + saliva	Enzyme + urea	Enzyme + saliva + urea
Protease 41					
% starch	79.35	79.35	79.35	79.35	79.35
% utilized	19.71	21.94	29.93	17.48	40.70
Rhozyme T-22					
% starch	79.35	79.35	79.35	79.35	79.35
% utilized	19.71	19.71	40.70	21.94	40.70
Protease 30					
% starch	79.35	79.35	79.35	79.35	79.35
% utilized	19.71	19.71	48.78	15.24	40.70
Rhozyme B-6					
% starch	79.35	79.35	79.35	79.35	79.35
% utilized	19.71	15.24	45.65	17.48	31.45

Table 17. The effect of the selective enzyme preparations on the utilization of the barley starch, with and without artificial saliva and urea additions.

Enzyme	Control	Enzyme only	Enzyme + saliva	Enzyme + urea	Enzyme + saliva + urea
Rhozyme T-22					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	64.11	49.76	64.11	51.37
Rhozyme A-4					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	44.65	58.66	44.65	58.66
Rhozyme P-11					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	43.07	51.37	37.72	55.98
Protease 30					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	43.07	57.48	37.72	51.37
Rhozyme 33					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	41.23	61.51	39.44	57.20
Protease 41					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	39.44	57.20	28.14	58.66

Table 17.--Continued

Diastase 70					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	37.72	52.92	41.32	54.47
Rhozyme S					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	37.72	49.76	41.32	51.37
Rhozyme H-39					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	35.82	55.73	41.23	52.71
Rhozyme B-6					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	35.82	55.73	35.82	55.73
Rhozyme W-15					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	30.06	55.73	41.23	46.31
Rhozyme J-25					
% starch	69.17	69.17	69.17	69.17	69.17
% utilized	35.82	30.04	41.32	30.04	44.79

Rhozyme P-11 and the Protease 30 each improved the starch utilization by 20.4 percent. The rest of the enzymes failed to improve starch digestibility to any appreciable extent. The samples to which the artificial saliva, urea, and a combination of both was added followed the same pattern as shown in Tables 11, 12, 14, 15, and 16, thus confirming once more the suggestion that the apparent improvement in starch digestion, so far observed, was due to the artificial saliva addition rather than to the enzymatic activity of the added enzymes.

GENERAL DISCUSSION

In order to obtain several comparisons on the extent of starch utilization from milo and barley by rumen microorganisms, a summary of all the trials in which milo and barley were studied is given in Table 18. The individual values used in this summary do not include samples which have been treated. The 29 samples of milo digested gave an average starch content of 80.46 percent with a standard deviation of ±1.99 as compared with the 10 barley samples that yielded 71.85 percent of starch with a standard deviation of ±1.87. The difference in starch content between the milo and barley samples can be attributed to the higher fiber and protein content of barley as compared to milo. The percent starch utilized for all milo samples was 19.46 with a standard deviation of ±7.04 while the percent starch utilization for the barley samples was of 37.24 with a standard deviation of ±5.90. These findings confirm the *in vivo* findings of Hale (21), Cadena (15), and Saba (33) that barley is more digestible to cattle than is milo. The studies reported herein suggest that the rate of starch utilization from milo is slower than for barley. On the basis of the five-hour fermentation period, the starch utilization from barley was approximately twice that from milo. The studies do not provide an answer as to why the difference occurs.

The data collected indicates that the addition of artificial saliva considerably improved the starch utilization from both milo and barley. The reason for this improvement is not known, but it may

be that the minerals were necessary for certain metabolic processes of the rumen microorganisms or perhaps the buffering action of the artificial saliva prevented the pH changes in the in vitro fermentation. If

Table 18. Summary of starch utilization from the milo and the barley.

	Milo	Barley
Number of samples	29	10
Percent of starch	80.46 ± 1.99[a]	71.85 ± 1.87
Percent utilized	19.46 ± 7.04	37.24 ± 5.90

a. Standard error of the mean

the pH in the fermentation medium changed drastically, the metabolic activity of the rumen microorganisms could be slowed considerably. However, if a comparison is made between milo and barley samples treated with artificial saliva, the difference in the rate of starch utilization between the two grains is similar to that noted with untreated samples. This suggests that the artificial saliva improves starch utilization from both grains and probably does not alter the poor utilization of milo starch relative to barley starch. With reference to previous in vivo trials from the Arizona Experiment Station, necessary minerals were included in the ration; yet a marked difference between milo and barley was observed.

The addition of urea contributed very little to the improvement of the starch utilization from milo and barley. This was especially noticed in the samples when both artificial saliva and urea were added. The percent starch utilized in such samples was not different from that of the samples with artificial saliva alone.

The addition of the commercial feed grade enzymes to the milo samples did not produce any appreciable effect on the starch utilization from milo. This is in agreement with Burroughs et al. (14), Kercher (22), Dyer and Templeton (18), Ralston, Church, and Oldfield (31), and Rea and Ross (32), who reported that the in vivo addition of enzymes did not produce any appreciable effect on the improvement of the digestibility of various grains.

The addition of enzymes to barley improved the utilization of barley starch in only four cases, but it is important to realize that when the artificial saliva and urea were added to the samples containing enzyme, similar results to the samples without enzymes were obtained, casting a doubt over the beneficial effect of these four enzymes on the starch utilization of barley.

It is suggested that the artificial rumen technique used in these studies is valuable in determining starch utilization from various grains provided there is a marked difference in the utilization of the starches in question. The technique appeared to have limitation in measuring small differences that might occur between varieties of one grain specie. A complicating problem is the difference in absolute values between artificial rumen runs. This can be attributed to the rumen liquid obtained on different dates.

Several different sources of milo and barley were studied but within each grain, starch utilization was similar. Of particular interest was the fact that the starch from the open-pollinated Hegari variety of milo was similar to that of the hybrid varieties of milo. This suggests that there are inherent physical structures in milo starch which slow down its rate of utilization by rumen microorganisms when compared to barley starch.

No satisfactory method of starch determination is available for determination of starch from grains which lend itself to rapid routine use. The starch determination method used in these studies also determines glucose, but presumably this is not a problem in an *in vitro* fermentation as glucose cannot be found in such fermentations. To measure small differences in starch utilization by rumen microorganisms, additional analytical techniques must be developed.

SUMMARY

The artificial rumen technique was used to determine the difference in starch utilization from milo and barley and from various sources of the two grains. The treatment effects studied were primarily the effect of artificial saliva, urea, and commercial feed grade enzymes on the utilization of starch from the two grains. The initial studies indicated the rumen fluid obtained from a fistulated steer on alfalfa hay should be centrifuged at 500 rpm for three minutes. The artificial rumen volume was 25 ml of centrifuged rumen fluid to which 100 mg of the grain in question was added. The fermentation period was 5 hours.

Regardless of source, starch utilization from barley was approximately twice that of milo during the same five-hour fermentation period. Milo from Arizona, Iowa, California, and Texas, together with Hegari milo, were compared. There was little difference in starch utilization from the various milos; however, the Arizona milo appeared to show the largest starch utilization among the ones tested.

The addition of the artificial saliva to the artificial rumen improved starch utilization from both grains, but it did not change the relative difference in starch utilization between the two grains.

The addition of urea had little effect. Of the 12 commercial enzymes tested only two resulted in improved utilization of starch from milo and four improved the utilization of starch from barley, when used without artificial saliva.

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