

FACTORS AFFECTING THE IN VITRO DIGESTIBILITY
OF MOIST SORGHUM GRAIN

By

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Bachelor of Science

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College Station, Texas

1967

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1969

SEP 29 1989

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ACKNOWLEDGMENTS

The author expresses his sincere appreciation to Dr. Robert Totusek, Professor of Animal Science, for his council and guidance during the course of this study and in the preparation of this thesis.

Appreciation is also extended to Dr. Joe V. Whiteman, Dr. J. E. McCroskey and Dr. I. T. Omtvedt, Professors of Animal Science, and Dr. Robert A. Morrison, Professor of Mathematics and Statistics, for their assistance in the interpretation of data and preparation of this thesis.

The author is also grateful to fellow graduate students at Oklahoma State University for their help and suggestions.

Acknowledgment is extended to Dennis Sullivan and Connal Addison for their assistance in the laboratory.

The author also wishes to acknowledge Farmland Industries for financial support of the research reported herein.

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INTRODUCTION

Sorghum grain constitutes a very large portion of the total quantity of cereal grain fed to feedlot cattle throughout the Southwest. Because of its price advantage and availability it will become even more widely used in the future.

Considerable research is being conducted at the present time to improve the utilization of sorghum grain by feedlot cattle. Chemical composition indicates that it has potential energy comparable to corn and superior to barley, but apparently the starch fraction is of a more complex nature and more resistant to digestion by rumen microorganisms. Current research is concentrated largely on processing methods.

Previous research has indicated that high moisture harvesting and reconstitution improves its value for cattle. However, the influence of many factors on the nutritional value of moist sorghum grain has not been elucidated. The purpose of this study was to determine the influence of several factors on moist sorghum grain by screening a large number of treatments with an in vitro system.

Four experiments were conducted to study levels of moisture in sorghum grain, time of oxygen free storage, environmental temperature during storage, physical form during reconstitution and storage, and dry matter loss during storage. Evaluation was on the basis of dry matter disappearance during in vitro fermentation.

REVIEW OF LITERATURE

Sorghum Grain

Riggs et al. (1959) reported that ground high moisture harvested sorghum grain (23% moisture) produced gains on fattening steers equal or superior to ground dry sorghum grain on 18% less dry matter from the grain and 12% less total dry matter. The unground high moisture sorghum grain was stored successfully without spoilage in an air-tight glass-lined silo. The high moisture grain fed in the whole form did not produce satisfactory results as it required 60% more feed per unit of gain than the dry grain. No difficulty was encountered in maintaining the steers on feed.

Franke et al. (1960) compared moist and dry sorghum grain (31 and 10% moisture) in a growing period and a fattening period. During the 112-day growing period while on a full feed of haylage and hay plus 4 lb. of grain, the cattle fed high moisture grain required 10% less feed per lb. of gain. During the 140-day fattening period the cattle on dry grain required 17.6% more feed per unit of gain. Rate of gain was not affected. A summary of the two periods showed that the moist grain was utilized 13% more efficiently than the dry grain.

King (1962) reported no significant differences in carcass characteristics between steers on moist and dry sorghum grain.

Riggs and Stilwell (1963) fed high moisture head chop and observed a similar significant improvement in feed efficiency. They

harvested the sorghum grain heads a few inches below the head with a modified silage cutter and stored this product in an air-tight structure. The chop contained 37% moisture and 70% grain which had a moisture content of 31%. This was compared to grain combined at 23% moisture. Rate of gain was similar while feed per 100 lb. gain was 844 and 956 lb. for the head chop and 23% grain, respectively.

Parrett and Riggs (1967) reported a 14.8% feed savings with reconstituted sorghum grain but for the first time observed no increase in efficiency from the high moisture harvested grain fed with cottonseed hulls. Feeding the high moisture harvested grain in an all-concentrate ration resulted in a 17.3% feed savings. The moisture levels were 10, 28, and 29.7% for dry, reconstituted, and high moisture harvested sorghum grains, respectively. The cattle fed moist grains gained slightly more than the control cattle.

McGinty et al. (1967) summarized seven trials with 273 cattle at the Texas Station. Dry, reconstituted, and high moisture harvested sorghum grains were compared. No significant differences in daily gain between cattle fed dry and moist sorghum grain were noted. In all cases the cattle fed ground moist grain required less dry matter per unit of gain than did those fed the dry grain. Improvement in dry matter efficiency ranged from 15 to 26% for the grain and from 8 to 15% for the total ration.

McGinty et al. (1967) compared reconstituted sorghum grain containing 29.72% moisture and dry milo containing 10.26% moisture and found the digestibility of all components of the reconstituted grain was significantly higher than for the dry grain. This improvement was approximately 29% for dry matter, organic matter, and non-protein

organic matter, and 16% for protein. In a second trial reconstituting the grain resulted in a 22% improvement in digestibility of protein and a 17% improvement in digestibility of all other components. McGinty concluded that the feed nutrients in the moist milo are evidently more available for digestive processes due to an alteration in the protein structure and/or the starch molecule to such an extent that it is more readily fermented by the rumen microorganisms or more highly digested in the small intestine.

Totusek et al. (1967) reconstituted milo in covered metal tanks to 27% moisture. The reconstituted grain was fed in comparison to coarsely ground milo. Reconstituting did not affect rate of gain but resulted in an improvement of 5.5% in feed efficiency.

Newsom et al. (1968) compared rolled and ground reconstituted sorghum grain (20%) to coarsely ground dry sorghum grain (12.6%) in a high concentrate ration. The grain was reconstituted by the addition of water while it was being augered into an air-tight storage structure. The reconstituted rolled grain compared to the coarsely ground dry grain resulted in a decrease in consumption (14.08%), with only a slight reduction in rate of gain (0.11%) and an increase in feed efficiency of 14.03%. The reconstituted ground grain produced a similar rate of gain as the dry grain but required 4.90% less feed per unit of gain.

A series of experiments comparing high moisture sorghum grain and dry sorghum grain was conducted at the Ft. Hays, Kansas Station (1961-64). Brethour and Duitsman (1961) ensiled sorghum grain containing 41% moisture in the whole and ground forms in a trench silo covered with black plastic. They reported little spoilage but the whole grain

became dark and appeared to have heated. The rate of gain and feed per cwt. gain (air-dry basis) were 3.09 and 933, 3.03 and 952, and 3.24 and 1048 lb., respectively, for unground high moisture, ground high moisture, and dry rolled grain.

Brethour and Duitsman (1962) reported similar results with high moisture sorghum grain (36%) when the experiment was repeated. Steers on the preground high moisture ensiled grain were 12% more efficient than the cattle fed dry grain. These steers also gained 2.76 lb. per day compared to 2.39 for steers fed grain ground after ensiling. Considerable loss was noted with the ensiled whole wet grain.

In a similar trial in 1963, Brethour and Duitsman ensiled high-moisture ground sorghum grain at two moisture levels, 27 and 36%. Average daily gain and feed required per 100 lb. of gain were 2.78, 1023; 2.78, 967; 2.73, 1118; and 3.03, 1126 lb., respectively, for 27% grain, 36% grain, finely ground dry grain, and coarsely ground dry grain. Less dry matter was required per unit of gain at 37% but the authors reported that 19% less dry matter was harvested at this stage of maturity than if it were left to dry. When harvesting was deferred until grain had dried to 27% moisture, dry matter yield was 6% less than that harvested at maturity. Gain was significantly higher for coarsely ground dry grain, but 10% more feed was required per unit gain with the dry rations.

Brethour and Duitsman (1964) reported no significant difference in gain or feed efficiency with 26% ensiled grain and dry rolled grain. Rate of gain and feed efficiency were 2.87 and 960, and 2.95 and 966 lb. for the ensiled and dry rolled grain, respectively.

Ely and Duitsman (1967) reported no significant difference in feed

efficiency or rate of gain of cattle fed soaked rolled sorghum grain compared to dry sorghum grain. The wet grain was soaked for 24 hr. in the proportion of .8 part water and 1.0 part milo. The wet grain contained 50% dry matter and was fed with sorghum silage and chopped alfalfa hay. Average daily gains were 2.31 and 2.42 lb. for the cattle on wet and dry sorghum grain, respectively.

Husted et al. (1966) indicated that the soaking of sorghum grain may increase digestibility of certain components. The wet grain was produced by soaking dry grain for 16 hrs. in water. The digestion coefficients for gross energy, N.F.E., protein, and T.D.N. were % 64.9, 75.4, 54.9, and 68.1, respectively, for soaked milo and 63.6, 70.9, 52.7 and 63.3, respectively for dry milo.

Corn

Henderson (1965) summarized 14 experiments in which high moisture harvested ground ear corn was compared to dry ground ear corn in cattle fattening rations. The average moisture content of the high moisture corn was 36% as compared to 16% for the dry corn. The cattle fed the high moisture ground ear corn required 10% less feed per unit of gain. The average daily gain was similar, but there was a slight advantage for the high moisture ear corn.

Beeson et al. (1956) reported an increase in efficiency for high moisture ground ear corn (32%) over dry ground ear corn (17%). The cattle were full fed ground ear corn and 3.7 lb. Purdue Supplement A. No significant difference was noted in daily gain but a 12-15% improvement in feed efficiency was reported.

Culbertson et al. (1957) and Beeson and Perry (1958) reported

similar results from two trials that indicate fattening beef cattle utilize high moisture ground ear corn (32%) 10-15% more efficiently than dry ground ear corn. The wet corn was ground and stored in a harvestore until fed. Differences in daily gain were not significant.

Research with high moisture shelled corn has indicated no significant improvement in feed efficiency over dry shelled corn. Kennedy et al. (1904) fed 35% moisture shelled corn and dry (14%) shelled corn to two groups of steers. The gains were similar but the high moisture group required 4% more feed per unit gain. Albert et al. (1960) reported no significant differences in daily gain or feed efficiency between high moisture shelled corn (31%) and dry shelled corn (15%).

Henderson (1965) summarized eight experiments which compared high moisture shelled corn to dry shelled corn. The cattle fed the high moisture shelled corn gained 5% less per day than those fed dry corn, but there was no difference in feed efficiency. The high moisture shelled corn averaged 30% moisture as compared to 17% for the dry corn.

Matsushima and Stenquist (1967) reported that as the moisture in shelled corn increased, daily consumption and rate of gain by feedlot cattle decreased. Rolled high moisture corn (32%), ground high moisture corn, ground dry shelled corn reconstituted to 30% just prior to feeding, and ground dry shelled corn were compared in a 134 day feeding test that resulted in daily gains of 2.64, 2.60, 2.56 and 2.80 lb., and feed per lb. of gain of 9.03, 6.68, 6.93 and 6.23 lb., respectively. The grain was fed to a maximum and all other constituents were kept constant.

Heuberger et al. (1959) compared 14, 24, 29, and 36% moisture corn and found only slight differences for the 14, 24, and 29% corn in

average daily gain and feed efficiency. A reduction of 14% in intake and feed efficiency was noted on the 36% moisture corn compared to the dry product. Average daily gain was 20% less for the cattle on the corn ensiled at 36% moisture.

Larson et al. (1966) reconstituted dry corn to 28% moisture and compared it to dry corn. The reconstituted grain was stored in an airtight silo for 23 days before feeding. All grains were rolled for the 70 steers on feed. No significant difference in feed efficiency was noted when the corn was fed twice daily but there was a 5.4% improvement in efficiency for the reconstituted corn when the corn was fed once daily.

Ross and Rea (1958) fed 27 and 15% moisture corn to feedlot lambs. The high moisture corn was stored in plastic bags before feeding. There was no significant difference in gain, grade, or yield. The lambs on the high moisture corn required 1.5% less feed per unit of gain.

Benjaman and Jordan (1960) also compared high moisture (30%) shelled corn, high moisture ear corn, and dry shelled corn for lambs. No significant difference in gain, intake, or feed efficiency were noted.

Mohrman et al. (1958) fed shelled corn containing moisture levels of 14.5, 25, 30 and 35% to 72 lambs. His results agree with those of others in that he found no significant difference due to moisture.

Barley

Fredrick et al. (1962) compared rolled dry and high moisture barley (30%). The high moisture barley was rolled when removed from an

air-tight silo immediately before feeding. The rolled high moisture barley increased average daily gain 0.2 lb. (statistically non-significant). The rolled dry barley was utilized 10% more efficiently.

Dinusson et al. (1964) made four comparisons at North Dakota over a 3-year period with high moisture (30%) and dry barley. Three experiments involved feeding calves for 252 to 294 days and one involved feeding yearlings for 147 days. Average daily gain for high moisture barley were 2.15 and 2.41 compared to 2.05 and 2.44 lb. for dry barley for calves and yearlings, respectively. The dry barley rations were 4.4 and 11.0% more efficiently utilized by calves and yearlings, respectively, than the moist barley rations.

Krall and Thomas (1967) reported on the basis of four trials, that high moisture barley rations were equal to dry barley rations in feed conversion. The high moisture groups gained slightly more per day.

In Vitro

In vitro techniques have been employed extensively in the past in evaluating forages and their relative nutritive values. The extent of in vitro techniques as a tool for measuring relative energy availability for concentrates has been limited but Kumeno et al. (1967), Albin et al. (1966) and Klett et al. (1967) agree that their use is helpful in searching for feed products which would be utilized more efficiently.

Kumeno et al. (1967) used an in vitro technique for estimating the nutritive value or digestibility of high energy mixed rations, using dry matter disappearance as the criterion. The values obtained after a 48 hr. fermentation were highly correlated with the estimated energy

digestibility of a wide range of mixtures of orchard grass or alfalfa with ground corn. Dry matter disappearance in vitro at 48 hr. was highly correlated ($r = 0.85$) with dry matter digestibility in vivo. Correlations of dry matter disappearance with total acid production resulted in r values of approximately 0.90.

Albin et al. (1966) used the in vitro technique to study digestibility of rations and feedlot performance of steers fed all-concentrate rations. His technique involved the use of both whole rumen fluid and resuspended bacterial cells as the inoculum. Samples were incubated in 50 ml. test tubes at 39°C for 24 hrs. The criteria for detecting differences in fermentation rates were digestion of dry matter, ether extract, starch, and gross energy. When compared to field trials, significant correlations were not found within each in vitro period, but the most consistent correlation coefficients were between per cent digestible dry matter (in vitro) and daily feedlot gain ($r = 0.88$); and per cent digestible dry matter (in vitro) and feedlot efficiency of feed utilization ($r = 0.99$).

Klett et al. (1967) compared the nylon bag, in vitro (strained rumen juice) and in vivo digestion techniques using rations consisting of various ratios of alfalfa hay to steamed rolled barley (4:0, 3:1, 2:2, 1:3, 0:4). When the means were pooled across rations, no significant differences were found among the 48 hr. nylon bag (71.9%), 24 hr. in vitro (65.5%) and in vivo (68.0%) dry matter digestibilities. The nylon bag dry matter digestion values at 12 and 24 hr. were significantly ($p < .05$) correlated with in vivo digestion of ether extract, energy, dry matter, cellulose and crude fiber.

MATERIALS AND METHODS

Four experiments were conducted to study the factors affecting in vitro digestibility of moist sorghum grain. The factors studied were moisture level, time of oxygen free storage, environmental temperature during storage, physical form during storage, and the effect of drying moist grain sorghum. The dry matter loss during storage was also determined.

Technique

An in vitro technique using dry matter disappearance as the basis was used to study the factors. This technique is a modification of the Tilley-Terry (1963) procedure.

This technique was employed since it permitted the digestion of a large number of samples concurrently, which is not possible with the nylon bag technique. Several preliminary trials were conducted to compare in vitro dry matter disappearance to feed efficiency values obtained in cattle feeding trials. Albin (1967) indicated a high correlation between in vitro digestion and feedlot performance.

The results of two comparisons between in vitro digestion and feedlot efficiency are given in Figures 1 and 2. Figure 1 shows the relationship between in vitro dry matter disappearance and feed per pound of gain of six differently processed sorghum grains which were fed at the Ft. Reno Experiment Station during 1967-68. Three in vitro trials were conducted at various times during the feeding period

Treatments:

- 1 - Coarse ground - dry
- 2 - Fine ground - dry
- 3 - Reconstituted, ground
- 4 - High moisture, ground
- 5 - Reconstituted, rolled
- 6 - High moisture, rolled

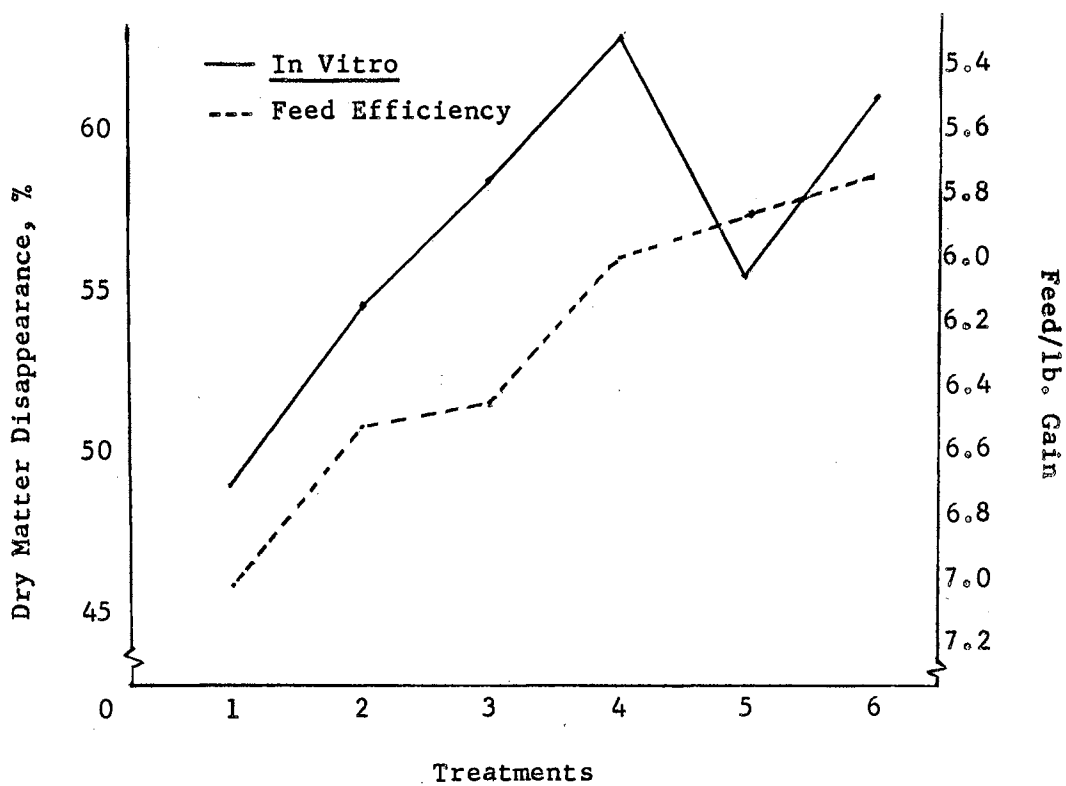


Figure 1. Comparison of In Vitro Dry Matter Disappearance to Feed Efficiency, Trial 1

Treatments:

- 1 - Dry sorghum grain
- 2 - High moisture harvested sorghum
- 3 - High moisture harvested corn

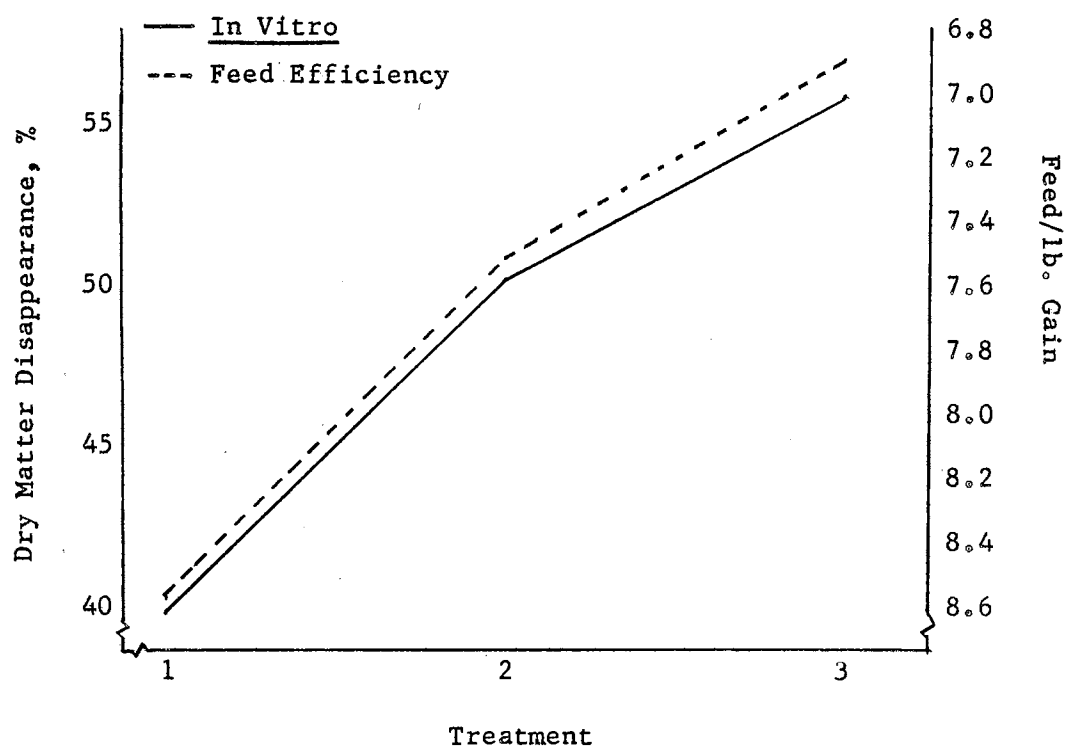


Figure 2. Comparison of In Vitro Dry Matter Disappearance to Feed Efficiency, Trial 2

using four samples per treatment. The plotted means are the average of the 12 samples for each treatment.

Figure 2 shows the comparisons of in vitro dry matter disappearance and feed efficiency of the three grains listed. The grains were fed in a feeding trial at Panhandle State College, Goodwell, Oklahoma during 1967-68. Correlations were not calculated on these data due to the lack of numbers, but a definite relationship between the two measurements is apparent.

The basic technique used to determine in vitro digestibility is given in Table I.

TABLE I
IN VITRO TECHNIQUE

Element	Level
Grain sample	1.0 g.
Artificial saliva	90.0 ml.
Rumen inoculum	25.0 ml.
Temperature	39°C.
Time of incubation	18 hours

The in vitro containers used were 250 ml. square glass centrifuge bottles. These wide-mouth bottles were fitted with bunsen valves to let gas escape while keeping the solution under anaerobic conditions. The grain samples were weighed into the bottles on an as-is basis but

adjusted so that each sample contained approximately 1.0 gm. of dry matter. Each grain sample was ground through a Wiley Mill with a 2 mm. screen and the sample for in vitro digestion was then taken.

The artificial saliva used was based on work by McDougal (1949) and is given in Table II.

TABLE II
COMPOSITION OF ARTIFICIAL SALIVA

Ingredient	Gm./liter of distilled H ₂ O
NaHCO ₃	9.80
KCL	0.57
CaCl ₂	0.04
Na ₂ HPO ₄ · 7H ₂ O	9.30
NaCL	0.47
MgSO ₄ · 7H ₂ O	0.12

Two liter quantities of this solution were mixed in advance of its use and stored under refrigeration until needed. Ninety ml. of artificial saliva solution were added to each in vitro bottle containing the 1.0 gram sample of grain. The bottles were then placed in a water bath with the temperature maintained at approximately 39°C.

While the bottles of sample and saliva solution were warming to a constant temperature, a quantity of rumen fluid was recovered from a

fistulated steer on a ration containing 83.4% sorghum grain. The steer was fed twice daily at the rate of 1.5 times the maintenance requirement. The rumen sample was taken 6-8 hours after the morning feeding of the day on which an in vitro trial was conducted. The rumen sample was dipped from the rumen through a fistula and placed into a thermos jug and immediately taken to the laboratory where it was filtered twice through six layers of cheesecloth. This was accomplished as quickly as possible to minimize bacterial loss. Only the liquid portion of each filtration was saved and used as the rumen inoculum for the in vitro bottles. Twenty-five ml. of the inoculum were pipetted into each bottle. The bottles were then saturated with CO₂ and returned to the water bath. The samples were incubated in the dark for 18 hr. and stirred twice during this period.

At the end of the 18 hr. incubation period 3 ml. of mercuric chloride were added to each bottle to stop microbial action. The bottles were then cooled for 2-4 hr. before being filtered. The samples were filtered with a buchner funnel and suction flask using no. 31 wattman, 12.0 cm. filter paper. Celite filter aid was used with the filter paper to help the filtering process. All contents were washed from the bottles with water onto the suction filter. The paper and contents were then placed in a petri dish, dried in an oven at 215°C., and weighed. Three blank in vitro bottles which contained no grain were included in each trial, and the average quantity of dry matter in the blanks was subtracted from the quantity of dry matter in all other samples. The "net" dry matter remaining after digestion was used in calculating dry matter disappearance.

Experiment I

This experiment was conducted to determine the effects of moisture, time, and temperature during storage on the dry matter disappearance of high moisture harvested sorghum grain during in vitro fermentation. A three-factor factorial was used so that main factors as well as interactions could be considered. The designated factors were A for time of storage (10, 20, and 30 da.), B for temperature (40°, 75°, and 110° F.) and C for moisture (13, 17, 22, 26, 30 and 35%). Table III shows the experimental design.

TABLE III
EXPERIMENTAL DESIGN OF EXPERIMENT I

		10 Da.						20 Da.						30 Da.					
		Moisture, %																	
		13	17	22	26	30	35	13	17	22	26	30	35	13	17	22	26	30	35
40° F	Block ^a 1																		
	2																		
	3																		
	4																		
75° F	1																		
	2																		
	3																		
	4																		
110° F	1																		
	2																		
	3																		
	4																		

^a Each block represents a separate in vitro trial

This experiment was blocked on time so each block represented a separate in vitro trial consisting of 54 treatments. Four trials were run which gave four measurements for each treatment.

The high moisture sorghum grain was harvested from a one-variety field. The composite sample for each moisture level was attained by harvesting at different times as the grain matured, starting when the grain contained 35% moisture. Each composite sample was composed of grain harvested from heads cut at random in a 15 acre block of NK-222 sorghum grain planted in June, 1967. The grain was grown on irrigated soil of a sandy-loam type. The grain was threshed from the heads with a portable Davis thresher. The composite sample was then returned to the laboratory where it was placed in 36 250 ml. plastic, air-tight bottles. The bottles were stored in three different environments (12 bottles in each) differing in temperature (40°, 75°, 110° F.) for three time periods (10, 20, and 30 da.). The 40° samples were stored in a refrigerator, the 75° samples at room temperature, and the 110° samples in an oven. After 10 days, one-third of the samples in each environment were removed and placed in a 0° F. freezer. This was repeated after each time interval until all samples were placed in the freezer to minimize biological action. This process was repeated with each composite sample of each moisture level, resulting in a total of 216 frozen samples ready for measurement.

When an in vitro trial was to be run, one bottle of each treatment was removed, allowed to thaw for one day and ground. The experiment was blocked on time with each block representing a trial so that one sample of each treatment was measured during each in vitro trial. Four trials were run; therefore, four measurements of dry matter disappear-

ance were made for each treatment. In the final analysis, the variation between samples within treatments was removed as block effect.

The analysis of variance is shown in Table IV.

TABLE IV
ANALYSIS OF VARIANCE,
EXPERIMENT I

Source	df
Total	215
Blocks	3
Treat. Comb.	53
A (Time)	2
B (Temperature)	2
C (Moisture)	5
AB	4
AC	10
BC	10
ABC	20
Error	159

Experiment II

The purpose of this experiment was to determine the effects of moisture, time, and temperature on the in vitro digestion of reconstituted sorghum grain. The time and temperature levels were the same as in Experiment I but the moisture levels tested were 15, 18, 23, 26, 30, and 34%. A three-factor factorial design similar to that in Experiment I was used. The sorghum grain was reconstituted to the desired moisture

The sorghum grain was reconstituted to the various moisture levels by soaking in water at 40°C. The samples of each moisture level were kept in air-tight plastic bottles at room temperature for the desired lengths of time and stored in a freezer at 0°F. until the 32 day samples were completed and frozen. Four in vitro trials were then conducted with each trial containing one sample of each treatment. Therefore, each trial contained 48 in vitro samples.

The analysis of variance is presented in Table VI.

TABLE VI
ANALYSIS OF VARIANCE,
EXPERIMENT III

Source	df
Total	191
Block	3
Treat. Comb.	47
A	1
B	5
C	3
AB	5
AC	3
BC	15
ABC	15
Error	141

In conjunction with this experiment, dry matter loss during the storage of the reconstituted grain was studied. Three samples of the treated grain in the whole and ground form for each moisture level were

checked for dry matter loss during the 16 and 32 day reconstitution periods. Total quantity of dry matter in each of the 48 bottles was determined at the start and conclusion of the 16 and 32 day time periods and net change in dry matter was calculated.

Experiment IV

This study was conducted to determine the effect of drying on the in vitro digestion of moist sorghum grain. Four treatments were studied: (1) dry, (2) reconstituted and ground, (3) reconstituted, ground, and dried, and (4) reconstituted, dried, and ground. Three trials were conducted; each trial consisted of 4 samples of each treatment. The analysis of variance is shown in Table VII.

TABLE VII
ANALYSIS OF VARIANCE,
EXPERIMENT IV

Source	df
Total	47
Reps	2
Treatment	3
Sampling	36
Error	6

The samples of treatments 2, 3, and 4 were reconstituted at the same time by soaking in 40°C. water for approximately 45 minutes to

produce grain containing about 30% moisture. The grain was then stored in air-tight plastic bottles for 30 days at which time they were frozen until in vitro dry matter disappearance was measured. Each of the four samples of each treatment in each in vitro trial was taken from a separate bottle.

RESULTS AND DISCUSSION

Experiment I

This experiment was designed to determine effects of time, temperature, and moisture and their interactions on in vitro dry matter disappearance of sorghum grain. Fifty-four total treatments were studied in this experiment; the results are given in Table VIII. The analysis of variance is presented in Table IX. The standard error of the mean was ± 1.46 .

Effect of Moisture

Moisture (13, 17, 22, 26, 30 and 35%) had a significant effect ($p < .01$) on in vitro dry matter disappearance. Dry matter disappearance remained constant or increased as moisture increased for all levels except the 30% moisture sample which was lower than the 26% sample at every time period and temperature. This may have been due to sampling error in taking the original sample from the field. Each sample was a composite of many samples which were taken by selecting sorghum heads at random over the field, but it is possible that this particular sample was taken from inferior heads in trying to obtain desired moisture content.

Dry matter disappearance increased only slightly at 17 and 22% moisture levels compared to 13%. The first substantial increase in digestion occurred between 22 and 26% moisture at all time and

TABLE VIII
EFFECT OF TIME, TEMPERATURE AND MOISTURE ON
DRY MATTER DISAPPEARANCE OF HIGH MOISTURE
HARVESTED SORGHUM GRAIN

Moisture (%)	<u>10 Da.</u>					
	13	17	22	26	30	35
40°F	39.4	41.3	46.2	48.3	47.8	49.6
75°F	39.9	44.4	47.1	50.0	47.0	55.8
110°F	40.4	41.8	43.9	49.8	46.7	57.3
<u>20 Da.</u>						
40°F	39.6	41.1	44.3	45.0	45.0	52.7
75°F	39.1	38.3	43.5	46.9	46.9	58.8
110°F	39.6	39.1	42.7	52.1	44.0	58.9
<u>30 Da.</u>						
40°F	40.2	42.4	42.8	44.4	42.9	50.0
75°F	42.0	39.2	39.9	48.3	43.3	55.9
110°F	38.2	36.4	37.4	50.4	46.1	57.3

temperature levels. The highest dry matter disappearance occurred at 35%, which also suggests that in vitro digestibility increases as the moisture content of the grain increases.

Effect of Time

The analysis of variance (Table IX) indicated that time (10, 20, and 30 da.) had no significant effect on dry matter disappearance. Therefore, there is little probability that time had a real effect on in vitro digestion. Figure 3 indicates this point in that the three

TABLE IX
EXPERIMENT I: ANALYSIS OF VARIANCE

Source	df	MS	F
Total	215		
Blocks	3		
Treat Comb.	53		
A (Time)	2	11.99	1.4007
B (Temperature)	2	25.08	2.9299*
C (Moisture)	5	1180.50	137.9088**
AB	4	8.31	.9708
AC	10	17.32	2.0234*
BC	10	36.90	4.3107**
ABC	20	2.11	.2464
Error	159	8.56	

* p < .05

** p < .01

lower levels of moisture seemed to slightly decrease in dry matter disappearance as time during storage increased while the three higher levels of moisture remained about the same. Only the grain with the highest level of moisture (35%) increased in digestibility during the 10 to 20 day period but it dropped during the next 10 days. These curves suggest a moisture and time interaction which was indicated in the analysis of variance (Table IX). This interaction suggests that increased moisture is required to maintain or increase starch availability with increased time. Higher moisture levels may be conducive to starch degradation during storage.

These data suggest that time and temperature were independent.

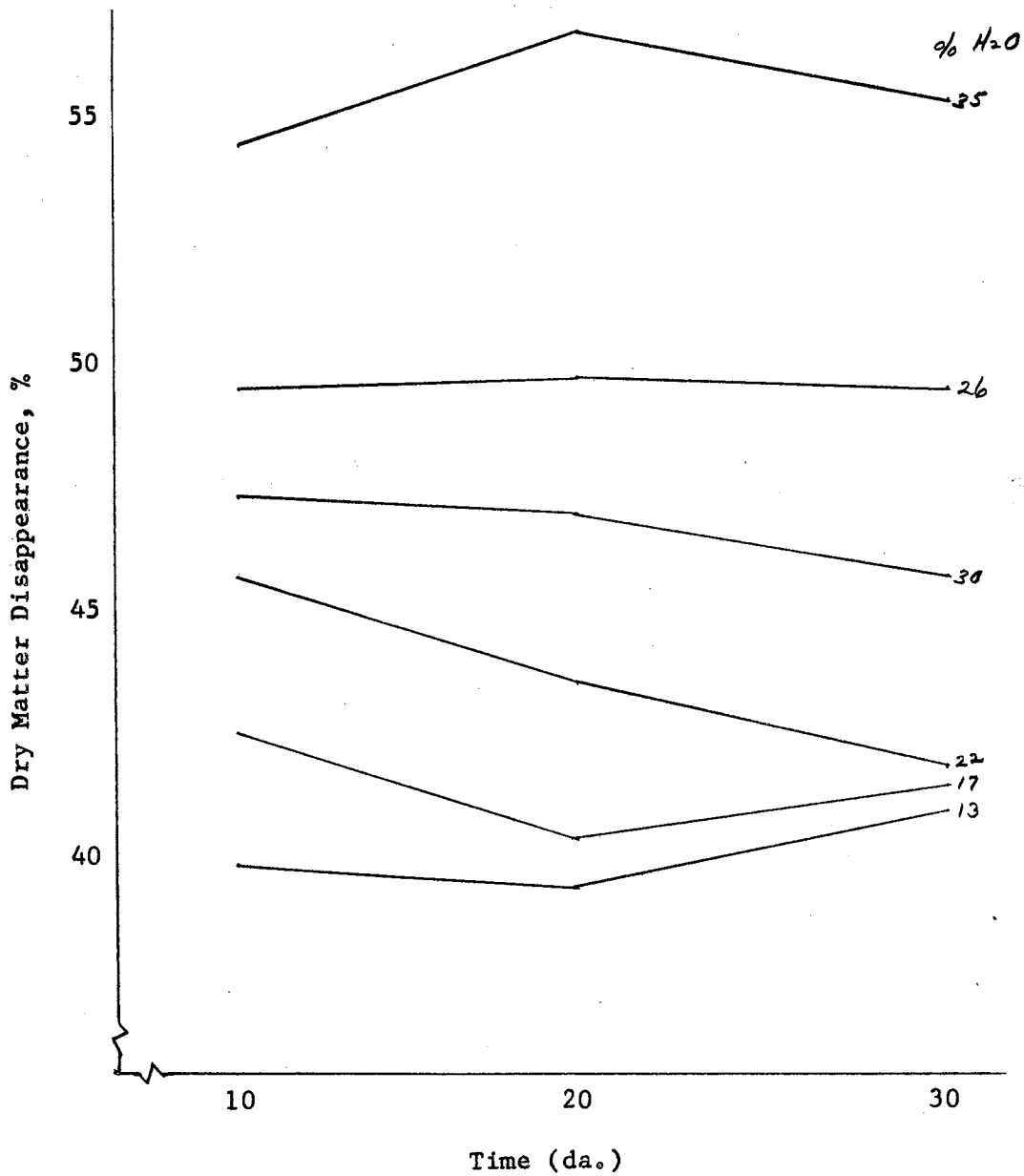


Figure 3. Effect of Storage Time and Moisture Content of Sorghum Grain on In Vitro Digestion

Effect of Temperature

Temperature (40°, 75°, and 110°F.) had a significant effect ($p < .05$) on in vitro dry matter disappearance. Figure 4 indicates the effect of temperature at each moisture level. In general, high temperature seemed to be detrimental at lower moisture levels and beneficial at higher moisture levels. Analysis of variance indicated an interaction between temperature and moisture ($p < .05$). Figure 4 indicates that as the temperature rose from the lower level (40°), the samples containing 13, 17, 22 and 30% moisture remained about constant or dropped in digestibility. However, the samples containing 26 and 35% moisture increased in dry matter disappearance as the temperature increased. Most of this overall increase was noted at the second level of temperature. Assuming that the 30% sample was a bad sample, these data indicate that a high storage temperature along with a high moisture level must be present in order to obtain maximum in vitro dry matter disappearance. Heat may serve as a catalyst in this instance if there is any type of fermentation or chemical breakdown taking place during the storage period. Therefore, high moisture harvested sorghum grain which has a moisture content above 26% and is stored in an oxygen free structure during the summer months may be utilized to a higher degree by fattening cattle than the same grain stored during the winter months.

The results of this experiment suggest that high moisture harvested grain should be harvested between 26 and 35% moisture for most efficient utilization. Sorghum grain containing much more than 35% moisture may not be fully matured in nutritional components. The period of time that it is stored seems to be of little importance in

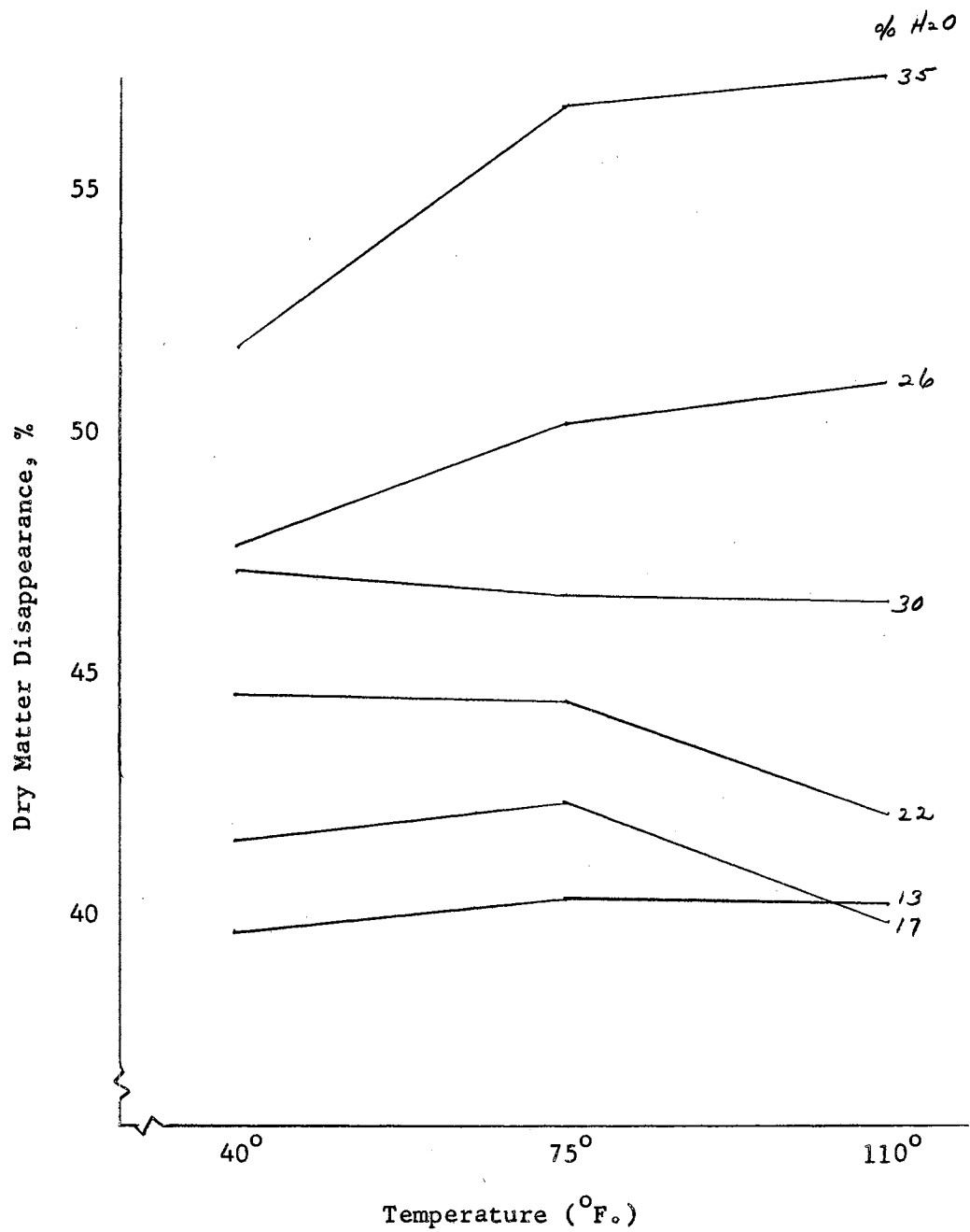


Figure 4. Effect of Storage Temperature and Moisture Content of the Sorghum Grain on In Vitro Digestion

increasing its utilization.

Experiment II

This experiment was conducted to determine the effects of moisture, time and temperature, and their interactions, on in vitro dry matter disappearance of reconstituted sorghum grain. The experimental design was identical to the one used in Experiment I, with a total of 54 treatment combinations being studied. The mean dry matter disappearance figures are presented in Table X. The analysis of variance is presented in Table XI. The standard error of the mean was ± 1.45 .

Effect of Moisture

Results were very similar to those found in Experiment I with high moisture harvested sorghum grain in that the dry matter disappearance increased progressively with each increase in moisture level in the grain. The analysis of variance indicated that moisture had a significant ($p < .01$) effect on dry matter disappearance. As noted from Table X dry matter disappearance was not increased greatly by reconstituting the grain to 18 or 23% moisture. However, there was a large increase at 26% and further increases at the 30 and 34% levels. These data suggest that the 26% moisture level may be a minimum to obtain the largest portion of the expected increase in utilization from reconstitution. It should be noted from Table X that digestibility at 23% was considerably lower than at 26% over all temperature and time levels even though there was only 3% difference in moisture. This was in close agreement with Experiment I. These in vitro results indicate maximum utilization at the highest moisture levels studied.

TABLE X
EFFECT OF TIME, TEMPERATURE, AND MOISTURE ON
DRY MATTER DISAPPEARANCE OF RECONSTITUTED
SORGHUM GRAIN

Moisture (%)	<u>10 Da.</u>					
	15	18	23	26	30	34
40°F	36.1	35.6	35.8	38.7	35.6	45.6
75°F	31.8	32.8	36.1	43.7	46.1	48.9
110°F	32.5	36.0	38.0	43.1	50.9	52.9
<u>20 Da.</u>						
40°F	33.9	38.0	39.5	42.0	43.1	45.2
75°F	36.2	37.1	39.5	46.7	49.3	52.0
110°F	34.5	36.2	41.5	49.5	54.0	54.1
<u>30 Da.</u>						
40°F	33.9	36.8	38.3	41.9	44.2	49.6
75°F	34.8	35.4	38.6	44.5	50.9	54.4
110°F	33.5	36.7	39.1	46.0	53.2	56.3

Feedlot trials are needed to determine if high moisture levels of grain are a limiting factor in dry matter intake by cattle.

Effect of Time

Time had a significant effect ($p < .01$) on dry matter disappearance as indicated in Table XI. Figure 5 shows the dry matter disappearance corresponding to each moisture level.

All the reconstituted samples tested had a higher dry matter disappearance at 20 days following reconstitution than at 10. This

TABLE XI
EXPERIMENT II: ANALYSIS OF VARIANCE

Source	df	MS	F
Total	215		
Blocks	3		
Treat. Comb.	53		
A (Time)	2	135.4800	16.1524**
B (Temperature)	2	266.4900	31.7719**
C (Moisture)	5	1633.0700	194.7005**
AB	4	5.4300	.6473
AC	10	12.6710	1.5107
BC	10	65.7630	7.8405**
ABC	20	4.8765	.5814
Error	159	8.3876	

** $p < .01$

indicates that additional breakdown of starch into a more available form may have occurred during this additional storage time.

Additional time after reconstitution above 20 days (30 da.) increased dry matter disappearance only at the 30 and 34% moisture levels. This indicates a trend toward a time and moisture interaction but such an interaction did not approach significance ($p < .05$) in the analysis of variance.

Figure 5 suggests that high levels of moisture may be essential with the added time to realize maximum digestibility.

Of particular interest in these data is the fact that the major portion of the increased dry matter disappearance from reconstitution is realized with the first 10 days of storage. This is illustrated in Figure 5 by the extrapolations (illustrated by the broken lines) from

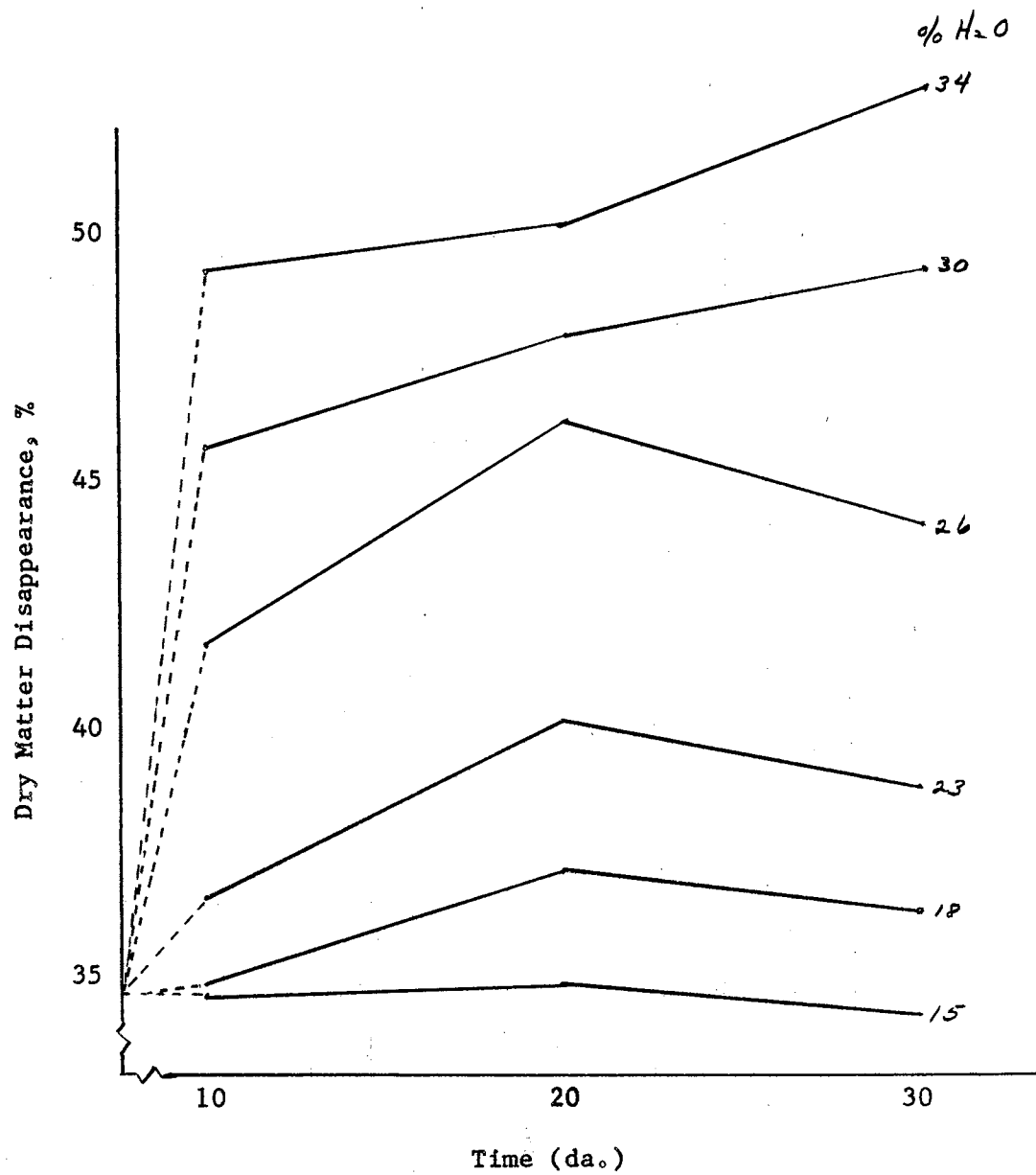


Figure 5. Effect of Storage Time and Moisture Content of Reconstituted Sorghum Grain on In Vitro Digestion

the mean digestibility (34.6%) of the dry (15% moisture) grain samples to the digestibility at each moisture level at 10 days. Dry grain reconstituted to 30% improved 11.1% in dry matter disappearance in 10 days and further improved only 3.7% during the next 20 days.

Effect of Temperature

The effect of environmental temperature during storage of reconstituted grain is presented in Figure 6. Analysis of variance (Table X) indicated that temperature had a significant effect ($p < .01$) on dry matter disappearance. However Table X also shows that there is a moisture and temperature interaction ($p < .01$). This indicates that digestibility is dependent on both these factors. Figure 6 illustrates this interaction in that the response curves for the moisture levels are not parallel. Grain containing 15, 18, and 23% moisture were affected very little by temperature during storage, while those containing 26, 30, and 34% moisture showed a considerable increase in dry matter disappearance with increased temperature during storage. Therefore, high moisture levels are required in conjunction with the higher storage temperatures to realize maximum in vitro digestibility. This seems feasible since moisture is required in fermentation or degradation processes. The additional heat may serve as a catalyst in the reactions which take place during storage. Chemical analyses are needed to determine reactions which take place during storage. The reactions may be a type of fermentation or a partial germination process. In germination, enzymes are activated which act on the starch fraction of the endosperm. B-amylase and α -amylase are two enzymes which break down starch to simple sugars. Therefore, during the

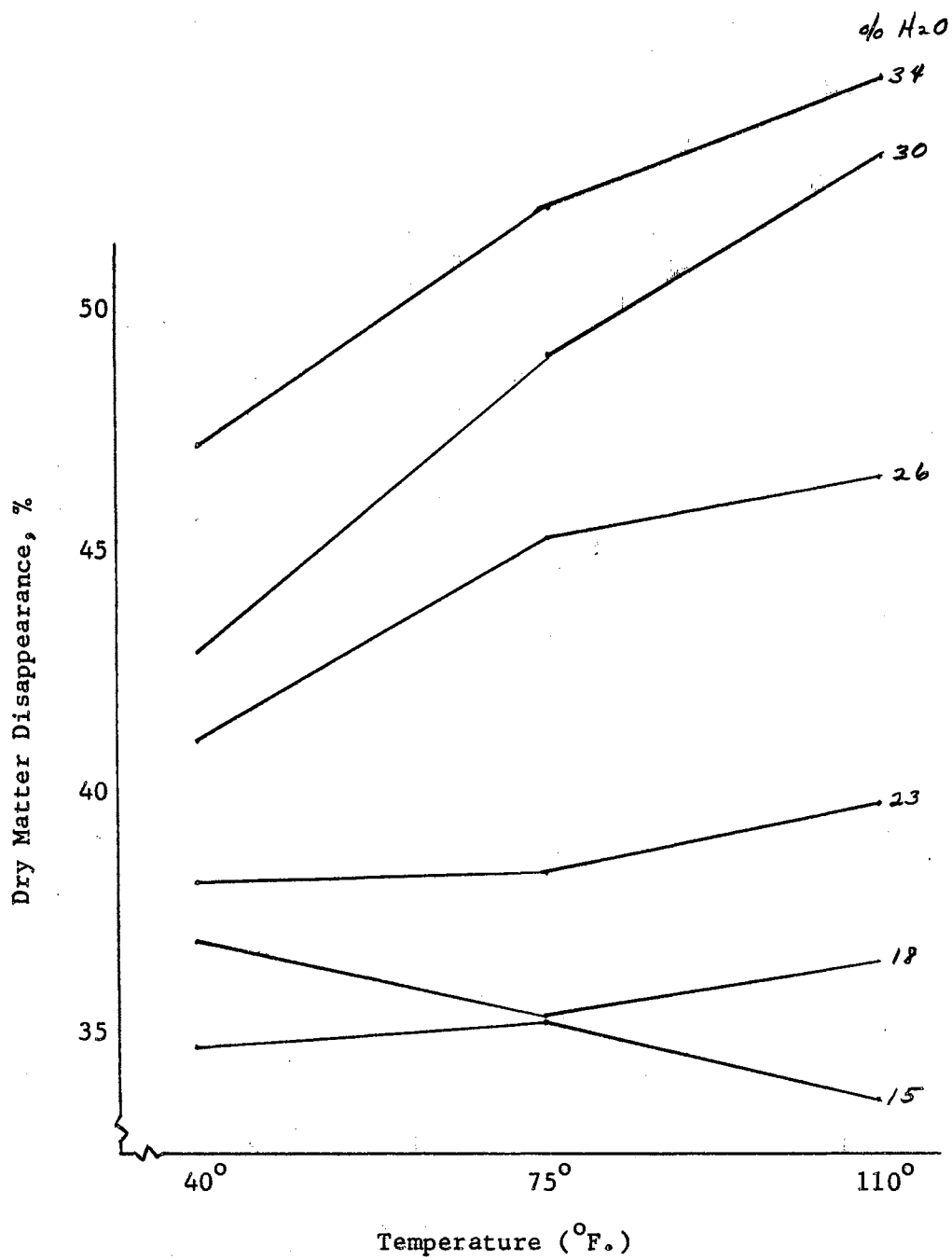


Figure 6. Effect of Storage Temperature and Moisture Content of Reconstituted Sorghum Grain on In Vitro Digestion

germination process of a normal seed, the starch fraction decreases and the simple sugars increase.

If there were partial germination during the storage of samples with adequate moisture, then there is a possibility that a small portion of these enzymes were activated causing a partial breakdown in starch. Hence, the in vitro bacteria could more readily utilize this fraction.

Experiment III

This experiment was designed to determine the influence of physical form of sorghum grain during reconstitution and storage (whole and ground), time of storage (1, 2, 4, 8, 16, and 32 da.) and moisture level (14, 21, 28, and 32%). The results are presented in Table XII. The analysis of variance (Table XIII) showed that all three main factors plus all the interactions were significant ($p < .01$). The standard error of the mean was $\pm .9$.

Effect of Physical Form

Table XII indicates the large difference in dry matter disappearance due to the physical form of the grain during reconstitution and storage. Little or no increase in dry matter disappearance was realized from reconstituting ground sorghum grain while reconstituting the whole grain resulted in a large increase in digestibility. This indicates that little beneficial fermentation occurs in the ground grain. The fact that whole grain is required for desirable reconstitution indicates that a partial germination may occur during storage following reconstitution. Amylase may be activated in the grain to

TABLE XII
EFFECT OF TIME AND MOISTURE ON IN VITRO DRY
MATTER DISAPPEARANCE OF WHOLE AND GROUND
RECONSTITUTED SORGHUM GRAIN

Da.	<u>Whole Reconstituted</u>					
	1	2	4	8	16	32
<u>Moisture, %</u>						
14	32.6	33.8	34.0	32.7	32.9	32.5
21	33.3	34.7	34.5	34.5	34.4	35.3
28	37.7	38.8	38.7	40.0	44.5	47.8
30	39.9	39.1	41.2	41.9	45.6	48.6
<u>Ground Reconstituted</u>						
14	32.1	33.7	32.2	31.6	32.2	33.1
21	32.6	31.2	31.5	30.9	32.1	32.2
28	32.6	32.9	32.8	31.5	33.8	33.7
32	33.8	34.4	33.7	32.7	33.8	35.5

break down some starch to simple sugars. If a fermentation process were taking place during storage, then a breakdown of starch in the ground grain should be noted as well as in the whole reconstituted grain. This evidence causes one to postulate that a partial germination process is taking place during storage.

Effect of Time

Figures 7 and 8 show the effect of time on dry matter disappearance at each moisture level of whole and ground sorghum grain, respectively. Time had little or no effect on ground reconstituted sorghum

TABLE XIII
EXPERIMENT III: ANALYSIS OF VARIANCE

Source	df	MS	F
Total	191	181	
Block	3	3	
Treat. Comb.	47	47	
A (Form)	1	1366.40	418.2814**
B (Time)	5	48.4760	14.8394**
C (Moisture)	3	351.2700	197.5305**
AB	5	17.4140	5.3308**
AC	3	216.0667	66.1422**
BC	15	15.6073	4.7777**
ABC	15	8.5913	2.6299**
Error	141	3.2667	

** p < .01

grain but it had a considerable effect on whole reconstituted sorghum grain at the high moisture levels. The increase in dry matter disappearance with time was very similar for the 28 and 30% moisture levels in the whole reconstituted grain. This was expected since the moisture levels were so similar. The highest moisture level was designed to be 35%, but it was not achieved in the reconstitution process.

As noted in Figure 7, a large portion (6%) of the total increase (15%) in dry matter disappearance of whole reconstituted sorghum grain at 30% moisture was achieved with 1 day in oxygen free storage. Generally, there was a steady rise in dry matter disappearance from 1 day to 32 days with the whole reconstituted sorghum grain at 28 and 30% moisture. The general appearance of the whole reconstituted grain at

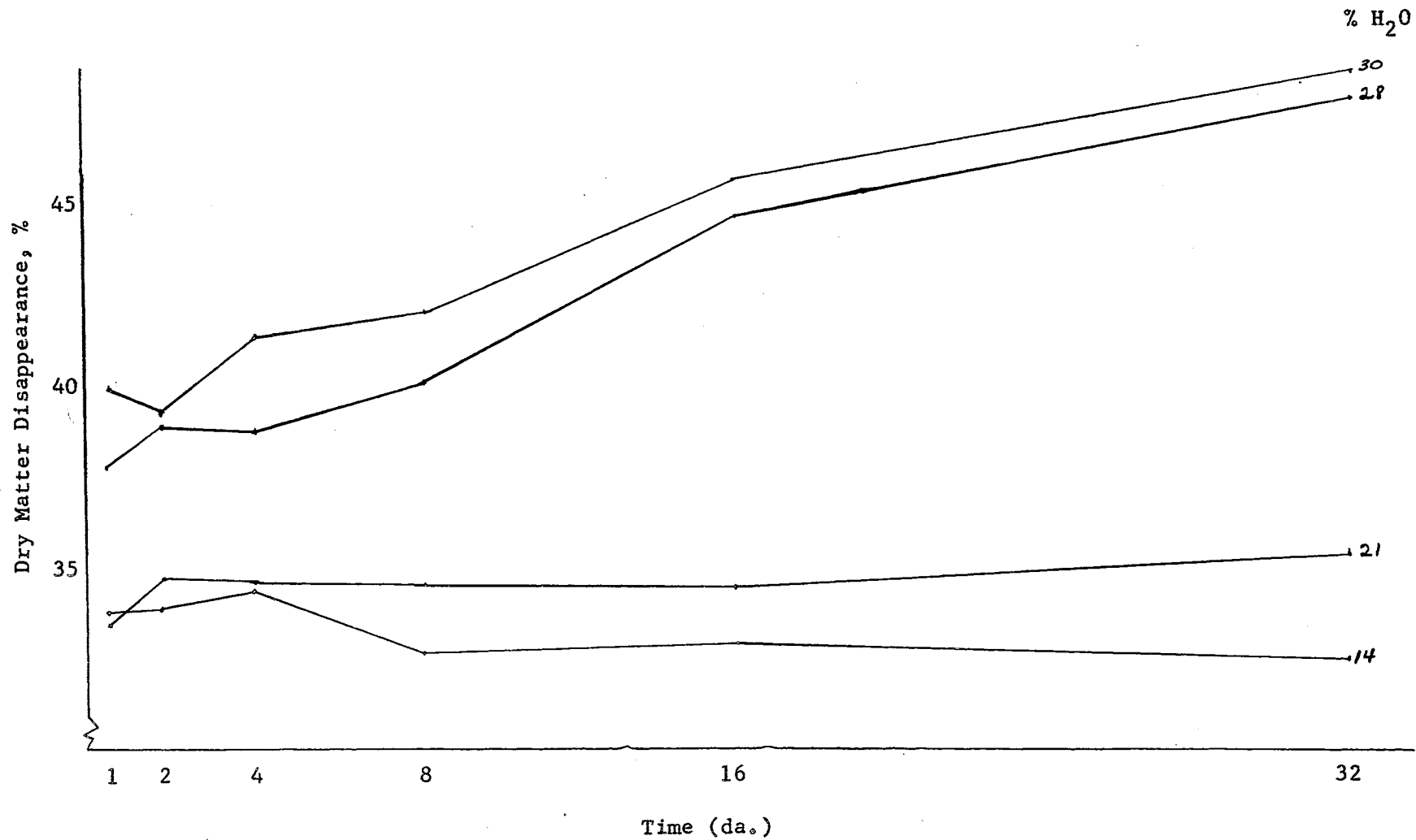


Figure 7. Effect of Time and Moisture Content of Whole Reconstituted Sorghum Grain on In Vitro Digestion

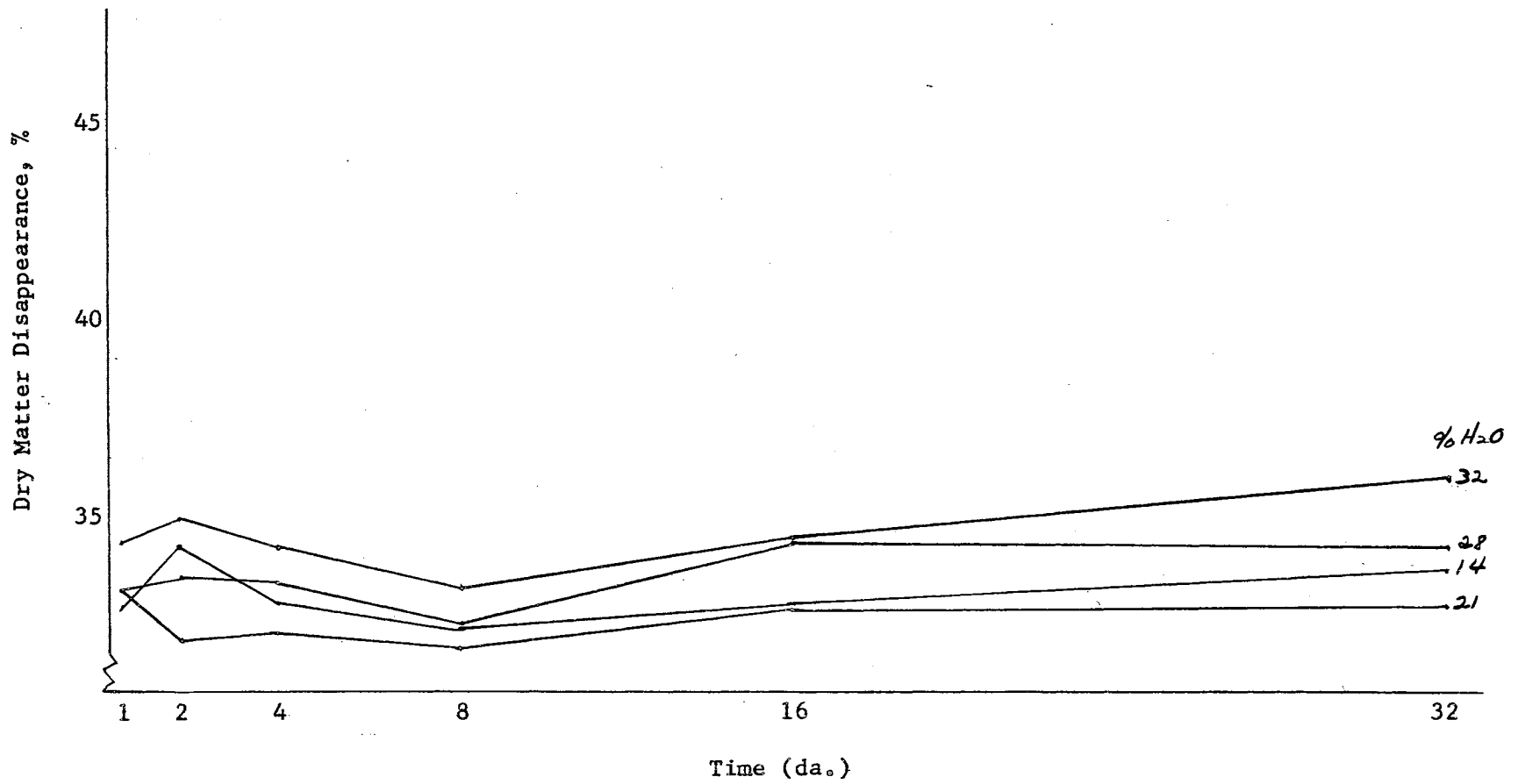


Figure 8. Effect of Time and Moisture Content of Ground Reconstituted Sorghum Grain on In Vitro Digestion

these moisture levels after it was ground was similar to the usual fluffy type texture of reconstituted grain. However, the endosperm portion was not as white in appearance as that from samples which were stored for a longer period of time. This suggests that the mere presence of moisture is not sufficient to obtain maximum results but that time is also required for the chemical changes to take place.

These data suggest that economics would play a large role in determining a satisfactory storage time since there is no abrupt increase in digestibility with time.

Effect of Moisture

Moisture had a significant effect ($p < .01$) on dry matter disappearance; however, it had little effect on sorghum grain reconstituted in the ground form (Figure 8). There was a trend toward increased dry matter disappearance with increased moisture. Analysis of variance (Table XIII) indicated a significant ($p < .01$) interaction between physical form and moisture. Therefore the effect of moisture is dependent on the physical form during reconstitution.

In agreement with Experiment II, little benefit was noted at the 21% level.

Dry Matter Loss During Reconstitution

This study was conducted in conjunction with Experiment III to determine the change in dry matter during storage following reconstitution. The mean percent change in dry matter for each treatment is shown in Table XIV. The data analyzed as a three-factor factorial experiment using sampling error as the error term in F tests since

TABLE XIV
 DRY MATTER LOSS OF RECONSTITUTED SORGHUM
 GRAIN DURING STORAGE, %

Moisture, %	Whole Reconstituted		Ground Reconstituted	
	16 Da.	32 Da.	16 Da.	32 Da.
14	0.067	0.190	0.660	0.833
21	-1.033	-0.700	-1.247	-1.753
28	-0.220	0.830	0.117	-0.823
30	0.583	1.063	-0.880	-0.090

there was no real measurement of error (Table XV).

Since the sampling error was so low, significance was high for several factors. The main point of interest in this study is that dry matter loss during storage was very low. Dry matter change ranged from + 1.06 to - 1.75%. This indicates that the loss during storage is very low as compared to the expected increase in utilization from feeding moist sorghum grain. The losses that occurred may have been due to the anaerobic fermentation taking place during storage, or they may reflect errors in technique, since they were of a random order. There was no pattern in these data indicating that the loss or gain was largely due to error in technique. Additional research concerning dry matter loss during storage is needed.

TABLE XV
ANALYSIS OF VARIANCE FOR DRY MATTER LOSS

Source	df	MS	F
Total	47		
A (Form)	1	5.1352	174.074**
B (Time)	1	0.0	0.0
C (Moisture)	3	5.6642	192.007**
AB	1	2.5704	87.132**
AC	3	3.1605	107.135**
BC	3	.0293	.993
ABC	3	1.4901	16.61 **
Error	32	.0295	

** p < .01

Experiment IV

This experiment was designed to determine the effect of drying reconstituted sorghum grain on in vitro dry matter disappearance. Results are given in Table XVI. The figures represent the mean dry matter disappearance of four subsamples for each replication.

The analysis of variance (Table XVII) indicated that treatment had a significant ($p < .01$) effect on dry matter disappearance. Figure 9 shows the comparison between treatments. As in previous experiments, reconstituting sorghum grain to 28.5% moisture (Treatment 2) resulted in an improved digestibility of about 15 percentage units.

The grain in Treatment 3, which had been reconstituted in the same original sample as that of Treatment 2, was dried to an air-dry basis after grinding before it was tested in the in vitro system. Treatment 3 resulted in digestibility slightly lower (1%) than that of the dry grain, but the difference was not statistically significant.

This suggests that moisture is not essential in the whole reconstituted grain if it is ground before feeding.

Treatment 4, which involved drying the grain before grinding, did not significantly change the dry matter disappearance compared to Treatment 1. The expected increase in digestibility due to Treatment 2 was apparently lost during the drying process. This principle is similar to the natural drying effect during the late steps of the maturing process of sorghum grain.

TABLE XVI
EFFECT OF DRYING SORGHUM GRAIN ON IN VITRO
DIGESTIBILITY

Rep.	Dry Ground	Recon. Ground	Recon. Ground Dried	Recon. Dried Ground
1	38.8	42.2	48.9	33.0
2	38.9	50.5	50.2	36.5
3	32.3	48.7	48.4	33.0
Mean	35.0	50.5	49.2	34.2

TABLE XVII
EXPERIMENT IV: ANALYSIS OF VARIANCE

Source	df	MS	F
Total	47		
Reps	2	47.005	4.3536*
Treatment	3	932.2900	86.3495**
Sampling	36	2.6150	.2422
Error	6	10.7967	

* $p < .05$

** $p < .01$

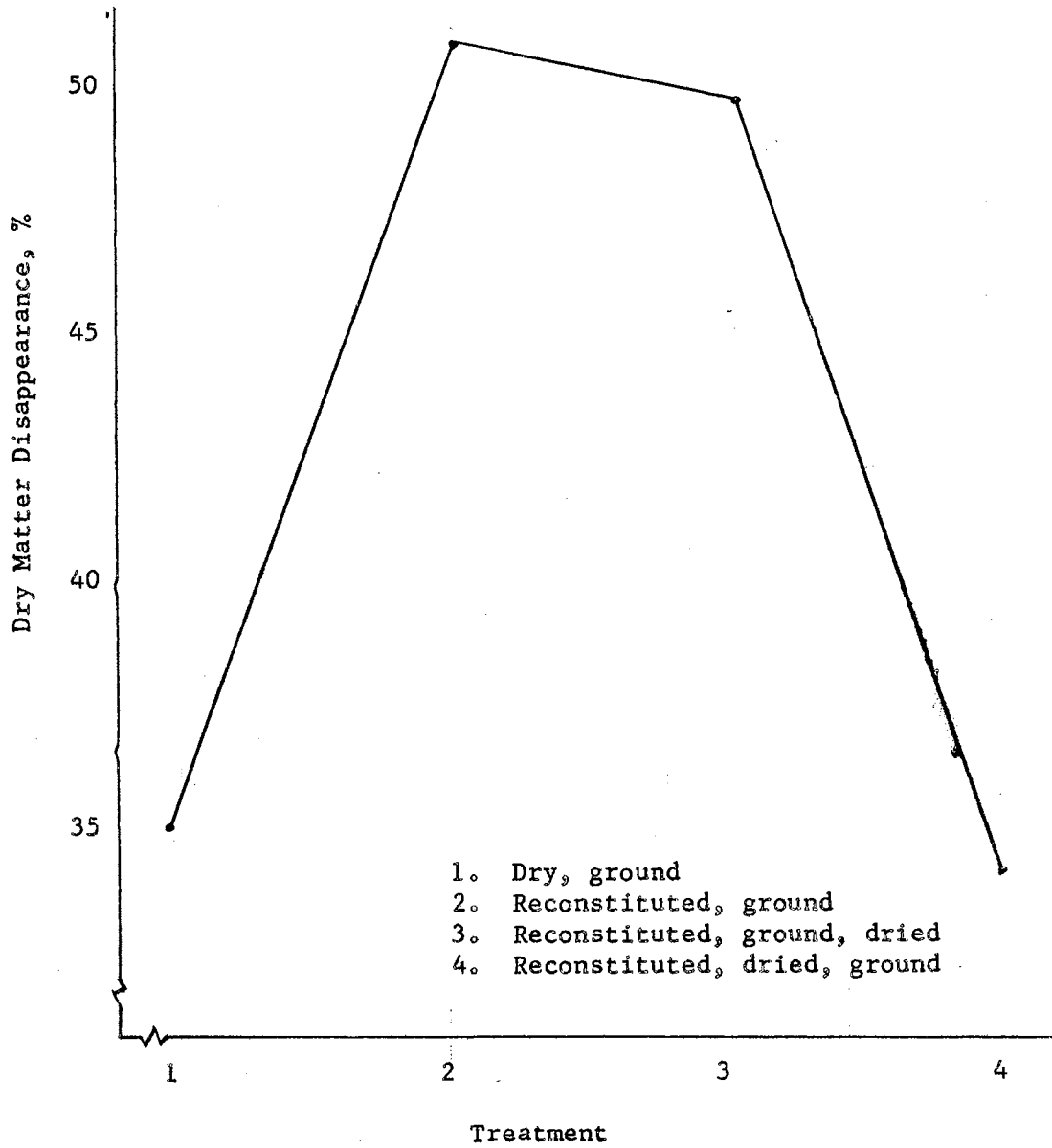


Figure 9. Effect of Drying Sorghum Grain on In Vitro Digestion

SUMMARY

Three experiments (I, II, III) were conducted to determine the effect of moisture level (13-35%), time of oxygen free storage following reconstitution (1-32 da.), environmental temperature during storage (40-110°F.), and physical form (whole and ground) during reconstitution and storage on in vitro digestion of moist sorghum grain. Experiment IV was conducted to investigate the effect of drying on reconstituted sorghum grain. In vitro digestion of sorghum grain was determined by measuring dry matter disappearance from the grain during fermentation with strained rumen fluid in the presence of artificial saliva, incubated at 39°C. for 18 hr. Change in quantity of dry matter during storage of reconstituted grain was also determined in one of the experiments.

Digestibility of both high moisture harvested and whole reconstituted sorghum grain increased significantly ($p < .01$) as the level of moisture increased over all time and temperature levels. There was a large increase in digestibility between 23 and 26% moisture with both types of moist sorghum grain, and maximum digestibility occurred at the highest moisture level tested (35%).

Significant interactions ($p < .05$) between moisture and temperature indicated that high environmental temperature (110°F.) and a high moisture level (26-35%) increased digestibility, but high temperature levels had little or no effect on grain containing low moisture levels (13-22%).

Increased storage time of high moisture harvested sorghum grain tended to be detrimental at the lower moisture levels ($< 26\%$) and had little or no effect at the higher moisture levels. Digestibility of reconstituted grain increased as the storage time increased from 1 to 32 days especially at the high moisture levels (30 and 34%). A large increase in digestibility was noted at 1 day following reconstitution, with considerable but diminishing increases to 10 days and from 10 to 20 days.

Interactions between storage time and temperature did not approach statistical significance (F values < 1.0) indicating that they are independent.

Reconstituting sorghum grain in the ground form did not improve its digestibility, but reconstituting the grain in the whole form significantly ($p < .01$) improved digestibility, compared to dry grain.

Drying whole reconstituted sorghum grain after it was ground did not result in a loss of its improved digestibility, whereas drying the grain in the whole form resulted in a total loss of the improved digestibility.

There was little or no dry matter loss during oxygen free storage of reconstituted sorghum grain.

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