EVALUATION OF PROCESSED CORN, MILO AND WHEAT BY *IN VITRO* DIGESTIBILITY, STARCH ANALYSIS AND GAS PRODUCTION

By

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Bachelor of Science

Abilene Christian College

Abilene, Texas

1970

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, 1973

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ACKNOWLEDGMENTS

The author expresses his sincere appreciation to Dr. Donald G. Wagner, Associate Professor of Animal Science, for his counsel and guidance during the course of this study and in preparation of this thesis. Appreciation is also extended to Dr. R. R. Johnson, Dr. J. E. McCroskey, Dr. L. J. Bush and Dr. R. R. Frahm for their help in the preparation of this manuscript.

Appreciation is also extended to Dr. R. D. Morrison, Professor of Mathematics and Statistics, for helpful suggestions and aid in conducting the statistical analysis of data.

The author is also grateful to fellow graduate students and student workers for their assistance in conducting this study.

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CHAPTER I

INTRODUCTION

More efficient methods of finishing cattle are needed to offset the increased cost of production and to supply the increased quantity of meat to an expanding human population. This must in part be accomplished by feeding nutrients to the animal which are more efficiently converted to edible products. Ruminants are not as efficient as the non-ruminants in converting cereal grain to edible product. If the microbial population of the ruminant can be supplied with nutrients that result in metabolic end products of higher caloric efficiency, the ruminant could be a more efficient utilizer of high carbohydrate diets.

Since cereal grains provide a large portion of the energy fed to finishing cattle, even small improvements in the efficiency of utilization are of major economic importance.

Many methods are available for the detailed study of carbohydrate fractions. However, there is a tremendous lack of knowledge as to the quality, quantity, and availability of carbohydrate fractions of cereal grains processed by different techniques.

The purpose of this study, therefore, was to study the starch fraction in cereal grains in relation to nutritional and digestibility studies done *in vitro*. Most of the energy in cereal grains is obtained

from starch. Since this starch must be broken down by enzymes, whether of mammalian or microbial origin, before it is used, information concerning the rate and extent of starch degradation should be helpful.

CHAPTER II

LITERATURE REVIEW

General

Although much is known about the chemistry of starch, very little is known about its availability in grain as a result of different grain processing methods.

The ripe grain of the common cereals consists of carbohydrates, nitrogenous compounds (mainly proteins), fat, mineral salts and water, together with small quantities of vitamins, enzymes and other substances, some of which are important nutrients in the animal's diet. Carbohydrates are quantitatively the most important constituents, forming about 83% of the total dry matter of wheat, corn, and sorghum grain (Kent, 1966). The carbohydrates present in cereal grains include predominately starch, and some cellulose, hemicelluloses, pentosans, dextrins, and sugars (Kent, 1966).

There are numerous recent reports in which various degrees of added moisture and pressure have been shown to increase the efficiency of utilization of cereal grains. However, results have not always been consistent, and the reasons for variable responses are not well understood (Parrot et al. 1969).

It is generally accepted that the starch granules from most plant species contain a mixture of two polysaccharides (Whelan, 1958). The major component, amylopectin, comprises 75-85% of most starches. It

has a branched structure in which chains containing an average of about 20-25 α-(1→4)-linked glucose residues are interlinked by α-(1→6)-glucosidic linkages to form a ramified or bush-like structure. Amylose, the minor component, is an essentially linear polymer of glucose containing more than 99% of α-(1→4)-glucosidic linkages (Radley, 1968).

Although the main structural features of the starch components are now well established, studies on the fine structure of the starch granule have yielded only limited and often conflicting information. Similarly, the individual enzymes which catalyze the synthesis of amylose and amylopectin have been the subject of many *in vitro* studies, but little is yet known of the mechanism of their action *in vivo* leading to the formation of a starch granule (LeLoire, 1961).

Starch occurs in plants in the form of granules, the size, shape, and striations of which are characteristic of the variety of starch (Reichert, 1913). The granules are usually associated with small quantities of non-carbohydrate materials, such as protein, fatty acid, and inorganic materials, and it is now known that of these only phosphorus, and probably a very small amount of protein, are chemically bound to starch (LeLoir *et al.* 1961).

Prior to 1939 great difficulty was experienced in fractionating starch. Starch solutions are unstable because of the tendency of colloidal particles to absorb impurities and because of the precipitation of the amylose component on standing. The fractionation methods used accomplished a partial separation of the starch components, although usually with attendant physical or chemical degradation (Radley, 1968).

Processing Methods for Cereal Grains

Cereal grains are valued for their high content of energy in the form of starch. Edwards and Curtis (1943) found that starch constituted 65 to 75% of the grain from 20 varieties of grains. Starch comprises 83% of the endosperm, 13.4% of germ, and 34.6% of the bran obtained by hand dissection of sorghum grain (Hubbard et al. 1950). Different types of starches are found in sorghums and other cereals. Leach (1965) reported that on heating in water, starch granules undergo gelatinization or disruption of their internal organization; they lose their birefringence, absorb water, and swell. He also reported gelatinization temperatures of sorghum starches extend from 68° to 76° C from initiation to complete gelatinization. On the other hand, gelatinization of corn starch occurs at 62° to 72° C, and the swelling powers of ordinary corn and sorghum starches are 24 and 22, whereas that of waxy corn and sorghum starches are 63 and 49, respectively, at 95° C.

Methods by which whole cereal grains may be processed for use in livestock feeding include, among others, grinding, crimping or dry rolling, reconstitution and micronizing. Only limited comparisons have been made between corn, wheat, and sorghum grain for fattening cattle. Totusek et al. (1963) have shown that grain and feed requirements for feedlot cattle had higher feed efficiencies on a corn ration compared to a sorghum grain ration. Hall et al. (1968), however, reported net energy values of corn and sorghum grain to be similar when fed to cattle. It has long been recognized, however, that sorghum grains must be processed prior to incorporation in feeds for cattle. During the process of rumination very little grain is apparently regurgitated to be remasticated. Any improvement in efficiency of utilization of the grain will be re-

flected in reduced expense.

Grinding

Grinding is one of the simplest and least expensive methods of preparing cereal grains for livestock feeds. The grinding may range from extremely fine to coarse. In general, results have been conflicting. Some studies have shown improved performance. The difference in results may be due in part to the types of ration and grains used and the fineness of grinding. Digestion studies with rations containing 78 to 85% sorghum grain have shown that there is no improvement in digestibility of fine ground grain over coarse ground or dry rolled grain (Husted et al. 1968; Buchanan-Smith et al. 1968).

Steevens (1971) reported cows fed very finely ground sorghum grain produced more milk ($P > .05$) and gained more body weight than cows fed medium or coarsely ground grain. Results from the Arizona Station, on the other hand, have shown that fine grinding reduces ($P < .05$) daily gain and increases feed requirements per unit of gain in beef cattle (Hubbert et al. 1962). They also reported excessive dustiness and fineness may reduce palatability and feed intake, in some cases, reducing animal performance. White et al. (1969) reported evidence that in beef cattle rations fine grinding of sorghum grain improves the digestibility compared with dry rolling or coarse grinding. Improved feed efficiency was obtained when very finely ground sorghum grain was compared to finely ground grain for fattening beef steers.

Grinding of shelled corn was thought to be essential for fattening cattle on a high concentrate ration. More recently, Hixon et al. (1969) have reported two experiments which discredit the traditional opinion

that corn must be ground for finishing cattle. In one trial, yearling steers weighing 328.9 kg. gained 16% more rapidly and required 8% less feed when fed whole shelled corn compared with those fed cracked shelled corn. In a second experiment, yearling steers fed whole shelled corn gained slightly faster than those fed ground corn, and the whole shelled corn ration was utilized with approximately 7% more gross efficiency than the ground shelled corn ration in which corn made up 50% of the high concentrate rations.

Dry Rolling

Dry rolled grain is prepared by passing the grain through a roller mill. Depending upon the rate of flow, weight of the rollers, and the tolerance between the rollers, the grain can be rolled to a consistency that resembles ground grain or one in which the kernel breaks into only a few pieces. Results of three trials at Oklahoma indicated no advantage for finely rolled milo over coarsely rolled milo (Totusek et al. 1968). These workers theorized that the grain particles resulting from rolling may be multifractured and, therefore, more susceptible to the entry of enzyme-containing fluid for digestion.

Martin et al. (1971) compared whole corn, ground corn, and rolled corn in feedlot rations for steers using sorghum silage as the roughage source. The results indicated very little difference in performance of cattle fed corn processed by the three methods. Rate of gain and feed conversion values were similar among treatment groups. However, it was noted that steers receiving whole corn were more difficult to keep on feed as compared to the other two groups.

Reconstitution

In this process dry grain is reconstituted to higher moisture levels of perhaps 20 to 35% moisture and stored in an air-tight unit for some period of time prior to feeding. Feeding trials have indicated that the efficiency (feed/unit of gain) of reconstituted sorghum grain is approximately 15% greater than for dry-rolled grain (Newson et al. 1968).

On the basis of present information it appears that a minimum period of 21 days is required before the necessary chemical changes take place within the reconstituted grain to permit optimum utilization of the nutrients (McGinty, 1968). He also reported that the grain should be reconstituted and stored as whole grain rather than as ground grain. The reason for this is not completely understood, but may be related to endogenous production of enzymes within the grain rather than to bacterial enzymes which develop during conventional ensiling. Florence et al. (1968) suggested that reconstitution destroys the proteinaceous matrix surrounding the starch granule. The effect of storage temperature upon reconstituted grain is not known. However, it appears that reconstituted sorghum grain stored at temperatures below 4°C has a feeding value similar to that of dry-ground grain (McGinty et al. 1968).

Many researchers (White and Totusek, 1969; Wagner and Schneider, 1970; Riggs and McGinty, 1970; Martin et al. 1971; Wagner, Christiansen, and Holloway, 1971) have noted improvements ranging from 6 to 18% in feed efficiency when beef steers were fed reconstituted and high moisture harvested sorghum grain as compared to dry-rolled or finely ground grain.

Ensiling of high moisture shelled corn or reconstituted high moisture corn has attracted a great deal of interest among beef cattle feeders because of possible storage and mechanical feeding advantages.

Perry et al. (1969) showed comparable 91 day gains of 2.47, 2.52, and 2.58 lbs. per day, respectively, on dry corn (10.6% moisture), ensiled high moisture corn (26.4% moisture), and ensiled reconstituted corn (18.2% moisture).

Riggs and McGinty (1970) compared early harvested and reconstituted moist sorghum grains with dry grain in seven cattle feeding experiments. Moist sorghum grain in whole kernel form was not satisfactory for finishing cattle, but in the ground form produced weight gains equal to that produced by ground dry grain. Reconstituting the grain to 25 to 30% moisture followed by storage for at least 21 days and grinding prior to feeding increased the digestibility of protein, dry matter, and organic matter from 16 to 29%. Oklahoma research indicated that the method of breaking the milo kernel after reconstituting and storing also affects the utilization of the grain (Newson, 1968; White and Totusek, 1969).

Christiansen and Wagner (1972) reported that reconstitution by two different methods produced little if any improvement in the nutritive value of wheat compared with dry rolling, when fed in high concentrate rations to finishing heifers.

Although limited, published reports on the value of wheat in feedlot rations are quite variable, but in some cases also imply a lower feeding value for wheat than some other grains. On the other hand, Totusek et al. (1968) noted somewhat, although not significantly, lower gains and feed efficiencies with wheat than milo. Further, performance was very similar between rations in which the concentrate was either all wheat or one-half wheat and one-half milo. Undoubtedly, the wide variations in the apparent feeding value of wheat are due to a variety of factors, including type of wheat fed, manner of wheat preparation, nature

of the ration and other variables.

The effect of length of storage period on reconstituted grain is not completely understood. It is probable that both temperature and moisture levels within the unit affect time of storage required (Newson and Totusek, 1968). Currently some feedlots are reconstituting ground or rolled sorghum grain and corn in pits; however, it is questionable that any advantage in feed efficiency is obtained over dry grinding or rolling by this method.

Micronized or Popped Grain

Sorghum grain can be micronized, and the finished product resembles popped corn. Limited feeding trials with micronized sorghum grain show it to be efficiently utilized by fattening cattle (Adame and Riggs, 1967).

Schake et al. (1970) evaluated micronized (dry heated and then rolled) and steam-flaked sorghum grain under commercial feedlot conditions in the Texas Panhandle. The rations included 15% roughage. Steers fed micronized grain went on feed more rapidly, and their feed intake was very slightly higher than for steers fed steam-flaked grain. Although feedlot performance, carcass weight, and carcass quality tended to favor the cattle fed micronized grain, those fed steam-flaked grain were slightly more efficient in feed conversion.

Hinders and Eng (1970) compared three sorghum grains varying widely in genetic type: (1) a red with waxy or glutenous starch, (2) a white with non-waxy or non-glutenous, and (3) a regular non-waxy red with relatively more soft, floury starch. The waxy or glutenous starch types differ distinctly from the non-waxy types in their starch struc-

ture. The percent gelatinization of the red waxy and the white non-waxy was similar and higher than that obtained in the regular red after processing as shown in Table I.

TABLE I

GELATINIZATION OF THREE TYPES OF SORGHUM GRAIN AFTER PRESSURE COOKING AND MICRONIZING

	Approximate Percent Gelatinization		
Processing Method	Red Waxy	White Non-Waxy	Reg. Red
Pressure Cooked	40	40	30
Micronized	25	25	12

The rate of enzymatic digestion of the three grains followed similar trends as the percent gelatinization. More specific values are shown in Table II.

TABLE II

GAS PRODUCTION FROM THREE TYPES OF SORGHUM GRAIN AFTER PRESSURE COOKING, MICRONIZING, STEAM-FLAKED, AND GRINDING

	Ml. of Gas Produced Per Gram Per Hour		
Processing Method	Red Waxy	White Non-Waxy	Reg. Red
Pressure Cooked	19.0	11.4	9.7
Micronized	9.8	9.7	4.7
Steam-Flaking	----	23.4	16.2
Ground	4.8	3.8	3.8

Estimation of Starch in Cereal Grains

Cereal grains are valued for their high content of energy in the form of starch. Starch content in cereal grains may be determined as reducing sugar after enzyme or acid hydrolysis. Determinations of available metabolizable carbohydrate which consists principally of glucose and fructose and the readily hydrolyzable carbohydrates, starch, sucrose, lactose, and maltose have been obtained by several methods. There are two official methods of the Association of Official Agricultural Chemists for the determination of starch in feeds, the diastase method and the direct acid hydrolysis method. The malt diastase method is slow and requires correction for a high blank due to the sugars in the malt extract. The acid method includes as starch the pentosans and other carbohydrate bodies which produce reducing substances under the conditions of the hydrolysis.

A comparison of the official methods, acid hydrolysis and malt diastase, was made by Fraps (1932). Also Etheredge (1941), and Walton (1932) have made similar comparisons. These workers have reported the highest results for "total" starch were secured by the A.O.A.C. method of acid hydrolysis. Friedemann et al. (1967) have reported that in all methods, reducing sugar is determined by ferricyanide (FeCn) reduction after clarification of the digest by $Zn(OH)_2$.

No biological methods have been devised for assessing the contribution of bacterial processes; none have been described for accurately estimating the carbohydrate which has been actually absorbed and is available for metabolism (Friedemann et al. 1962). The general method for the determination of starch content of products containing large quantities of proteinaceous matter by conversion into dextrose by means

of diastase and acid hydrolysis is tedious and at times unreliable.

In Vitro Dry Matter Disappearance

Increasing use has been made of in vitro systems to evaluate grain digestion or utilization by rumen microorganisms. In vitro fermentation methods have been used to study utilization of starch by rumen microorganisms (Salisbury, Hoeffer, and Luecke, 1961; Moore, Johnson, and Dehority, 1962; Loper, Little, and Mitchell, 1966). The methods as used by them required starch measurements following the incubation period.

Neuhaus and Totusek (1969) studied the influence of moisture level (13 to 35%), time of oxygen-free storage (1 to 32 days), and environmental temperature during storage (4-43°C.) on the in vitro digestibility of high moisture harvested (HMH) and reconstituted sorghum grain. In vitro digestibility of milo was determined in a series of experiments in which sorghum grain samples were incubated for 18 hours at 39°C. with strained rumen fluid in the presence of artificial saliva. Significant ($P < .05$) interactions between moisture level and temperature indicated that high temperature at a high moisture level increased digestibility; high temperature had little effect at low moisture levels. At high moisture levels digestibility increased markedly at 1 day followed by a gradual increase to 32 days. Whole reconstituted sorghum grain was significantly ($P < .01$) improved in digestibility, but ground reconstituted sorghum grain was not improved at all.

Volatile Fatty Acid Production

While glucose is the major source of energy in the monogastric animal, the adult ruminant receives its energy mainly from the volatile

fatty acids, acetic, propionic, and butyric acid (Jones et al. 1970).

Baile and Mayer (1969) showed that a close relationship exists between rate of injection of acetate, propionate or a mixture of volatile fatty acids and the depression of feed intake in goats. Injection rates in the experiment were adjusted to nearly match the physiological rates of production following introduction of readily fermentable feedstuffs. It seemed likely that the feed depressing effect of the volatile fatty acids can play a significant role in the overall regulation of energy balance since: (1) they are important energy sources to the ruminant, (2) their rates of production increase with feeding, and (3) they are the first and most immediate products of the digestive process to be absorbed. Butyrate was totally ineffective as a depressant.

Riggs (1970) noted significant differences were found in volatile fatty acids of rumen samples from cattle fed differently treated grains. Acetic and isovaleric acids both showed significantly higher values, but propionic acid showed significantly lower values in samples from cattle fed the original grain than in those from cattle fed heat treated grains. In the case of valeric acid, cattle fed 100 % popped grain showed a significantly higher level than did those fed the other grains. The acetic: propionic acid ratio also was significantly wider in the case of the original grain group than in the others. This group showed a ratio of 1.82 : 1 as compared with 0.88 : 1, 1.01 : 1 and 1.06 : 1 for the cattle fed "normal run" or total heat treated grain, 100-percent popped, partial and non-popped grains, respectively. Expressed in terms of estimated net energy (ENE) values, which may be a more meaningful measure of the energy available for productive purposes, the point of maximal energy intake is at about 65% concentrates (Zeremski et al. 1965).

Rate of Enzymatic Digestion

Greater susceptibility of different grains to various types of processing techniques can significantly affect the time required to produce a given degradation of starch. Albin and Sherrod, (1971) compared twenty-eight different samples of sorghum grain grouped according to endosperm type: floury (40% or less corneous type starch); intermediate (40 to 60% corneous type starch); corneous (60% and above corneous type starch; waxy (Near 100% amylopectin); and commercial run (mixture of all starch types). Gas production from yeast utilization of sugars (as they were produced by amyloglucosidase digestion of starch) was used as an indicator of starch availability for each grain. It was reported that the in vitro gas production technique gave acceptable estimates of digestible energy of rations containing different endosperm types. A similar experiment reported by Hinders and Eng (1971) comparing two selections of waxy (A), three selections of non-waxy, corneous endosperm (B), three selections of non-waxy, floury endosperm (C), and two selections of brown seed coat bird resistant (D) sorghum grain was conducted on raw grain and on samples that were exposed to infra-red heat and immediately ground. The milliliters of gas produced per hour for raw ground grain samples A, B, C, and D were 5.1, 2.4, 2.9, and 1.2, respectively.

McGinty (1968) also found highly significant correlations of gas production with dry matter (0.94) and starch (0.95) disappearance in vitro. Significant correlations of in vitro gas production with dry matter digestibility in vivo (r = 0 79 and 0.91 in two experiments) were also obtained. As a result, McGinty (1968) concluded that in vitro gas production provides a quick, reliable method for estimating relative

differences in dry matter digestibility of grains.

Trei et al. (1970) reported that steam processing and flaking milo or barley significantly increased gas production over the untreated grain. Also increasing flake flatness of milo increased gas production. This parallels the results of digestion trials with milo (Husted et al. 1968) in that flaking of the milo appeared necessary once it had been steamed. On the basis of gas production in in vitro trials, it might be expected that steam processing and flaking of barley would increase its utilization.

CHAPTER III

MATERIALS AND METHODS

General

An alcohol soluble carbohydrate experiment (Experiment I), two *in vitro* dry matter disappearance studies (Experiments II and III), and a gas production study (Experiment IV) were conducted to determine the effects of various processing techniques on corn, milo, or wheat.

Alcohol Soluble Carbohydrates

Experiment I

Alcohol soluble carbohydrate (expressed as percent reducing sugar) determinations were made on corn, milo, and wheat which were dry ground or reconstituted at 22% and 32% moisture in either the whole or ground form. The milo used was Northrup King-222 grown at the Fort Reno Experiment Station, and the wheat was a hard red winter variety (Triumph) grown at the same station. The corn was a commercial source from the Oklahoma State University feed mill.

A randomized complete block design was used as shown in Table III, in which blocks consisted of ethanol and isopropanol extractions.

All grains were cleaned of trash using a sieve cleaner which removed most foreign matter and cracked grain prior to reconstitution and final analysis. The three grains were processed as follows:

TABLE III

EXPERIMENT I: FORM OF THE ANALYSIS OF VARIANCE

Source	d.f.
Total	119
Blocks	1
Treatments	14
Grains	(2)
Processing Methods	(4)
Grains x Processing Methods	(8)
Blocks x Treatments	14
Experimental Error[1]	90

[1]Error term used to test treatments

(1) Whole dry grain.

(2) Reconstituted in whole form at 32% moisture for 21 days.

(3) Reconstituted in ground form at 32% moisture for 21 days.

(4) Reconstituted in whole form at 22% moisture for 21 days.

(5) Reconstituted in ground form at 22% moisture for 21 days.

Reconstituted grains were prepared by determining the dry matter content of each grain and then adding sufficient water to raise the moisture content to either 22 or 32%. The grain was mixed in a small bucket until the desired quantity of water was absorbed by the

grain. The reconstituted grains were then placed in air-tight plastic bags, flooded with CO_2 before sealing, and held for 21 days at room temperature (approximately 20°C). The grains were then mixed with dry ice and ground through a laboratory Wiley mill (20 mesh screen). The dry ice was used to keep the Wiley mill cool while grinding the grain samples and, perhaps, from alternating the starch granules by heat in the grinding process.

Extraction was in either 20% absolute-ethanol or 40% isopropyl-alcohol. The grain was then subjected to B-amylase. The procedure used was that reported by Friedmann et al. (1967) as revised by Johnson and McGeehon (1970)[1] (Appendix Table XXIII). The principal objectives of this procedure were to 1) present a choice simple method whereby the nutritionist may determine the available carbohydrate, either in a single analysis of the sample, or in a separate assay of the soluble sugars and starch, to 2) suggest improved methods for calculating the total available hexose or carbohydrate, and their calorie equivalents, from the raw titration data and to 3) relate a fraction of the carbohydrate to the total crude carbohydrate.

In Vitro Dry Matter Disappearance

Technique

A modification of the two-stage in vitro rumen fermentation procedure, as described by Tilley and Terry (1963) and modified by Schenider (1971), was used. The general method is shown in Table IV.

---

[1]Dr. R. R. Johnson and Mike McGeehon, Oklahoma State University.

TABLE IV

IN VITRO TECHNIQUE

Element	Level
Grain Sample	0.4 g. (D.M.)
Artificial Saliva	22.0 ml.
Rumen Inoculum	8.0 ml.
Temperature	39°C.
Time of Incubation	12 and 24 Hrs.

Before inoculation with fermentation media, all grain samples, except where designated, regardless of experimental treatment, were prepared in the following manner:

(1) Ground through a laboratory Wiley mill (20 mesh screen) except where otherwise indicated.

(2) Approximately .4 gm. samples on a dry matter basis were weighed into numbered 50 ml. centrifuge tubes which were dried at 100°C.

(3) Inoculated with fermentation media and allowed to digest for either 12 or 24 hr. as indicated.

The composition of the artificial saliva used is shown in Table V.

Four-liter quantities of the artificial saliva solution were prepared, saturated with CO_2, and warmed to 39°C. prior to mixing with whole rumen inoculum. The inoculum source steer was fed a ration containing 84% grain twice daily at approximately 1.5 times maintenance. Rumen contents were dipped from the rumen with a small beaker, filtered through two and then six layers of cheesecloth and placed into a thermos

jug. The rumen fluid was then taken to the laboratory where it was gassed with CO_2 as quickly as possible to minimize bacterial loss. Seven hundred twenty-six milliliters of the rumen fluid were then mixed with two liters of warmed artificial saliva, and CO_2 was bubbled through the mixed media. Temperature was maintained at 39°C. and solids were kept in suspension by a heated magnetic stirring plate.

TABLE V

COMPOSITION OF ARTIFICIAL SALIVA

Ingredient	Gm./Liter of Distilled H_2O
$NaHCO_3$	9.8
Na_2HPO_4	3.7
KCL	0.57
NaCl	0.47
$MgSO_4$ $7H_2O$	0.12
$CaCl_2$	0.04

Five milliliters of the mixed media were pipetted into each substrate tube to moisten the feed and prevent floating of feed particles when greater quantities of fluid were added. An additional 25 ml. of the buffered inoculum were then pipetted into each tube. Following inoculation, the unfilled portion of each tube was immediately flooded with CO_2 and stopped with a #6 stopper. All stoppers contained a 2 mm. hole to allow fermentation gas to escape. The tubes were then incubated in a water bath at 39°C in the dark for either 12 or 24 hours as designated. The tubes were stirred at least two times during this period.

Seven pre-weighed tubes containing only 30 ml. of the saliva and rumen inoculum mixture (no grain) were incubated simultaneously as blanks. Both the blank and the substrate containing tubes were removed from the water bath in a random order in which they were entered and centrifuged at 2200 rpm for 10 minutes. Five milliliters were pipetted out of two random samples of each treatment in Experiment II for VFA analysis. All the remaining supernatant solution in each tube was decanted, 25 ml. of distilled water was added and centrifugation was repeated. After the supernatant was again decanted, the tubes were placed in a drying oven at 100°C for 24 hours.

Upon removal from the oven, the tubes were allowed to cool in dessicators and then weighed. Percent dry matter disappearance was determined by the following formula:

$$100 - \left[\frac{(\text{Dry tube} + \text{total D.M.}) - (\text{Dry tube} + \text{avg. D.M. wt. of blanks})}{\text{Original Grain Sample}} \times 100\right]$$

In Vitro - Experiment II

Treatments

Corn was reconstituted in the whole form using different moisture levels, storage temperatures and storage times as indicated:

(1) Dry whole corn.

(2) 22% moisture, storage temperature 4°, stored 7 days.

(3) 22% moisture, storage temperature 4°, stored 14 days.

(4) 22% moisture, storage temperature 4°, stored 21 days.

(5) 22% moisture, storage temperature 4°, stored 28 days.

(6) 22% moisture, storage temperature 22°, stored 7 days.

(7) 22% moisture, storage temperature 22°, stored 14 days.

(8) 22% moisture, storage temperature 22°, stored 21 days.

(9) 22% moisture, storage temperature 22°, stored 28 days.

(10) 32% moisture, storage temperature 4°, stored 7 days.

(11) 32% moisture, storage temperature 4°, stored 14 days.

(12) 32% moisture, storage temperature 4°, stored 21 days.

(13) 32% moisture, storage temperature 4°, stored 28 days.

(14) 32% moisture, storage temperature 22°, stored 7 days.

(15) 32% moisture, storage temperature 22°, stored 14 days.

(16) 32% moisture, storage temperature 22°, stored 21 days.

(17) 32% moisture, storage temperature 22°, stored 28 days.

The experimental design was a completely randomized design with factoral arrangement of treatments as shown in Table VI. The analysis of variance components with all corn treatments excluding the control is shown in Table VII. The control was omitted in this design due to unequal numbers in the analysis.

Volatile Fatty Acid Determination

Five milliliters of rumen fluid supernatant were obtained from two *in vitro* tubes at random. The supernatant was mixed with one milliliter of 25% metaphosphoric acid containing 2.1248 mg/ml. of 2-ethyl-butyric acid and retained in an ice bath for 30 minutes. The samples were then centrifuged at 2200 rpm for 20 minutes. Five microliters of the supernate were injected directly into a six-foot U-shaped column of 28 percent carbowax, 20M TPA on 60/80 chromasorb W.[2] A Bendix, series 2500, chromatograph[3] was used for the VFA analysis. The detector, injection

[2]Wilkens Instrument and Research, Inc., Walnut Creek, California.

[3]Bendix Process Instrument Division, Ronceverte, West Virginia.

TABLE VI

EXPERIMENT II: EXPERIMENTAL DESIGN SHOWING NUMBER OF SAMPLES PER TREATMENT FOR EITHER 12 OR 24 HRS

		Processed Corn																
Moisture		22%								32%								
Temperature		4°C				22°C				4°C				22°C				
Days Stored		7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28	
Replicates	Control																	Total Number
1	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	119
2	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	119
3	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	119
4	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	119
5	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	119
6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	119
	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	714

TABLE VII

EXPERIMENT II: FORM OF THE ANALYSIS OF VARIANCE

Source	d.f.
Total	671
Replications	5
Moisture	1
Days	3
Temperature	1
Moisture x Days	3
Moisture x Temperature	1
Days x Temperature	3
Moisture x Days x Temperature	3
Replicates x Treatment	75
Replicates x Moisture	5
Replicates x Days	15
Replicates x Temperature	5
Replicates x Moisture x Days	15
Replicates x Moisture x Temperature	5
Replicates x Days x Temperature	15
Replicates x Moisture x Days x Temperature[1]	15
Error	576

[1]Error term used to test treatments.

and column temperatures were maintained at 250, 225 and 140°C, respectively. Calculation of the volatile fatty acids in each sample were simplified by the utilization of a computer program based on peak height, retention time, and attenuation.

In Vitro - Experiment III

Treatments

The treatments investigated in this in vitro trial are indicated below:

(1) Dry whole wheat.

(2) 22% reconstituted wheat, stored 21 days.

(3) 32% reconstituted wheat, stored 21 days.

(4) Dry whole milo.

(5) 22% reconstituted milo, stored 21 days.

(6) 32% reconstituted milo, stored 21 days.

(7) Dry rolled milo, ground through a laboratory Wiley mill 20 mesh screen.

(8) Dry rolled milo.

(9) Micronized milo, ground through a laboratory Wiley mill 20 mesh screen.

(10) Micronized milo.

Treatments 1-6 were the same as those used in Experiment I. It should be noted that the milo treatments were all of the same variety (Northrup King 222) and obtained from the same location; however, dry and reconstituted (22 and 32%) milo were collected from the 1970 harvest, while rolled and micronized milo were collected from the 1971 harvest.

The experimental design was a randomized complete block as shown in

TABLE VIII

EXPERIMENT III: EXPERIMENTAL DESIGN SHOWING NUMBER OF SAMPLES PER TREATMENT FOR EITHER 12 OR 24 HRS

Replicates	Processed Wheat			Processed Milo							Total Number
	Dry Control	22% Wheat	32% Wheat	Dry Control	22% Milo	32% Milo	(20 mesh) Dry Rolled	Dry Rolled	(20 mesh) Micronized	Micronized	
1	7	7	7	7	7	7	7	7	7	7	70
2	7	7	7	7	7	7	7	7	7	7	70
3	7	7	7	7	7	7	7	7	7	7	70
	21	21	21	21	21	21	21	21	21	21	210

Table VIII.

The analysis of variance components for the 10 treatments are shown in Table IX.

TABLE IX

EXPERIMENT III: FORM OF THE ANALYSIS OF VARIANCE

Source	d.f.
Total	209
Replicates	2
Treatments	9
Replicates x Treatments[1]	18
Sampling Error	191

[1]Error term used to test treatments

Gas Production

Experiment IV

Technique. The gas production method used was adapted from Sandstedt et al. (1962) and revised by Hinders and Eng (1969). The gas produced from the grain sample was by an enzymatic digestion (amyloglucosidase) of the starch portion in which yeast was used as the prime energy source during the six-hour digestion period. The technique of this method is indicated below:

(1) Grain samples were ground in a laboratory Wiley mill (20 mesh screen) unless otherwise designated.

(2) Eight-tenths gram ground grain sample (dry matter basis) was

weighed and transferred to a 50 ml. Erhlenmeyer flask.

(3) One-fourth gram Fleischmann's dry yeast and 10 ml. of amyloglucosidase solution (0.25 gram of amyloglucosidase per 250 ml. water) were added to the flask containing the processed grain sample.

(4) The Erhlenmeyer fermentation flask was connected in an airtight manner to an inverted 50 ml. burette filled with .1 N HCl containing methylene orange as indicator to form a manometric apparatus.

(5) The flasks were then placed in pulsating water bath which was thermostatically controlled at 39°C.

(6) The flasks were shaken twice during their duration in the water bath.

(7) The quantity of gas produced (milliliter per gram of dry matter) was measured every hour for six continuous hours by the quantity of liquid displaced.

Trial 1 and 2. The treatments compared were the same as those used in the two in vitro dry matter disappearance experiments (Experiments II and III).

CHAPTER IV

RESULTS AND DISCUSSION

Alcohol Soluble Carbohydrate Experiment

Experiment I

This experiment was conducted to determine the percent reducing sugars, as measured by ethanol and isopropanol extractions, of dry and reconstituted forms of corn, wheat and milo

Mean percent reducing sugar from ethanol and isopropanol extractions and standard errors of alcohol soluble carbohydrates for the different forms of reconstituted corn, wheat and milo are shown in Table X. Actual percent dry matter for the various treatments are also given in this table. The analysis of variance is shown in Table XI. Comparisons of means were made by Duncan's Multiple Range Test.

Method of grain preparation produced a significant effect ($P < .01$) on the level of reducing sugar. Corn which was reconstituted in the whole form at 32% moisture had a significantly ($P < .05$) higher level of reducing sugar, as measured by both ethanol and isopropanol extractions, than all other grain treatments Corn reconstituted in the ground form at 22% moisture had a significantly lower ethanol soluble carbohydrate level ($P < .05$) than the other corn treatments. Furthermore, corn reconstituted, whole or ground, at 22% moisture had a significantly lower ($P < .05$) isopropanol soluble carbohydrate level than the other treat-

TABLE X

MEAN PERCENT REDUCING SUGAR OF ALCOHOL SOLUBLE CARBOHYDRATES FOR DIFFERENT PROCESSED FORMS OF CORN, MILO AND WHEAT

Treatment	Ethanol Extractions[1]	Isopropanol Extractions[1]	Actual D.M. (%)
	------% on D.M. Basis------		
Dry Wheat	.72^{cdk}	.74^{bck}	89.4
Recon. Gr. Wheat (32%)	.99^{fgl}	1.19^{dl}	65.2
Recon. Whole Wheat (32%)	.97^{fgkl}	.76^{bck}	65.6
Recon. Gr. Wheat (22%)	.70^{bcdk}	.66^{bk}	78.0
Recon. Whole Wheat (22%)	.90^{efgkl}	.71^{bck}	76.6
Dry Corn	.70^{bcdk}	.63^{bk}	89.6
Recon. Gr. Corn (32%)	.74^{cdek}	.87^{ckl}	66.5
Recon. Whole Corn (32%)	1.38^{in}	1.69^{em}	66.5
Recon. Gr. Corn (22%)	.39^{aj}	.19^{aj}	78.1
Recon. Whole Corn (22%)	.85^{defkl}	.35^{ajk}	76.1
Dry Milo	1.04^{ghl}	.88^{ckl}	88.9
Recon. Gr. Milo (32%)	.55^{abjk}	.67^{bk}	65.3
Recon. Whole Milo (32%)	1.16^{hm}	1.28^{dl}	67.6
Recon. Gr. Milo (22%)	.40^{aj}	.21^{aj}	78.0
Recon. Whole Milo (22%)	.63^{bcjk}	.31^{ajk}	79.1
Standard Errors	.12	.09	

[1] abcdefghi: Values in the same column without a common letter differ significantly ($P < .05$).

jklmn: Values in the same column without a common letter differ significantly ($P < .01$).

TABLE XI

EXPERIMENT I: ANALYSIS OF VARIANCE FOR ETHANOL AND ISOPROPANOL ALCOHOL SOLUBLE CARBOHYDRATES

Source	d.f.	M.S.	F
Total	119		
Blocks	1	.19	
Treatments	14	.36	3.6[1]
Grains	(2)	.28	2.8
Processing Methods	(4)	.90	9.0[1]
Grains x Processing Methods	(8)	.11	
Blocks x Treatments	14	.06	
Experimental Error[2]	90	.10	

[1]Significant ($P < .01$).

[2]Error term used to test treatments.

ments. In general, reconstituting corn in the whole form at 32% moisture produced an increase in percent reducing sugar; whereas, reconstituting corn in either the ground form or at a low moisture level (22%) produced either no increase or only a small increase in percent reducing sugar over dry corn.

Milo which was reconstituted in the whole form at 32% moisture contained the highest level of reducing sugar, as measured by both ethanol and isopropyl extractions, of all milo treatments and was significantly higher ($P < .05$) than all other reconstituted milo treatments.

For wheat, treatment differences for alcohol soluble sugar content were, in general, smaller than for corn and milo. However, a few significant differences were obtained. All forms of reconstituted wheat except ground reconstituted wheat (22%) contained a significantly higher ethanol soluble carbohydrate content ($P < .05$) than dry ground wheat. The reconstituted ground wheat (32%) treatment showed a higher ($P < .05$) isopropanol soluble carbohydrate level than all other wheat treatments, with no difference among any of the other treatments.

Corn and milo reconstituted in the whole form at 32% moisture showed a higher trend in reducing sugars compared to corn and milo ground prior to reconstitution. This indicates that some of the change may be a result of partial starch hydrolysis as in germination. However, starch hydrolysis may not take place if the physical integrity of the whole kernel is disrupted by grinding (van Overbeck, 1966). The increase in alcohol soluble carbohydrates observed in this experiment in whole reconstituted milo and corn support observations by other workers that reconstitution of sorghum grain or corn in the whole form increases the efficiency of feed utilization by finishing cattle and the rate of

in vitro digestibility (Neuhaus and Totusek, 1971; Totusek *et al.*, 1967; McGinty and Riggs, 1967).

Buchanan-Smith *et al.*, (1968) reported an increase in amount of reducing sugars from about 0.3% in dry grain to 1% in whole reconstituted grain. These results support suggestions made by other workers that the starch granule is altered (Riggs and McGinty, 1970) and that there is a larger amount of starch available for digestion (Florence and Riggs, 1968) in reconstituted grain. More research in the area of carbohydrate alteration and availability in reconstituted grains is needed.

In Vitro Dry Matter Disappearance

Experiment II

This experiment was conducted to determine the effects of moisture level (22% and 32%), storage temperature (4° and 22°C) and storage time (7, 14, 21 and 28 days) during reconstitution on *in vitro* dry matter disappearance of sixteen forms of reconstituted whole corn.

Mean values and standard errors for dry matter digestibility during 12 and 24 hour incubation periods are given in Figures 1 and 2, respectively. The analysis of variance is presented in Tables XII and XIII for the 12 and 24 hour incubation periods, respectively.

The moisture content of reconstituted corn during storage had a significant effect ($P < .01$) on *in vitro* dry matter disappearance during both the 12 and 24 hour incubation periods (Table XII and XIII). As noted in Figures 1 and 2, corn reconstituted at 32% moisture, in general, showed a trend for a higher *in vitro* digestibility than corn reconstituted at 22% moisture. Neuhaus (1967) reported that dry matter disappearance increased only slightly at 17 and 22% moisture levels compared

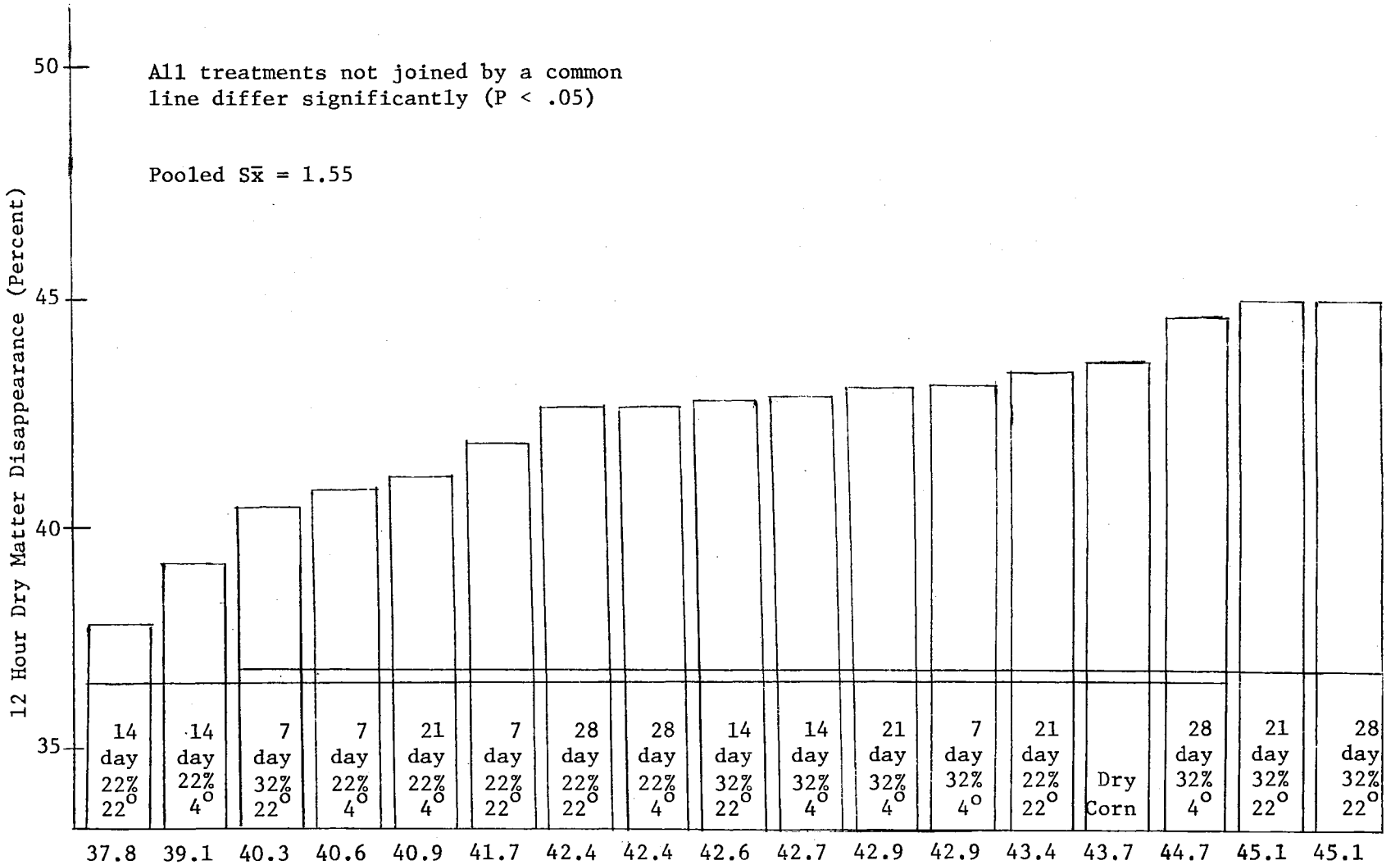

Figure 1. In Vitro Dry Matter Disappearance of Reconstituted Corn During 12 Hour Period

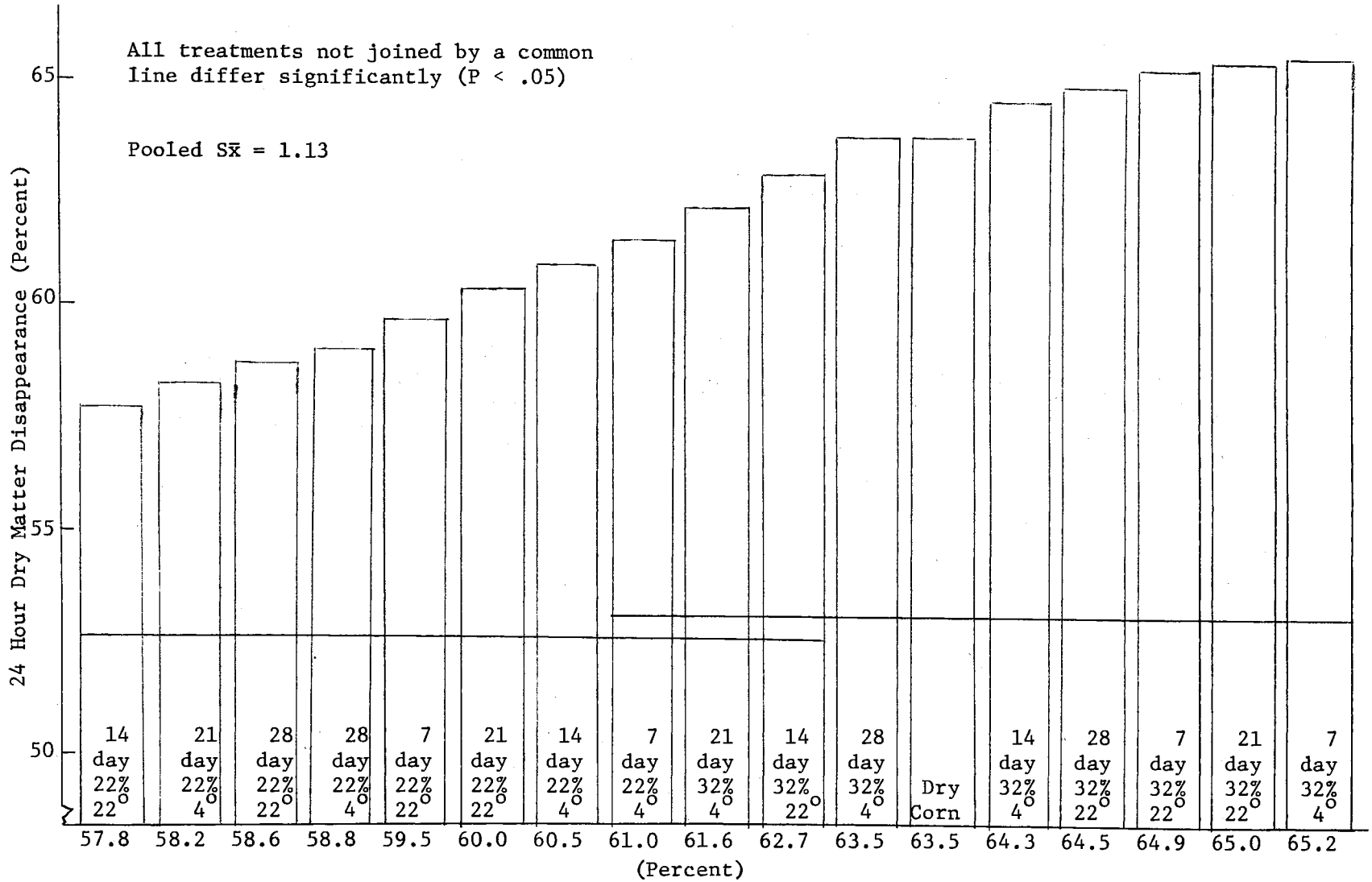

Figure 2. In Vitro Dry Matter Disappearance of Reconstituted Corn During 24 Hour Period

TABLE XII

EXPERIMENT II: ANALYSIS OF VARIANCE FROM *IN VITRO* DRY MATTER DISAPPEARANCE DURING 12 HOUR PERIOD

Source	d.f.	M.S.	F
Replicate	5	4190.28	
Moisture	1	969.60	9.56[2]
Days	3	317.83	3.13[3]
Moist x Days	3	109.31	
Temperature	1	3.69	
Moist x Temp.	1	8.19	
Days x Temp.	3	91.62	
Moist x Days x Temp.	3	60.39	
Rep. x Moist	5	277.18	
Rep. x Days	15	102.53	
Rep. x Moist x Days	15	129.77	
Rep. x Temp.	5	64.02	
Rep. x Moist x Temp.	5	39.15	
Rep. x Days x Temp.	15	102.71	
Rep. x Moist x Days x Temp.	15	45.14	
Tube (Rep. Moist Days Temp.)	576	10.33	
Rep. x Treatment = Error[1]	75	101.40	

[1]Error term used to test treatments.

[2]Significant ($P < .01$).

[3]Significant ($P < .05$).

TABLE XIII

EXPERIMENT II: ANALYSIS OF VARIANCE FROM IN VITRO DRY MATTER DISAPPEARANCE DURING 24 HOUR PERIOD

Source	d.f.	M.S.	F
Replicate	5	6207.97	
Moisture	1	3675.44	68.03[2]
Days	3	76.08	
Moist x Days	3	10.61	
Temperature	1	.14	
Moist x Temp.	1	67.07	
Days x Temp.	3	173.02	
Moist x Days x Temp.	3	.67	
Rep. x Moist	5	197.00	
Rep. x Days	15	37.67	
Rep. x Moist x Days	15	32.38	
Rep. x Temp.	5	22.54	
Rep. x Moist x Temp.	5	21.24	
Rep. x Days x Temp.	15	45.78	
Rep. x Moist x Days x Temp.	15	74.07	
Tube (Rep. Moist Days Temp.)	576	8.16	
Rep. x Treatment = Error[1]	75	54.03	

[1]Error term used to test treatments.

[2]Significant ($P < .01$).

to 13% in milo. In his trials with milo, the first substantial increase in digestion occurred between 22 and 26% moisture at all time and 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.

The analysis of variance (Tables XII and XIII) indicated that the temperatures (4° and 22°C) used during reconstitution of corn in this experiment had no significant effect ($P > .05$) on dry matter disappearance. Contrary to these data, Neuhaus (1967) reported temperature (40°, 75° and 110°F.) had a significant effect ($P < .05$) on in vitro dry matter disappearance of reconstituted milo. Neuhaus found high temperature to be detrimental at lower moisture levels and beneficial at higher moisture levels.

Length of storage (7, 14, 21 and 28 days) during reconstitution had a significant effect ($P < .05$) on in vitro dry matter disappearance during the 12 hour incubation period (Table XII). As illustrated in Figure 1, corn which was reconstituted for a longer time tended, in general, to have a greater in vitro dry matter digestibility. Time did not have a significant effect during the 24 hour incubation period. In agreement with these data, (Neuhaus, 1967; Schneider, 1971) reported all the reconstituted samples tested had a higher dry matter disappearance at 20 days following reconstitution than at 10 days. These data indicate that longer storage time during reconstitution may be conducive to greater starch degradation during storage. The changes which occur in grain during reconstitution may be similar to those which take place in the kernel during germination. Ingle et al., (1964) indicated that in corn, during the first days of germination, the embryo is activated and

holds most of the water taken up by the kernel.

When a longer digestion was allowed a higher percentage of the sample was digested, possibly diminishing treatment differences during the 24 hour incubation period.

Volatile Fatty Acid Production

At the end of the 12 and 24 hour *in vitro* incubation periods the tubes containing the sixteen forms of reconstituted corn were sampled and analyzed for volatile fatty acid productions.

Molar percentages and μmoles per milliliter for acetic, propionic and butyric acids, total concentration of volatile fatty acids, and standard errors are reported in Table XIV. Although there were some significant differences among treatments, there was no consistent trend for acetic:propionic acid ratios with respect to moisture level during reconstitution. However, the level of acetic acid tended to be higher than either propionic or butyric acids. Isovaleric and valeric acids were not included because of the small values obtained and the amount of technique error that therefore could be associated with them.

In Vitro Dry Matter Disappearance

Experiment III

This experiment was designed to determine the influence of several dry processed and reconstituted forms of sorghum grain and wheat on *in vitro* dry matter disappearance.

Mean percent dry matter disappearance and standard errors are illustrated graphically in Figures 3 and 4 for 12 and 24 hour incubation periods, respectively. The analysis of variance is shown in Tables XV

TABLE XIV

VOLATILE FATTY ACIDS FOR SIXTEEN RECONSTITUTED CORN TREATMENTS IN EXPERIMENT II

Treatment			Time[1] (Hr.)	Acetic[2] µmoles/ml	Acetic[2] %	Propionic[2] µmoles/ml	Propionic[2] %	Butyric[2] µmoles/ml	Butyric[2] %	Total VFA[2] µmoles/ml
22% Moisture	4°	7 day	12	49.4	54.6	21.8	24.2^{ab}	13.9	15.3^{c}	90.4^{kl}
			24	67.2	57.6^{g}	24.2	21.0	16.6	14.3^{j}	116.1^{mn}
		14 day	12	51.4	54.8	22.8^{f}	24.6^{ab}	13.5	14.8^{c}	92.9^{kl}
			24	52.6	54.2^{h}	22.3	22.9	14.7	15.3^{ij}	95.9^{n}
		21 day	12	48.3	53.2	22.4	24.6^{ab}	13.5	15.1^{c}	89.6^{kl}
			24	56.9	54.2^{h}	23.3^{f}	22.6	16.3	15.5^{ij}	104.7^{n}
		28 day	12	50.9	57.8	21.9	25.0^{ab}	10.4	12.0^{d}	87.7^{kl}
			24	74.1	55.1^{h}	28.1^{e}	22.3	19.5	14.9^{ij}	131.2^{m}
	22°	7 day	12	47.5	57.6	17.6	21.2^{b}	12.6	15.3^{c}	82.5^{l}
			24	55.5	56.7^{gh}	19.9^{f}	20.9	14.5	14.7^{j}	97.0^{n}
		14 day	12	48.7	52.6	25.2	26.5^{a}	13.5	14.5^{cd}	93.4^{kl}
			24	79.5	56.9^{g}	28.7^{e}	21.7	19.8	14.6^{j}	136.8^{m}
		21 day	12	43.1	51.5	21.9	26.5^{a}	13.0	15.9^{c}	82.9^{l}
			24	72.2	57.1^{g}	25.9^{e}	21.5	18.0	14.5^{j}	124.5^{mn}
		28 day	12	54.9	54.8	25.9	26.0^{a}	13.2	13.5^{d}	99.6^{k}
			24	70.9	55.3	27.2^{e}	21.6	18.9	15.0^{ij}	126.8^{mn}

TABLE XIV (Continued)

Treatment		Time[1] (Hr.)	Acetic[2] µmoles/ml	Acetic[2] %	Propionic[2] µmoles/ml	Propionic[2] %	Butyric[2] µmoles/ml	Butyric[2] %	Total VFA[2] µmoles/ml
32% Moisture	4° 7 day	12	51.6	54.3	24.9	25.4^{ab}	13.5	14.5^{cd}	95.4^{k}
		24	82.4	54.0^{h}	29.5^{e}	21.5	29.5	17.4^{i}	151.3^{m}
	14 day	12	51.8	56.1	22.6	24.7^{ab}	12.6	13.7^{cd}	91.9^{kl}
		24	56.6	55.6^{h}	21.6^{f}	22.7	13.4	14.2^{j}	98.7^{n}
	21 day	12	41.9	49.8	24.2	28.3^{a}	13.1	15.7^{c}	84.5^{l}
		24	56.4	56.6^{gh}	21.4^{f}	21.9	14.3	14.2^{j}	98.9^{n}
	28 day	12	43.6	53.4	20.1	25.2^{ab}	12.2	15.5^{c}	80.5^{l}
		24	59.1	58.4^{g}	19.4^{f}	19.9	14.6	14.6^{j}	99.9^{n}
	22° 7 day	12	44.9	53.3	21.2	24.8^{ab}	13.3	15.8^{c}	84.7^{l}
		24	66.9	57.5^{g}	23.5^{f}	21.3	15.2	13.5^{j}	113.7^{mn}
	14 day	12	43.4	52.5	21.7	25.6^{a}	12.6	15.6^{c}	82.8^{l}
		24	61.1	55.6	22.2	20.7	15.7	15.2^{ij}	106.9^{n}
	21 day	12	49.1	56.2	18.9	22.9^{b}	12.1	14.8^{c}	84.9^{l}
		24	67.9	57.0	23.6	21.5	16.6	14.4^{j}	116.1^{mn}
	28 day	12	50.0	52.4	25.7	26.5^{a}	13.9	14.9^{c}	95.6^{k}
		24	58.9	57.1^{g}	21.5^{f}	21.0	15.2	14.7^{j}	102.7^{n}
Standard (Error) ($S\bar{x}$)		12	4.3	1.8	2.5	1.3	.8	.6	6.7
		24	6.3	.9	2.4	.7	2.3	.6	11.3

[1]Time: In Vitro Incubation Time. [2]Volatile Fatty Acids and Total VFA (ab; cd; ef; gh; ij; kl; mn): Means with different letters as paired differ significantly ($P < .05$) according to Duncan's multiple range test.

TABLE XV

EXPERIMENT III: ANALYSIS OF VARIANCE FOR 12 HOUR DIGESTION OF WHEAT AND MILO

Source	d.f.	M.S.	F
Total	209		
Blocks	2	1657.26	
Treatments	9	1484.79	515.55[1]
Block x Treatment[2]	18	2.88	
Sampling	191	13.68	

[1]Significant ($P < .01$).

[2]Error term used to test treatments.

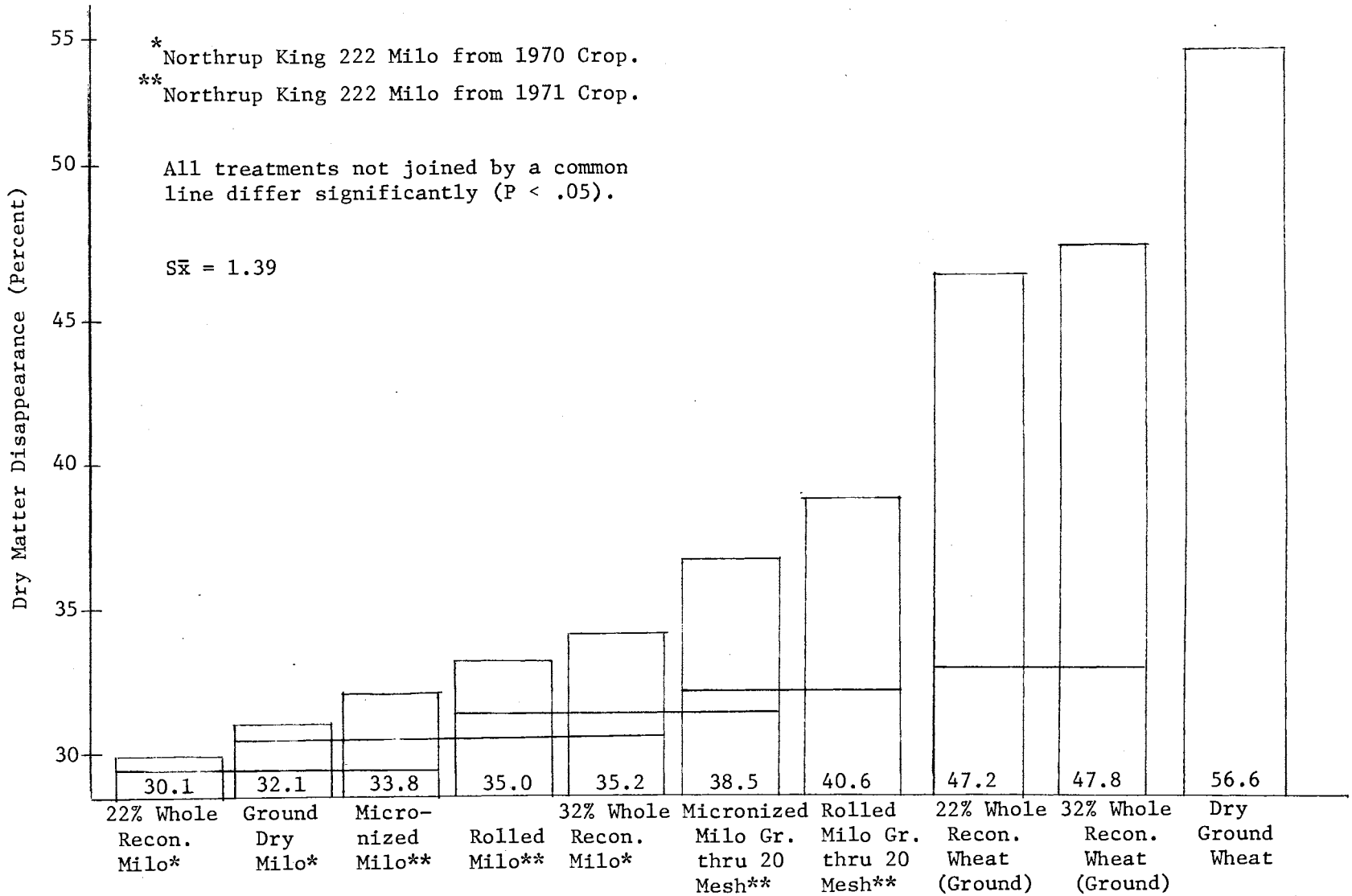

Figure 3. In Vitro Dry Matter Disappearance of Wheat and Milo During 12 Hour Incubation Period

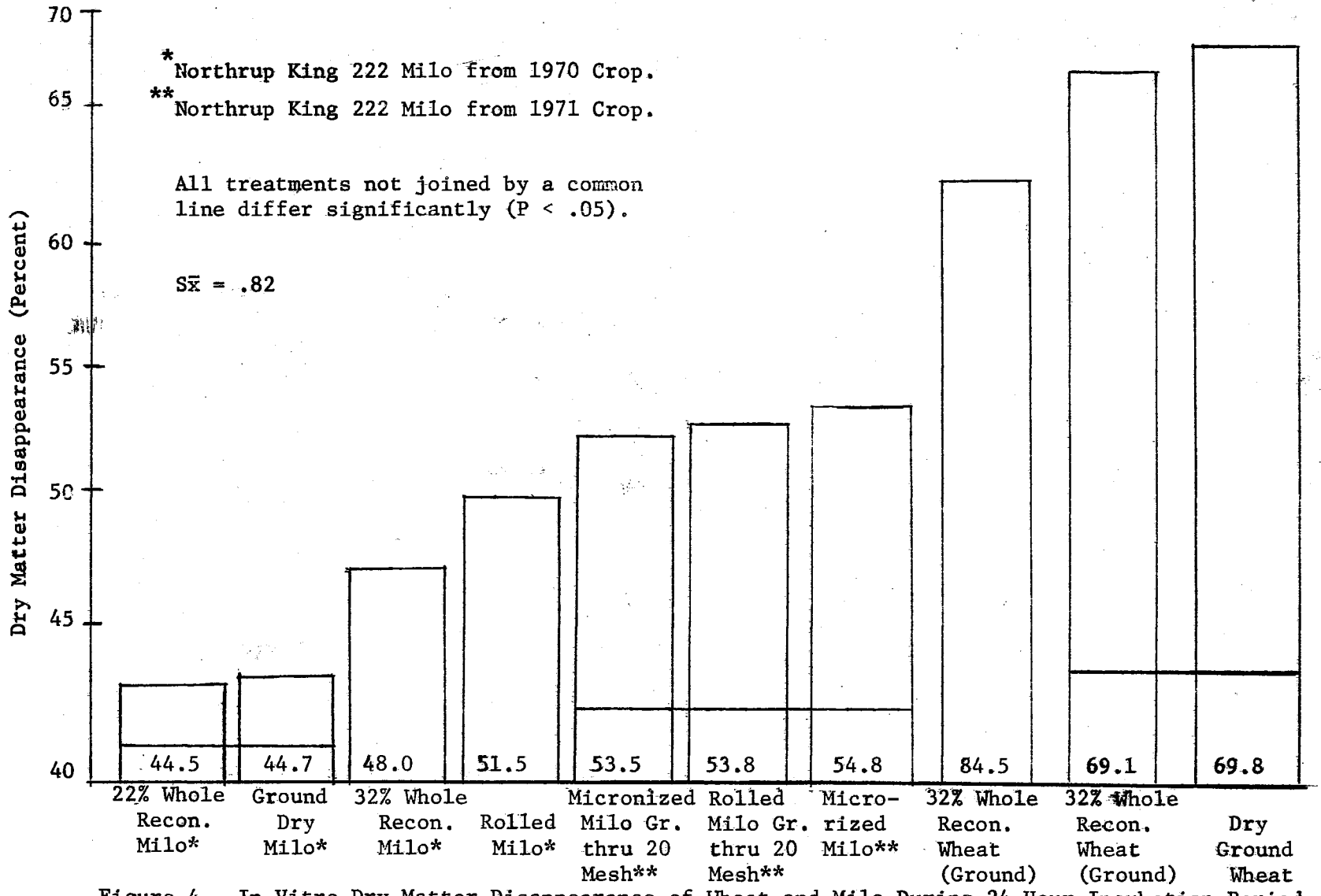

Figure 4. In Vitro Dry Matter Disappearance of Wheat and Milo During 24 Hour Incubation Period

and XVI.

The three wheat treatments used in this *in vitro* comparison were the same ones used in the starch analysis study (Experiment I). The three wheat treatments had higher ($P < .05$) *in vitro* dry matter disappearance than all seven of the milo treatments. These results are in agreement with those reported by Schneider, (1971). The fact that wheat was digested *in vitro* more readily than the milo also indicates that the starch portion of wheat is in a more easily utilized form than in milo.

Dry ground wheat had a higher ($P < .05$) *in vitro* disappearance during the 12 hour incubation period than the 22% and 32% moisture reconstituted wheat treatments. The two reconstituted wheat treatments, however, were not significantly different ($P > .05$). The same general trend existed in the 24 hour incubation period, although the differences between the dry and the reconstituted (22%) wheat treatments were not significant ($P > .05$). The higher disappearance obtained for the dry ground wheat might be the result of greater surface area in the dry ground wheat. Whole reconstituted wheat which is ground or rolled after storage tends to produce a somewhat gummy textured product.

The seven milo treatments were from the same variety (Northrup King 222) and obtained from the same location (Fort Reno Experiment Station). The dry ground and reconstituted (22% and 32%) milo treatments (Figures 3 and 4) were produced from milo collected from the 1970 harvest year, while the two rolled and two micronized milo treatments were produced from milo collected from the 1971 harvest year. The dry ground and reconstituted (22% and 32%) milo treatments were the same grain samples used in the alcohol soluble carbohydrate study (Experiment I). The two

TABLE XVI

EXPERIMENT III: ANALYSIS OF VARIANCE FOR 24 HOUR DIGESTION OF WHEAT AND MILO

Source	d.f.	M.S.	F
Total	209		
Blocks	2	1374.47	
Treatments	9	1839.44	26.29[1]
Block x Treatment[2]	18	69.96	
Sampling	191	4.73	

[1]Significant ($P < .01$).

[2]Error term used to test treatments.

dry rolled and the two micronized milo treatments were prepared from the 1971 crop year since the micronizing equipment was installed prior to this time. Since these were some of the first micronized milo samples produced at Oklahoma State University, the micronized milo treatments were investigated in this *in vitro* dry matter disappearance study together with processing treatments which had been shown to produce treatment differences in other studies. Experiment I had been conducted prior to this time; therefore, there was a confounding of milo treatments by harvest year.

In vitro dry matter disappearance during the 24 hour incubation period (Figure 4) showed dry ground milo and 22% reconstituted whole milo values to be lower ($P < .05$) than all of the other milo treatments. In agreement with work by Schneider (1971), the 32% whole reconstituted milo produced a significantly higher dry matter disappearance ($P < .05$) than either dry ground milo or the 22% whole reconstituted milo treatments. Also Neuhaus (1967) reported milo reconstituted in the whole form had significantly ($P < .01$) improved digestibility compared to dry grain. Results of the determination of alcohol soluble carbohydrate (Experiment I) support these data. Furthermore, a similar trend was noted during the 12 hour incubation period (Figure 3). The rolled and micronized milo treatments were significantly higher ($P < .05$) than the dry ground and reconstituted (22% and 32%) milo treatments during the 24 hour incubation period. This improvement may have been due, however, to differences in the milo as a result of different crop years.

However, valid milo treatment comparisons could be made for the two dry rolled and two micronized milo treatments coming from grain produced in the same harvest year. During the 12 hour incubation period rolled

and micronized milo ground through a 20 mesh screen prior to in vitro incubation produced a higher in vitro dry matter disappearance than the same treatments not preground through a 20 mesh screen before in vitro incubation (Figure 3). Grinding the rolled and micronized milo treatments through a 20 mesh screen likely increased the surface area which probably accounts for the more rapid in vitro digestion of these materials during the 12 hour incubation period. During the 12 hour incubation period, there were no significant differences between the dry rolled and micronized milo treatments when neither sample was preground through a 20 mesh screen. Likewise, there was no significant difference between the dry rolled and the micronized milo treatments when both samples were preground through a 20 mesh screen before in vitro incubation. Thus, pregrinding of the sample through a 20 mesh screen prior to incubation resulted in a significant increase in dry matter disappearance compared to samples not ground through the 20 mesh screen in the 12 hour incubation period. However, micronizing and dry rolling did not yield a significant difference in in vitro dry matter disappearance during the 12 hour digestion period.

During the 24 hour incubation period, however, the micronized milo treatment did produce a significantly ($P < .05$) higher dry matter disappearance than the dry rolled milo when the samples were not preground before incubation. When the samples were preground, no significant treatment difference existed. The dry rolled and micronized milo samples were preground for two treatments in this trial to permit representative sampling of these feeds of large particle size.

Gas Production - Experiment IV

Trial I: Gas Production of Processed Corn

This trial was designed to determine the influence of moisture level (22% and 32%), storage time (7, 14, 21 and 28 days) and storage temperature (4° and 22°C) of reconstituted whole corn on the rate of gas production. The corn treatments are the same ones which were analyzed for *in vitro* dry matter disappearance in Experiment II.

Mean values and standard errors for gas production from reconstituted corn stored 7, 14, 21 and 28 days are summarized in Tables XVII, XVIII, XIX and XX, respectively, as measured by the milliliters of gas produced per hour per gram of sample dry matter. The data were analyzed by three methods: (1) pooling all hours (hour 1 through 6), Appendix Table XXIV, (2) pooling all hours after the first hour (omitting hour 1), Appendix Table XXV, and (3) analyzing for each hour as illustrated in Tables XVII, XVIII, XIX, XX and graphically in Figures 5, 6, 7, and 8. When these data were analyzed by pooling all hours, there was no significant difference in the rate of gas production ($P > .05$) between treatments. This was also true when all hours after the first were pooled and analyzed. The purpose for analyzing these data by the second method, which involved omitting hour 1, was to eliminate the large and unexplained variation which occurred in hour 1. However, when these data were analyzed by hour (third method), a significant ($P < .01$) difference was found between treatments.

Gas productions by hour for the various reconstituted corn treatments (22% - 4°; 22% - 22°; 32% - 4°; 32% - 22°) stored 7 days are illustrated in Figure 5 and in Table XVII. During the first hour, the

TABLE XVII

EXPERIMENT IV: GAS PRODUCTION MEANS FROM RECONSTITUTED CORN STORED 7 DAYS

Moisture Temperature Hours	22%		32%		Control	Pooled Standard Errors
	4°	22°	4°	22°		
1[1]	8.0[b]	12.7[c]	5.6[ab]	3.3[a]	4.5[a]	1.8
2[1]	5.7[ab]	8.2[b]	6.3[ab]	5.8[ab]	4.3[a]	1.6
3	6.7	8.3	7.1	6.7	4.6	1.2
4	6.1	6.5	7.8	7.3	4.6	1.5
5	5.4	6.6	7.5	6.5	4.7	1.1
6[1]	6.0[ab]	6.0[ab]	7.0[ab]	8.4[b]	4.9[a]	1.3

[1]Any two means across the same line without a common superscript letter differ significantly, ($P < .05$).

TABLE XVIII

EXPERIMENT IV: GAS PRODUCTION MEANS FROM RECONSTITUTED CORN STORED 14 DAYS

Moisture Temperature Hours	22%		32%		Control	Pooled Standard Errors
	4°	22°	4°	22°		
1[1]	9.4[b]	8.4[b]	4.9[a]	5.3[ab]	5.0[a]	1.8
2	6.3	6.0	4.2	5.2	4.5	1.6
3	6.5	6.5	4.7	6.5	4.3	1.2
4	6.3	6.4	6.3	8.6	5.8	1.5
5	6.1	6.3	7.1	8.2	6.1	1.1
6	5.1	6.0	5.1	6.9	5.6	1.3

[1]Any two means across the same line without a common superscript letter differ significantly, ($P < .05$).

TABLE XIX

EXPERIMENT IV: GAS PRODUCTION MEANS FROM RECONSTITUTED CORN STORED 21 DAYS

Moisture Temperature Hours	22%		32%		Control	Pooled Standard Errors
	4°	22°	4°	22°		
1	8.5	9.1	6.3	9.6	7.3	1.8
2	5.8	8.5	5.8	7.8	5.2	1.6
3	5.3	6.0	6.4	6.0	4.6	1.2
4[1]	6.0^{ab}	7.8^{ab}	4.9^{a}	9.1^{b}	5.6^{ab}	1.5
5[1]	6.9^{ab}	8.2^{ab}	7.7^{ab}	9.8^{b}	6.5^{a}	1.1
6[1]	5.3^{a}	6.4^{a}	8.0^{ab}	11.5^{b}	5.2^{a}	1.3

[1]Any two means across the same line without a common superscript letter differ significantly, ($P < .05$).

TABLE XX

EXPERIMENT IV: GAS PRODUCTION MEANS FROM RECONSTITUTED CORN STORED 28 DAYS

Moisture Temperature Hours	22%		32%		Control	Pooled Standard Errors
	4°	22°	4°	22°		
1[1]	14.5^{b}	5.8^{a}	6.5^{a}	5.0^{a}	5.0	1.8
2	7.7	5.7	7.0	6.4	4.9	1.6
3[1]	8.3^{b}	5.7^{ab}	7.5^{ab}	6.9^{ab}	4.1^{a}	1.2
4	7.4	5.9	8.4	7.4	5.9	1.5
5	5.1	4.8	5.8	6.2	3.9	1.1
6	5.1	4.5	6.6	7.5	4.2	1.3

[1]Any two means across the same line without a common superscript letter differ significantly, ($P < .05$).

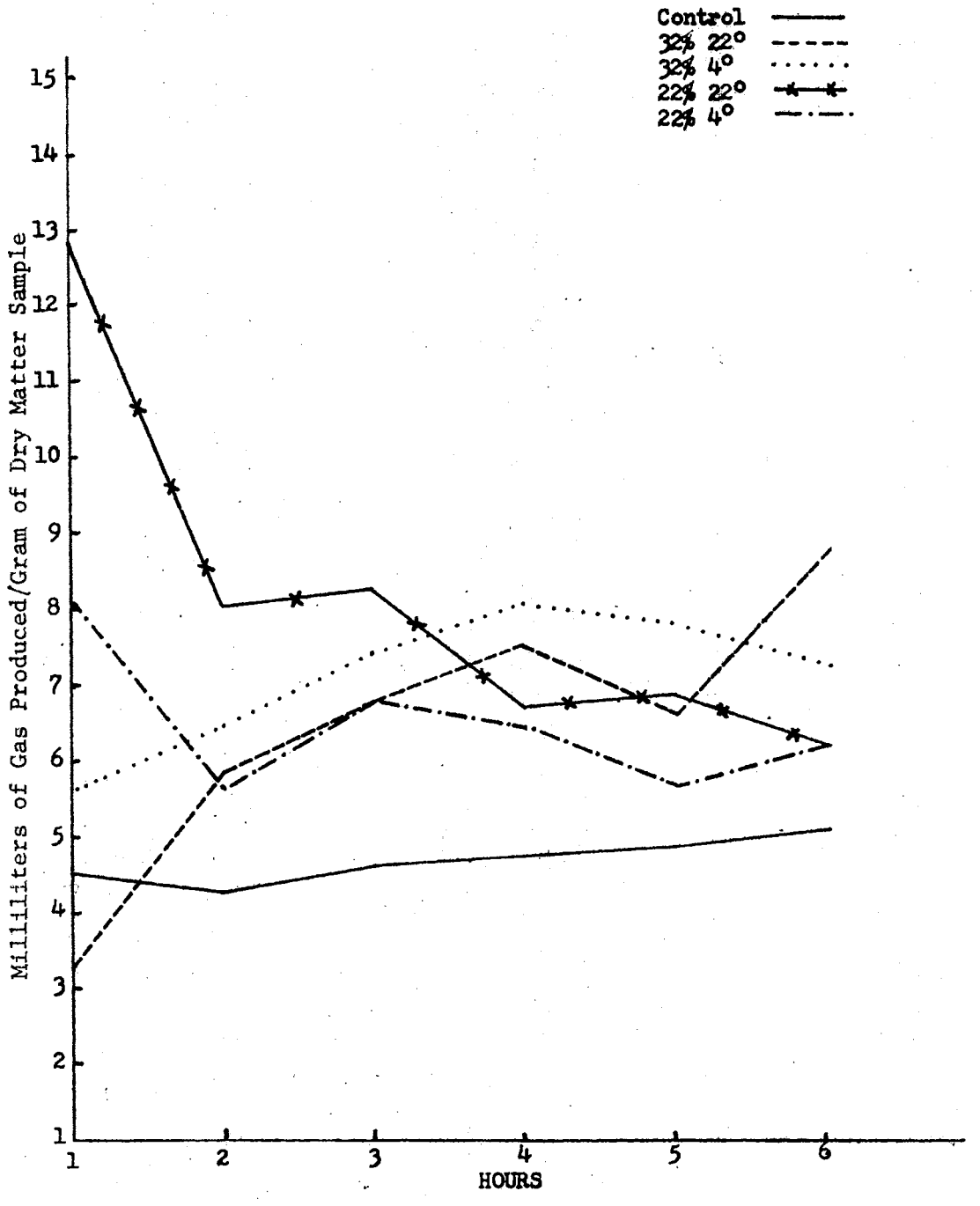

Figure 5. Gas Production From Reconstituted Corn Stored 7 Days

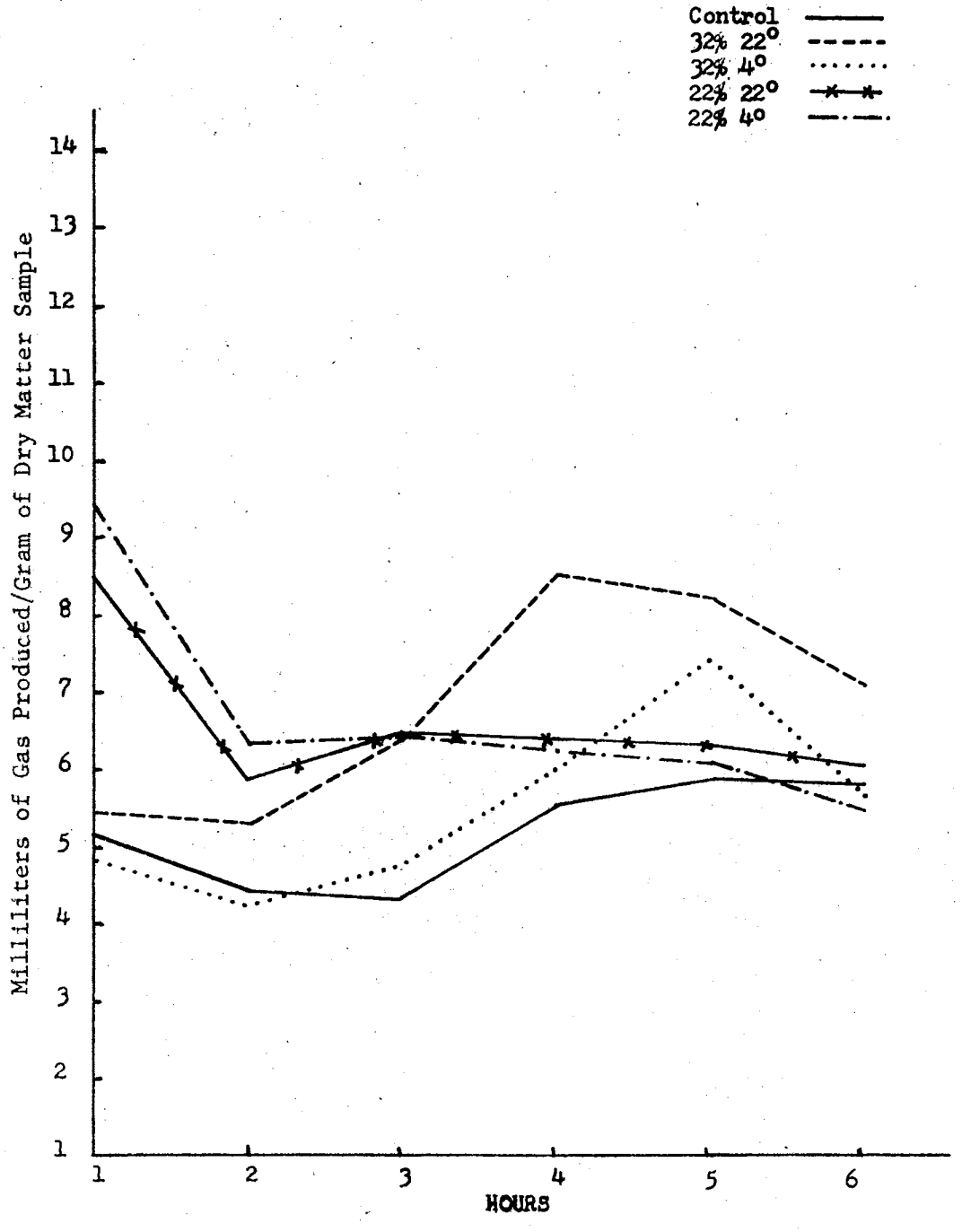

Figure 6. Gas Production From Reconstituted Corn Stored 14 Days

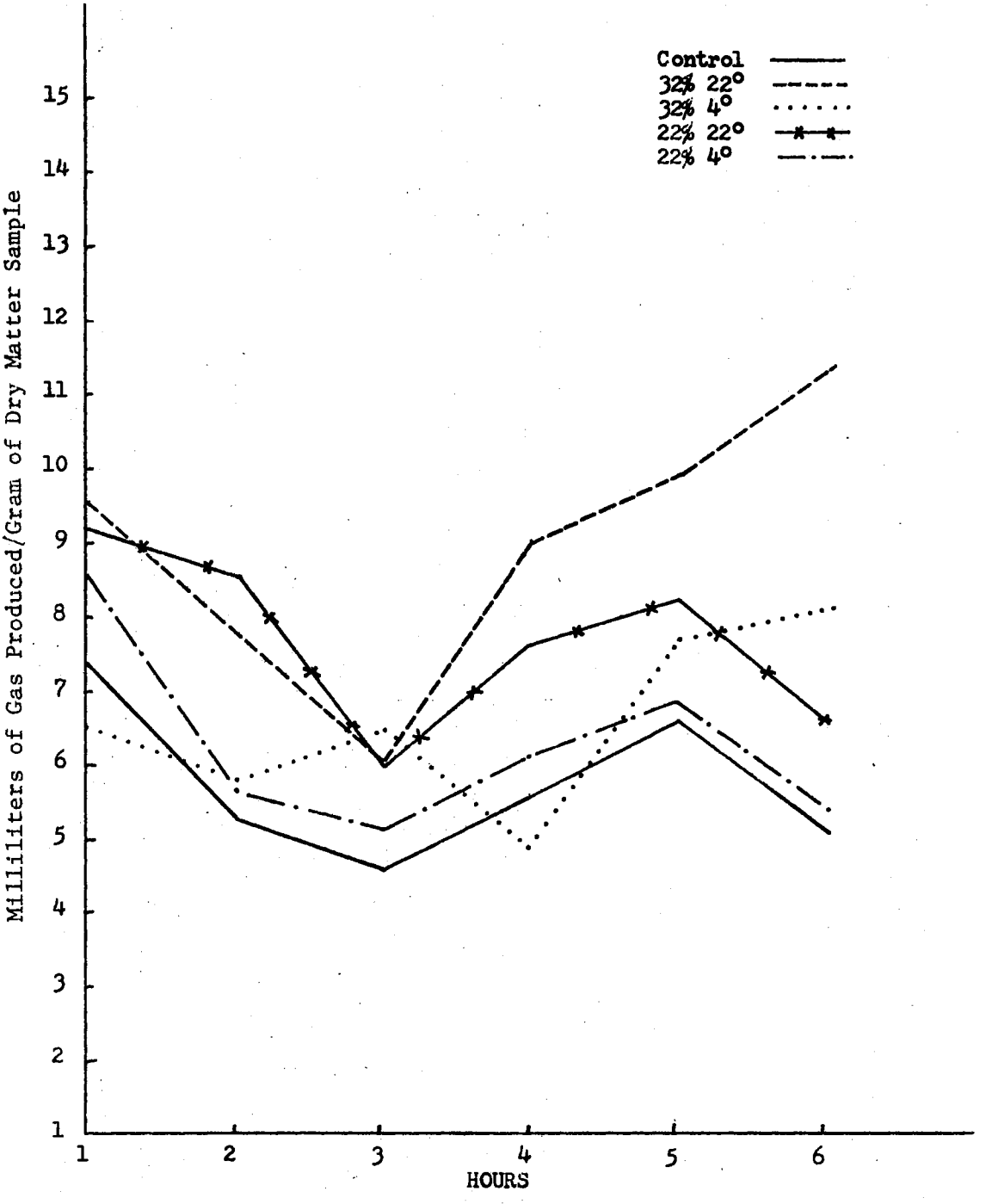

Figure 7. Gas Production From Reconstituted Corn Stored 21 Days

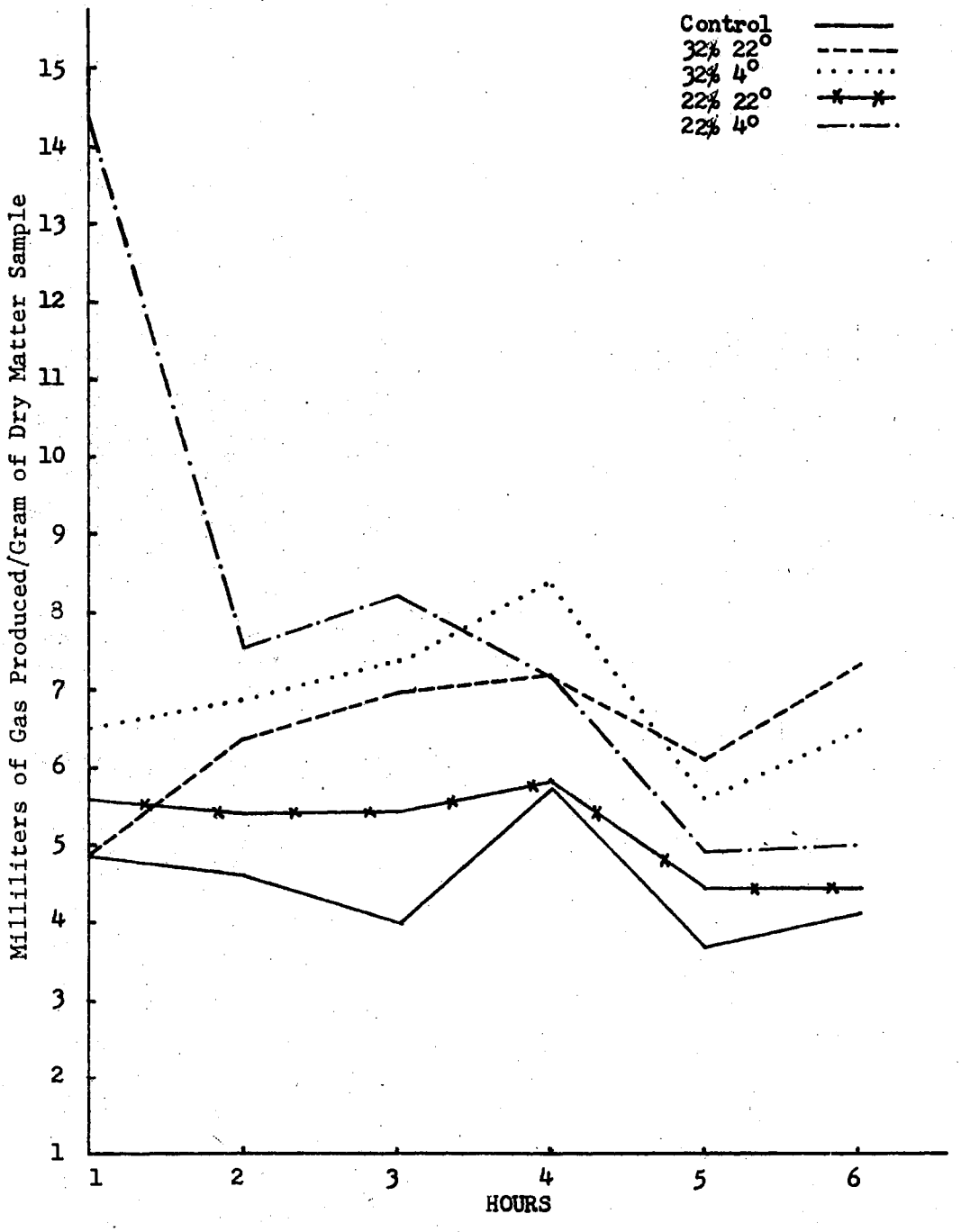

Figure 8. Gas Production From Reconstituted Corn Stored 28 Days

rate of gas production for the 22% - 22° treatment was superior ($P < .05$) to all other 7 day treatments, with the 22% - 4° reconstituted corn showing a significantly higher ($P < .05$) gas production than the dry whole corn and the 32% - 22° reconstituted corn treatments. During hour one no difference existed between dry corn and either the 32% - 4° or 32% - 22° reconstituted corn treatments. At the second hour, the 22% - 22° reconstituted corn showed a significantly higher ($P < .05$) gas production than dry whole corn. However, there was no statistical significant difference ($P > .05$) among any of the four reconstituted corn treatments. Furthermore, during hours 3, 4 and 5 there were no significant differences among any of the treatments ($P > .05$). During hour 6, however, the 32% - 22° reconstituted corn showed a significantly higher ($P < .05$) rate of gas production than dry whole corn. There were no significant differences between the four reconstituted corn treatments, although gas production rates during hour 6 tended to favor the two 32% moisture treatments.

As illustrated in Figure 6 and in Table XVIII, the reconstituted corn stored for 14 days showed 22% - 4° and 22% - 22° reconstituted corn to have a significantly higher ($P < .05$) gas production during the first hour compared to the dry whole corn and the 32% - 4° reconstituted corn treatments. The 32% - 22° reconstituted corn was not significantly ($P > .05$) different from the other treatments during hour 1. There were no significant differences in gas production among any of the treatments during hours 2, 3, 4, 5 and 6 for the corn stored 14 days (Table XVIII).

For reconstituted corn stored 21 days, there were no significant differences in gas production between any of the treatments during hours 1, 2, and 3 (Table XIX). However, during the fourth hour of gas produc-

tion the 32% - 22° reconstituted corn treatment had a higher ($P < .05$) value compared to the 32% - 4° reconstituted corn. The other treatments at the fourth hour of gas production showed no significant difference ($P > .05$). During the fifth hour of gas production, only the 32% - 22° reconstituted corn showed a significantly higher ($P < .05$) value when compared to that of the dry whole corn. During the sixth hour of gas production, the 32% - 22° reconstituted corn showed a superior ($P < .05$) value when compared to all treatments, with the exception of the 32%-4° reconstituted corn which was not significantly different ($P > .05$) from any of the treatments.

For reconstituted corn stored 28 days (Table XX) no significant differences ($P > .05$) were found between any of the treatments for hours 2, 4, 5 and 6. However, during hour 1 the 22% - 4° reconstituted corn produced a higher ($P < .05$) quantity of gas than all other treatments. Also, the 22% - 4° reconstituted corn was superior ($P < .05$) to the dry whole corn during hour 3.

Even though treatments showed no significant F value ($P > .05$) for gas production when hours 1 through 6 were pooled, there appeared to be some trends. In general, the 22% reconstituted corn treatments produced greater gas production during the first hour with lower levels per hour thereafter. The reverse was generally true for the 32% reconstituted corn treatments. As noted in Figures 5, 6, 7 and 8, the 32% - 22° reconstituted corn treatment produced a greater quantity of gas per hour during the sixth (final) hour than any other treatment for all four storage times (7, 14, 21 and 28 days). In the <u>in vitro</u> dry matter disappearance study of these same treatments (Experiment II), moisture during reconstitution produced a significant effect ($P < .05$) on dry

matter disappearance, with the 32% moisture treatments yielding the highest dry matter disappearance. Moreover, as depicted in Figures 5, 6, 7 and 8, all four reconstituted corn treatments (22% - 4°, 22% - 22°, 32% - 22°), with few exceptions, produced more gas during each hour (1, 2, 3, 4, 5 and 6) than did dry corn.

Trial II: Gas Production of Wheat and Milo

This trial was designed to determine the influence of several dry processed and reconstituted forms of milo and wheat on *in vitro* gas production. The milo and wheat treatments were the same as those used in *in vitro* experiment III except that the dry rolled and micronized milo treatments which were preground through a 20 mesh screen before *in vitro* incubation were deleted in this gas production study.

Mean values of gas produced per hour per gram of dry matter for various milo and wheat treatments are shown in Table XXII. The analysis of variance (Table XXI) shows treatments and hours to be highly significant ($P < .01$).

The micronized and dry rolled milo treatments produced more gas per hour for hours 1 through 6 than all other wheat and milo treatments, with the differences being significantly higher ($P < .05$) for these two treatments during hour 1, 2 and 3. In general, the micronized milo treatment showed a trend for a slightly greater total gas production for each hour than the dry rolled milo, with the micronized milo treatment producing a significantly higher ($P < .05$) quantity of gas than dry rolled milo during the first two hours. Likewise, in *in vitro* Experiment III, the micronized milo treatment produced a significantly higher ($P < .05$) 24 hour dry matter digestibility than the dry rolled milo

treatment. Since the micronized milo produced gas more readily than the dry rolled milo, possibly the starch portion of micronized milo was in a more utilizable form than in the dry rolled milo. The milo treatments were all of the same variety (Northrup King 222) and obtained from the same location; however, as stated previously (Page 46), the dry ground and reconstituted (22% and 32%) milo treatments were produced from milo collected from the 1970 harvest year, while the dry rolled and the micronized milo treatments were collected from the 1971 harvest. Some differences might be expected due to the year in which the milo was harvested.

In comparing the dry ground, 32% moisture whole reconstituted and 22% moisture whole reconstituted milo treatments from the 1970 harvest year, there were no significant treatment differences in gas production, ($P > .05$). Nevertheless, as noted in Table XXII, both the 22% and 32% reconstituted milo treatments produced more gas during each of the six hours, with only one exception, than did the dry ground milo. The 32% reconstituted milo treatment showed a general trend for a larger hourly gas production during hours 5 and 6 compared to hours 1 and 2, with the reverse trend appearing to exist for the 22% reconstituted milo treatment. The same general observation was true for gas production for reconstituted corn treatments in Experiment IV - Trial I.

The three wheat treatments (dry ground, 32% whole reconstituted and 22% whole reconstituted) showed no significant difference in gas production. Although not significant, as was true for the milo treatments, both of the reconstituted wheat treatments produced more gas during each hour, one through six, than did the dry ground wheat treatments. Similar observations were apparent for reconstituted corn treatments compared

to dry corn in Experiment IV - Trial II.

TABLE XXI

EXPERIMENT IV: ANALYSIS OF VARIANCE FROM IN VITRO GAS PRODUCTION OF WHEAT AND MILO

Source	d.f.	M.S.	F
Total	191	28.17	
Replicate	3	90.99	
Treatment	7	360.73	178.09[2]
Hours	5	70.64	34.87[2]
Rep. x Treatment	21	145.28	
Rep. x Hours	15	8.14	
Treatment x Hours	35	46.87	
Rep. x Treatment x Hours[1]	105	2.03	

[1]Error term used to test treatments.

[2]Significant ($P < .01$).

TABLE XXII

EXPERIMENT IV: MILLILITERS OF GAS PRODUCED PER HOUR PER GRAM OF DRY SAMPLE FOR MILO AND WHEAT TREATMENTS (TRIAL II)[3]

Hours[2]	Dry Ground Wheat	32% Whole Recon. Wheat	22% Whole Recon. Wheat	Dry Ground Milo	32% Whole Recon. Milo	22% Whole Recon. Milo	Micro-nized Milo	Rolled Milo	Pooled Standard Errors
1[3]	3.3[a]	5.0[a]	5.5[a]	3.0[a]	3.0[a]	5.2[a]	28.6[cd]	12.0[b]	1.7
2[3]	3.2[a]	6.2[a]	5.7[a]	2.5[a]	3.6[a]	5.1[a]	23.0[cd]	16.9[b]	1.1
3[3]	3.4[a]	6.0[a]	5.7[a]	3.0[a]	4.4[a]	5.0[a]	10.5[b]	9.3[b]	1.0
4	3.3	5.7	4.9	2.7	4.9	4.5	7.9	7.4	1.4
5	3.6	5.3	4.3	3.8	5.5	4.6	8.2	7.4	1.3
6	3.2	5.3	3.4	3.5	5.5	4.1	7.5	7.2	1.2

[1]Volumes are means of eight samples.

[2]Hours allowed to Digest.

[3](abc) Means on the same line without a common superscript letter differ significantly, ($P < .05$). (d) means on the same line without a common superscript letter differ significantly, ($P < .01$).

CHAPTER V

SUMMARY

An alcohol soluble carbohydrate experiment (Experiment I), two *in vitro* dry matter disappearance studies (Experiment II and III), and a gas production study (Experiment IV; Trial I and II) were conducted to determine the effects of various processing techniques on corn, milo and wheat.

Alcohol soluble carbohydrate determinations (expressed as percent reducing sugar) were made on dry and reconstituted (ground and whole) corn, wheat and milo. Method of grain preparation produced a significant effect ($P < 01$) on the level of reducing sugar. Corn which was reconstituted in the whole form at 32% moisture had a significantly ($P < .05$) higher level of reducing sugar, as measured by both ethanol and isopropanol extractions, than all other grain treatments. Reconstituting corn in either the ground form or at a low moisture level (22%) produced either no increase or only a small increase in percent reducing sugar over dry corn.

Milo which was reconstituted in the whole form at 32% moisture contained the highest level of reducing sugar of all milo treatments and was significantly higher ($P < .05$) than all other reconstituted milo treatments.

All forms of reconstituted wheat other than ground reconstituted wheat (22%) contained a significantly higher ethanol soluble carbohy-

drate content ($P < .05$) than dry ground wheat. The reconstituted ground wheat (32%) treatment showed a higher ($P < .05$) isopropanol soluble carbohydrate level, with no difference among any of the other treatments. In general, method of processing had less effect on wheat than on corn and milo.

Experiment II was conducted to determine the effects of moisture level (22% and 32%), storage temperature (4° and 22°C) and storage time (7, 14, 21 and 28 days) on reconstituted whole corn as measured by *in vitro* dry matter disappearance. The moisture content of reconstituted corn during storage had a significant effect ($P < .01$) on *in vitro* dry matter disappearance during both the 12 and 24 hour incubation periods. Corn reconstituted at 32% moisture, in general, showed a trend for a higher *in vitro* digestibility than corn reconstituted at 22% moisture. Length of storage during reconstitution had a significant effect ($P < .05$) on *in vitro* dry matter disappearance during the 12 hour incubation period, with longer storage times, in general, producing higher *in vitro* dry matter digestibility. During the 24 hour incubation period, however, storage time of reconstituted corn had no significant effect on dry matter digestibility. Storage temperature produced no significant effect ($P > .05$) *in vitro* dry matter disappearance.

Experiment III was designed to determine the influence of several dry processed and reconstituted forms of sorghum grain and wheat on *in vitro* dry matter disappearance. All wheat treatments (dry ground, 22% and 32% reconstituted) had higher ($P < .05$) *in vitro* dry matter digestibilities than the seven milo treatments. Dry ground wheat showed a higher ($P < .05$) *in vitro* digestibility during the 12 hour incubation period than the 22% and 32% moisture reconstituted wheat treatments.

The two reconstituted wheat treatments, however, were not different ($P > .05$). The 32% whole reconstituted milo produced a significantly higher ($P < .05$) digestibility than the 22% reconstituted milo during the 12 hour incubation period and, furthermore, produced a higher digestibility ($P < .05$) than either dry ground milo or the 22% whole reconstituted milo treatments during the 24 hour incubation period. Moreover, during the 24 hour incubation period, the micronized milo treatment produced a significantly ($P < .05$) higher digestibility than dry rolled milo when the processed milo samples were not preground through a 20 mesh screen before incubation.

No significant ($P > .05$) difference existed among reconstituted corn treatments (same treatments as in Experiment II) for gas production when hours 1 through 6 were pooled, in Trial I of Experiment IV. However, when the data were analyzed by hour, a significant ($P < .01$) difference was found between treatments. In general, the 22% reconstituted corn treatments produced greater gas production during the first hour with lower levels per hour thereafter. The reverse was generally true for the 32% reconstituted corn treatments.

In Trial II of Experiment IV, micronized and dry rolled milo treatments produced more gas per hour for hours 1 through 6 than all other wheat and milo treatments, with the differences being significantly higher ($P < .05$) for these two treatments during hour 1, 2, and 3. Moreover, during the first two hours, micronized milo produced a significant greater ($P < .05$) quantity of gas than dry rolled milo. Although the differences were not significant ($P > .05$) reconstituted whole milo (22% and 32%) and reconstituted whole wheat (22% and 32%) treatments showed a trend for greater gas production during hours 1 through 6 than

did the dry ground milo and dry ground wheat treatments. No explanation is known at the present time why wheat and milo responsed differently.

CHAPTER VI

GENERAL DISCUSSION

The most promising method of improving starch availability and the utilization of the energy of cereal grains, and thereby feed efficiency, is in the area of grain processing. Simple processing has always been practiced to facilitate grain digestion from methods of processing of the grain to crack its structural carbohydrate coat and expose the starchy endosperm. This study was initiated to compare the effects of several different methods of milo, wheat and corn preparation. Three laboratory analyses were performed on each of the three grains in an attempt to compare one grain to another. These three procedures were *in vitro* dry matter disappearance, soluble starch analysis and gas production, when incubated by either pure amylolytic enzymes or by rumen microbes.

Results obtained from the alcohol soluble carbohydrate experiment by measuring only a small soluble fraction of the total starch of grains showed that reconstitution increased solubility of starch in corn and milo compared to dry forms, but produced no change in wheat. Reconstitution does not result in starch gelatinization. However, if an aqueous suspension of starch is heated the granules do not change in appearance until a certain critical temperature is reached. At this temperature some of the granules undergo rapid swelling and lose their crystalline structure. Different grain starches exhibit large differences in re-

ducing sugars, due perhaps, first, to the great differences in the relative content of amyloses and amylopectins among starches and therefore, to the relative number of α-1,4- and α-1,6-linkages and, second, to the degree of alteration undergone by the starch during preparation from the natural source. For best results with reconstituted grain, this experiment suggests that grains, in general, should be reconstituted whole, stored under oxygen-limited conditions and then ground rather than being ground prior to reconstitution.

The results of Experiment II and Experiment IV, Trial I indicate that it would be nutritionally feasible to reconstitute corn when fed to ruminants. Results of moisture level (22% and 32%), storage temperature (4° and $22^{\circ}C$) and storage time (7, 14, 21 and 28 days) during reconstitution of corn indicated an increased dry matter disappearance and a faster rate of gas production with reconstituted corn. In this situation it is very possible that the constitution of the protein matrix could have a strong effect on the digestibility of the starch. This could in part explain the increased starch digestibility found by a number of workers using reconstituted grains. During the process of reconstitution perhaps, loosening the starch granule in its protein matrix occurs. Therefore, it is a matter of economics as to whether reconstituted grains can compete as an energy source in ruminant rations.

The results of Experiment III (in vitro dry matter disappearance) and Experiment IV, Trial II (gas production) indicates a inconsistent response with milo and wheat. Wheat responsed better than milo in the in vitro dry matter disappearance study while milo gave the best response in the gas production study. Know explanation is known at the present

time why wheat and milo responded differently in the two experiments. However, in the gas production study, micronized and dry rolled milo produced more CO_2 than wheat. In micronization it would seem that the starch granules are expanded rapidly by the vaporization of internal moisture and then are fixed in the expanded form by drying.

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A P P E N D I X

TABLE XXIII

DETERMINATION OF SOLUBLE CARBOHYDRATES

(1) Transfer a sample containing not more than 800 mg. soluble sugars (500 - 5000 mg. sample) to a dry 100 ml. volumetric flask. Add 1 g. NaCl and either 20 ml. absolute ethanol or 40 ml. isopropanol. Let stand for 10 minutes mixing frequently.

(2) Add sufficient water to bring the volume to about 90 ml. Keep at 20° for 60 minutes, mixing frequently by rotation. Add water to the mark, mix, adjust to the mark again and mix.

(3) Add 200 mg. Celite; continue to extract for 30 minutes with frequent mixing at 20°C.

(4) Filter through Whatman No. 54 filter paper. Cover the funnel to prevent evaporation of alcohol.

(5) Prepare several reagent blanks by same procedure.

(6) Transfer 50 ml. of filtrate (at 20°) to a 250 ml. volumetric flask. Make a mark with a grease pencil at 50 ml. line.

(7) Add small amount of talcum, 1-2 drops Octanol and 50-60 ml. water. Remove ethanol by boiling on a hot plate until volume is 40-45 ml. Cool. Watch flask during boiling, adding 1-2 drops Octanol when foaming is noted.

(8) Add 5 ml. 0.4 M acetate buffer and 5 ml. enzyme preparation. Incubate 6 hours in a 50° C. water bath.

(9) Add 10 ml. $ZnSO_4$ solution and 2-3 drops phenophthalein indica-

tor. While rotating the flask, rapidly add 0.5 N NaOH until precipitation of $Zn(OH)_2$ begins. Thereafter carefully add the alkali until the contents are fairly pink.

(10) Wash down the sides of the flask and add 0.5 N H_2SO_4 drop-by-drop until the solution is colorless. Dilute to the mark, let stand 10 minutes, mixing frequently, and filter through Whatman No. 54. Filtrates may be stored in the refrigerator at this stage but preferably no more than 24 hours.

(11) Transfer exactly 2, 3, 4, and 5 ml. of the samples and blanks to the bottom of 29 x 200 mm text tubes. Best results are obtained when the tube contains 3-3.5 mg. glucose. Cover the tubes with glass marbles or small beakers. Add water to bring volume to 5 ml.

(12) Add exactly 5 ml. 0.04 M FeCy reagent, mix immediately by rotation and incubate exactly 30 minutes at 80°C.

(13) Cool rapidly in running water to 20-25°.

(14) Prepare 5 ml. water blanks with each analysis.

(15) Remove the cover. Add 1 ml. KI solution and 5 ml. $ZnSO_4$ acetic acid reagent, mixing gently after each addition. Cover the tubes immediately after last addition to prevent loss of I_2. Let stand at least 20 minutes with occasional mixing.

(16) Titrate with 0.01 N thiosulfate until almost colorless. Add first few ml. around sides of tube to prevent loss of I_2. Add 0.5 ml. starch indicator, wash down walls with stream of water and titrate drop-by-drop until the color is pure white.

(17) The reducing sugar titration procedure can be standardized using 2 to 4 mg. glucose in the 5 ml. volume.

Calculation: Percent reducing sugar expressed as glucose can be

calculated by:

$$\%R_s = aT \left[\frac{200V}{vW}\right]$$

where

T = ml. difference between thiosulfate titration of blank and sample solution.

a = mg. glucose equivalent per ml. thiosulfate.

$$\text{i.e. } \left(\frac{\text{mg standard glucose}}{\text{T (standard)}}\right)$$

V = ml. final volume of digest clarified with $Zn(OH)_2$ (250 ml. in this case).

v = ml. aliquot of filtrate taken for analysis.

W = mg. weight of sample.

The factor 200 comes from 2 (only 50 ml. first filtrate used) and 100 to convert to percent.

TABLE XXIV

EXPERIMENT IV: TREATMENT MEANS FROM GAS PRODUCTIONS WHEN POOLING ALL HOURS (HOUR 1 THROUGH 6)

(%) Moisture	Days	(°C) Temperatures	Means[1]
22%	07	4°C	6.34
22	07	22	8.08
22	14	4	6.63
22	14	22	6.61
22	21	4	6.33
22	21	22	7.64
22	28	4	7.83
22	28	22	5.13
32	07	4	6.93
32	07	22	6.36
32	14	4	5.41
32	14	22	6.86
32	21	4	6.48
32	21	22	8.95
32	28	4	6.86
32	28	22	6.43

[1]Treatment means per hour for six hours which did not differ significantly at ($P > .05$).

TABLE XXV

EXPERIMENT IV: TREATMENT MEANS FROM GAS PRODUCTIONS WHEN POOLING ALL HOURS AFTER THE FIRST HOUR (OMITTING HOUR 1)

(%) Moisture	Days	(°C) Temperatures	Means[1]
22%	07	4°C	5.99
22	07	22	7.15
22	14	4	6.08
22	14	22	6.24
22	21	4	5.86
22	21	22	7.35
22	28	4	6.54
22	28	22	5.02
32	07	4	7.19
32	07	22	6.97
32	14	4	5.51
32	14	22	7.15
32	21	4	6.49
32	21	22	8.84
32	28	4	6.93
32	28	22	6.72

[1]Treatment means per hour for six hours which did not differ significantly at ($P > .05$).

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