INFLUENCE OF GRAIN PROCESSING FACTORS ON THE IN VITRO FERMENTATION RATE BY A MIXED SUSPENSION OF RUMEN MICROORGANISMS

by

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A Dissertation Submitted to the Committee on

AGRICULTURAL BIOCHEMISTRY AND NUTRITION

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1966

THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by John Earl Trei

entitled Influence Of Grain Processing Factors On The In Vitro Fermentation Rate By A Mixed Suspension Of Rumen Microorganisms.

be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

William H. Hale — May 27, 1966
Dissertation Director — Date

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SIGNED: John E. Frei

ACKNOWLEDGMENTS

The author wishes to express deep appreciation and gratitude to his major professor, Dr. W. H. Hale, for his perpetual guidance and assistance during the course of this study and the preparation of this dissertation.

Grateful acknowledgment is also extended to Drs. Brent Theurer, Bruce Taylor and A. R. Kemmerer for suggestions in the preparation of this dissertation.

Sincere appreciation is also due Dr. Brent Theurer, Mr. Hassan Osman, Mr. Wayne Figroid, Mr. Stephen Mehen and Mr. Tom Husted for supplying the data for the additional *in vitro* and *in vivo* observations presented.

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ABSTRACT

The effect of steam processing, pressure cooking and flaking of grains on *in vitro* gas production rate was evaluated with a mixed suspension of rumen microorganisms for a three hour incubation period. Values recorded were expressed as ml. gas produced per gram of dry matter incubated, which were highly correlated ($r = .95$) with *in vitro* dry matter disappearance.

Preliminary data was collected on the development of the experimental technique. All samples were ground through a 20 mesh screen prior to incubation. *In vitro* gas production varied with species and varieties of untreated grain. High amylose corn fermented at significantly slower rate than regular corn (18.0 ml. vs. 40.3 ml.). Gas production from milo and barley preparations was 65 ml. and 79 ml., respectively, which parallels data from *in vivo* digestion trials for these two grains.

Steam processing without flaking milo decreased gas production when compared to the untreated grain (59.1 ml. vs. 43.8 ml.). Steam processing and flaking milo and barley significantly increased gas production over the respective untreated grains (57.2 ml. and 76.0 ml. vs. 84.1 ml. and 104.4 ml.). Increasing flake flatness with milo resulted in increased gas production (untreated 62.6 ml., poor flake 54.1 ml., good flake 75.6 ml. and excellent flake 81.4 ml.). The same

trends were observed with similarly flaked barley (41.8 ml, 52.6 ml, 66.7 ml and 79.2 ml). However, with milo there appeared to be a practical limit regarding increasing gas production by increasing flake flatness.

Pressure cooked milo not flaked (4.2 kg./cm.^{2} for 1 min.) was not different from the untreated grain. Gas production of steam processed flaked and pressure cooked flaked milo was significantly increased over untreated grain (93.7 ml and 96.8 ml vs. 72.2 ml). A similar effect was noted with steam processed flaked and pressure cooked (4.2 kg./cm.^{2} for 1 min.) flaked barley (104.6 ml and 99.1 ml vs. 67.2 ml) indicating that the desirable effect on the grains was the same by both processes.

Cooking with increasing increments of pressure of whole milo without flaking increased gas production (untreated 30.7 ml, 1.4 kg./cm.^{2} 27.1 ml, 2.8 kg./cm.^{2} 29.9 ml, 4.2 kg./cm.^{2} 38.1 ml, 5.6 kg./cm.^{2} 44.8 ml). However additional increases in the gas production rate were obtained by flaking the 5.6 kg./cm.^{2} grain (62.3). A minimum of pressure with a moist heat treatment (steam processing) and the formation of a good flake are as beneficial as pressure cooking at 4.2 kg./cm.^{2} and then flaking.

Wheat and high amylose or regular corn responded similarly to pressure cooking at 2.8 - 4.2 kg./cm.^{2} and flaking. Autoclaving ground milo without subsequent flaking reduced gas production rates compared to untreated grain (42.7 ml vs. 59.6 ml).

Various expanded starch milo preparations (popped or boiled) significantly increased gas production (79.9 ml. vs. 20.8 ml. for untreated). All results indicate that the grain must be satisfactorily flaked after the whole grain has been moist heat treated to obtain optimum utilization. This screening technique apparently offers a good indication of rate of rumen fermentation and a rapid evaluation of the effect of processing on grains.

INTRODUCTION

Due to the development of large scale commercial cattle fattening operations, the demand for cattle finished at lighter weights, mechanization of feed processing and handling, and relative costs of nutrients in roughage and concentrates there has been a distinct trend toward high concentrate rations. If cattle feeding based on high concentrate rations is to continue, improved efficiency must be obtained in view of world food shortages. Even in the United States there is a growing concern over the competition for grains between cattle and monogastric animals.

One method of improving efficiency in the feedlot on high concentrate rations is by increasing the digestibility or utilization of the starch fraction of the ration, since quantitatively it is the most important fraction of grains. The processing of grain to render the starch more available to the ruminant appears promising for improving the value of high concentrate rations. Any improvement in the utilization of the ration due to grain processing will be reflected in overall ration efficiency since the grain portion comprises the major part of the feed on high concentrate rations.

Recent investigations with the moist heat treatment of grains have resulted in improved feedlot performance of fattening steers at the Arizona Experiment Station. It is believed that some form of moist heat

treatment along with proper rolling renders the starch fraction of the grain more available to the rumen microorganisms and/or the animal.

It has been reported that the increases in digestibility of the nitrogen free extract fraction of moist heat treated grain probably accounts for the improved performance in the feedlot. Since a large portion of the starch on a high concentrate ration disappears in the rumen, rumen digestion appeared to be one area to investigate factors which may increase the rate of starch utilization by the animal. It is realized that the rumen is an inefficient site in the gastro-intestinal tract for increasing the quantity of carbohydrate digested due to energy lost in the form of carbon dioxide and methane gas. However with the increases in gas production, there are associated increases in total volatile fatty acids from which are derived the major proportion of the ruminants energy.

There are currently no adequate in vitro (screening) techniques for quantitatively estimating rumen starch digestion by steers. Consequently the need arose for an expedient technique for evaluating the influence of various grain processing factors for predicting their value to the ruminant animal.

This research was initiated to establish the validity of the in vitro gas production technique with a mixed suspension of rumen microorganisms in predicting the digestion and utilization of grains as influenced by several processing methods.

LITERATURE REVIEW

Much empirical and scientific information is available on the relative feeding values of various grains. However, during the past 20 years new varieties of grain have been developed, high rates of fertilization used, more irrigated grain produced, and new harvesting and storing methods have been introduced. The effect of these factors on the feeding value of grains is yet undetermined. An example to illustrate the problem mentioned is that textbooks on feedstuffs list the feeding value of milo as being greater than that of barley when fed to beef cattle (51). However with grains produced in the Southwest, cattle feeders observed that performance on and feed efficiency of high milo rations were inferior to barley. Later experimental trials and feeder observations showed that milo and barley have different feeding values for the fattening steer with barley being the more useful grain (24, 12). These differences in grain values became even more evident when maximum production was attempted. Among the possibilities that may explain this difference are lowered protein utilization and a more slowly or less digestible nitrogen free extract of milo. Saba et al (60) reported the lowered digestibility of the milo nitrogen free extract was apparently due to the lower digestibility of the milo starch. Cadena (12) with in vivo

nylon bag studies showed a significantly greater disappearance of dry matter during the first eight hours with barley than with milo.

Hale and Taylor (25) using a defined condition of steaming and rolling of milo and barley reported the treated barley increased gains and feed efficiency by 10 and 11 percent respectively over dry rolled milo. Also steam processing milo resulted in improved gain and reduced feed requirement when compared to dry rolled milo.

Saba (59) found that cooking milo for an average of 9 hours at 180°F with 2 parts water to 1 part milo added to palatability and therefore increased intake resulting in greater average daily gain than with dry rolled milo or dry rolled barley. Furthermore, it appeared that cooking aided in breakdown and availability of the milo starch as shown by a significant increase in nitrogen free extract digestibility of cooked grain. However, cooking apparently denatured the protein of milo which resulted in a significant decrease from 41.8 to 33.5 percent in crude protein digestion.

With studies on improvement of barley for chicks by water treatment, Thomas et al (72) concluded that microbial fermentation played a role and that enzymes or other factors produced by the microorganisms brought about the nutritional improvement of barley.

Newland et al (57) reported processing corn by steam cooking finely ground grain at 250°F for thirty minutes and rolling decreased palatability with both steers and lambs. Although daily gains on processed

corn were no different from ground shelled corn, he reported a significantly narrowed ruminal acetate to propionate ratio and an increase in feed efficiency when heat processed corn was fed to either species.

Jordan (41) reported similar effects on weight gain and grain consumption with lambs fed steam cooked corn at 180°F for 5 minutes, extruded through a wenger expansion system and flaked at the extrusion die. However, from the slight increase in efficiency of feed utilization with heat processed flaked corn fed in conjunction with hay, he conduded efficiency was not of sufficient magnitude to compensate for the increased costs in ration preparation.

Garrett, Lofgreen, and Hall (21) reported that for fattening cattle daily weight gains and feed efficiency were favorably influenced by steam pressure processing the grain for 1.5 minutes at 20 pounds per square inch and rolling. All grains (barley, corn, and milo) reacted similarly to pressure processing.

Steaming corn for 12 minutes at 200°F and flaking decreased feed requirements by 5-10 percent according to Matushima (46). However, if the steamed corn was cracked rather than flaked, feed requirements were increased by 8 percent over cracked corn not steamed. Daily gains were similar on cracked and flaked corn.

Woods (81) reported that when the corn in a steer ration containing 72 percent grain was completely gelatinized feed consumption was reduced to approximately one half that of cattle fed cracked corn. The corn

was processed at a temperature of 380°F and extruded from a die at 400 to 500 pounds per square inch. He reported excessive levels of lactic acid in the rumen of steers fed the gelatinized corn which may be responsible for decreased feed consumption.

Hale et al (31) using defined conditions of moist heat treatment of grain reported steam processing and flaking milo or barley increased daily gain and feed intake over the dry rolled milo or barley rations, respectively. The steam processed flaked grain was prepared by subjecting the milo to low-pressure, high moisture steam for approximately 25 minutes before rolling. The chamber temperature averaged 99°C and the final moisture content of the flake from the roller was 17.8 percent. The steam-processed flaked barley was similarly treated but the final moisture level averaged 13.8 percent. Processing increased feed efficiency of milo but not of the barley ration. Furthermore steam processing and flaking milo significantly increased the digestibility of the nitrogen free extract and total digestible nutrients of a 77 percent grain ration. The total digestible nutrients of milo were increased by 8 percent but protein digestibility was not significantly affected. It was suggested by Hale (28) that the pressure and heat applied during rolling to attain a thin flake with the hot moist grain further affects the availability of the starch which is to some extent independent of the steam processing. Furthermore he suggested that the desirable effects of

treated grain by steam processing or pressure cooking could be lost by an unsatisfactory rolling process.

The favorable influences of grain processing reported by the above mentioned authors may be partially explained from reports by Balch et al (6), Armstrong and Blaxter (34), Ensor, Shaw, and Tellechea (19) and Shaw (66). These workers have indicated that moist heat processed starch and grain could result in a greater proportion of propionic acid being produced during fermentation in the rumen. Furthermore, Blaxter and Wainman (8) reported greater efficiency of utilization of food energy for growth and fattening for those rations which give rise to greater amounts of propionic acid compared to acetic acid.

Borchers (10), Booker, Behan, and McMeans (9) and Sandstedt et al (63) in studies with rats and chicks suggest that selection of certain grain hybrids such as high amylose corn for industrial purposes results in high amylose starch that is less digestible by monogastrics. One example is recent work by Borchers (10) who reported normal corn starch was 95 percent digestible by rats but high amylose corn starch had a digestibility of only 66-77 percent. Ackerson (1) found many intact starch granules in the feces of chicks fed high amylose corn.

Sandstedt et al (63) observed a similar effect when feeding high amylose corn to rats substantiating in vitro enzyme digestion data and reported high amylose corn was resistant to amylolytic enzymes. However, digestion of amylose was not related to amylose content per se

but to a specific gene which was necessary for production of high amylose and also responsible for the high resistance of the starches to enzyme action. On the other hand, genes associated with an intermediate amylose appeared also to be associated with high susceptibility to digestion indicating a definite genetic effect on digestibility.

Reasons for susceptibility or lack of susceptibility to enzyme action are still obscure. The answer probably lies in the structure of the starch granules, i.e. in differences in the bonding between the starch molecules; and possibly, also in anomolous linkages with the starch molecules (63).

With newly developed hybrids that have starch contents which were resistant to microbial and intestinal enzymatic attack, new methods of pretreatment of the starch fraction of the grain were attempted. Baker, Morrise, and Bruce (5) demonstrated that physical degradation either by heating or grinding potato starch, enhanced bacterial attack. Salisbury, Hoeffer, and Luecke (61) showed that moist heat treatment of corn starch brings about hydration of the starch and that hydrated starch is more rapidly digested by rumen microorganisms.

The first in vitro rumen fermentation procedures were developed primarily for the study of nutritive value of roughages. As a result major contributions to the understanding of cellulose digestion and roughage utilization in the rumen have occurred (39). However, with the emphasis on high energy feeding programs for ruminants in which

starch is the primary source of energy, more interest has developed in in vitro procedures for studying starch digestion by the rumen microorganisms. Moore, Johnson, and Dehority (49) described an in vitro method designed for studying starch digestion which utilized the anthrone procedure of Dreywood (15) and Morris (50) for estimating starch disappearance. However many inherent problems such as pentose interferance have been reported with this method (76, 38, 13).

Cadena (12) with in vitro centrifuged rumen liquor studies found the amount of barley starch digestion was five times greater than milo starch during the first two hours of incubation. Vargas (75) also found 18 and 43 percent in vitro starch digestion for milo and barley respectively for a specified evaluation period using raw centrifuged rumen fluid.

Recently Loper, Little and Mitchell (45) have described a procedure utilizing a gravimetric technique for estimating starch digestion and other nutrients by rumen microorganisms in vitro. Results obtained by their gravimetric procedure were highly correlated ($r = 0.94$) with results obtained with the more arduous anthrone procedure.

According to Hungate (35) significant parameters of the rumen fermentation are: (a) rate of fermentation, (b) rate of digestion of various components of feed, and (c) rate of microbial synthesis. The rate of fermentation can be followed by measuring inefficiency in the conversion of substrate to products. El Shazly and Hungate (18)

utilized a constant volume pressure manometric technique for studying fermentation capacity as a measure of the net growth of rumen microorganisms. This technique has some application to starch fermentation studies since growth of microorganisms is a function of substrate fermented(yields of bacteria are proportional to adenosine triphosphate derivable during fermentation)(35) and therefore a function of amount of fermentation products formed. They reported this technique was quicker than Warburg respirometric methods for measuring gas production.

Hopkins, Story and Daugherty (33) reported satisfactory results with total gas production from in vitro bacterial fermentations as a measure of relative microbial metabolism. Their in vitro method was used for studying fertilization rates with hays.

Smith (67) with an undefined in vitro system reported favorable results from pressure processing grains in commercial cookers. As pressure was increased from atmospheric to 60 psi a dramatic increase in conversion of milo starch to dextrose, increased solubility, moisture absorption, and increased ration bulk was observed. He also reported increases in total volatile fatty acids, and rate of volatile fatty acids production, dry matter digestibility and the ratio of propionic to acetic produced by microorganisms. Hunter (36) reported an increase in total volatile fatty acids, increase in ratio of longer chain to shorter chain fatty acids, and greater conversion of starch to sugar due to pressure cooking. He stated that by rupturing the fibrous, or waxy coating the

energy portion of the grain can thus be made more available to the rumen microorganisms resulting in increased feed efficiency and better utilization. This can be done either mechanically or by moisture and heat. His tests conducted with milo, barley, and corn in pressure cooking chambers indicated significantly less starch in feces of beef cattle as pressure was increased from atmospheric to 30-35 psi with no further response with higher pressure.

Salisbury, Hoeffer, and Luecke (61) reported moist heat treatment of corn starch incubated in vitro with centrifuged rumen fluid increased the rate of disappearance of readily hydrolyzable dry matter. Autoclaving the ground corn without the addition of water or a commercially heated corn showed a decrease in the rate of digestion of the readily hydrolyzable dry matter. These results are in keeping with the concept that the application of moist heat to starch or starchy feeds brings about hydration of the starch and that hydrated starch is digested more rapidly by rumen microorganisms than untreated starch.

Expansion (the actual swelling of material) is another method of grain treatment in which the conditions of pressure, moisture, and temperature required to ensure expansion are also conditions sufficient to cook the materials (79).

To consider the effect of expansion upon cereal grain in general Williams and Baer (79) defined it as a method of inflating a material and causing it to swell. This is accomplished by subjecting the material

to high mechanical pressure in the presence of super-heated liquid followed by a sudden relief (release) of pressure, causing the material to expand. It was found that grain expansion also provides sufficient temperature to denature proteins but does not damage the heat sensitive amino acids such as lysine. However, some proteins which are soluble in the native state lose their solubility and coagulate after being denatured. They reported no decrease in nutritive value of protein. Haenlein et al (22) with cattle feeding trials reported that expansion improved the digestibility of feeds and that the cattle gained more when fed expanded grains.

It has been reported that the starch of cereal grains is gelatinized during expansion and this causes the crystal structure to be lost due to breaking of secondary bonds (47).

Any physical modification of starches causes granular destruction, disruption in ordered arrangement, and gelatinization with a concomitant loss of birefringence. The heat moisture treatment of starch alters these physical properties of the starch (47).

According to Matz (47) five effects can occur during flaking and cooking: (1) starch gel slightly hydrolyzed, (2) protein may undergo browning reaction (interaction of protein and sugars), (3) enzymatic reactions are stopped, (4) dextrinization of sugars (conversion of starch milo to dextrose), and (5) carmelization of sugars at high temperatures.

As mentioned before there is a difference in the availability of starch due to genetic factors and much is unknown about how these various factors effect utilization by animals. For example, Whistler (78) reports that residual amylopectin in high amylose starch is not normal highly branched amylopectin as in normal starch but rather it contains only relatively few branches and thus is not as detrimental to films, plastics, or coatings that are made from the starch as is normal amylopectin. Zuber (82) gives variations of amylose-amylopectin ratios in starch for various species. The range in amylose content is 0-7 percent for waxy samples, 17-27 percent for wheat, 20-36 percent for regular corn, 23-28 percent for sorghum, and 24-27 percent for barley. However, there are 70 percent amylose corns available.

Annison and Lewis (3) reported both origin and physical state of starch markedly influence its rate of degradation by rumen microorganisms. However, the effect of source and modification of starch of grains on the relative feeding value to livestock is still not understood.

The starch granule is composed of linear and branched starch molecules associated by hydrogen bonding either directly or through water hydrate bridges to form radially oriented micelles or crystalline areas of various degrees of order (42). The over all strength of the micellar network which is dependent on the degree of association and the molecular arrangement controls the behavior of starch in water. It has been found by Leach, McCowen and Schoch (42) that the swelling

pattern of the starch is greatly influenced by the species and its associated characteristic bonding forces. Corn, milo, and barley starches show a limited two stage swelling indicative of two sets of bonding forces which relax at different temperatures. In contrast potato starch undergoes very rapid and unrestricted swelling at relatively low temperatures, indicating weak and uniform bonding. Both swelling power and solubility were greatly reduced by moist heat treatment. The proportion of water added was adjusted to permit hydration but prevent gelatinization. The above is attributed to an increase in the strength of associative bonding within the granule.

Leach and Schoch (43) also studied the dissolving action of various amylases on granular starches. Two general patterns of enzymatic solubilization by alpha amylase were observed: (a) extensive erosion and fragmentation of corn and sorghum starches and their waxy counterparts, and (b) selective granule by granule destruction of potato and most of the other starches, suggesting the former starches have a porous granule structure accessible to the enzyme while potato starch is less permeable. He showed enzyme susceptibility was not influenced by physiochemical considerations of micellar structure, internal molecular association, or type of crystallinity but an inherent property of the starch that accounts for the greater digestion. However, Matz (47) reports gelatinized or modified starch is rendered more susceptible to enzyme action in that partially gelatinized starch is used for fermentation

of grits by the brewing trade. Furthermore it is stated that the degree of structural damage that has occurred in milling is important for enzymes to convert starch to sugars for metabolism during fermentation with yeast. Matz (47), Booker, Behan, and McMeans (9), and Sandstedt (62) have reported that both the processes of cooking and grinding starch damage the granules and usually render them more susceptible to bacterial attack.

In general from the literature reviewed some forms of pretreatment of grains appears to enhance starch utilization and increase the value of grain for fattening cattle. However, the best method of treatment and/or the desired product (degree of molecular disruption, degradation, extent of modification of the starches) for optimum starch utilization by rumen microorganisms and/or the animal are not known.

EXPERIMENTAL PROCEDURE

I. In Vitro Analytical Procedure:

Since the products of fermentation in the rumen are either gases or acids which release a gas from the rumen bicarbonate, the rate of rumen fermentation can be measured by the rate of gas production. Furthermore, El Shazly and Hungate (18) have shown that short term in vitro organism growth is quite similar to comparable samples within a dialysis sac in the rumen. It is recognized that gas production from rumen fermentation represents a loss of energy to the ruminant. However, the ratio of methane to volatile fatty acids produced remains relatively constant so that any measurable increase in methane production will be associated with increased formation of volatile fatty acids which satisfy a large portion of the ruminants energy requirements. However, this ratio does not remain strictly constant, since the proportion of methane increases slightly with time after feeding, but this increase is small. Also changes in ration affect the ratio of methane to volatile fatty acids, but if the ration is constant the proportion remains constant, and the method remains valid (35).

According to Hungate (35) .58 mole of methane appears for each mole of hexose fermented (for starch and cellulose this would be 162 grams dry weight). The actual determined ratio, which is not much

different than the calculated ratio, was 55.3:31.5:13.2 for acids, carbon dioxide and methane, respectively. Therefore, it appears that gas production measurements are valid since the ratio of total gas to acids produced remains relatively constant.

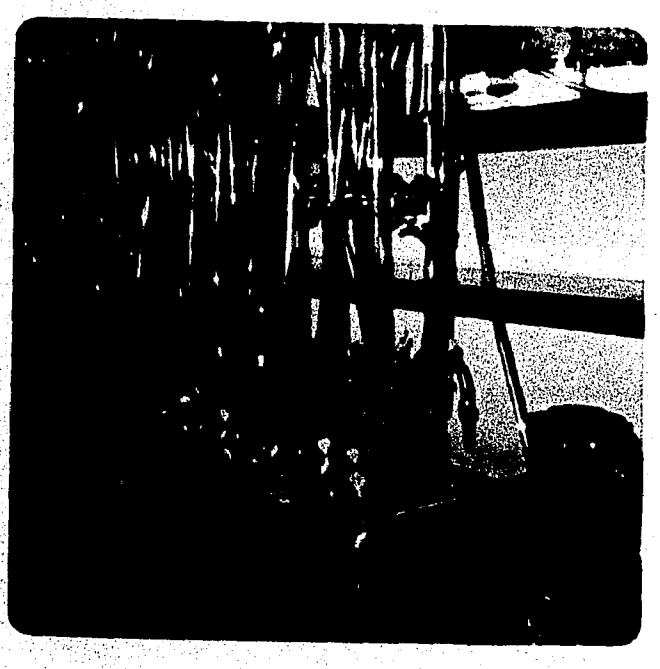
A. Laboratory equipment

A rectangular stainless steel tank (10" x 50" and 9" deep), heater circulator, carbon dioxide tank, 125 ml. erlenmeyer flasks fitted with gas outlet stoppers and tubing connected to water manometers, continuous flow centrifuge, pH meter and other miscellaneous equipment were employed in the in vitro fermentation studies. The description of the in vitro apparatus will be clarified upon observation of the photographs presented in Figure 1. The in vitro fermentation apparatus although modified considerably to accommodate the conditions and available equipment is similar to that used by Hopkins, Story and Daugherty (34).

B. In vitro collection procedure

The method of preparing the bacterial inoculum was that of Johnson (39). The first extraction of rumen fluid was discarded and the rigorously pressed ingesta was retained. Four hundred fifty grams of ingesta was placed in a pre-warmed thermos jug and 900 ml. of preheated carbon dioxide saturated phosphate buffer (Table 1) was added. After being transported to the laboratory the microorganisms

a. Before Fermentation



b. After Fermentation

Figure 1. In vitro fermentation apparatus: water bath, water heater and regulator, water manometers, erlenmeyer flasks and connecting tubing.

Table 1. Composition of phosphate buffer.

Reagent	Grams
Na_2HPO_4	1.059
KH_2PO_4	0.436
Dist. H_2O	1000.0

were filtered through six layers of cheese cloth and collected in a continuous flow centrifuge[1] at 18,000 rpm. The microorganisms were then resuspended in 1125 ml. of artificial saliva (Table 2) and incubated with the addition of CO_2 for thirty minutes. These quantities of inoculum were sufficient for incubating 15 samples. The pH of the mixed suspension was adjusted to 6.9 with sodium carbonate when necessary before the inoculum was added to the flasks containing the grain (substrate). The buffering capacity of the artificial saliva was sufficient to hold pH above five even when maximum fermentation was attained. Loper, Little and Mitchel (45) reported that nutrient media containing minerals and nitrogen buffered at a pH 6.8 provided for satisfactory corn starch digestion in vitro when using an all glass system. Precautions were taken to insure a homogeneous suspension

1. Automatic Servall superspeed centrifuge.

Table 2. Composition of artificial saliva medium in grams per liter of distilled water.

Sodium phosphate (monobasic)	4.8
Sodium bicarbonate	4.8
Potassium chloride	.7
Sodium chloride	.7
Magnesium sulfate	.2
Calcium chloride	.07
Urea[a]	1.2

[a]Added immediately before use.

during the transfer of 75 ml. processed rumen inoculum into each flask. Further precautions for protecting the bacteria from temperature change during processing of fluid were not taken since Doetsch, Robinson and Shaw (14) has shown bacterial counts were not lowered by exposure to room temperature up to eight hours or by aeration up to four hours with agitation. However with the precautions taken only slight changes in temperatures were encountered.

C. In vitro method

Two and four tenths grams (equivalent 2.0 grams dry matter) of processed grain were ground through a 20 mesh screen in a laboratory Wiley mill and weighed into a 125 ml.erlenmeyer flask. After the grain and mixed suspension of rumen microorganisms were added, the flask contents were gently mixed and placed in the water tank maintained at 39°C. The flasks were saturated with CO_2 and after waiting 10 minutes to allow the fermentation gases to expel the air, the flasks were connected to water manometers by means of vinyl-tubing and one holed rubber stoppers. The manometer tubes were approximately 60 cm. long and 2.2 cm. inside diameter. The manometric fluid consisted of water adjusted below a pH of one with 25 ml. H_2SO_4 per gallon to prevent the gas from dissolving in the fluid and methyl orange was added as an indicator to facilitate the recording of gas production measurements. Vinyl-tubing similar to that connecting the flasks and the top of the manometer were connected to the bottom of each manometer tube and extended into a receiving flask. This tubing could be lowered to the level of the manometric fluid as fermentation proceded. Therefore, atmospheric and manometer pressures were equalized before readings were taken with this leveling device. No trouble with siphoning was encountered.

After the initial mixing there was a periodic agitation of the flask contents throughout the fermentation period. However, El Shazly and

Hungate (18) reported that continuous shaking is not necessary since there are so many nuclei for gas bubble formation within the contents that equilibration between gas and liquid phases occurs without agitation.

Readings were taken at hourly intervals up to 3 to 6 hours with grains incubated with grain and roughage inoculum respectively. The volume of fluid displaced in each manometer was used as a measure of gas produced during the fermentation period. All values were corrected for gas produced by the control (75 ml. of rumen inoculum) and expressed as ml. per gram of dry matter incubated. The amount of dry matter added by the inoculum varied by days however an average of one tenth gram was normally added by the inoculum.

II. Determination of Dry Matter Disappearance:

At the end of the desired fermentation, the samples were filtered through a number 4 Whatman disc filter and dry matter disappearance values were calculated based on the dry weight incubated and corrected for the dry matter added by the 75 ml. of mixed suspension of rumen microorganisms. Simple correlations between dry matter disappearance and gas production values were calculated.

III. Microscopic Examination of the Modified Starch:

Various quantitiative methods of estimating modified or damaged starch, gelatinization and etc. were reviewed such as Sandstedts

enzymatic susceptibility test (62), the colorimetric method with iodine (32), and the birefringence test.[1] Three different criteria have been used to detect the gelatinization temperature: loss of birefringence, increase in optical transmittancy and rise in viscosity. Measurement of the loss of birefringence is the most sensitive, accurate, and reproducible technique for determining the initial gelatinization of starch. Methods based on the other two criteria lack sensitivity (44). However, for a quick means of obtaining an estimate of the extent of starch conversion or modification without the use of more elaborate equipment necessary for obtaining precise data the Congo red method by Jones (40) was utilized to obtain gelatinization estimates. These estimates were compared with those obtained by the birefringence test and found to be quantitatively similar.

IV. Experimental Animals.

Fistulated Hereford steers maintained on high concentrate or roughage rations were used to supply the necessary rumen fluid. Rumen ingesta was collected 2-3 hours after the morning feeding for steers on high concentrate rations and 5-6 hours after feeding for the roughage inoculum source. The feeding and collection schedule was held relatively constant since the population of bacteria varies with the time after feeding (11).

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1. Hunter and Wagner (unpublished procedure).

V. Grain Preparation:

For these experiments, various varieties of milo, barley, corn, and wheat were used to study the influence of grain processing on the in vitro fermentation rate.

Steam processing of the grain consisted of subjecting the grain to wet steam in a tempering chamber for 20 minutes at 210°F. and then rolled. The grain processed in this manner contained 18-20 percent moisture.

The pressure cooked grains were prepared by cooking the grains in a home made pressure cooker for specified times and pressures. The grain was then flaked with the same roller as the steam heated grains. Any desired level of moisture could be added to the grains by increasing time held and/or steam pressure in the cooker. Some pressure cooked samples were prepared in commercial cookers designed for this type of feed preparation.

Also various samples of high amylose corn,[1] high amylopectin corn,[1] and high amylopectin milo[2] were available for in vitro evaluation.

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1. Obtained from American Maize Products Co., Watseka, Illinois.

2. Obtained from Lindsey Grain Co., Lubbock, Texas.

RESULTS AND DISCUSSION

Phase I: Preliminary Work:

The first phase of this research and the data collected involves the development of the *in vitro* technique for studying the rate of grain fermentation by measuring the volume of gas produced and the influence grain processing had on the rate of *in vitro* starch digestion. Since this technique was modified to suit the conditions and equipment available several factors had to be evaluated before the ultimate goal of examining the effect of grain processing could be accomplished. Initially such factors as the effect of sample weight, particle size incubated, source of inoculum, and other mechanics of performing the determination were studied. In order to obtain quantitatively large amounts of gas production with this apparatus yet utilize short term incubation periods relatively large samples of grain were incubated.

Perez and Story (55) found that gas production during the first 6 hours of *in vitro* hay fermentation was from the soluble carbohydrate fraction of the feed and the remainder of the fermentation period from cellulose. Therefore, when evaluating the starch fraction of grain it appeared appropriate to study the rate of gas production with relatively short incubation periods. An additional advantage of short term incubations is that fermentation rates are not likely to be limited due

to the build up of end products. However, according to Annison (2) even the most effective artificial rumen is unlikely to maintain the normal pattern of rumen flora for more than a few hours. Although, Loper, Little and Mitchell (45) using the gravimetric technique evaluated rate of fermentation over an 8 hour incubation period with a washed cell suspension of rumen microorganisms for studying moderate alterations in starch digestion and found that the incubation length had a highly significant influence on starch digestion. The response was linear from 0-8 hours in their series of experiments. With the in vitro gas production method maximum fermentation rate or values were not attempted or obtained but rather the relative rates of grain fermentation were measured. Therefore all gas production values reported were after a 3 or 6 hour period of fermentation depending on whether grain or hay inoculum was used.

This method appears to be more advantageous than the Warburg respirometric method or other micro-techniques for measuring gas production since larger samples can be used, rate of gas production can be measured immediately, and sampling error is less due to larger samples taken.

A. Sample weight

Table 3 shows the effect of sample weight incubated within the one to three gram range upon the rate of gas production and percent dry

Table 3. Effect of sample weight on *in vitro* gas production.[a]

Grain	Grams Dry Matter	Dry Matter Disappearance %	Ml. gas/gm D. M. Inc.[b]
Milo	1.0	30.9	64.9[c]
	1.5	30.4	63.7[c]
	2.0	28.9	60.7[c,d]
	2.5	26.9	56.4[d]
	3.0	24.5	51.5[e]

[a]Average values based on two runs.

[b]Milliliters gas produced per gram dry matter after three hours' incubation with grain inoculum.

[c,d]Means with the same superscript are not significantly different by Duncan's Multiple Range Test of significance between means ($P < .05$).

matter disappearance. No significant reduction in the rate of fermentation was noted with 75 ml. of rumen fluid when between one and two grams of substrate were incubated with grain inoculum. With subsequent trials 2 gram samples were used. It was assumed this sample size provided a "limiting substrate" so that *in vitro* rates of fermentation could be studied (35).

B. Particle size

The effect of particle size is shown in Table 4. Only small differences in gas production or dry matter disappearance were observed between grains ground through 40 mesh or 20 mesh screen in a laboratory Wiley mill and incubated with roughage inoculum. Due to the difficulty in grinding some grains through the smaller mesh size all subsequent samples were ground through the 20 mesh screen. Also from this table a marked difference in the untreated milo and barley rate of gas production was noted.

Table 4. Effect of particle size on in vitro gas production.

Grain	Mesh Size	Dry Matter Disappearance %	Ml.gas/gm. D. M. Inc.[a]
Milo	40	25.6	30.9
	20	23.9	28.5
Barley	40	30.2	57.0
	20	27.5	60.4

[a]Milliliters of gas produced per gram dry matter after six hours incubation with roughage inoculum.

C. Source of inoculum

Table 5 gives the effect of source of inoculum. There was a moderate advantage, in total gas production from in vitro fermentations when obtaining inoculum from the milo steer when compared to the microbial activity of the barley steer. There was considerable variation in fermentation rate from inoculum sources of different steers on the same ration (Table 28). This effect was also reported by Bezeau (7) while studying in vitro cellulose digestion. He reported highly significant difference existed between the activity of inocula from donor cows on the same ration. Furthermore with repeated sampling a high daily variation was observed with the same steer (Table 28). However there was a highly significant reduction in the rate of fermentation when inoculum was collected from a hay steer (Table 5). This effect of the amylase activity of rumen fluid was reported by Templeton and Dyer (69). They found as the percent of concentrate in the ration increased the amylase activity increased significantly over the range studied which was 0, 50, and 80 percent concentrate. To further account for the difference in amylase activity, Van Der Wath (74) with an in vitro system found no amylase activity on hay ration until one and one-half hours later than on a grain ration. Therefore to obtain satisfactory large amounts of gas production with grain substrates when an inoculum was obtained from a roughage steer 6 hours rather than 3 hours values are reported.

Table 5. Influence of inoculum source on in vitro gas production.

Grain		Source of Inoculum		
		Milo Steer	Barley Steer	Hay Steer
		ml[a]	ml[a]	ml[a]
Milo	Untreated	57.2	56.4	9.2
	Steam processed flaked	84.1	72.6	8.8
Barley	Untreated	76.0	63.4	21.1
	Steam processed flaked	104.4	86.0	20.4

[a]Ml of gas produced/gram of dry matter after three hours' incubation.

In order to show the effect of milo or barley treatment, an inoculum with high amylase activity is necessary. This effect can be noted from Table 5 when comparing the marked difference between treated and untreated grains incubated with an inoculum from grain steers as compared to the lack of difference if the inoculum was from a hay steer.

D. Experimental design

A series of preliminary tests indicated a high correlation between gas production and dry matter disappearance (Table 27) and there was very close agreement of gas production from individual flasks on the same treatment (Table 21-standard deviation). From Table 21 on milo

flake experiment it can be observed from the analysis of variance that replicating of samples within a run were not necessary due to the small measured sampling error term. Also like treatments responded similarly over trials (runs) as shown in Tables 21-25, inclusive, yielding a small experimental error (T x R interaction). However, there was a significant run effect. It was deemed more important to replicate runs in order to reduce the magnitude of this effect and decrease the number of samples per treatment incubated per run. Therefore, one sample per treatment and several replications of runs were used in the remaining experiments. This design decreased the source of variation due to trials and when grain inoculum was used runs were non-significant (Tables 24 and 25).

Coefficients of variation range between 5-10 percent with standard errors of the mean ranging from .192 - 1.21 (Table 21 to 25, inclusive). Therefore, a difference between 0.50 and 3.0 ml. of gas was necessary for detecting significant differences (.05 level of probability) in treatment means depending on error degrees of freedom available (68).

Phase II: Influence of Species and Variety of Grain on the In Vitro Gas Production

The grains shown in Table 6 were incubated with a roughage inoculum source so the magnitude of differences between species, varieties,

Table 6. Influence of species and variety of grain on in vitro gas production.

Grain	Origin	Treatment	Ml. gas/gram D. M. Inc.[a]
Amylose corn	Midwest		24.7[b]
Milo	Texas		30.3[c]
	Wilcox		30.6[c]
	Sacramento		32.0[c]
	Tucson		34.0[c]
Hegari	Arizona		31.9[c]
Amylopectin corn	Midwest		34.4[c]
Corn	Midwest		35.3[c]
Barley		Dehulled	55.3[d]
		Untreated	59.2[d,e]
		Pearled	62.0[e]
		Rolled	62.7[e]

[a]After six hours with roughage inoculum.

[b, c, d, e]Means with the same superscript are not significantly different by Duncan's Multiple Range Test of significance between means ($P < .05$).

etc. observed are not as great as might be expected with inoculum from a grain steer. There were no significant differences between varieties or sources of milo, hegari or corn. The high amylose corn fermented at significantly slower rate than all other grains shown. Likewise, gas production at the end of 6 hours fermentation period for all barleys was significantly ($P < .05$) higher than for all other grains considered.

Table 7 shows additional comparisons of untreated grains with a grain inoculum. In this trial, gas production from milo was greater than from barley and corn although none were significantly different from each other. Again the amylose corn showed significantly ($P < .05$) slower rate of fermentation than regular corn by gas production values. High amylopectin corn and milo were intermediate but the amylopectin corn significantly ($P < .05$) lower of the two.

Phase III. Influence of Steam Processing Milo and Barley on In Vitro Gas Production

The effect of steam processing and flaking milo and barley on the in vitro gas production rate is shown in Table 8. Steam processing and flaking either milo or barley significantly ($P < .05$) increased gas production when compared to untreated grain and all four treatments are significantly ($P < .05$) different from each other. As usually observed gas production from untreated barley was significantly ($P < .05$) greater than the untreated milo.

Table 7. Comparison of *in vitro* gas production of untreated grains.

Grain	Dry Matter Disappearance %	Ml.gas/gm. D.M.Inc.[a]
Milo	16.7	45.1[b]
Amylopectin milo	13.1	31.0[c]
Barley	18.8	41.8[b]
Corn	19.1	40.3[b]
Amylose corn	10.3	18.0[d]
Amylopectin corn	12.3	22.7[d]

[a]After three hours' fermentation with grain inoculum.

[b, c, d]Means with the same superscript are not significantly different by Duncan's Multiple Range Test of significance between means ($P < .05$).

Table 8. Effect of steam processing milo and barley on gas production.

Grain	Treatment	Ml.gas/gm. D. M. Inc.[a]
Milo	Untreated	58.5[b]
	Steam processed flaked	69.8[c]
Barley	Untreated	80.9[d]
	Steam processed flaked	96.9[e]

[a]Three hour incubation with grain inoculum.

[b, c, d, e]Means with the same superscripts are not significantly different with Duncan Multiple Range Test of significance between means ($P < .05$).

Phase IV: Influence of Flatness of Flaking Moist Heat Treated Grain on Gas Production

A. Table 9 shows the influence of the flatness of milo flakes on the gas production. Steaming milo without rolling caused a significant decrease ($P < .05$) in gas production compared to the untreated milo. As observed later, this depression was not always significant or observable with pressure cooked grains at 4.2kg./cm.^{2} if the cooked grain was not flaked (Table 13 and Figure 4). The poor flake was no better than untreated milo. Furthermore after steaming, a medium flake was necessary to obtain any significant improvement in the fermentation rate of the grain over the untreated grain. By rolling

Table 9. Influence of flaking milo on in vitro gas production.

Treatment	Ml.gas/gm. D. M. Incubated[a]
Untreated	41.7[b]
Steam processed (not flaked)	31.6[c]
Steam processed (poor flake)	45.8[b, d]
Steam processed (medium flake)	50.0[d]
Steam processed (fine flake) not ground	60.7[e]
Steam processed (fine flake)	65.6[e]

[a]Incubated six hours with roughage inoculum.

[b, c, d, e]Means with the same superscript are not significantly different by the Duncan Multiple Range Test of significance between means ($P < .05$).

the flake still flatter as is represented by the fine flake, whether ground or unground, gave significantly higher gas production values than any of the other treatments presented.

From Table 10, numbers 6 through 0, represent increasing degrees of flake flatness (thinness) for milo on its rate of gas production. The significant effects as previously mentioned are observeable here except a greater range of flake flatness or degrees or rolling are shown. It can be noted as mentioned in Table 9 a poor to medium flake was necessary, as represented by sample number 4, before a significantly

Table 10. Effect of flatness of flaking steam processed milo on *in vitro* gas production.

Treatment	% Gelatinization	Ml. gas/gm. D. M. Inc.[a]
Untreated	-	62.6
Steam processed (not rolled)	-	51.1
SP poor flake 6[b]	10	54.1
SP 5	25	58.8
SP medium flake 4	40	75.6
SP excellent flake 3	55	81.4
SP 2	70	79.5
SP 1	80	79.9
SP very flat flake 0	90	79.1

[a]Incubated three hours with grain inoculum.

[b]No. 6 through 0, increasing degrees of flake flatness.

faster rate of gas production was obtained than with the untreated milo grain. Once an excellent flake is formed, there appeared to be little advantage to producing a flatter flake as far as improving gas production rates. These differences in fermentation rates are graphically shown in Figure 2.

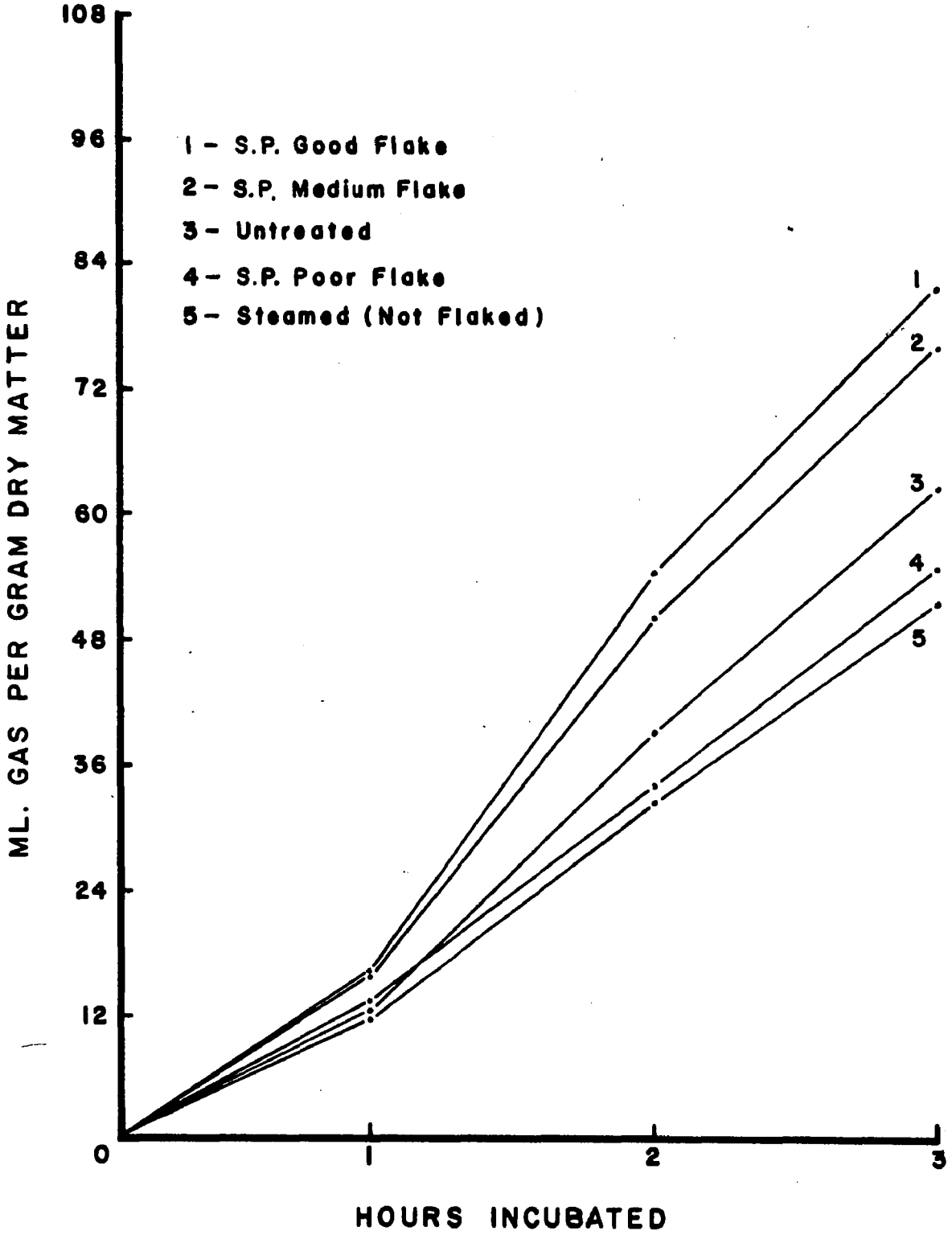

FIGURE 2: IN VITRO FERMENTATION CURVE FOR MILO PREPARATIONS

B. Table 11 shows the influence of varying degrees of flaking barley with the associated dry matter disappearance values and the estimated percent gelatinization values. The effect of flatness of flaking of barley was similar to that of milo. However, with barley there was an advantage to increasing flake flatness to the thinnest flake possible. The rates of fermentation for various barley preparations are shown graphically in Figure 3.

C. A comparison of effect of steam processed milo and barley medium flake ground and unground is shown in Table 12. Again gas production from untreated milo was less than untreated barley as normally observed with representative samples of each. There was no difference in gas production with steam processed flaked milo ground or unground. However, with the barley flake, the steam processed grain had to be ground to obtain the advantage of steam processing and flaking. This may account for the observed additional increase in rate of fermentation obtained with the progressively thinner flakes of barley whereas with milo once a certain flake flatness was obtained no significant advantage in rate of fermentation was observed in going beyond that point. This effect noticed between the grains may be due to inherent physical characteristics of the grains themselves rather than treatment differences on the starch granules.

Table 11. Effect of flatness of flaking steam processed barley on *in vitro* gas production.

	% Gelatinization	% Dry Matter Disappearance	Ml.gas/gm. D. M. Inc.[a]
Untreated	10-15	18.8	41.8
Steam processed (6) av. flake[b]	10-15	20.2	52.6
Steam processed (5)	25-30	22.3	62.6
Steam processed (4)	25-30	25.1	66.7
Steam processed (3)	75	25.3	67.7
Steam processed (2)	75	26.2	72.9
Steam processed (1) very flat flake	75-80	29.1	79.2

[a]Three hours incubation with grain inoculum.

[b]No. 6 through 1 represent increasing flake flatness.

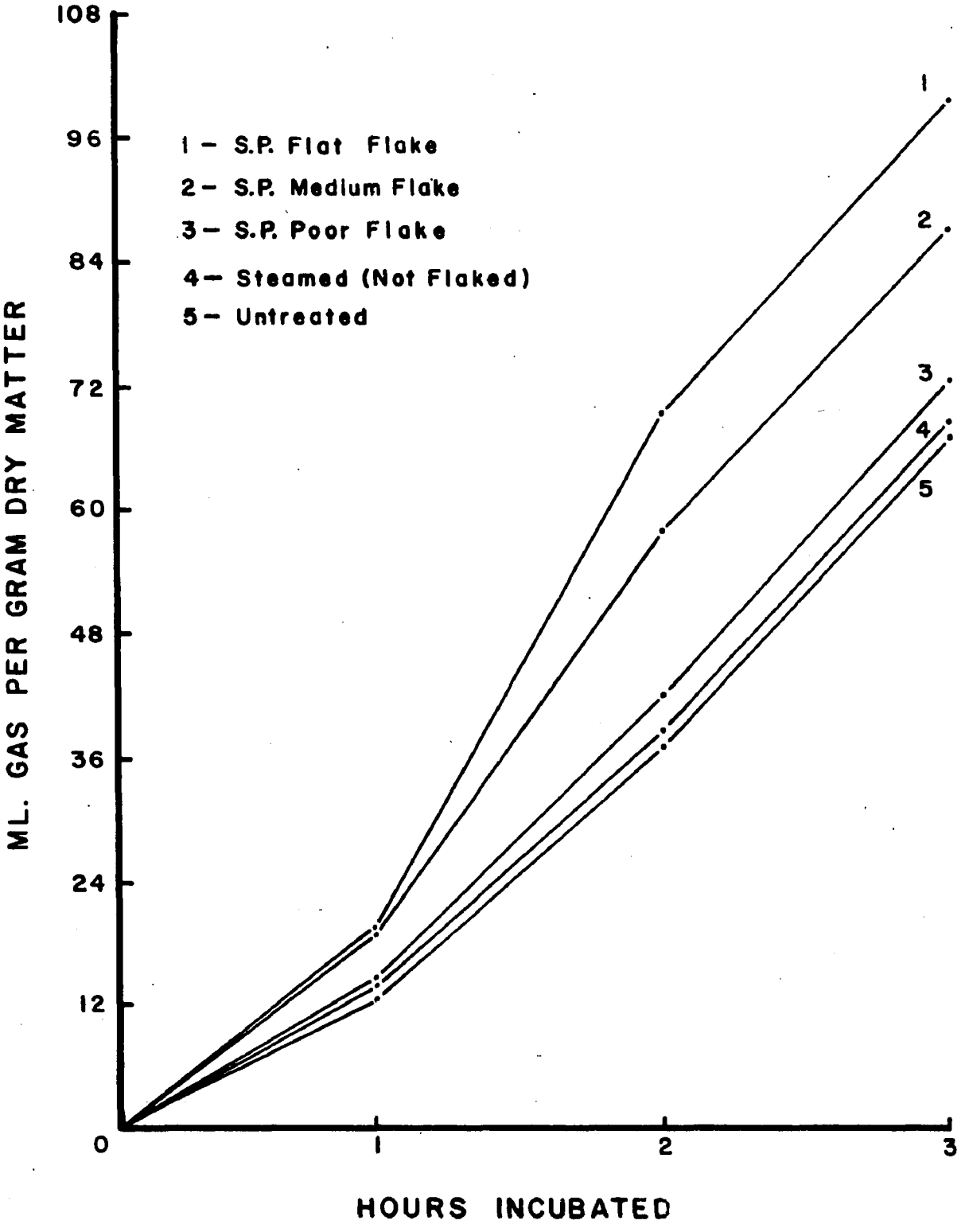

FIGURE 3: IN VITRO FERMENTATION CURVE FOR BARLEY PREPARATIONS

Table 12. Effect of grinding after flaking on *in vitro* gas production.

Grain	Treatment	Ml. gas/gm. D. M. Incubated[a]
Barley	Untreated	66.0
	Steam processed flaked (not ground)	60.7
	Steam processed flaked (ground)	80.3
Milo	Untreated	55.7
	Steam processed flaked (not ground)	76.8
	Steam processed flaked (ground)	78.7

[a]Incubated with grain inoculum for three hours.

Phase V: Influence of Pressure Cooking Grains on the *In Vitro* Gas Production

A. Effect of pressure cooking milo:

Table 13 shows the effect of pressure during cooking of whole milo without rolling on the rate of gas production. There was a progressive increase in quantity of gas produced per gram dry matter incubated as pressure (kg. /cm.^{2}) was raised. From the rates of gas production, it appears that at least 2.8 kg. /cm.^{2} is necessary to equal the untreated in fermentation rate if the grain is not flaked. This does not imply that

grain cannot be pressure cooked at 2.8 kg./cm.^{2} or less if properly flaked and be equal in value to grain cooked at higher pressure and rolled. It is apparent from the table that cooking at 5.6 kg./cm.^{2} yielded additional increases in the gas production rate and a further improvement in fermentation rate could be obtained by flaking. However, it appears that greater increases in the fermentation rate can be accomplished by flaking than by cooking with pressure only, unless much higher pressures or longer cooking times are used. Furthermore, cooking at higher temperatures may be disadvantageous due to the possibilities of protein destruction (58, 59, 77) and increased power costs of providing higher boiler temperatures.

Table 13. Effect of cooking pressure with milo on _in vitro_ gas production.[a]

Pressure Kg./cm.^{2}	Dry Matter Disappearance %	Ml.gas/gm. D. M. Inc.[b]
0	16.0	30.7
1.4	15.5	27.1
2.8	14.9	29.9
4.2	16.6	38.1
5.6	19.1	44.8
5.6 flaked	21.9	62.3

[a]Cooked whole grain.

[b]Three hour incubation with grain inoculum.

Figure 4 shows comparisons of steam processed, conventional pressure cooked (4.2 kg./cm.^{2} for 1 minute) dry rolled, and untreated milo. There appeared to be a slight improvement of the dry rolled over the untreated. As observed before with steam processed (not flaked) milo, a significant depression exists in rate of fermentation compared to untreated milo. However, the flaked steam processed grain produced more than twice as much gas as the steamed processed unflaked milo. Pressure cooking at 4.2 kg./cm.^{2} for 1 minute without rolling did not depress rate of fermentation compared to untreated as normally occurred with unflaked steamed grain. Furthermore a minimum of pressure with moist heat treatment (steam processing) and the formation of a good flake are as beneficial as pressure cooking at 4.2 kg./cm.^{2} for 1 minute and then flaking.

Rates of gas production for grains were generally the greatest between the first and second hours of incubation when grain inoculum was used (Figures 2, 3, and 4).

B. Effect of pressure cooking various feed grains

There was a significant increase ($P < .05$) in the rate and the total volume of gas produced at termination of a 3 hour in vitro fermentation period by pressure cooking several grains (Table 14) over the untreated samples. By increasing the time and/or pressure during cooking the rate of gas production was greatly increased. Furthermore, as shown

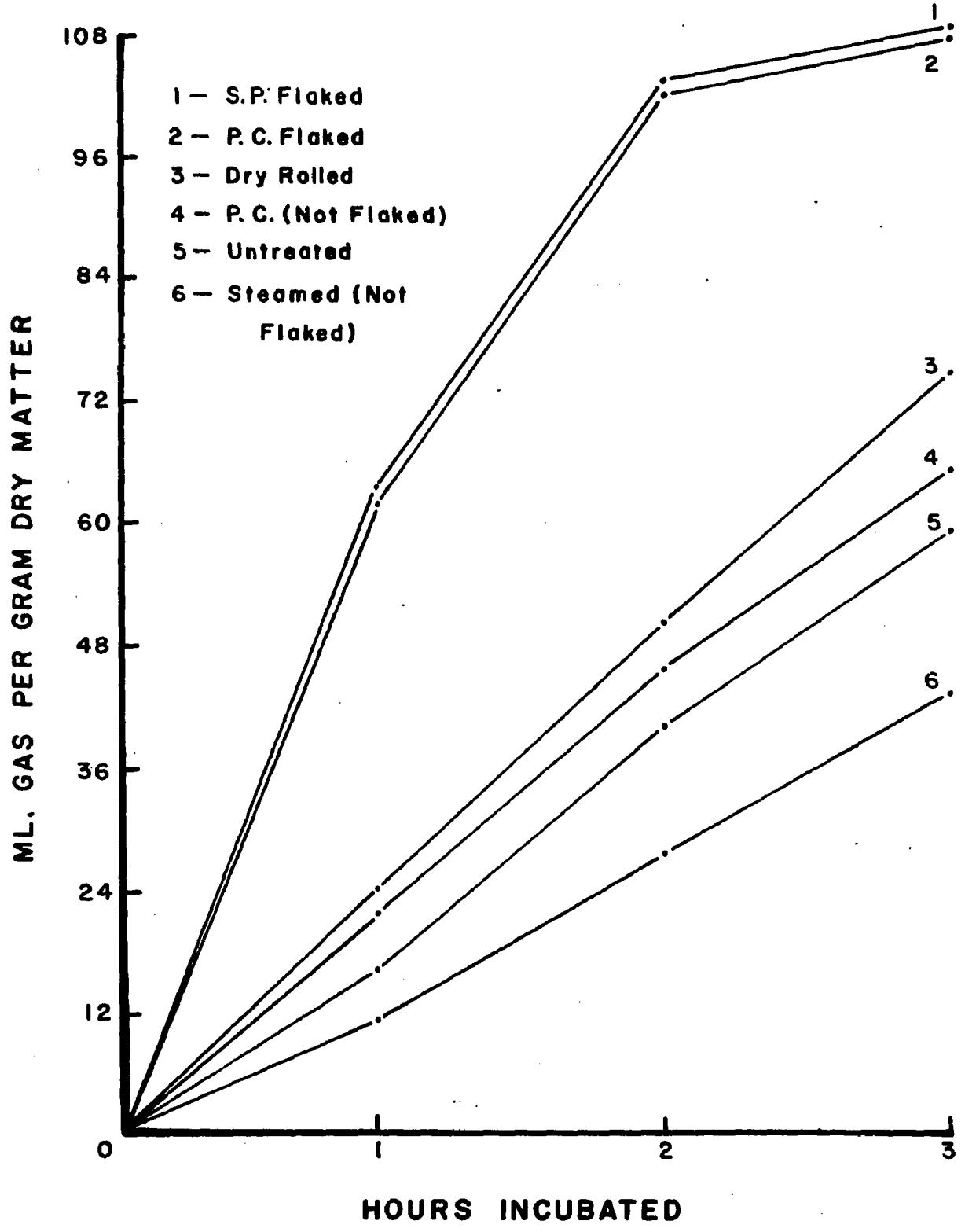

FIGURE 4: IN VITRO FERMENTATION CURVE FOR MILO PREPARATIONS

Table 14. Effect of pressure cooking various grains on *in vitro* gas production.

Grain	Treatment	Conditions		Ml.gas/gm. D. M. Inc.[a]
		Kg./cm.^{2}	Min.	
Milo	Untreated	-	-	66.8
	Pressure cooked	4.2	1 (not flaked)	48.8
	Pressure cooked	4.2	1 (flaked)	86.0
	Pressure cooked	4.2	2 1/2 (flaked)	95.2
Corn	Untreated	-	-	48.8
	Pressure cooked	4.2	1 (not flaked)	44.2
	Pressure cooked	4.2	1 (flaked)	61.8
	Pressure cooked	4.2	5 (not flaked)	44.9
	Pressure cooked	4.2	5 (flaked)	65.7
Amylose corn	Untreated	-	-	26.5
	Pressure cooked	4.2	5 (not flaked)	22.3
	Pressure cooked	4.2	5 (flaked)	35.3

Table 14--Continued.

Wheat	Untreated	-	-	52.6[b]
	Pressure cooked	2.8	1 (flaked)	68.7
	Pressure cooked	4.2	1 (flaked)	87.2
	Pressure cooked	4.2	1 (flaked twice)	97.2
Barley	Untreated	-	-	67.2
	Pressure cooked	2.8	1 (flaked)	99.1

[a]Incubated for three hours with grain inoculum.

[b]Remaining values in table cannot be directly compared with the values above the dotted line as they are in different in vitro runs.

before much of the improvement in volume of gas produced appears to be accomplished by the rolling process after pressure cooking.

C. Effect of cooking time on ground milo at constant pressure

The effect of cooking small samples of ground milo in the laboratory with a minimum of pressure (1.1 kg./cm.^{2}) for varying lengths of time are shown in Table 15. The pressure cooked ground milo was added to the in vitro system without drying. Cooking for 15 minutes approached but did not equal the untreated milo in gas production, further indicating

Table 15. Influence of pressure cooking unflaked ground milo on *in vitro* gas production.[a]

Cooking time Minutes	Ml. of gas produced per gram of D. M.[b]
0	67.2
1	44.9
2	51.5
3	53.0
4	52.6
6	56.8
15	58.8
15 (oven dried)	64.5

[a]All samples cooked at 1.1 kg./cm.^{2}

[b]Incubated for three hours with grain inoculum.

that flaking is necessary once the grain has been steamed. Oven drying the milo cooked for 15 minutes appeared to increase gas production. A similar effect in gas production has been shown before in that low pressure cooking or steam heating without flaking appears to depress or not significantly increase fermentation rate compared to the untreated grain.

Phase VI: Influence of Autoclaving Milo with Various Moisture Levels on Gas Production

Leach (44) stated that the micellear areas of the starch granule could be dispersed by autoclaving, which would appear to increase rate of enzymatic attack on the starch granule. However the hydrogen bonding in starch granules is not by strong direct association of linear and branched molecules but through hydrate bridges. The moist heat treatment of starch may dissociate the hydrate water and induce rearrangement to a strong micellar pattern inter-connected by linear molecules, or recrystalize in a molecular latice thereby reducing swelling power and solubility due to the greater associative bonding within the granule.

Patrick (54) found autoclaving caused marked lowering in the feeding value of barley. Salsbury, Hoeffer and Luecke (61) also found that commercially dried or autoclaved ground corn without the addition of water resulted in a decrease in the rate of digestion of the readily hydrolyzable dry matter. The feeding of a 60 percent finely ground and steam rolled milo ration to cattle decreased daily gain (. 10 lb.) and increased feed requirements per 100 pounds of gain (90 lb.) when compared to the unsteamed finely ground milo (56).

The same effect was observed by in vitro on gas production (Tables 15 and 16). Autoclaving finely ground milo in general depressed rate of gas produced but the magnitude of depression depended on amount

Table 16. Effect of autoclaving ground milo with various levels of moisture and methods of handling on *in vitro* gas production.[a]

Total Grain Moisture %	Method of Storage	Ml.gas/gm. D. M. Inc.[b]
10	Refrigerated	42.5
	Quick dried	45.3
	Air dried	41.1
20	Refrigerated	47.5
	Quick dried	54.5
	Air dried	51.6
30	Refrigerated	42.7
	Quick dried	56.8
	Air dried	50.6
40	Refrigerated	29.5
	Quick dried	55.8
	Air dried	33.8
Air dry	Untreated	59.6

[a]Autoclaved at 1.2 kg./cm.^{2}, 123°C.

[b]Incubated for three hours with grain inoculum.

of moisture present and how the sample was handled after treatment until incubation in vitro. The least decrease in gas production from untreated grain was noted on those samples with 20-30 percent total moisture when autoclaved and quick dried after autoclaving. It is known that exhaustive drying can produce cracks or damage within the starch granule. The 20-30 percent moisture level may be critical for producing a greater degree of damaged starch and disruption in the molecular orientation of the granule when heated. Furthermore this level of moisture has appeared to be essential before the rolling process to obtain the advantage of steam processing of the grain. However, if moisture and heat does not physically alter the granular structure of starch it may be that the moisture provides the effect of wetability. However too much water in the fissures of the starch granule may inhibit penetratability by the enzymes.

Phase VII: Influence of Various Physical and Mechanical Treatments of Milo on Gas Production

Table 17 shows the effect of soaking, boiling and popping milo. Boiling or popping greatly increased gas production over the control.

In the soaking experiments with milo, there was no evidence of fermentation at room temperature as the refrigerated samples were not different in gas production from the unrefrigerated sample. The fineness of grind in a hammermill effects the rate of fermentation above certain

Table 17. Influence of various physical and mechanical treatments of milo on *in vitro* gas production.[a]

Method of Treatment	Conditions	Ml.gas/gm. D. M. Inc.
Untreated	-	65.3
Dry heated	Whole - 180°F	45.7
Boiled (submerged in water) 3 min.	Ground	79.9
Boiled - 15 min.	Ground	101.4
Popped	Ground	79.9

Effect of soaking milo for 18 hours on gas production.

Treatment	Particle Size	Storage Temperature	Ml.gas/gm. D. M. Inc.
Soaked ground	Medium	80°F	68.4
Soaked ground	Medium	20°F	73.3
Soaked ground	Coarse	20°F	48.8
Soaked ground	Cracked	20°F	34.9
Soaked whole	20 mesh	80°F	40.3
Soaked whole	20 mesh	20°F	41.9

[a]All treatments in the table were in same *in vitro* runs or incubated with grain inoculum for three hours.

limits of particle size. However below the previously specified fineness in a laboratory Wiley mill(particle size of 20 mesh) there was no marked difference as shown with the sensitivity of this in vitro apparatus (Table 4). Furthermore, there was no advantage to soaking whole grains for the times indicated but it appears that a portion of the grain was lost during the filtering and grinding of the soaked whole grain as evidenced by lowered gas production compared to untreated. It appears that various physical processes can alter the starch fraction so that it may ferment more rapidly. Such outside factors are moisture, heat, time and mechanical breaking. However some evidence suggests that as the temperature of aqueous starch is increased above the gelatinizing temperature the granules swell and lose their ability to return to birefringence. However granules that merely gelatinize appear to retain their ability to return to the spherocrystalline state (return to birefringence)(47). Furthermore some retrogradation can occur within the swollen granule during aging.

Phase VIII: Influence of Isolated or Purified Starch from Various Sources on In Vitro Gas Production and a Comparison with Native Starch

In Table 18 the effect of isolated and native starch incubated with grain inoculum for 2 hours are shown. Isolated amylopectin produced gas at faster rate than corresponding isolated amylose samples.

Table 18. Comparison of in vitro gas production with isolated and native starch.

Substrate	Source	Ml.gas/gm. D. M. Inc.[a]
Milo (untreated)	Texas	56.8
Amylose	N. B. C.[b]	63.4
Amylose	70%	71.0
Amylopectin	N. B. C.[b]	78.7
Amylopectin	100%	84.1
Corn starch	Argo	81.4
Potato starch	-	76.0

[a]Incubated with grain inoculum for two hours.

[b]Nutritional Biochemicals Corporation.

Enzymatic studies with these fractions have also indicated a significantly slower rate of hydrolysis of the highly linear amylose fraction compared to the highly branched amylopectin (63). Isolation starch appeared to produce more gas than native grain starch possibly because of alteration of starch upon sisolation or due to physical obstruction of the starch by other material as the concentrations of starch were approximately equivalent between the sources before incubation.

Phase IX: Correlation of In Vitro Gas Production and Dry Matter Disappearance

Correlation coefficients for percent dry matter disappearance and gas production are shown in Table 27. The correlation for grains incubated for 6 hours with roughage inoculum was r = .89. However, with the shorter fermentation period of 3 hours with a grain inoculum source, the correlation was .94. It appears that there is not a constant volume of gas produced per gram of dry matter digested among different grain sources because a .97 value was obtained when a simple correlation was run on treatments within processed barley only. However, the amount of dry matter solubilized and loss upon filtration may account for the difference. Theoretically it should be possible to account for all dry matter disappearance by the volatile fatty acids and gas produced. However, gas production was found to be highly correlated with dry matter disappearance in vitro and therefore gas production measurements appear to be a more expedient technique in time and convenience than the gravimetric technique for procuring information on the in vitro rate of starch fermentation.

Phase X: Effect of Gelatinization of Starch on the In Vitro Gas Production

Estimates of gelatinization of the starch for various degrees of flaking of milo and barley are shown in Tables 10, 11 and 20. The evidence is not clear cut with barley, but with milo little additional

advantage is obtained once a good to excellent flake is formed in attempting to go beyond this point in flatness of flake or apparent associated percent gelatinization. Steam processed milo samples (30 to 40% gelatinized) were equivalent in gas production rates to those samples that were 75 - 80 percent gelatinized. This level of gelatinization for pressure cooked and flaked milo appears adequate for optimum gas production, also. Although the extent of gelatinization corresponds to the increased flatness of the flake, it may not be directly responsible for the increased rate of fermentation of the processed grain but only an associated effect of rolling.

Although the extent to which the grains should be treated with moist heat is not known, results by Pope, Harber and Waller (57) indicate that complete gelatinization of milo starch is detrimental to cattle gains. They also state that the lowered feed consumption of gelatinized milo caused lowered gains and the adverse effect was attributed to faster rate of fermentation and higher acid concentrations in the rumen. The gelatinized product was prepared by steaming ground milo at 180°F, pelleting, drying and regrinding the pelleted grain.

Similar results were obtained by Woods and Debie (80) with gelatinized corn.

Phase XI: Comparison of Effect of Milo Preparations by In Vitro and In Vivo Studies

In Table 19 the relative values of various milo preparations from in vivo and in vitro studies are shown. Only absolute values within rows are comparable. However, relative differences of preparations between rows can be prepared. All in vitro values for rate of grain utilization i.e. enzymatic starch digestion, gas production and volatile fatty acid production by rumen microorganisms parallel one another for the milo preparations studied. It was observed that steam processing and production of an excellent flake increased the rate of enzymatic pancreatin digestion compared to the untreated milo. Total volatile fatty acids produced in a given period of time also were significantly increased with a concomitant increase in gas production. Furthermore, the ratio of acetic to propionic acid was somewhat lowered which is necessary for promoting efficient beef production since propionic acid is metabolized with greater efficiency than acetic acid. By pressure cooking at 4.2 kg./cm.^{2} for one minute and the formation of an excellent flake, a similar advantageous effect on the rate of enzymatic starch digestion occurs as shown by the increased starch digestion and gas production values. However it can be noted that low pressure moist heat treatment (steam processing) without flaking or the production of a poor flake was no better or frequently not as good as untreated grain when comparing any of the tabular measures of fermentation rates. This again

Table 19. In vitro and in vivo evaluation of milo preparations.

Item		Untreated	Steam Processed			Pressure Cooked (4.2 kg./cm.^{2})	
		Dry Rolled or Fine Ground	Not Flaked	Poor Flaked	Excellent Flaked	Not Flaked	Excellent Flaked
Gas Production (Ml./gm. D. M. Inc.)	(73)[a]	72.2	-	-	93.7	74.9	96.8
	(73)	62.6	51.1	54.1	81.4	-	-
Enzymatic Starch Digestion (%)	(53)	17.8	12.9	16.0	34.8	-	-
	(53)	-	-	-	-	25.1	47.2
Volatile Fatty Acid Production							
Total V. F. A. (Micromoles/gm. D. M.)	(70)	1558	1308	1783	2038	-	-
Ratio of C_2/C_3	(70)	1.16	1.17	1.14	1.07	-	-
Nitrogen Free Extract Digestibility (%)	(48)	78.5	-	-	87.1	-	86.6
	(37)	70.9	-	73.3	83.8	-	-

Table 19-- Continued

Nylon Bag Study								
Dry Matter Disappearance (%)	(26)	31.9	-	29.9	49.4	-		-
	(28)	21.9	-	-	49.3	-		49.1
Feedlot Trial								
Av. Daily Gain (kg)	(27)	1.25	-	-	1.38	-		-
Feed efficiency	(27)	848	-	-	812	-		-
(kg./kg. gain)	(30)	865	-	844	763	-		-

[a]Number in parentheses refer to reference cited list.

emphasizes the importance of adequate flaking after moist heat treatment of the grain.

The in vivo data closely parallels the in vitro data discussed. Nitrogen free extract digestibilities with fattening cattle similarly reflect the significantly better utilization of the excellent flaked steam processed or pressure cooked grain compared to dry rolled, fine ground, or poor flaked milo. Furthermore in the two digestion trials cited, the significant improvement in nitrogen free extract digestion was reflected in digestible gross energy, and total digestible nutrients which were both significantly improved. Rate of dry matter disappearance from the nylon bag studies support the other in vivo trials and the in vitro data presented. However the real value of the relative improvement of moist heat treated and excellent flaked grain is shown by the increased gain and feed efficiency with fattening cattle. Average daily gain was increased from 1.25 kg. on dry rolled to 1.38 kg. on an excellent flaked grain ration. Corresponding data of feed required to produce 100 kg. of gain were reduced from 848 to 812 with steam processed flaked milo. In a similar feeding trial, feed requirements of excellent flaked steam processed milo ration was decreased to 763 compared to 865 for dry rolled or 844 for steam processed poor flaked ration. These feedlot trials indicate the relative advantage in gain and feed efficiency of satisfactorily flaking steam processed grain compared to dry rolled grain. This advantage is of primary concern for more

efficient and profitable beef production. Furthermore the *in vitro* gas production results with the processed grains closely parallel the feedlot trials with these processed grains.

SUMMARY

The effect of steam processing, pressure cooking, and flaking of milo and barley on the *in vitro* gas production rate was evaluated with a mixed suspension of rumen microorganisms for a three hour incubation period. Values quoted are for gas production, however they are highly correlated with dry matter disappearance values and a good indication of the rate of rumen fermentation. Values were recorded as milliliters of gas produced per gram of dry matter incubated. Much of the data collected concerns the development of the experimental technique such as quantity of substrate, source of inoculum, particle size, length of incubation, and experimental design, and differences in amylase activity at collection time as indicated by gas production rates.

In vitro gas production was affected by species and varieties of grain. High amylose corn fermented at a reduced rate compared to regular corn. Rates of gas production from barley preparations exceeded those from equivalent milo samples which parallel data from *in vivo* digestion trials with these grains. Steam processing and flaking milo or barley significantly increased gas production over the respective untreated grains. Increasing the flatness of the barley or milo flake by rolling significantly increased gas production of moist heat treated grain indicating that flaking is extremely important in the

processing of steam treated grains. Steam processed or pressure cooked milo at 4.2 kg. per cm.^{2} for 1 minute but unflaked was inferior or not significantly greater than untreated grain.

Steam processed flaked and pressure cooked flaked milo at 4.2 kg. per cm.^{2} gave similar significantly increased gas production over the untreated indicating that the desirable effect on the grains was approximately the same by both processes. Increasing increments in pressure (0 to 5.6 kg. per cm.^{2}) without flaking significantly increased rate of in vitro fermentation. However additional increases in gas production were obtained by flaking after cooking at all pressures. Increased flake flatness and its apparent associated increased gelatinization to an extent increased gas production rates. All results indicate that the grains must be satisfactorily flaked after the whole grain has been treated with moist heat to obtain optimum utilization.

Autoclaving ground milo without flaking reduced gas production rates while various expanded milo preparations (popped or boiled milo) significantly increased gas production rates.

This in vitro screening technique appears to offer a quick estimation of the rate of digestion and an effective guide to the relative feeding value of processed grains. The in vitro gas production data closely parallel in vitro enzymatic digestion and animal performance with fattening cattle fed processed grains. Furthermore, the data suggests the validity

of measuring the maximal fermentation rate *in vitro* as an indicator of animal performance when fed processed grains.

OBSERVATIONS AND CONCLUSIONS

1. The in vitro gas production technique with a mixed suspension of isolated rumen microorganisms appears to offer a quick screening tool for estimating rate of digestion of processed grains.

2. The rate of in vitro fermentation is affected not only by processing but by specie and variety of grain.

3. Rate of gas production from barley preparations exceeded that from equivalent milo samples. This parallels results of animal digestion trials with milo and barley.

4. Increasing the flatness of the flake by rolling increased gas production indicating that flaking is extremely important in the processing of steam treated grains. Feeding trials have shown a flat flake is much more efficient in promoting gains than a poor flake.

5. Steam processed flakes and pressure cooked flakes gave similar gas production values indicating that the desirable effect on the grain was approximately the same by both processes.

6. Cooking under pressure up to 5.6 kg. per cm.^{2} without flaking significantly increased rate of gas production. However, additional increases in fermentation rates could be obtained by flaking after cooking at 5.6 kg. per cm.^{2}

7. Higher gas production was obtained by pressure cooking at 4.2 kg. per cm.^{2} or higher and flaking than with steam processed grains. However, increased processing costs and possible protein damage at these pressures may limit the treatment of grains at higher pressures.

8. The treatment of grains by conventional autoclaving caused a marked reduction in rate of in vitro gas production possibly due to the heat moisture treatment inducing a rearrangement in the molecular lattice with a greater degree of associative bonding within the starch granule.

9. It appears that various other physical processes such as popping, boiling, etc. can alter the starch granule so that the rate of fermentation is increased.

10. The rate of gas production from isolated starch appeared to be greater than from native starch sources. Isolated amylose fermented at a slower rate than amylopectin fraction as similarly observed with these starch fractions in their native state.

11. Gas production values were highly correlated with dry matter disappearance and therefore are an effective aid for estimating the in vitro fermentation rate of grains.

12. Gelatinization up to approximately 30 to 40 percent was associated with increasing gas production. Gelatinization over 40 percent with processed milo appeared not to increase gas production.

13. All results suggest that a well formed flake is necessary after the grain has been moist-heat treated.

14. The in vitro gas production data has been closely correlated with in vivo performance of steers when evaluating the influence of grain processing factors and suggests that this in vitro technique can be used as a successful guide in predicting the relative value of processing methods and feeding value of the grain for cattle.

15. The data on the rate and total in vitro gas production with processed grains closely parallel in vitro starch digestion with pancreatin, thereby indicating that an enzymatic system is being dealt with and one of the determinants of digestion rate of grain starch depends on the rate of enzymatic attack on the starch granule.

APPENDIX

APPENDIX

Table 20. Influence of temperature, pressure, duration of cooking and degree of flaking of grain on in vitro gas production.

Grain	Processing Method	Temperature (Degrees)	Pressure (kg./cm.^{2})	Time (Minutes)	Flatness of Flake	Gelatinization %	Ml. Gas[a]
Milo	P.C.[b]	200	4.2	6	Not flaked	25	74.9
	P.C.	145	4.2	6	Poor flaked	50	96.8
	P.C.	145	4.2	1	Medium flake	50-60	89.9
	Steam processed	210	1.4	20	Medium flake	15-25	93.7
Barley	P.C.	200	2.8	1	Medium flake	25-30	99.1
Barley[c]	Untreated				Not flaked	10	41.8
	Steam processed				Poor flake	10-15	52.6
	Steam processed				Medium flake	25-30	66.7
	Steam processed				Medium-good flake	25-30	62.7

Table 20--Continued.

Steam processed	Good flake	75	67.7
Steam processed	Flat flake	75	72.9
Steam processed	Very flat flake	75+	79.2

[a]Ml gas produced/gram dry matter incubated with grain inoculum after three hours incubation.

[b]Pressure cooked.

[c]Barley values determined on latter in vitro trial: absolute values are not directly comparable with pressure cooked milo and barley above.

APPENDIX

Table 21. Analysis of variance for milo flake experiment, as shown in Table 9.

Source	df	SS	MS
Runs	2	23.72	11.86 *
Treat.	5	317.36	63.47 **
RXT	10	19.63	1.96 NS
Error	18	1.33	.07
Total	35	362.04	

*, ** Significant at the .05 and .01 levels, respectively.

C V = 10.92 $S\bar{x}$ = .572

APPENDIX

Table 22. Analysis of variance for species and varieties, gas production after six hours incubation, as shown in Table 6.

Source	df	SS	MS
Runs	3	5.94	1.98 **
Grain	11	93.45	8.50 **
Error	33	4.85	.15
Total	47	104.34	

** Significant at the .01 level.

C V = 9.14 $S\bar{x}$ = .192

Table 23. Analysis of variance for effect of autoclaving milo, as shown in Table 16.

Source	df	SS	MS
Runs	2	111.24	55.62 ***
Treatment	12	200.15	16.68 ***
Error	24	12.67	.53
Total	38	324.06	

*** Significant at the .005 level.

C V = 5.94 $S\bar{x}$ = .420

APPENDIX

Table 24. Analysis of variance for steam processing milo and barley, as shown in Table 8.

Source	df	SS	MS
Runs	2	10.36	5.18
Treatment	3	163.75	54.58 **
Error	6	7.81	1.30
Total	11	181.92	

** Significant at the .01 level.

C V = 5.72 $S\bar{x}$ = .658

Table 25. Analysis of variance for steam processed and pressure cooked milo, as shown in Table 14.

Source	df	SS	MS
Runs	2	9.53	4.77
Treatment	2	106.11	53.06 *
Error	4	17.68	4.42
Total	8	133.32	

* Significant at the .05 level.

C V = 9.53 $S\bar{x}$ = 1.214

APPENDIX

Table 26. Analysis of variance data for steam processed milo and barley and steam processed and pressure cooked milo trials.

	Replication Means		
	R_1	R_2	R_3
Steam processed milo and barley exp.	18.70	20.13	20.95[a]
Steam processed, pressure cooked milo trial	20.63	22.50	23.03[a]

[a]Means underscored by the same line are not significantly different by Duncan's Multiple Range Test at .05 level of probability.

APPENDIX

Table 27. Coefficients of determination and correlation coefficients of percent dry matter disappearance (X) and gas production (Y) as shown in Table 7 for several species and varieties of grain, Tables 6, 11, 13 and 29 for processed grains and Table 11 for processed barley, only.

Correlations on	Number	Corrected Sum Squares			r^2	r
		X^2	XY	Y^2		
Untreated grains[a]	36	2,157.2	381.4	85.5	.788	.89
Treated grains[b]	26	479.6	1,663.2	6,535.4	.882	.94
Processed barley[b]	6	71.7	239.6	847.0	.946	.97

[a]Determined after a six hour incubation with roughage inoculum.

[b]Determined after a three hour incubation with grain inoculum.

APPENDIX

Table 28. Variation in gas production due to steer and ration differences.[a]

Item	RATION		
	BARLEY		MILO
	Steer A	Steer B	Steer C
	ml.[b]	ml.[b]	ml.[b]
Ground milo	55.7	66.8	79.9
	56.8	59.1	72.2
	44.9	67.2	68.4
	63.4	62.6	73.5
Steer and ration $\bar{x}$	55.2	63.9	73.5

[a]Values reported represent daily sampling variation.

[b]Ground milo incubated for three hours with the indicated inoculum sources.

APPENDIX

Table 29. In vitro evaluation of grain: Dry matter disappearance and gas production data.

Grain	Treatment	Ml.gas/gm. D. M. Inc.[a]	D. M. D.[b]
Milo	Untreated	25.4	13.9
Milo	Steam processed (regular flake)	42.9	18.3
Milo	Steam processed (flat flake)	46.0	20.1
Milo	Steam processed (very flat flake)	51.5	20.7
Milo	Steam flaked	41.9	18.1
Corn	Steam flaked	37.5	15.0

[a]Incubated for three hours with grain inoculum.

[b]Dry matter disappearance values.

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