INFLUENCE OF GRAIN PROCESSING ON NITROGEN SOLUBILITY AND *IN VITRO* ENZYMATIC STARCH DIGESTION OF BARLEY AND MILO

by

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ABSTRACT

Initial starch content of various grains was determined by hydrolyzing grain samples in 10% HCl (modified A. O. A. C. procedure). In vitro starch digestion was determined by incubating ground samples (80 mesh) with an amylolytic enzyme solution for 30 min. In these studies pancreatic enzymes of both bovine and porcine origin were used. The Nelson-Somogyi procedure was used to determine the reducing sugars resulting from the acid hydrolysis and the maltose released from the enzyme digestion. Nitrogen solubility was determined in artificial saliva and autoclaved rumen fluid. The solvents were adjusted to pH 6.0 before using. The average per cent starch digestion and total soluble nitrogen of barley were significantly higher than milo. Steam processing or pressure cooking lowered the nitrogen solubility in both grains. Steaming or pressure cooking at 20 psi for 1 min. (without rolling) significantly decreased in vitro starch digestibility of milo and barley. Flaking the grains after steaming or pressure cooking increased ($P < .05$) in vitro starch digestion of both grains. Digestibility increased with increased degree of rolling (flake flatness). Similar results were obtained with both enzyme sources. Maximum increase in digestion over the untreated grains was 173% for steam processed flaked milo and 126% for steam processed flaked barley.

INTRODUCTION

During recent years commercial feedlots capable of handling many thousands of cattle per year have come into existance. This reflects that cattle feeding is a growing and highly competitive business. A total of 346,000 head were on feed in Arizona, January 1, 1966; approximately 650,000 head were fed during 1965. In 1965 gross sales from Arizona feedlots were $160,000,000 and cattle are Arizona's leading source of agricultural income (6).

Feedlot cattle have a high requirement for grain and today the common feedlot finishing ration will contain about 70-75% concentrate. As the grain portion of these finishing rations makes up the major part of the feed and represents the major total feed cost, methods of improving the nutritive value of these grains are of great concern to the cattle feeding industry. Any increase in efficiency from improving the utilization of these grains in a fattening ration could greatly enhance profits.

Milo and barley are the chief feed grains for fattening cattle in the Southwest and long ago most of the cattle feeders recognized the need to reduce the hard and flinty milo kernel to a smaller size by grinding or rolling in order to improve its utilization by cattle. Surveys have shown

that on a national average more than 60% of the commercial feed grain processors have roller mills (52).

Today new methods of processing grains become available as the older methods of grinding or cracking appear to be inadequate for present day performance and efficiency. These new methods have emphasized the importance of finding the best way to process the grain for fattening cattle. The method which holds the greatest promise is the moist-heat treatment of grains plus rolling or flaking. This can be accomplished by either steaming or pressure cooking the grains.

A series of feeding and digestion studies with cattle conducted at the Arizona Experiment Station has shown that the feeding value of barley is superior to milo. This is in contrast to the accepted feeding values listed in most nutrition textbooks for milo and barley. These trials suggested that the nitrogen free extract (NFE) and protein fractions of the milo were the factors limiting its nutritional value.

In the usual analysis of feedstuffs starch falls in the NFE portion. As the NFE in cereals is primarily starch, these studies suggested that the starch of milo might be less digestible than that of barley by rumen microorganisms and/or digestive enzymes.

The Arizona workers showed that steam processing and flaking milo significantly increased the digestibility of NFE, and hence the dry matter, but the protein digestibility of milo was not appreciably affected by steam processing and flaking. Since the NFE constitutes the major

portion of both grains the true digestibility of this fraction will be a major influence in the overall energy value of the grains. This increase in NFE digestibility of milo may be due to increased availability of the starch.

Since the grain protein in these finishing rations may constitute a significant part of the total ration protein, it becomes apparent that the digestibility of this fraction will be of great influence in the overall nutritive value of the grain. The lowered digestibility of milo protein as compared to barley was unexplainable in the Arizona studies.

Several studies conducted to evaluate the factors affecting the utilization of feed proteins by ruminants suggest that solubility of the feed protein could be one of the important factors determining its nutritional value to these animals.

The purpose of the studies reported in this thesis was to determine the susceptability of milo and barley starch to in vitro enzyme attack which may offer another means for explaining the apparent poor utilization of the NFE of milo and the effect of grain processing on the availability of barley and milo starches to the ruminant animal.

The other area studied was to determine the solubility of milo and barley proteins in different solvents as an aid in explaining the differences in protein digestibility. The preliminary investigations were concerned with finding the factors which affect the solubility of the feed proteins.

LITERATURE REVIEW

Carbohydrate Studies

In the usual proximate analysis of feedstuffs starch falls in the nitrogen free extract (NFE) fraction. This NFE portion, which constitutes mainly starch in grains, is determined by difference. The nutritive value of milo and barley in most standard nutrition textbooks shows milo to have more total digestible nutrients than barley (44, 46).

Morrison (44) lists milo grain with 70.7% NFE and digestion coefficient of 91%; for barley the corresponding values are 66.6% NFE and digestion coefficient of 92%. The metabolizable energy values for milo and barley with poultry, as given by Titus (64) are 1552 and 947 calories per pound of grain, respectively.

Contrary to the above values Schneider (57) listed separate digestibility values of both milo and barley for various species of livestock. In the case of cattle he gave a digestion coefficient of NFE for milo of 83% and 91% for barley.

On the basis of those first reported values the expected performance and feed efficiency for fattening cattle fed high milo rations should be higher than for those fed barley.

Bidwell, Bopst and Bowling (12) who conducted a study on the physical characteristics and chemical composition of the milo kernel reported 78.72% NFE, 68.52% starch and 3.95% pentosans. Lechuga (37) using a modification of the method of Dubois et al. for starch determination of milo and barley reported an average starch content of 80.46% and 71.85% for milo and barley, respectively.

Hale (22) reported that barley rations increased gains of cattle by 8% and improved feed efficiency by 11% over comparable milo rations. It appeared that the gain difference between the two grains was greatest at higher grain levels.

In digestion trials with cattle Saba (53) showed that the NFE and protein fractions of milo were less digestible than the corresponding fractions of barley. These differences could account for the lower performance and feed efficiency noted in the group feeding trials reported previously by Hale (22). Cadena (14), Saba (53) and Lechuga (37) suggested that the lowered NFE digestibility of the milo was apparently due to the lower digestibility of the starch of milo.

Cadena (14) conducted artificial rumen studies and reported that barley starch digestion was five times greater than that of milo during the first three hours of fermentation. At nine hours of fermentation the difference was only 2 1/2 times. Supporting his results with in vivo nylon bag studies he found a greater disappearance of barley dry matter

during the first eight hours. However between eight and 24 hours milo dry matter disappearance approached the barley dry matter disappearance.

Employing the artificial rumen technique Lechuga (37) showed that the per cent starch utilized by rumen microorganisms was 19.46 for milo, while the per cent starch utilized of barley was 37.24. Regardless of the grain source, starch utilization of barley was approximately twice that of milo during the five hour fermentation period.

Several excellent reviews are available on the subject of fermentation and metabolism in the ruminant animal (4, 10, 38). In general carbohydrates are hydrolyzed in the rumen to simple sugars which are further metabolized by the rumen microorganisms with production of volatile fatty acids which serve as a major energy source for the animal. Some carbohydrates may be incorporated into microbial organisms and some may also pass out of the rumen and be digested in the lower tract.

In sheep fed a hay ration, Heald (26) showed that the quantity of carbohydrates stored in the microorganisms and passing into the duodenum was nutritionally insignificant. Certain investigators (Baker et al. 8, Van Der Wath, 66) suggested that a large portion of the carbohydrates ultimately utilized by the ruminant animals were made available via a bacterial polysaccharide.

Indirect measurement of the rate of starch digestion posterior to the rumino-reticular cavity of calves by determining the blood reducing

sugar level (Larsen et al., 34), revealed very little starch breakdown in diets containing 52.2% corn or 56.0% starch. Weller and Gray (68) found that increasing the amount of starch fed to sheep from 3 to 150 gms produced only a small increase (1 to 8 gms) in starch passing through the abomasum. He also found that when starch taken from the abomasum was incubated from one to three hours with abomasal fluid, no change was noted, suggesting that starch is not degraded in the abomasum. Other work (Phillipson and McAnally, 48) indicated that very little carbohydrate in its ingested form reaches the lower gastrointestinal tract when sheep were fed 100 gm of glucose, starch or cellulose. However, the steers fed various levels of corn in the ration (20%, 40%, 60% and 80% of the ration) by Karr, Little and Mitchell (32) showed that the absolute amount of starch passing out of the rumen increased considerably at higher levels of corn intake. No literature could be found on the extent of starch digestion in the intestinal tract of adult ruminant.

Studying the digestive action on starch of extracts of the duodenal glands of domestic animals, Bergman, Dukes and Yarborough (11), found that the extracts from ox and sheep contain an active amylolytic enzyme. Huber et al. (28) reported that the levels of pancreatic amylase in young calves were quite variable, being lowest at one day, nearly tripling by eight days of age and remaining relatively constant thereafter.

Apparently considerable uncertainty exists as to the activity and actual quantitative production of amylase in the ruminant intestine.

Moreover the amount of starch, especially in high grain rations, that escape fermentation and passes into the small intestine in the ruminant animal is not yet known.

Conflicting evidence is obtained from a review of the literature on differences in susceptability of different starch sources to enzyme action. Many hypotheses have been put forward by various researchers. Many studies have dealt with the effect of granule size, viscosity, swelling power and gelatinization temperature on the properties of various starches.

Sandstedt et al. (55) showed a wide variation between the raw starches in their susceptability to the action of pancreatic alpha-amylase. He suggested that the resistance to digestion was not directly associated with amylose content, but presumably the genetic selection for high-amylose content has resulted in simultaneous selection for starch having altered properties with respect to susceptability to amylolytic attack.

Ackerson (2) working with one day old chicks and Borchers (13) working with rats confirmed that there was no relationship between the per cent of amylose in high-amylose corn and the in vivo digestibility of the starch.

Leach and Schoch (36) who determined solubilization of various granular starches with bacterial alpha-amylase found there was no preferential attack on either the linear or the branched starch fraction. They concluded that the enzyme susceptability of granular starches was

not influenced by the physiochemical consideration of micellar structure, internal molecular association or type of crystallinity; but it might be that the more susceptable granules possess pores or a coarse sponge-like structure with openings of sufficient size to admit the enzyme molecules.

Van Der Wath (66) studying the rate of bacterial disintegration of starch granules of different cereals concluded that there was a definite linear relationship between the log. of the time of digestion and the diameter of the granule.

Ball and Schwimmer (9) in an attempt to correlate the size of the starch granules with their susceptability to breakdown by enzymes reported there was no correlation between such susceptability with either granule size or phosphorus content of the raw starches studied. Stamberg and Bailey (60) working with the action of amylases on raw wheat starches showed that there was no correlation between the phosphorus content of the wheat starches and enzyme susceptability. They suggested that the observed variation in enzyme susceptability of the raw starch granules was due primarily to differences in granule structure.

The granule sizes for sorghum starch and corn are 15.0 microns and 9.2 microns respectively as reported by Schoch and Maywald (56). On the other hand the granule size of barley starch is 10-35 microns as reported by Stamberg and Bailey (60). Morrison (44) lists phosphorus contents of 0.28, 0.40 and 0.28 per cent for milo, barley and corn, respectively.

The effect of various moist-heat treatments on grain or starch utilization have been reported by several workers. Pope et al. (49) reported that steaming milo at 170-180°F for about two min., whether applied before or after rolling or grinding, appeared to have little if any beneficial effect. In another report Pope, Harper and Waller (50) showed that steam-heating ground milo at 180-270°F for about ten seconds so as to gelatinize the starch resulted in lowered feed intake and reduced daily gains of calves self-fed rations containing 55 per cent of the treated grain. Feed efficiency was not affected by heat treatment as compared to ground, unheated milo.

Salsbury, Hoeffer and Luecke (54) showed that application of moist-heat to corn starch brought about hydration of the starch and more rapid digestion by rumen microorganisms than was true for untreated starch.

Results from the Texas Experiment Station (51) indicated that milo ensiled when containing high levels of moisture produced equal steer gains to those fed dry milo. However feed efficiency was markedly improved with the ensiled milo.

With cattle fed milo rations Saba (53) reported an improvement in starch digestibility when the grain was cooked in an oil-jacket grain cooker at 180°F and five hours for the evening feed and 14 hours at the same temperature for the morning feed.

In several cattle trials conducted at the University of Arizona Experiment Station Hale et al. (25) reported that steam processing milo

at low-pressure high moisture steam for approximately 20 min. at 205-210°F and flaking improved gains by 10% and reduced feed requirement by 5%. This was attributed to the significantly improved digestibility of the dry matter, NFE and gross energy over dry rolling. Digestibility of NFE was increased from 69.2% to 78.5%. A similar comparison for steam-processed barley showed an increase in daily gain and feed intake, but there was no improvement in feed efficiency over dry rolling. Feedlot trials reported by Hale and Taylor (24) showed that short steaming for three to five minutes improved milo little if any over the conventional dry rolling process.

In nylon bag studies conducted to determine the significance of the flatness of the flake, Hale (23) showed a 22% greater dry matter disappearance from the extra flat than regular rolled milo. The poorly rolled steam-processed milo proved to be no better than the dry rolled milo.

Garrett, Lofgreen and Hull (19) reported that both daily weight gain and feed efficiency were favorably influenced by pressure cooking grains for 1.5 min. at 20 psi. However cooking the grains at 60 psi for 1.5 min. lowered feed intake compared to dry rolled. All grains, barley, milo and corn reacted similarly to the treatment. They suggested the possibility that steam pressure cooking of grain could be carried too far, that is there may be an optimum condition which should not be exceeded for maximum benefit.

Many studies have dealt with the effect of swelling power and gelatinization on the properties of starches. The term gelatinization is now appearing frequently in the literature of grain processing for fattening cattle.

Whistler and Paschall (69) define gelatinization as "the subjection of an aqueous suspension of starch to the influence of heat or appropriate chemicals which weakens the micellar network within the granule by disrupting hydrogen bonds. This permits further hydration and irreversible granule swelling, a process termed gelatinization." He suggested that the major factor that controls the swelling behavior and gelatinization of starch is the length and character of the micellar network within the granule which in turn is dependent upon the degree and kind of association.

Schoch and Maywald (56) have defined the gelatinization temperature as "the point at which the starch granules lose their polarization crosses when heated in a swelling medium." The various starches have different gelatinization temperatures which are fairly uniform within any given variety.

An excellent review was given by Leach, McCowen and Schoch (35) in an attempt to explain the theory of the structure of various starch granules and their differences in the pattern of swelling and solubility powers. They reported that milo starch showed an initial gelatinization then a period of restricted swelling and finally a second rapid swelling

when treated with a heat-moist treatment. This behavior was attributed to two sets of bonding forces which relax at different temperature levels.

The literature reviewed suggests that the NFE of milo is less digestible than that of barley and that steam processing and flaking milo significantly increased its NFE availability to the rumen microorganisms and/or the animals digestive enzymes. Differences in the structure of the starch granules of different grains may be the main factor affecting their susceptability to digestive enzymes and their different pattern of behavior to heat-moist treatment.

Nitrogen Solubility

Morrison (44) lists milo and barley with digestion coefficients for crude protein of 78% and 79%, respectively. Schneider (57) separated digestibility values of both milo and barley for various species of livestock. With cattle he gave coefficients of digestion for crude protein as 57% for milo and 75% for barley. In digestibility trials with cattle fed milo and barley rations, Cadena (14) reported 51% digestibility for milo crude protein and 64% for barley crude protein.

Observation of the above reveals a discrepancy between the three sets of values. Since the grain protein may constitute quantitively a great portion of the total crude protein in today's high concentrate rations for fattening cattle the true digestibility of this fraction will have a major influence in the overall protein value of the ration. Recent interest in the

processing of grains to improve performance and feed efficiency of fattening cattle make it essential to determine with digestion trials what nutritive fractions of the grains are affected by the specific processing methods.

In digestion trials with cattle fed rations containing 77% dry-rolled or steam-processed milo, Hale (23) reported that digestibility of crude protein was 49.6% for dry-rolled milo and 51.4% for flaked steam-processed milo.

Mehen (42) conducted digestion trials with yearling steers fed rations containing 77% milo which had been processed in various ways. The apparent crude protein digestibility was 58.7%, 55.5% and 53.3% for dry-rolled, steam-processed-flaked, and pressure cooked at 40 psi for one min. and flaked, respectively. In other digestion trials with cattle fed rations containing 85.6% dry-rolled or steam-processed barley the digestibility of the crude protein was 68.9% and 64.9%, respectively.

Husted (30) in digestion trials with cattle fed rations containing 77% steam-processed or dry-rolled milo reported 56.9% and 52.7% coefficients of digestibility for crude protein, respectively.

Keating et al. (33) conducted digestion trials with sheep that were fed rations containing 94.7% dry-rolled milo or 98.7% dry-rolled barley. These workers found a digestion coefficient of 70.0% for dry-rolled milo crude protein and 69.1% for dry-rolled barley. In other studies, rations containing 65% dry-rolled milo, 80% dry-rolled barley or 65%

cooked milo (milo cooked in an oil jacketed feed cooker at 180°F for five hours) were fed to cattle. The protein digestibility was 66.0%, 41.8% and 33.5% for barley, milo and cooked milo, respectively.

With all grain rations containing 91.7% dry-rolled milo or 98.7% dry-rolled barley fed to cattle, Saba (53) reported that the crude protein digestion coefficients were 55.2% and 77.1% for milo and barley, respectively. The crude protein digestibility was significantly lower (33.5%) in a ration containing 65% milo which had been cooked in an oil jacketed feed cooker at 180°F for five hours. The reason for the lowered digestibility of the milo protein as a result of cooking was not explained but these workers suggested that it might be related to lower solubility of the crude protein in the cooked material.

The solubility of various nitrogen sources has been implicated in nitrogen utilization in ruminants by various workers. When sheep were fed a partially purified diet to which zein contributed 94% of the total nitrogen, McDonald (41) found that approximately 40% of the zein was utilized by ruminal microorganisms for the synthesis of their own proteins. Zein was highly insoluble in aqueous solution and when a suspension was warmed to body temperature, it formed a glutenous, fibrous mass thus reducing the surface area available for enzymatic attack.

Nitrogen-balance experiments conducted by Chalmers, Cuthbertson and Synge (15) showed that casein which was highly soluble in aqueous

media gave a negative nitrogen-balance when fed to ewes, in spite of the fact that its apparent digestibility was 94%. It was found possible to process casein by the addition of N-sodium hydroxide that led to better utilization as shown by a positive nitrogen-balance. The processed casein gave less ammonia concentration in the rumen. The treatment of casein with alkali gave it a rubbery consistency which rendered it less soluble in the aqueous media. Their data indicated that the highly soluble nitrogen of casein was rapidly coverted to ammonia in the rumen and that ammonia was lost through ruminal absorption.

Solubility of milo and corn proteins in 0.02N sodium hydroxide was reported by Absher et al. (1) as 48% and 79%, respectively.

The nutritional significance of soluble nitrogen of several feed proteins for ruminants was investigated in its relationship to in vitro ammonia production, in vitro cellulose degradation, in vivo digestibility, nitrogen balance and lamb performance by Little, Burroughs and Woods (39). Solubility was determined in rumen fluid, distilled water and 0.02N sodium hydroxide. Solubility in any one solvent was not related to the solubility in other solvents, however the solubilities of nitrogen in 0.02N sodium hydroxide were higher than in the other two solvents. Nitrogen solubility of soybean oil meal in all three solvents was reduced by heating in a forced air oven at 110°C for 24 hours. Corn gluten meal, heated soybean meal and zein were particularly low in soluble nitrogen in autoclaved rumen fluid. Purified zein which was insoluble in distilled

water had a 3% solubility in rumen fluid and 99% in 0.02N sodium hydroxide. It was found that the highest correlation existed between per cent nitrogen soluble in rumen fluid and level of free ammonia at two hours and *in vitro* cellulose digestion. In contrast to the *in vitro* experiments, no differences were indicated in lamb performance between the regular and heated soybean oil meals. Lambs fed corn gluten meal gained significantly less than lambs fed soybean oil meal. However, no apparent differences in protein digestibility or nitrogen retention were detected when lambs were fed corn gluten meal or regular soybean meal (solubility in rumen fluid 13% vs. 19%) as protein sources in a semi-purified ration.

Whitelaw, Preston and Dawson (70) compared nitrogen retention in lambs of ground nut, heated ground nut and fish meal with the nitrogen solubility of these protein sources in 1 M sodium chloride solution. Nitrogen retention was inversely related to solubility. Fish meal, which was the least soluble, had the greatest nitrogen retention, followed by the heated ground nut and the untreated ground nut which had the greatest nitrogen solubility.

Tagari, Assarielli and Bondi (62) showed that the main factor determining the different efficiencies of processed and non-processed soybean meals fed to lambs was their solubilities in rumen fluid. Toasting soybean meal at 120°F for 15 min. reduced its solubility from 61% to 13% in rumen fluid. *In vitro* and *in vivo* experiments showed a striking decrease in the

amount of ammonia liberated by rumen microorganisms from heated soybean meal in comparison with the untreated.

In studying the effect of autoclaving at 121°C and 1.05 Kg./cm^2 of pressure or steaming at atmospheric pressure at 95°C on cottonseed meal, Danke et al. (16) showed that both autoclaving and steaming decreased nitrogen solubility in 0.02N sodium hydroxide. Cottonseed meal did not respond to the two types of heat treatment to the same degree. Autoclaving reduced solubility from 98.6% to 40% whereas steaming for 100 min. reduced solubility to only 73.5%.

Experiments conducted by Sherrod and Tillman (59) to determine the effect of varying the processing temperature upon the nutritive values of solvent extracted soybean or cottonseed meals for sheep showed that both autoclaving under 15 lb. steam pressure at 250°F for 45 min. or 90 min. reduced their solubilities in 0.02N sodium hydroxide. Autoclaving the meals resulted in decreased ruminal ammonia production, protein digestibility and urinary nitrogen losses, and an increase in nitrogen retention. In another separate trial, autoclaving cottonseed meal with the same procedure for 240 min. reduced nitrogen solubility drastically from 78.2% to 25.5% and resulted in reduced nitrogen retention (58).

In vitro studies were conducted by Henderickx and Martin (27) to determine the factors influencing the breakdown and conversion of feed proteins. They reported that the rate of breakdown was directly

proportional to the solubility of the tested proteins in a mineral mixture. Their studies of the influence of pH on the degradation of proteins revealed that maximum in vitro degradation was at pH 6.8.

It was possible that an explanation of those reported results can be obtained by considering the relationship between heat and protein denaturation. A general review of the properties of proteins was given by Fruton and Simmonds (18). They reported that heat or high pressure, or a combination of both, would cause protein denaturation. One of the distinctive consequences of denaturation of a protein is a disorganization of its internal structure and a decrease in solubility. Moreover, they reported that solubility of the proteins varies greatly with the pH of the solution and the salt concentration.

Mirsky and Pauling (43) in an attempt to present structure theory of protein denaturation suggested that as a result of increase in temperature or attack by reagents the side-chain hydrogen bonds are broken down leaving the protein molecule free to assume any one of a very large number of configurations and this is evident by the striking change in its solubility.

A survey of the literature suggests that physical properties of proteins may be indicative of their feeding values for ruminants. The proteins with a high solubility were degraded in the rumen to ammonia at a rate too rapid for efficient utilization. Several studies have shown that heat treatment of various protein sources has altered nitrogen

solubility and digestibility or retention in ruminants. Increasing use of steam-processed or pressure cooked milo and barley grains raises the question as to the effect of these heat treatments on the solubility and digestibility of the protein in these grains.

EXPERIMENTAL PROCEDURE

These procedures include studies involving nitrogen solubility and in vitro starch digestion of untreated grains as well as the influence of various processing treatments on these criteria.

The specifications of the moist-heat treatments of the milo and barley reported in these studies were as follows: the steam-processed (SP) grains were subjected to low pressure-high moisture steam in a steel tempering chamber for approximately 20 min. The temperature of the steamed grains reached 205-210°F. The grains were rolled after steaming at this temperature. In this thesis, steamed grains are differentiated from steam-processed grains in that steam processing denotes subsequent rolling of the grains after steaming. The moisture content of the milo was between 18% and 20% as it left the rollers while that of barley was approximately 15%.

The other method studied was by pressure cooking (PC) milo and barley at various pressures for one min. This one min. was the actual cooking time of the grains at the specific pressure. The time taken to reach the specified pressure for both grains was: 20 psi, 2 min.; 40 psi, 3 1/2 min.; and 60 psi, 5 min. In the case of milo which was pressure cooked at 80 psi it took 7 1/2 min. to reach the specified

pressure. The effect of pressure cooking was studied both with and without subsequent rolling of the grains following the pressure treatment.

In both moist-heat treatments the so called poor rolling was done by just cracking the grains through the rollers. For the extra flat flake the white starch portion was ivory instead of white and the flake was so thin as to be slightly perforated. This extra flat flaking would not be obtained when grains were pressure cooked at 60 psi or higher. The regular rolled product was intermediate in flake flatness where most of the starch material was visible.

Data were subjected to analysis of variance according to Steel and Torrie (61). Differences among three or more means were tested by Duncan's (17) multiple range test. Abbreviations appearing in analysis of variance tables are explained in the Appendix.

In order to evaluate the in vitro starch digestion of barley and milo by combining the different trials, preliminary investigations were run to determine the error encountered between trials. The results showed that the error between trials was negligible and that enzymatic digestion of a grain sample was highly reproducible between trials (See Appendix, Tables 28, 29 and 30).

Total Starch Determination

No satisfactory method is available for the determination of starch in feedstuffs which lends itself to rapid laboratory routine use. In the

proximate analysis of the feedstuffs, starch falls in the nitrogen-free extract (NFE) fraction. This NFE portion, which constitutes mainly starch in grains, is determined by difference. So in this adopted method most of the preliminary analysis was done to account for the errors in the precision of determining the total starch content of the tested grains.

The total starch content of the tested grains was determined using a modification of the A. O. A. C. method (5). Approximately 1 gram of grain sample was boiled for 2 1/2 hours with 200 ml. distilled water and 20 ml. hydrochloric acid (10% Hcl., sp. gr. 1.125) in a flask provided with a reflux condenser. The solution was then cooled. Eleven ml. of 45% sodium hydroxide was added to neutralize about 90% of the hydrochloric acid used. Other workers have shown that glucose is extremely sensitive to alkaline solutions. Noyes et al. (45) showed that alkaline and even neutral solutions gave low results with solutions of pure glucose, while slightly acid solutions gave nearly correct results. The solution was then filtered through a dry filter paper and made up to 500 ml. in a volumeteric flask.

The amount of glucose produced in the hydrolysis of the starch was determined by the Nelson-Somogyi method (47). One ml. of the solution was deproteinized by addition of 12 ml. of a zinc-hydroxide solution and 12 ml. of a barium sulphate solution. An aliquot of the zinc hydroxide-barium sulphate filtrate (0.5 ml.) was heated with an alkaline-copper

reagent for 30 min. in a boiling water bath, and after cooling treated with a special Arsenomolybdate color reagent. (The chemical composition of the latter two reagents are given in Hawk's Physiological Chemistry, 47.) The solution was diluted to 10 ml. with distilled water and the color developed was read with an Evelyn Colorimeter. The wave length of the colorimeter was set at 540 millimicrons. The blank, which was prepared in the same manner using 1 ml. of water in place of the sample, was set at 100 per cent transmission. The per cent transmission readings of the samples were converted to optical densities and the glucose content of the samples was calculated from a standard glucose curve. The total glucose was expressed as milligrams glucose produced per gram of sample (dry matter basis).

Calculations:

mg. glucose/0.5 ml. sample = optical density of sample (O.D.) x constant, k (k = mg. glucose/O.D. unit from standard curve)

mg. glucose/.5 ml. sample x 2 = mg. glucose/1 ml. filtrate

mg. glucose/1 ml. filtrate x 25 = mg. glucose/25 ml. filtrate = mg. glucose/1 ml. of original sample

mg. glucose/1 ml. of sample x 500 = mg. glucose/gm. of sample

Therefore: mg. glucose/gm. sample = O.D. x k x 2 x 25 x 500.

Preliminary investigations were run to determine the per cent recovery of glucose and the glucose-starch conversion factor with this method. Four samples of pure glucose were run with the same procedure

described for starch digestion. The results obtained are given in Table 1. On the average, 91.29% of the glucose was recovered by this method.

Table 1. Glucose recovery from acid hydrolysis.

	Mg. digested	Mg. recovered[1]
Trial 1	1000	905.9
Trial 2	1000	920.2 939.0
Trial 3	1000	904.5 892.5
Trial 4	1000	910.6 917.4
Average	1000	912.9

1. Average of two readings.

For the determination of the glucose-starch conversion factor, which was reported by A.O.A.C. as 0.9 (5), soluble starch, potato starch and corn starch were taken as controls and digested with the same outlined procedure. The results of the average conversion factor of glucose-starch found in this manner are presented in Table 2.

Table 2. Determination of glucose-starch conversion factor.[1]

Sample	Sample weight[2] mg.	Glucose[3] mg.	Conversion factor[4]
Soluble starch	877.2	947.3	0.926
Potato starch	882.8	952.3	0.927
Corn starch	919.4	992.9	0.926
Average			0.926

1. Average of four determinations.
2. Dry matter basis.
3. Mg. glucose determined from the standard curve divided by 0.9129, which is the recovery factor for glucose.
4. Mg. starch digested divided by mg. of glucose.

The data shows that 100 parts of glucose found with this procedure represent 92.6 parts of starch in the original sample. The conversion factor did not change with type of starch digested. For ease of calculation the mg. glucose determined from the standard curve was converted directly to starch equivalents by multiplying by 1.014.

Soluble Carbohydrate

The soluble carbohydrate in the tested grains was determined in a similar manner described by Huber, et al. (28). One gram of grain sample was mixed with 25 ml. water and the mixture was shaken with a

mechanical shaker for approximately two hours. A protein-free filtrate was prepared from the supernatent which contained soluble carbohydrate. This protein-free filtrate was analyzed for content of reducing sugar by the same method described for determination of total starch content. The total reducing sugars were expressed as milligrams glucose produced per gram of sample (dry matter basis).

Determination of Enzymatic Starch Digestion

In vitro starch digestion was determined using amylolytic activities of pancreatin (porcine origin) and lyophylized bovine pancreas.

Porcine pancreatin [1]

One hundred milligrams of the tested grain, which had been ground through a laboratory Wiley mill using an 80 mesh screen, were incubated with 0.4 ml. of enzyme solution and 5 ml. distilled water. Each 0.1 ml. of the enzyme solution contained 1 mg. of pancreatin to give an enzyme-starch ratio of 1:25 as specified. The tubes were incubated for 30 min. at 50°C in a water bath and slowly agitated with a mechanical shaker. The amplitude of shaking was adjusted to the minimum necessary to maintain the starch granules in suspension. After the 30 min. digestion the tubes were placed in a boiling water bath for 10 min. to inactivate the enzyme, and then filtered after centrifuging at 2500 rpm (1320 xg) for one min.

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1. Source: Nutritional Biochemical Corp., Cleveland 28, Ohio. Activity: 1 part will digest 25 parts starch. Pancreatin was of porcine origin.

Determination of maltose produced by amylase activity was measured according to the Nelson-Somogyi method outlined previously in starch determination. The amount of maltose produced was calculated from a maltose standard curve (Figure 1). Per cent-starch digestion was defined as milligrams of maltose per 100 mg. of grain starch (dry matter basis).

Calculations:

mg. maltose/0.5 ml. filtrate = optical density (O. D.) of sample x constant, k (k = mg. maltose/O. D. unit from standard curve)

mg. maltose/0.5 ml. filtrate x 2 = mg. maltose/1 ml. filtrate

mg. maltose/1 ml. filtrate x 20 = mg. maltose/20 ml. filtrate = mg. maltose/1 ml. original sample

mg. maltose/1 ml. of original sample x 5.4 ml. incubation media = total mg. maltose/100 mg. of sample

$$\text{Per cent-starch digestion} = \frac{\text{total mg. maltose/100 mg. sample}}{\text{100 mg. sample x \% starch (dry matter basis)}}$$

Bovine pancreas (lyophilized)[1]

For determination of bovine pancreatic amylase digestion on the tested grains, a modification of the method described by Huber et al. (29) was used. A homogenate was prepared by comminuting the tissue and 0.2% sodium chloride (1:8 dilution) in a Serval omnimixer (approximately

1. Source: Nutritional Biochemical Corp., Cleveland 28, Ohio. Ratio of fresh to dry weight is 4:1.

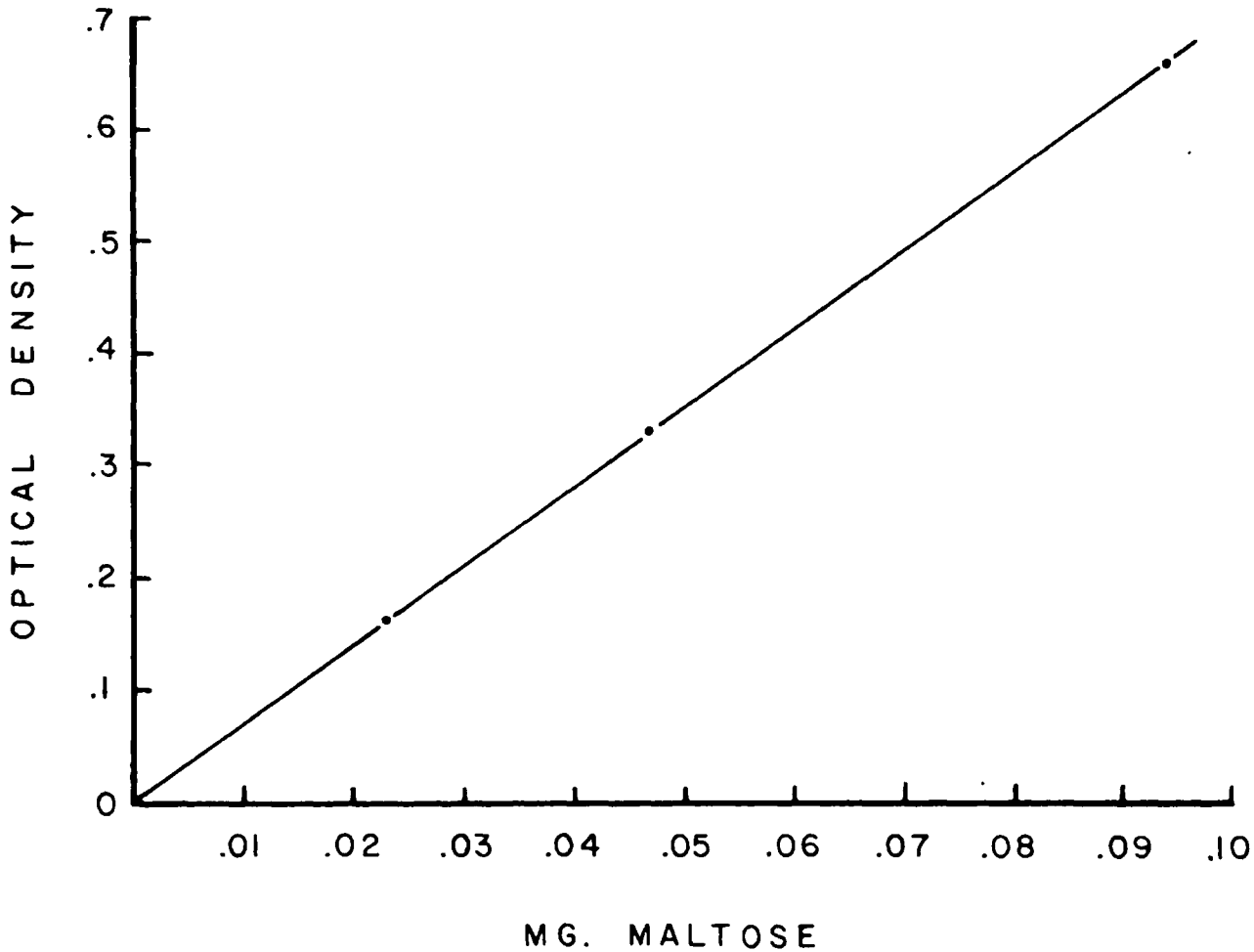

FIGURE 1. MALTOSE STANDARD CURVE.

1,400 rpm) for one min. Four ml. of tissue homogenate were incubated in a water bath for 30 min. at 40°C with 100 mg. of the sample suspended in 10 ml. of phosphate buffer, pH 6.8 (2.69 gm of Na_2HPO_4 and 6.38 gm KH_2PO_4/liter). Mg. of maltose produced per 100 mg. of the sample were calculated by the same procedure outlined for pancreatin.

Initial studies were conducted to determine the effect of pH, temperature and concentration of the solutions on activities of pancreatin and bovine pancreas. It was found that optimum starch digestion with pancreatin occurred when 5 ml. of distilled water as compared to the same amount of phosphate buffer (pH 6.8) and a temperature of 50°C were used. The optimum conditions of bovine pancreas activity were found to be 10 ml. phosphate buffer (pH 6.8) and temperature of 40°C.

Nitrogen Solubility

Trial 1

Initial studies were conducted to determine the effect of pH and salt concentration on the nitrogen solubility of various grains in artificial saliva and autoclaved rumen fluid. The pH of the solvents was adjusted in the range 4-12 using a pH meter by 1 N hydrochloric acid and 0.02 N sodium hydroxide. Concentrations of 0.5%, 1.0%, 2.0%, 3.0% and 4.0% (expressed as gm/100 ml. distilled water) of sodium chloride solutions were used as solvents to study the effect of the salt concentration on the nitrogen solubility. The soluble nitrogen content of milo, milo gluten

meal, cottonseed meal, barley and hegari was determined using a modification of the extraction procedure of Lyman, Chang and Couch (40). Approximately 5 grams of the feed (ground through 40 mesh screen) were placed in a 300 ml. Erlenmeyer flask with 150 ml. of the appropriate solvent. Several glass beads were added to curb foaming. The solutions were shaken on a platform shaker for approximately two hours and then centrifuged at 2400 rpm (1270 xg) for ten min. and the supernatant filtered through coarse filter paper. The soluble nitrogen in the supernatant fluid and the total nitrogen of the grains were determined by the Kjeldahl procedure (5). The soluble nitrogen was expressed as a percentage of total grain nitrogen.

The composition of the artificial saliva is shown in the Appendix (Table 31). The autoclaved rumen fluid was prepared by collecting rumen fluid before the morning feeding from a fistulated steer fed alfalfa hay. It was then strained through 16 layers of cheesecloth, poured into 40 ml. centrifuge tubes and centrifuged at 2400 rpm (1270 xg) for 15 min. The supernatant fluid was autoclaved at 15 psi for 20 min.

Trial 2

This trial was conducted to determine the salt concentration of rumen fluids from fistulated steers fed straight alfalfa hay, milo, barley, or a high concentrate rations. The composition of the high concentrate ration is listed in Table 3.

Table 3. Percentage composition of the high concentrate ration. [1]

Ground alfalfa	5.00
Cottonseed hulls	15.00
Milo	68.95
Cottonseed pellets	4.50
Molasses	5.00
Dicalcium phosphate	0.50
Urea	0.50
Salt	0.50
Ground limestone	0.05

1. Ten gm. Vitamin A-10-P added per cwt. of ration furnishing 1,000 I. U. Vitamin A/lb.

The rumen fluid samples were obtained three hours after the morning feeding. These were strained immediately after collection through 16 folds of cheesecloth. Duplicate 25 ml. test tubes of the fluid were taken from each rumen sample.

The salt concentration of the samples was determined by the direct indicating bridge procedure (65). The conductivity cell was rinsed and filled with the sample and the electrical conductivity of the solution was read from the bridge in millimhos (mmho) per centimeter at 25°C by balancing with the main dial. The salt concentration of the samples

were read directly from standard sodium chloride curve. Concentrations of sodium chloride of 0.5, 0.6, 0.7, 0.8, and 0.9 gm./100 ml. distilled water were prepared as standard solutions. The electrical conductivities of these various solutions, which are given in Table 4, were read and plotted in a graph (Figure 2).

Table 4. Electrical conductivity of sodium chloride solutions.

Sodium Chloride gm./100 ml. distilled water	Millimhos/cm
0.5	7.0
0.6	8.0
0.7	9.0
0.8	10.0
0.9	11.0

Trial 3

This trial was conducted to determine the soluble nitrogen content of milo, barley, and hegari in artificial saliva and autoclaved rumen fluid. Both solvents were adjusted to pH 6.0 before using since rumen pH varies and steers fed high barley or milo rations have a rumen pH of approximately 6.0 three hours after feeding.

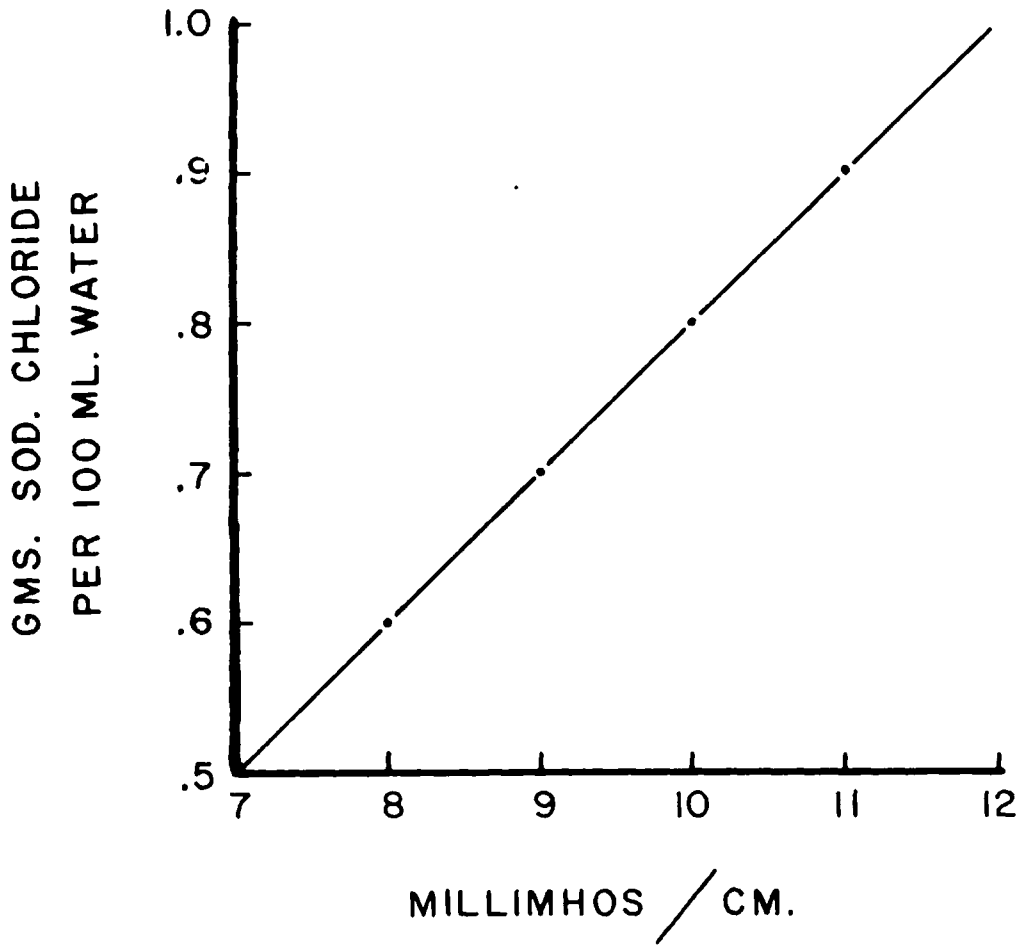

FIGURE 2. CONCENTRATION OF SODIUM CHLORIDE SOLUTIONS IN PER CENT AS RELATED TO ELECTRICAL CONDUCTIVITY.

To determine the effect of different grain treatments on the solubility of the nitrogen, the nitrogen solubility of milo, barley and hegari samples which had been processed with different treatments was determined in artificial saliva and autoclaved rumen fluid. The steam processed or pressure cooked grains were prepared in a similar manner to that described previously. Soluble nitrogen and total nitrogen were determined by the same procedure outlined in Trial 1.

RESULTS AND DISCUSSION

In the routine analysis of feedstuffs the nitrogen free extract (NFE) which comprises the sugars and starches in grains is determined by difference. It is represented by the figure obtained when the sum of moisture, ash, crude protein, ether extract and crude fiber of a feed is substracted from 100. No satisfactory method of starch determination is available which lends itself to rapid routine use.

The results presented in Table 5 represent a comparison study of two samples of milo and barley between the per cent NFE found with the usual proximate analysis procedure and the per cent starch determined by the method outlined previously. The average per cent NFE of the two milo samples was 7 percentage units higher than the average per cent starch determined by this method, while it was only 4.4 percentage units higher in the case of barley. Since NFE was determined by difference instead of directly, it includes the cumulative errors of the other determinations and thus is not an exact value. Further the NFE in these grains might include small amounts of other complex polysaccharides which were by no means completely hydrolyzed in the starch determinations. Of course, possible errors in the starch determination could also be involved in these differences. These factors may account for those observed differences in the two procedures.

Table 5. Proximate analysis of milo and barley.[1]

Sample	Crude Protein %	Ether Extract %	Crude Fiber %	Ash %	NFE %	Starch %
Wilcox milo	10.36	2.75	2.71	1.69	82.49	75.49
Texas milo	10.83	3.21	2.83	1.86	81.27	74.29
Davis barley	10.62	1.52	7.77	2.78	77.31	72.05
Barley	9.97	1.48	7.31	2.81	78.43	74.97

1. All values are on dry matter basis and represent an average of duplicate determinations.

Soluble Carbohydrates

Preliminary investigations were run to determine the amount of soluble sugars in barley and milo and the effect of grain processing on them. Some workers (21) have suggested that these soluble sugars may have an important role in the utilization of steam-processed grains by fattening cattle. Results presented in Table 6 revealed that these soluble sugars were really of very minor importance when considering the total carbohydrate in these grains. Milo contained 1.0% soluble sugars while barley contained only 0.6%. Steam-processing the grains decreased the per cent soluble sugars in both grains to approximately 0.3%.

Table 6. Determination of soluble sugars in milo and barley.

Sample	Number of Duplicate Samples	Percent Soluble Sugars[1]
Milo		
Untreated	2	1.06
Dry rolled	1	1.02
Steam-processed, regular rolled	1	0.32
Pressure cooked (60 psi, 1 min.) rolled	1	0.55
Barley		
Untreated	1	0.62
Dry rolled	1	0.68
Steam-processed, regular rolled	1	0.32

1. Dry matter basis.

In Vitro Starch Digestion of Various Grains by Porcine Pancreatin

Digestibility of untreated grains

The per cent starch in various samples of milo and barley were determined as outlined in the experimental procedure and are summarized in Table 7. The eleven milo samples averaged 77.7% starch on a dry matter basis while the eight barley samples averaged 73.1%. The difference in starch content between milo and barley could be attributed to the higher fiber content of barley compared to milo.

Table 7. Average per cent starch digestion of milo and barley by porcine pancreatin. [1]

Sample	Number of Duplicate Samples	Per cent Starch in Grain Sample[2]	Per cent Digestion
Milo	11	77.67 ± 2.19	16.68 ± 1.25
Barley	8	73.07 ± 2.28	21.84 ± 1.61

1. Values are given with standard error of the mean.

2. Dry matter basis.

Analysis of Variance

Source of Variation	df	SS	MS	F test
Between grains	1	269.99	269.99	1111.07[1]
Samples/grains	17	84.45	4.97	20.45[1]
Within samples	19	4.61	.243	
Total	37	359.05		

1. $P < .01$

Per cent starch digestion of the eleven milo samples and eight barley samples indicated that on the average 31% more barley starch was digested ($P < .01$) than milo starch by porcine pancreatin (Table 7). There was a variety difference in per cent starch content and

susceptability to in vitro enzyme digestion in both grains. Detailed results of per cent starch content and per cent digestion for all milo and barley samples are shown in the Appendix (Tables 32, 33).

These findings may explain the in vivo results of Hale (22) who found that barley rations increased gains of cattle by 8% and improved feed efficiency by 11% over comparable milo rations. In digestion trials with cattle Saba (53) showed that NFE and protein fractions of milo were less digestible than the corresponding fractions of barley. Employing the artificial rumen technique Cadena (14) and Lechuga (37) reported that barley starch was digested more than that of milo by the rumen microorganisms.

The comparative studies on the starch digestion of various untreated grains presented in Table 8 indicate that barley starch is more susceptable to enzyme attack than Texas milo, high amylopectin milo, corn, and high amylose or high amylopectin corn.

The digestion rate of the starch from corn closely paralleled that of Texas milo and this agrees with the results found by Cadena (14) using the artificial rumen technique. The per cent starch digestion of Texas milo was 8.8% greater than ($P < .05$) high amylopectin milo. Similarly the increase in starch digestion of corn ($P < .05$) was 8.4% and 11.1% greater than the high amylopectin and high amylose corns, respectively. Starch digestion was similar for high amylopectin corn and milo, and high amylose corn.

Table 8. Comparative per cent starch digestion by porcine pancreatin of various untreated grains.[1,2]

Sample	Per cent Starch in Grain Sample[3]	Per cent Starch Digestion
Barley	74.84	22.23[a]
Davis Barley	72.05	21.64[a]
Texas Milo	73.31	18.10[b]
High amylopectin milo[4]	74.44	16.63[c]
Corn	71.99	17.84[b]
High amylopectin corn[5]	74.18	16.46[c]
High amylose corn[5]	71.82	16.06[c]

1. Values represent an average of duplicate determinations.
2. Values with same superscript are not significantly different $P < .05$
3. Dry matter basis.
4. Obtained from Lindsey Grain Co., Lubbock, Texas.
5. Obtained from American Maize Products Co., Watseka, Ill.

Analysis of Variance

Source of Variation	df	SS	MS	F test
Treatment	6	75.82	12.64	68.32[1]
Error	7	1.30	.185	
Total	13	95.65		

	2	3	4	5	6	7
SSR	3.03	3.18	3.27	3.33	3.37	3.39
LSR	0.92	0.97	0.99	1.01	1.02	1.03

1. $P < .01$

The difference in the extent of enzyme digestion of those various starches suggest that there may be differences in the structure of the various starch granules. These results are in close agreement with Leach and Schoch (36) who determined solubilization of various granular starches with Bacterial Alpha-amylase (24 hrs., 50°C, 0.5% enzyme). The per cent solubilization of the various starches were: sorghum, 50.2%; corn, 49.5%; and high amylose corn, 14.0%. Borchers (13) working with rats, found that high amylose corn starch had a digestibility of 66% to 77%, whereas normal corn was 95% digested. Sandstedt et al. (55) also demonstrated that a sorghum containing 5% amylose was digested to a greater extent with pancreatic enzyme than a sorghum containing 25% amylose.

Microscopic examination of the disintegration of two varieties of pea starches, one of high amylose content (75%) and the other of low amylose content (25%) revealed that the high amylose starch was practically unaffected whereas the other starch disintegrated quite rapidly under the action of alpha-amylase plus maltase (Balls and Schwimmer, 9).

Various workers (9, 60, 66) have indicated that the enzyme susceptability of different granular starches was not influenced by their granule size, phosphorus content or swelling behaviour. The answer may lie in the suggestions of both Leach and Schoch (36) and Sandstedt et al. (55) that the more susceptable granules possess pores

or coarse sponge-like structures with openings of sufficient size, which must be characteristic of the particular starch species, to admit the enzyme molecules. Another suggestion is that the differences are in the bonding between the starch molecules and possibly also in anomalous linkages within the molecules.

The possibility that the ratio of amylopectin to amylose, which is unknown in these grains, is a factor in the high susceptability of barley starch should not be overlooked. At present the reasons explaining the differences in susceptability of various starches to enzyme attack are still obscure.

Influence of steaming and pressure cooking without rolling

The results of steaming and pressure cooking milo and barley without rolling on starch digestion are presented in Tables 9 and 10. Pressure cooking at 20 psi or steaming significantly ($P < .05$) decreased *in vitro* starch digestion of both grains compared to the untreated grains. Pressure cooking at 40 psi still decreased significantly ($P < .05$) the barley starch digestion while starch digestion for the milo subjected to 40 psi for 1 min. was similar to the untreated milo. There was an increase in per cent starch digestion of 37% and 78% over the untreated milo with pressure cooking (without rolling) at 60 psi and 80 psi, respectively ($P < .05$) while there was 45.4% increase in starch digestion of barley by pressure cooking at 60 psi.

Table 9. Per cent starch digestion of untreated, steamed and pressure cooked milo by porcine pancreatin.[1, 2, 3]

Sample	Per cent Starch Digestion
Untreated	16.17[a]
Steamed	12.41[b]
Pressure cooked at 20 psi	13.39[b]
Pressure cooked at 40 psi	15.53[a]
Pressure cooked at 60 psi	22.21[c]
Pressure cooked at 80 psi	28.84[d]

1. Grains were processed without rolling.
2. Average of duplicate determinations.
3. Means with same superscript letters are not significantly different ($P < .05$).

Analysis of Variance

Source of Variation	df	SS	MS	F test
Treatments	5	394.11	78.82	151.57[1]
Error	6	3.15	.52	
Total	11	397.26		

	2	3	4	5	6
SSR	3.46	3.58	3.64	3.68	3.68
LSR	1.81	1.87	1.90	1.92	1.92

1. $P < .01$

Table 10. Per cent starch digestion of untreated, steamed and pressure cooked barley by procine pancreatin.[1, 2, 3]

Sample	Per cent Starch Digestion
Untreated	21.42[a]
Steamed	17.84[b]
Pressure cooked 20 psi	17.02[b]
Pressure cooked 40 psi	18.62[b]
Pressure cooked 60 psi	31.13[c]

1. Grains were processed without rolling.
2. Average of duplicate determinations.
3. Means with same superscript letters are not significantly different ($P < .05$).

Analysis of Variance

Source of Variation	df	SS	MS	F test
Treatments	4	261.55	65.39	89.57[1]
Error	5	5.38	0.73	
Total	9	266.93		

	2	3	4	5
SSR	3.64	3.74	3.79	3.83
LSR	2.67	2.74	2.78	2.81

1. $P < .01$.

The decrease in in vitro starch digestion of milo by steaming without rolling might explain the reported feeding trials by Oklahoma workers (50). Daily gains of calves fed ground milo which had been steam heated at 180-270°F for about 10 seconds were reduced as compared to calves receiving ground milo. Similar results were obtained at the Kansas Experiment Station (31).

It may be that steaming, or pressure cooking at 20 psi causes an increase in the strength of associative bonding within the starch granules making them less susceptable to enzyme attack.

Influence of steam processing and flaking

The results of in vitro starch digestion with steam-processing and flaking barley in three different trials are shown in Table 11. Steaming without rolling decreased ($P < .05$) starch digestion as in the previous trials. Poor rolling did not increase starch digestion over the untreated grain. However digestibility was 17% higher than the untreated barley. Per cent starch digestion of the steam processed regular flake was significantly different ($P < .05$) from the untreated grain. Flat flaking increased the per cent starch digestion significantly ($P < .05$) by an average of 39% over the regular rolled barley. The average per cent maximum increase in digestion was 126% over the untreated (51.18% vs. 22.69%).

The results shown in Table 12 for the per cent starch digestion of three samples of steam processed and flaked milo revealed nearly the

Table 11. Effect of flaking on steam processed barley.[1, 2, 3]

Samples	Untreated	Steamed	Steam Processed		
			Poor Flake	Regular Flake	Flat Flake
1	25.93	19.82	22.10	34.75	-
2	21.41	17.39	27.42	40.01	52.25
3	20.71	17.84	29.92	40.04	50.12
Average	22.69[a]	18.35[b]	26.48[c]	36.77[d]	51.18[e]

1. Values are average of duplicate determinations.
2. Means with same superscript letters are not significantly different ($P < .05$).
3. Values represent per cent starch digestion.

Analysis of Variance

Source of Variation	df	SS	MS	F test
Samples	2	210.66	105.33	9.78[1]
Treatment/samples	11	3214.18	292.20	27.13[1]
Duplicates/treatment	14	150.74	10.77	
Total	27	3575.58		

	2	3	4	5
SSR	2.92	3.09	3.15	3.22
LSR	3.07	3.24	3.31	3.38

1. $P < .01$.

Table 12. Effect of flaking on steam processed milo.[1, 2, 3]

Samples	Untreated	Steamed	Steam Processed		
			Poor Flake	Regular Flake	Flat Flake
1	15.39	10.46	12.59	28.34	37.04
2	15.80	10.66	14.72	32.74	48.95
3	16.79	13.55	15.92	32.83	36.89
Average	15.99[a]	11.55[b]	14.41[a, b]	31.30[c]	40.96[d]

1. Values are average of duplicate determinations.
2. Means with same superscript letters are not significantly different ($P < .05$).
3. Values represent per cent starch digestion.

Analysis of Variance

Source of Variation	df	SS	MS	F Test
Samples	2	73.88	36.94	2.17[1]
Treatment/samples	12	3797.82	316.48	18.62[2]
Duplicate/treatment	15	254.96	17.00	
Total	29	4126.66		

	2	3	4	5
SSR	2.92	3.09	3.15	3.22
LSR	3.80	4.02	4.10	4.19

1. Non-significant at .05 level of probability.
2. $P < .01$.

same pattern increase with the degree of flaking. The per cent of digestion of the flat flake was 31% greater than that of the regular rolled milo. The maximum per cent increase in starch digestion was 156% over the untreated grain.

The average starch digestion of steam processed regular flaked barley was 17% higher than that of milo, while the flat flaked barley was 25% greater than the steam processed flat flaked milo. It seems that steam processing and flaking affected the milo starch availability to enzyme attack more than the barley starch but it still does not make it equivalent to barley in this regard.

Summary of in vitro starch digestion of all trials with steam processed and various degrees of flaking milo and barley are presented in Table 13. These data show the same trend as discussed before that the rate of starch digestion was primarily governed by the degree of flaking after properly steaming the grains. Flat flaking resulted in an increase of 157% and 134% in starch digestion over the untreated milo and barley, respectively. Rolling the dry grain (without steaming) gave similar digestion to milo just cracked after processing.

The results of the comparison of per cent bushel weight of whole grain with various degrees of flaking of steam processed milo and barley are given in Table 14. These weight measurements were taken after the grains were air-dried overnight. Since it is difficult to define

Table 13. Summary of *in vitro* starch digestion of steam processed and flaked barley and milo by porcine pancreatin.[1, 2]

Treatment	Milo (Number of Duplicate Samples)	Milo % Starch Digestion	Barley (Number of Duplicate Samples)	Barley % Starch Digestion
Untreated	7	16.61	4	21.84
Steamed	5	11.68	3	18.35
Dry rolled	2	15.09	-	-
Steam processed: Just cracked	3	15.14	1	22.69
Poor flake	3	15.51	3	26.48
Regular flake	5	31.54	4	39.23
Flat flake	6	42.75	2	51.18
Maximum % increase in digestion over untreated		157		134

1. Values are average of duplicate determinations.

2. Average starch content for milo and barley were 77.67% and 73.07%, respectively.

Table 14. Comparsion of per cent bushel weight of whole grain with various degrees of flaking steam processed barley and milo[1]

Sample number	Milo			Barley		
	1	2	3	1	2	3
Untreated (whole)	100.0	100.0	100.0	100.0	100.0	100.0
Steam processed: poor flake	64.1	65.2	-	71.2	67.3	-
Regular flake	46.7	52.7	56.0	52.7	52.6	59.1
Flat flake	33.4	49.4	-	27.9	28.8	-

1. Values represent weight/volume of processed grains as a per cent of weight/volume of the untreated or whole grain.

the degree of flaking by visual aid it was thought that measurements of bushel weight may be a possible way to do that. It is apparent that the flaked grains are of much lower density than the original grains. Bushel weights of steam processed regular flaked milo and barley were approximately 50% of the original grains. Flat flaking reduced the per cent bushel weight of milo and barley to approximately 40% and 28%, respectively, as compared to the whole grains.

These findings suggest that the degree of flaking after properly steaming milo and barley governs their starch availability to enzyme attack. The degree of flaking may be more important for milo than barley. It appears that flaking is the key factor in the increased starch

digestibility of these grains. In fact, steaming without flaking is useless and even deterimental in the utilization of their starches. These studies confirm the nylon bag studies reported by Hale (23) where the flatness of the flake influenced the utilization and dry matter disappearance of steam processed milo. The fact that all grains were ground finely through an 80 mesh screen makes the surface area relatively unimportant in evaluating the different starch digestibilities due to flaking. These studies are also in agreement with the feedlot studies reported by Hale (23).

In digestion trials with cattle Husted (30) reported that the NFE digestion coefficients of dry rolled, steam processed regular rolled and steam processed poor rolled milo were 70.93%, 83.82% and 78.76%, respectively.

The significantly improved in vitro starch digestion with steam processing and flat flaking barley suggests an improved availability to the rumen microorganisms and/or the beef animal. This is in contrast to the findings of Hale (23) who reported that steam processing and rolling barley improved its physical characteristics which resulted in greater feed intake in fattening trials with beef cattle, but did not alter its nutritional quality. The latter effect appeared true in that feed efficiency was similar for cattle fed dry rolled and steam processed barley. In digestion and growth trials with cattle Mehen (42) found no significant difference between dry rolled and steam processed barley for

any of the coefficients, average daily gains, feed conversion or feed consumption.

Influence of pressure cooking and flaking

The effect of degree of flaking after pressure cooking milo at 20, 40, 60 and 80 psi for 1 min. is shown in Table 15. Pressure cooking at 20 psi and 40 psi without rolling decreased the starch digestibility over the untreated milo. As in previous trials pressure cooking at 60 psi and 80 psi without rolling increased *in vitro* starch digestion compared to the untreated grain. Pressure cooking at 20 psi prior to poor rolling gave the same result as pressure cooking at 20 psi without rolling. Poor rolling after pressure cooking at 60 psi and 80 psi significantly ($P < .05$) increased the starch digestibility. Per cent starch digestion of pressure cooked milo at 20, 40, and 60 psi and regular flaked was significantly ($P < .05$) different from the unflaked grain. Pressure cooking at 20, 40 and 60 psi with flat flaking did not greatly affect the rate of starch digestion over the regular rolled grain at their respective pressures. The maximum increase in starch digestion when milo was pressure cooked and flat flaked at 80 psi was 186% over the untreated (47.76% vs. 16.72%). Starch digestion of milo pressure cooked at 80 psi without rolling was equivalent to or similar to digestion of milo pressure cooked at 20 psi or 40 psi with flat flaking. There was no difference between pressure cooked milo at 20 psi with

Table 15. Effect of flaking on pressure cooked milo at 20, 40, 60 and 80 psi[1, 2] (Per cent starch digestion of untreated, 16.72)

Treatment[3]	Not Flaked	Poor Flake	Regular Flake	Flat Flake
20 psi	14.02[a]	14.17[a]	25.84[c]	28.46[d,e]
40 psi	14.68[a]	-	26.61[c,d]	29.61[d,e]
60 psi	22.59[b]	27.74[c,d,e]	42.37[g]	40.92[g]
80 psi	29.08[d,e]	37.95[f]	-	47.76[h]

1. Values are average of duplicate determinations.
2. Means with same superscript letters are not significantly different ($P < .05$).
3. Pressure cooked for 1 min. after reaching given pressure.

Analysis of Variance

Source of Variation	df	SS	MS	F test
Treatment	3	1405.20	468.40	18.92[1]
Processing/treatment	10	1175.91	117.59	4.75[2]
Samples/process	14	346.70	24.76	
Total	27	2927.81		

1. $P < .01$
2. $P < .05$

either regular or flat flaking and milo pressure cooked at 40 psi with the same degrees of flaking.

It seems that this will not explain the results reported by Garrett, Lofgreen and Hull (19) that both daily gain and feed efficiency with cattle were favorably influenced by pressure cooking and rolling milo, corn and barley for 1.5 min. at 20 psi over those which were cooked at 60 psi for 1.5 min. and rolled.

The results of pressure cooking barley at 20, 40, and 60 psi with the various degrees of flaking are seen in Table 16. Again pressure cooking at 20 and 40 psi without rolling decreased the starch digestibility of barley over the untreated grain. Pressure cooking at 60 psi without rolling increased *in vitro* enzymatic starch digestion compared to the untreated barley. The per cent starch digestion of the three different treatments of pressure cooked barley revealed the same pattern in increase with the degree of flaking. Flat flaking after pressure cooking at 20, 40 or 60 psi resulted in 14.2%, 9.3%, and 10.8% increase in starch digestibility over regular rolling at the same pressures, respectively. The per cent maximum increase in starch digestion was 123% over the untreated barley (48.30% vs. 21.68%). Pressure cooking at 60 psi without rolling was equivalent to pressure cooking at 20 psi and flat flaking. Starch digestibility of barley pressure cooked at 60 psi with regular or flat flaking was not increased over similarly flaked barley cooked at 40 psi. There was a significant difference ($P < .05$)

Table 16. Effect of flaking on pressure cooked barley at 20, 40 and 60 psi[1,2] (Per cent digestion of untreated, 21.68)

Treatment[3]	Not Rolled	Poor Flake	Regular Flake	Flat Flake
20 psi	17.27[a]	-	29.01[b]	33.80[c]
40 psi	18.57[a]	30.83[b]	43.55[e]	47.04[f]
60 psi	34.29[c]	38.37[d]	43.09[e]	48.30[f]

1. Average of duplicate determinations.
2. Means with same superscript letters are not significantly different ($P < .05$).
3. Pressure cooked for 1 min. after reaching given pressure.

Analysis of Variance

Source of Variation	df	MS	SS	F test
Treatment	2	1016.29	508.14	14.60[1]
Processing/treatment	8	1835.56	229.44	6.59[1]
Samples/processing	11	382.82	34.80	
Total	21	3234.67		

1. $P < .01$

between pressure cooking barley at 20 psi with regular or flat rolling and the other two treatments. The observed variations in enzyme susceptability between milo and barley may be due primarily to differences in the effect of the specific treatment on the degree of alteration of the structure of their starch granules. There are insufficient studies pertaining to the value of pressure cooking grains on feedlot performance. According to these studies milo had to be pressure cooked at 60 psi or higher for one minute and flaked to equal starch digestion of properly flaked steam processed milo.

These data leave no doubt that a well formed flake is necessary to obtain maximum starch digestion of steamed processed or pressure cooked milo or barley. This implies that the effect of either steaming or pressure cooking these grains to improve their utilization by fattening cattle could be lost by an unsatisfactory rolling process. So well established methods of quality control for moist-heat treatments are necessary to produce a consistent desirable product. It seems that one way of quality control is by measurement of per cent bushel weight of the resulting product.

In Vitro Starch Digestion of Milo and Barley by Bovine Pancreas

The per cent starch digestion of steam processed and rolled milo or barley with a homogenate of bovine pancreas versus porcine pancreatin is shown in Table 17. Steaming both milo and barley without rolling significantly ($P < .05$) decreased the starch digestion by bovine

Table 17. Comparison of per cent starch digestion of steam processed and flaked milo and barley by bovine pancreas and porcine pancreatin.[1, 2]

Sample	Enzyme Source	Untreated	Steamed	Steam processed	
				Regular Flake	Flat Flake
Milo	Porcine Pancreatin	16.79	13.55	32.83	48.95
	Bovine Pancreas	13.30[a]	7.19[c]	23.02[d]	36.65[f]
Barley	Porcine Pancreatin	21.45	17.39	40.01	52.25
	Bovine Pancreas	17.22[b]	10.10[c]	29.64[e]	41.18[g]

1. Average of duplicate determinations.

2. Means with same superscript letters are not significantly different ($P < .05$).

Analysis of Variance of Per Cent Starch Digestion by Bovine Pancreas

Source of Variation	df	SS	MS	F test
Treatments	7	2202.24	314.61	749.91[1]
Error	8	3.36	.42	
Total	15	2205.60		

	2	3	4	5	6	7	8
SSR	3.26	3.39	3.47	3.52	3.55	3.56	3.56
LSR	1.49	1.55	1.59	1.61	1.62	1.63	1.63

1. $P < .01$.

pancreas compared to the untreated grains. This decrease was much greater than porcine pancreatin on either milo or barley. Starch digestion of both grains by bovine pancreas was significantly increased ($P < .05$) by degree of flaking. Regular flaking increased the starch digestion of milo and barley by 73% and 71.1%, respectively, over the untreated grains. The starch digestibility with the steam-processed, flat flaked milo and barley was 176% and 136% higher than the untreated grains, respectively. Enzymatic starch digestion with the homogenate was very similar to that obtained by the solutions of pancreatin. However, the per cent starch digested by the homogenate was somewhat inferior to the latter.

There appears to be no doubt that the two methods of moist-heat treatment to barley and milo (steam processed or pressure cooked and flaking) aided in availability of their starches to in vitro enzyme attack. The degree of flaking of the hot moist grain is the key factor influencing this availability. This increase in starch digestion is probably due to the chemical change of the moist starch granules with heat and pressure. The above findings suggest that as more pressure is applied through the roller to these hot moist grains, the more the micellar network within the starch granule is weakened by disrupting the hydrogen bonds and hence increasing their susceptability to enzyme activity.

The many reports appearing on the value of moist-heat treatments to grains in cattle fattening rations could be explained through these

findings by the increased availability of their starches to rumen-microorganisms and/or digestive enzymes. Starches may be digested in two ways in the ruminant animal: some may pass unattacked or in the form of protozoal polysaccharides to the small intestine and undergo normal digestion as in the other monogasteric animals; while the major part is subjected to bacterial fermentation in the rumen. The proportion that is accounted for by each of these routes especially in high concentrate rations remains to be established. The presence of amylases in the bovine rumen fluid is well established (Baker, Nasr and Morrice 7, Templeton and Dyer, 63). Although pancreatic amylases were used in these studies, they should act in the same way as microbial amylases. Thus they would act similarly in revealing such patterns in starch digestion as well.

This laboratory method of studying the susceptability of various starches by enzymes may be of great help in evaluating the nutritive value of various grains and the effect of processing for the live animal. It seems that various grains behave differently to the various processing methods. Thus there is a need to specifically define the treatments applied to various grains so that results of experiments could be evaluated.

Nitrogen Solubility

Trial 1

Preliminary investigations were conducted in this trial to determine the effect of pH and salt concentration on nitrogen solubility. The results of the effect of pH on nitrogen solubility of various protein sources in artificial saliva are presented in Table 18 and graphed in Figure 3. The results indicate that the nitrogen solubility in artificial saliva of milo gluten meal, cottonseed meal, and steam processed regular flaked milo and barley were slightly affected by the change of pH from 4.0 to 7.0. From pH 10.0 to 12.0, the nitrogen solubility of milo gluten

Table 18. Effect of pH on per cent total soluble nitrogen of various protein sources in artificial saliva.[1]

Sample	pH Range						0.02N NaOH
	4.0	5.5	7.0	10.0	11.0	12.0	
Milo gluten meal	7.6	7.9	8.0	13.8	20.4	34.2	38.1
Cottonseed meal	5.6	7.1	8.5	10.3	12.8	40.6	34.4
SP, regular[2] flaked milo	4.2	4.4	4.2	5.2	7.1	11.4	-
SP, regular[2] flaked barley	11.1	11.6	11.8	17.8	20.2	46.6	-

1. Values are average of duplicate determinations.
2. Steam processed.

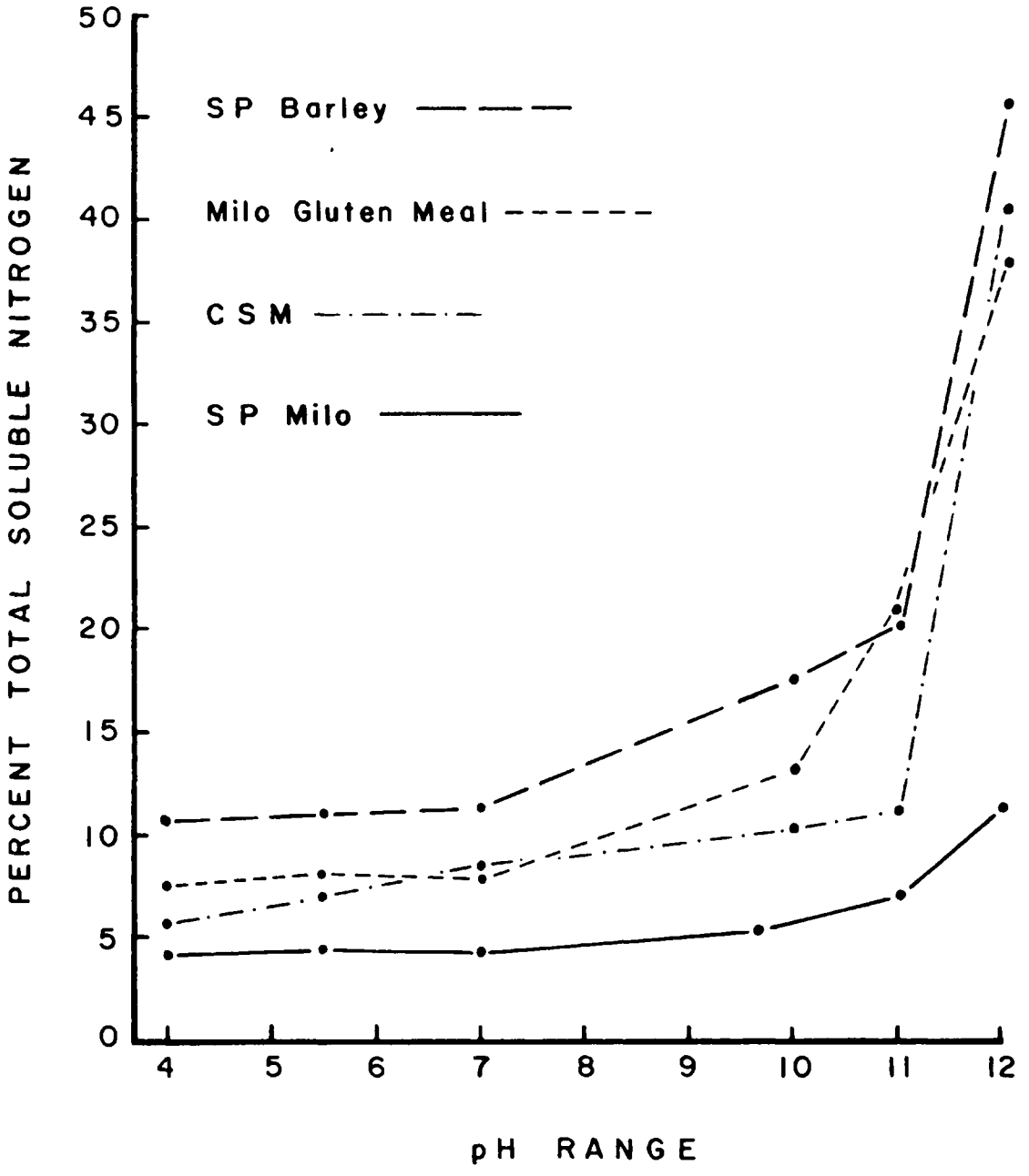

FIGURE 3. EFFECT OF pH ON PER CENT TOTAL SOLUBLE NITROGEN.

meal, cottonseed meal and steam processed barley was highly increased. At the same pH range steam processed milo showed a gradual increase in its nitrogen solubility. The maximum solubility was at pH 12 and represented increases of 350%, 625%, 171% and 320% in nitrogen solubility for milo gluten meal, cottonseed meal, and steam processed milo and barley, respectively.

The high nitrogen solubility of milo gluten meal and cottonseed meal in 0.02N sodium hydroxide which had a pH of 11.9 might explain the high percentages of total soluble nitrogen of various proteins reported by Little, Burroughs and Woods (39), Sherrod and Tillman (58) and Danke et al. (16). The results of these trials showed that the solubility of nitrogen increased steadily as the pH of the solution was increased. But the most pronounced increase was on the alkaline side especially at pH 10-12. Steers fed high barley or milo rations have a rumen pH of approximately 6.0 three hours after feeding. This was the reason why the pH of the studied solvents were usually adjusted to pH 6.0 before using. It would be meaningless to report nitrogen solubility in solvents of such high pH. In vitro studies conducted by Henderickx and Martin (27) to determine the optimum pH for protein degradation and microbial protein synthesis showed that maximum protein degradation was occuring at pH 6.5 while the optimum pH for protein synthesis was 7.0.

The results of nitrogen solubility of steam processed, regular flaked milo and barley in autoclaved rumen fluid adjusted to pH 5.0 to 7.0 are presented in Table 19. The nitrogen solubility in autoclaved rumen fluid showed a different pattern with change of pH. There was an increase of 63% and 60% in nitrogen solubility at pH 7.0 of steamed processed milo and barley, respectively, compared to pH 5.0. This pH range (5.0-7.0) appears more critical with autoclaved rumen fluid as the solvent than with the artificial saliva.

The results of the studies of the influence of sodium chloride concentration on the nitrogen solubility of steam processed, regular rolled milo and barley are presented in Table 20. The concentration of up to 3% sodium chloride did not affect the nitrogen solubility of steam processed milo compared to that in distilled water. But the 4% concentration significantly decreased ($P < .05$) nitrogen solubility of milo. The soluble nitrogen of steam processed barley was significantly ($P < .05$) increased in 0.5% sodium chloride as compared to distilled water. Its solubility was further increased ($P < .05$) in 1% and 2% sodium chloride solutions. The sodium chloride concentrations of 3% and 4% decreased ($P < .05$) the nitrogen solubility of steam processed barley compared to that in distilled water.

It is apparent that the solubility of milo and barley proteins differs greatly with salt concentration. However, the greatest change in

Table 19. Effect of pH on per cent total soluble nitrogen of steam processed (SP) regular flaked milo and barley in autoclaved rumen fluid.[1,2]

Sample	pH Range 5.0	5.5	6.0	6.5	7.0
SP, regular rolled milo	4.1[a]	4.1[a]	5.4[a,b]	5.5[b]	6.7[b]
SP, regular rolled barley	13.5[a]	15.5[b]	18.3[c]	20.9[d]	21.6[d]

1. Values are average of duplicate determinations.

2. Means on the same line bearing different superscript letters are significantly ($P < .05$) different.

Analysis of Variance

Source of Variation	df	Mean Squares Milo	Barley
Among treatments	4	3.02[1]	24.34[1]
Within treatments	5	1.35	.104

1. $P < .05$.

Table 20. Effect of different sodium chloride concentrations on per cent total soluble nitrogen of steam processed (SP) regular flaked milo and barley.[1,2]

Sample	Distilled Water	Salt Concentration[3]				
		0.5%	1%	2%	3%	4%
SP, regular flaked milo	4.1[a]	4.2[a]	4.4[a]	4.7[a]	4.0[a]	1.6[b]
SP, regular flaked barley	9.4[a]	12.4[b]	14.9[c]	16.1[c]	4.0[d]	2.7[e]

1. Values are average of duplicate determinations.

2. Means on the same line bearing different superscript letters are significantly ($P < .05$) different.

3. Grams per 100 ml. distilled water.

Analysis of Variance

Source of Variation	df	Mean Squares	
		Milo	Barley
Among treatments	5	2.69[1]	62.72[1]
Within treatments	6	0.138	.222

1. $P < .05$.

solubility occurred at the 3 to 4% sodium chloride levels. Fruton and Simmonds (18) explained this phenomena by the fact that the solubility of a protein is affected by the ionic strength of the solutions. At higher ionic strengths the solubility of a protein is decreased and the protein is said to be "salted out."

Trial 2

The previous trial showed that the salt concentration of a solution had an effect on the nitrogen solubility especially at the higher concentrations. This trial was conducted to determine the salt concentration of rumen fluid from steers fed different types of rations.

The results of the determination of the salt concentration of rumen fluid samples from steers fed different rations are presented in Table 21. The differences between the salt concentration of the four rumen fluid samples from those steers were not great, ranging from .63% to .72%. The salt concentration of the artificial saliva which was 1.1%, was higher than the rumen fluid samples. These differences in salt concentrations would appear to have little effect on the solubility of the nitrogen, since the data presented in Table 21 showed only fairly small differences in nitrogen solubility between .5% and 1% sodium chloride concentrations.

Trial 3

The per cent total soluble nitrogen of various samples of barley and milo in artificial saliva is presented in Table 22. The per cent total soluble nitrogen of barley was significantly ($P < .01$) higher than that

Table 21. Determination of salt concentrations of rumen fluid samples from steers fed different rations.[1, 2]

Trials	Milo		Barley		High Concentrate		Roughage Ration	
	Mmho cm	NaCl %	Mmho cm	NaCl %	Mmho cm	NaCl %	Mmho cm	NaCl %
1	8.0	.60	8.5	.65	10.0	.80	9.0	.70
2	8.5	.65	9.0	.70	8.5	.65	9.5	.75
3	8.0	.60	8.0	.60	9.0	.70	9.5	.75
4	9.0	.70	7.5	.55	8.5	.65	9.0	.70
Average		.64		.63		.70		.72

1. Values are average of two readings.

2. Concentration is equivalent to sodium chloride gm./100 ml. water.

Table 22. Per cent soluble nitrogen of milo and barley in artificial saliva (pH 6.0).[1]

Sample	Number of duplicate samples	Per cent crude protein dry matter basis	Per cent soluble nitrogen
Untreated milo	9	11.18 ± 1.15	9.3 ± 1.4[2]
Untreated barley	5	10.62 ± 0.73	25.1 ± 1.9[2]

1. Values are average of duplicate determinations.

2. $P < .01$.

Analysis of Variance

Source of Variation	df	SS	MS	F test
Between grains	1	1609.57	1609.57	8942.0[1]
Samples/grains	12	62.11	5.18	28.78[1]
Error	14	2.58	0.18	
Total	27	1674.26		

1. $P < .01$.

of milo. There was a 170% increase in soluble nitrogen of barley over that of milo.

The nitrogen solubility of barley and milo in autoclaved rumen fluid is shown in Table 23. The rumen fluid was obtained from a steer maintained on alfalfa hay. The per cent total soluble nitrogen of barley was 1.5 times greater ($P < .01$) than that of milo. Solubilities of barley and milo nitrogen in artificial saliva were closely related to those in the autoclaved rumen fluid.

The lower nitrogen solubility of milo crude protein in both artificial saliva and autoclaved rumen fluid may be one of the factors which contribute to the low crude protein digestibility of milo compared to that of barley in the digestion trials reported by Hale (23), Cadena (14) and Mehen (42). The work of Little, Burroughs and Woods (39) suggested that the nitrogen soluble in rumen fluid was closely related to in vitro ammonia production and cellulose digestion. According to McDonald (41) ammonia represents an important intermediate in the rumen digestion of dietary proteins. The amount of ammonia formed was dependent on the nature of that dietary protein. Free ammonia was rapidly formed from casein where as the insoluble protein, zein, was slowly attacked in the rumen. The extent of conversion of zein, which was water-insoluble, to microbial protein was about 40% in sheep fed a partially purified diet. With the artificial rumen technique, Annison (3) reported that casein,

Table 23. Per cent soluble nitrogen of milo and barley in autoclaved rumen fluid (pH 6.0).[1]

Sample	Number duplicate samples	Per cent crude protein dry matter basis	Per cent soluble nitrogen
Untreated milo	5	11.28 ± 1.06	10.9 ± 1.2[2]
Untreated barley	4	9.85 ± 0.70	27.6 ± 0.8[2]

1. Values are average of duplicate determinations.

2. $P < .01$.

Analysis of Variance

Source of Variation	df	SS	MS	F test
Between grains	1	4641.81	4641.81[1]	22103.8[1]
Samples/grains	7	24.83	3.55[1]	16.90[1]
Error	9	1.88	0.21	
Total	17	4668.52		

1. $P < .01$.

arachin and soybean protein, which were highly soluble in aqueous solution, were extensively degraded. But bovine albumin, wheat gluten and zein were only slightly degraded with the washed suspensions of rumen bacteria. Lyman, Chang and Couch (40) reported correlations between nitrogen solubility in 0.02N sodium hydroxide and nutritive value of cottonseed oil meal for chicks. In digestibility trials with lambs Woods et al. (71) reported that protein digestibility was lower in the cottonseed meal of low nitrogen solubility than that of high nitrogen solubility (soluble N, 30% vs. 66%). Their nitrogen solubilities were determined in 0.02 N sodium hydroxide.

The results of the effect of steaming without rolling or steam processing and flaking on the nitrogen solubility of barley, milo and hegari are presented in Table 24. Nitrogen solubility in artificial saliva of all grains was reduced by steaming without rolling. There was 45%, 26% and 29% diminution in the nitrogen solubility of milo, barley and hegari, respectively. The degree of flaking after steam processing did not alter the solubility as compared to the steamed grain (without rolling). The processing effect on the soluble nitrogen of milo in autoclaved rumen fluid (Table 25) showed the same trend. Steaming without rolling decreased the nitrogen solubility of milo by 58%. Steam processing with regular or flat flaking increased nitrogen solubility slightly over steaming without rolling or steam processing with a poor flake.

Table 24. Influence of steam processing and flaking on per cent total soluble nitrogen of milo, barley and hegari in artificial saliva (pH 6.0).[1]

Samples	Untreated	Steamed, without rolling	Steam Processed		
			Poor Flake	Regular Flake	Flat Flake
Milo	11.8 ± 0.1	6.5 ± 0.2	6.8 ± 0.2	6.8 ± 0.1	6.4 ± 0.1
Barley	28.1 ± 0.9	20.8 ± 0.9	19.9 ± 0.8	18.1 ± 0.1	21.5 ± 0.0
Hegari	8.6 ± 0.06	6.1 ± 0.1	5.0 ± 0.2	-	5.1 ± 0.1

1. Values are average of duplicate determinations.

Table 25. Influence of steam processing and flaking on per cent total soluble nitrogen of milo in autoclaved rumen fluid (pH 6.0).[1]

Samples	Untreated	Steamed, without rolling	Steam Processed		
			Poor Flake	Regular Flake	Flat Flake
Milo	11.5 ± 0.3	6.9 ± 0.3	6.9 ± 0.1	9.8 ± 0.1	8.0 ± 0.0

1. Values are average of duplicate determinations.

The results of the influence of pressure cooking on per cent total soluble nitrogen of milo and hegari in artificial saliva are presented in Table 26.

Table 26. Influence of pressure cooking on per cent total soluble nitrogen of milo and hegari in artificial saliva (pH 6.0).[1]

Sample	Untreated	60 psi, 1 min. without rolling	60 psi, 6 min. without rolling	60 psi, 6 min. regular flake
Milo	9.1 ± 0.3	7.3 ± 0.08	6.6 ± 0.0	7.0 ± 0.1
Hegari	8.6 ± .06	6.3 ± 0.05	6.0 ± 0.1	6.1 ± 0.3

1. Values are average of duplicate determinations.

Pressure cooking at 60 psi for 1 min. without rolling decreased the nitrogen solubility by 20% and 26% compared to the untreated milo and hegari, respectively. Pressure cooking at 60 psi for 6 min. slightly decreased the soluble nitrogen of milo but it had little effect on hegari. Pressure cooking at 60 psi for 6 min. and regular flaking slightly increased nitrogen solubility of milo but had no effect on the hegari as compared to pressure cooking at 60 psi for 1 min. without rolling.

A summary of the nitrogen solubility of milo or barley samples which had been processed with different treatments in artificial saliva and autoclaved rumen fluid is presented in Table 27. The nitrogen

Table 27. Summary of soluble nitrogen studies with untreated or processed milo and barley in artificial saliva and autoclaved rumen fluid (pH 6.0).[1]

Sample	Artificial Saliva		Autoclaved rumen fluid	
	No. duplicate samples	Per cent soluble nitrogen	No. duplicate samples	Per cent soluble nitrogen
Milo				
Untreated	9	9.3 ± 1.4	5	10.9 ± 1.2
Dry rolled	2	10.8 ± 0.8	1	13.6 ± 0.02
Steamed, without rolling	3	5.3 ± 0.9	2	6.2 ± 0.8
Steamed, regular flaked	5	5.4 ± 0.8	3	5.5 ± 0.7
Steamed, flat flaked	2	6.9 ± 0.02	2	7.3 ± 0.05
Barley				
Untreated	5	25.1 ± 1.9	4	27.9 ± 1.3
Dry rolled	2	22.3 ± 2.1	1	23.9 ± 1.6
Steamed, without rolling	3	20.1 ± 0.6	3	21.6 ± 0.6
Steamed, regular flaked	3	16.8 ± 3.4	2	17.7 ± 0.8
Steamed, flat flaked	1	21.5 ± 0.0	1	22.4 ± 0.3

1. Values are average of duplicate determinations.

solubility in artificial saliva was closely related to the solubility in autoclaved rumen fluid. Steaming (without rolling) milo or barley decreased the nitrogen solubility by 43% and 20% in artificial saliva and by 43% and 22% in autoclaved rumen fluid, respectively. The variable effect of steam processing and flaking on the nitrogen solubility of both milo and barley could not be attributed to the specific degree of flaking. It is interesting to note that dry rolling milo slightly increased solubility over the untreated grain. This data was not analyzed statistically due to variations in the number of the samples and the samples themselves.

The results indicate that steam processing or pressure cooking the grains decreased their nitrogen solubilities. This may be explained by the findings of Fruton and Simmonds (18) that heat or high pressure or a combination of both would cause denaturation. One of the distinctive consequences of this denaturation of a protein was a decrease in its nitrogen solubility. Mirsky and Pauling (43) reported that denaturing of native protein by heat or pressure was due to breaking of hydrogen bonds, leaving the protein molecules to rearrange in different configurations.

In the digestion trials in cattle reported by Hale (23), protein digestibility was not affected by steam processing and flaking milo. In the digestion trials conducted by Mehen (42), crude protein digestibility was 58.8%, 55.3%, and 53.3% for dry-rolled milo, steam processed or pressure cooked milo at 40 psi for 1 min., respectively. In other

digestion trials with cattle fed rations containing dry-rolled or steam processed barley the digestibility of the crude protein was 68.9% and 64.9%, respectively. It seems that in both these studies there was a tendency for lower protein digestibility with steam processing. Contrary to that Husted (30) in digestion trials with cattle fed steam-processed or dry-rolled milo reported 56.9% and 53.0% coefficients of digestibility for crude protein, respectively.

Several investigators have shown that proteins with high solubility will be degraded in the rumen to ammonia at rates too rapid for efficient utilization by ruminants. The beneficial effect of reducing the solubility of casein by heating was first recognized by Chalmers, Cuthbertson and Synge (15) and confirmed by Whitelaw, Preston and Dawson (70), who found considerable advantage in the use of heat-treated ground-nut meal in comparison with the untreated ground-nut. With the artificial rumen technique, Warner (67) showed that increasing degrees of heat treatments of ground-nut meals decreased the nitrogen solubility in both .05N sodium hydroxide and 5% sodium chloride solutions. This decrease in nitrogen solubility resulted in decreased susceptability to proteolysis by the washed suspensions of bacteria from the rumen of sheep. Little, Burroughs and Woods (39) found that lambs fed low quality roughage rations containing either regular or heated soybean at 7%, 10% or 13% protein levels performed equally well. Sherrod and Tillman (59) reported that both autoclaving or steaming soybean

and cottonseed meals reduced their nitrogen solubilities in 0.02N sodium hydroxide. The results of the reduced solubility were reduced ruminal ammonia, digestibility of their proteins, urinary nitrogen losses, and an increased nitrogen retention.

It seems that there is a critical ratio of soluble nitrogen to total nitrogen for efficient rumen function. Although other studies indicate that the protein digestibility was not affected largely by processing milo or barley, it might be that this reduced nitrogen solubility has an effect on nitrogen retention in ruminant animals. Unfortunately nitrogen balance studies with cattle comparing barley versus milo or the effect of grain processing have not been conducted in recent years.

SUMMARY

Laboratory studies were conducted to determine in vitro enzymatic starch digestion of milo and barley grains. Additional studies were conducted to determine factors affecting nitrogen solubility of these grains in artificial saliva and autoclaved rumen fluid. The pancreatic enzymes of porcine and bovine origin were used. The same techniques were used to determine the influence of various grain processing methods on both areas studied. The initial studies indicated that the soluble carbohydrates in barley and milo were 0.6% and 1.0%, respectively. Steam-processing and flaking decreased the per cent soluble carbohydrates to 0.3% in both grains. These low values suggest that soluble carbohydrates are of very minor importance in the utilization of these grains by steers.

Initial starch content of the grains was determined by hydrolyzing the grain samples in 10% HCl (modified A. O. A. C. procedure). In vitro starch digestion was determined by incubating ground samples of the grain (80 mesh) with the enzyme solution for 30 minutes. The Nelson-Somogyi procedure was used to determine the reducing sugars resulting from the acid hydrolysis and the maltose released by the enzyme digestion.

Nitrogen solubility was determined by shaking the respective grains in the appropriate solvents for 2 hours. These solvents were adjusted to pH 6.0 before using. Preliminary investigation showed that pH and salt concentration had a marked influence on nitrogen solubility. However, in the biological range of pH 5.0 to 7.0, solubility seemed to be quite stable.

The average per cent starch digestion of 8 samples of barley was significantly higher than the average of 11 milo samples (21.8% vs. 16.7%). Nitrogen solubility of barley in autoclaved rumen fluid was 27.6%; for milo, 10.9%. On the average barley contained 1.7 times more soluble nitrogen than milo in artificial saliva, and 1.5 times more in autoclaved rumen fluid. Solubility in both solvents was closely related.

Steaming or pressure cooking with or without flaking decreased ($P < .05$) the nitrogen solubility of milo, barley and hegari.

Steaming or pressure cooking at 20 psi for 1 minute (without rolling) decreased in vitro starch digestion of milo and barley compared to the untreated grains. Starch digestion was increased 37% and 45% over the untreated milo and barley, respectively, by pressure cooking (without rolling) at 60 psi for 1 minute. There was a 78% increase in starch digestibility in milo by pressure cooking at 80 psi for 1 minute without rolling.

Flaking the grains after steaming or pressure cooking for 1 minute significantly ($P < .05$) increased the *in vitro* starch digestion of both grains. Digestibility increased with increased degree of rolling (flake flatness). Steam-processing and very flat flaking increased starch digestion of milo from 16.6% to 42.8%, while digestion of barley was increased from 21.8% to 51.2%. The average starch digestion of steam-processed flat flaked barley was 20% greater than that of similarly treated milo. Milo had to be pressure cooked at 60 psi for 1 minute and flaked to equal starch digestion of properly flaked steam-processed milo. Similar results were obtained with both enzyme sources.

There appears to be no doubt that the two methods of moist-heat treatment for barley and milo (steam-processing or pressure cooking and flaking) increased the availability of their starches to *in vitro* enzyme attack. The degree of flaking of the hot moist grain is the key factor influencing this availability. In fact, steaming without flaking was useless and even detrimental to *in vitro* starch utilization. This implies that the effect of moist-heat treatment to these grains to improve their utilization by fattening cattle could be lost by an unsatisfactory flaking process.

APPENDIX

EXPLANATION OF ABBREVIATIONS

d f	degrees of freedom
S S	sum of squares
MS	mean square
S S R	significant studentized range
L S R	least significant range

APPENDIX

Table 28. Per cent starch digestion of the same processed milo in two different trials by porcine pancreatin.[1]

	Untreated Milo	Steam Processed	
		Regular Rolled	Flat Rolled
Trial #1	16.51	32.36	39.32
Trial #2	16.52	33.40	38.14

1. Average of duplicate determinations.

Analysis of Variance

Source of Variation	df	SS	MS	F test
Between trials	1	.35	.35	
Treatment/trials	4	1107.01	276.75	282.40[1]
Error	6	5.88	.98	
Total	11	1113.24		

1. $P < .01$.

APPENDIX

Table 29. Per cent starch digestion of the same untreated milo in three different trials by porcine pancreatin.[1]

Trials	Per cent Digestion
1	16.79
2	17.05
3	16.32

1. Average of duplicate determinations.

Analysis of Variance

Source of Variation	df	SS	MS	F test
Between trials	2	.56	.28	2.54[1]
Within trials	3	.35	.11	
Total	5	.91		

1. Non-significant at .05 level of probability.

APPENDIX

Table 30. Per cent starch digestion of the same untreated barley in three different trials by porcine pancreatin.[1]

Trials	Per cent Digestion
1	21.95
2	21.84
3	21.26

1. Average of duplicate determination.

Analysis of Variance

Source of Variation	df	SS	MS	F test
Between trials	2	.47	.25	1.08[1]
Within trials	3	.69	.23	
Total	5	1.16		

1. Non-significant at .05 level of probability.

APPENDIX

Table 31. Composition of artificial saliva.

	Grams/liter distilled water
Sodium phosphate (monobasic)	4.8
Sodium bicarbonate	4.8
Potassium chloride	0.7
Sodium chloride	0.7
Magnesium sulphate	0.2
Calcium chloride	0.07

APPENDIX

Table 32. Per cent starch content and enzyme starch digestion of milo samples by porcine pancreatin.[1]

Whole Milo	% Starch Dry Matter	Maltose/ 100 mg. Sample	Maltose/ 100 mg. Dry Matter	Per cent Starch Digestion[2]
June 5, '65	78.67	12.33	13.38	17.01
July 16, '65	80.50	14.18	15.98	19.85
Aug. 27, '65	80.18	11.88	13.15	16.41
Sept. 3, '65	80.08	10.64	11.87	14.81
Oct. 22, '65 (Marana)	78.00	11.66	14.89	19.08
Oct. 25, '65 (Texas)	74.29	12.44	13.25	17.84
Oct. 25, '65 (Willcox)	75.36	11.09	11.87	15.75
Nov. 22, '65	76.30	10.87	12.06	15.80
Nov. 23, '65	79.49	10.90	12.22	15.38
Dec. 28, '65	76.19	10.82	11.83	15.54
Jan. 8, '65	75.38	11.23	12.17	16.16
Average	77.67	11.63	12.97	16.69

1. Values are average of duplicate determinations.
2. Dry matter basis.

APPENDIX

Table 33. Per cent starch content and enzyme digestion of barley samples by porcine pancreatin.[1]

Whole Barley	% Starch Dry Matter	Maltose/ 100 mg. Sample	Maltose/ 100 mg. Dry Matter	Per cent Starch Digestion[2]
Aug. 18, '65	74.02	17.87	19.19	25.93
Oct. 22, '65	74.32	15.03	16.18	21.77
Oct. 25, '65	74.97	15.04	15.88	21.18
Oct. 25, '65 (Davis)	72.05	14.80	15.59	21.64
Nov. 23, '65	73.99	14.61	15.75	21.29
Dec. 28, '65	68.65	12.47	13.56	19.75
Jan. 8, '66	71.90	14.63	15.89	22.09
Mar. 1, '66	74.68	14.10	15.47	21.16
Average	73.07	14.83	15.95	21.85

1. Values are average of duplicate determinations.

2. Dry matter basis.

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