STUDIES ON CEREAL STARCHES AS CARBOHYDRATE SOURCES IN A UREA-CONTAINING LIQUID SUPPLEMENT

by

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INTRODUCTION

In many areas, the only available feeds for a winter type beef ration are low quality roughages and pasture. The protein and energy contents of these forages are low and result in a loss of weight and condition throughout the wintering period. The increasing cost of grains has caused dairy rations with lower plant protein content. The economic feasibility of using plant protein as the total nitrogen source to supplement wintering cattle and dairy animals is rapidly declining. This, in turn, is increasing the use of non-protein nitrogen in supplements to replace some of the protein supplied by plants.

Liquid supplements are increasing in popularity in the livestock industry to aid against animal weight loss and high cost of plant protein. Liquid feeds, for ruminants, generally contain urea for a nitrogen source and molasses for a carbohydrate source. A new liquid supplement, however, incorporates a hydrothermally processed starch-urea mixture.

The objectives of these studies were to examine the effects and characterists of this starch-urea liquid supplement during storage and to examine the effects of processing technique on the liquid supplement.

LITERATURE REVIEW

Grain Composition

The most widely used cereal grains as livestock feed in the United States today are corn and grain sorghum. Corn is composed of 82% endosperm, 12% germ, 5% pericarp and 1% tip cap (29). Table 1 shows the proximate analysis of yellow dent corn.

The main source of energy in corn is supplied by the endosperm, which is composed of two regions. The regions are the hard or horny endosperm, which contains relatively small starch granules embedded in a thick protein matrix; and the soft or floury endosperm, which is composed of large starch granules embedded in a thin, weak protein matrix (76). It has been hypothesized that upon drying, the cytoplasmic protein ruptures leaving void areas. This causes opaqueness in the soft endosperm due to light refraction. The hard or translucent endosperm is believed to be absent of void spaces, preventing light refraction (16). Results from a scanning electron microscope study have shown that starch granules in hard endosperm of corn are tightly packed, polygonal shaped and contained in a continuous protein matrix. It was also found that the soft endosperm, in contrast to hard endosperm, contains nearly round starch granules, which are not tightly packed, resulting in intergranular air spaces (55).

Approximately 10% of the whole corn kernel is composed of protein. Classes of proteins and their relative amounts found in the endosperm of corn are: 3.2% albumins, 1.5% globulins, 47.2% prolamines and 35.1% glutelin (29).

TABLE 1. PROXIMATE ANALYSIS OF YELLOW DENT CORN[a]

Component	Mean %	Range %
Dry matter	89.0	87-91
Protein (N% X 6.25) (W.B.)	10.0	9.3-10.7
Ether extract (W.B.)	4.4	4.0-4.8
Crude fiber (W.B.)	2.2	2.1-2.3
Ash (W.B.)	1.2	0.9-1.5
Starch (D.B.)	72.0	64-78

[a]Inglett, G. E. (29).

The lipid portion of corn is composed of fats, waxes, phosphatides, cerebrosides, steroids and caratenoids. These comprise between 4.0 and 4.8% of the kernel by weight.

A minor carbohydrate in corn is sugar, which involves 1.0 to 3.0% of the kernel. Approximately 75% of the total is sucrose, with smaller amounts of D-glucose, D-fructose and raffinose (29).

Corn contains approximately 2.1 to 2.3% crude fiber. Most of the fiber is found in the paricarp. The paricarp contains approximately 40% hemicellulose (84).

Less than 1% of the grain sorghum in the United States is used for milling purposes (24,26). However, grain sorghum ranks third in cereal grain production in the United States, and is the second most important cereal grain fed to livestock. Grain sorghum is similar to corn in many ways. The proximate analysis is shown in table 2 for grain sorghum.

The endosperm is the energy storage center in grain sorghum. As in corn, the endosperm is composed of two major regions: the hard and soft endosperms (75). A scanning electron microscope study showed that grain sorghum endosperm is much like that of corn in regard to starch granule shape and compaction (26). However, the starch granule in corn is slightly smaller (10 μ) than in grain sorghum (15 μ) (75).

A major difference between corn and grain sorghum endosperm is that of location of prolamines. Prolamines are small spherical-shaped protein bodies which are embedded in the protein matrix of the hard and soft endosperms in grain sorghum. By contrast, these protein bodies are found in corn's hard endosperm, but not in the soft endosperm (26).

Protein levels in sorghum are more variable than in corn, making it difficult to control formulation (40). Normally, grain sorghum has a higher

TABLE 2. PROXIMATE ANALYSIS OF SORGHUM GRAIN[a]
(Moisture Free Basis)

Component	Average %	Range %
Moisture content	15.50	11-20
Protein (N% X 6.25)	13.99	11-15
Ether Extract	3.47	3.1-4.9
Crude fiber	1.93	---
Ash	1.89	1.6-2.2
Starch	68.52	56.97-70-93

[a]Wall, J. S. and C. W. Blessin (75).

protein level than corn. Protein classes found in grain sorghum and their approximate amounts are: albumins, 5.2%; globulins, 9.3%; prolamines, 44.5% and glutens, 34.6% (75).

Grain sorghum contains 3.6% lipids, which is lower than in corn. The endosperm contains 13% of the total oil in the kernel, the germ 76% and the bran 11 percent (75),

Fiber content in grain sorghum is similar to that of corn, averaging between 2 and 3 percent. Almost all pentosans are found in the bran portion of grain sorghum. Sugars in grain sorghum range from 0.9 to 2.0%, with sucrose being most prominent, as in most cereal grains (75).

The grain sorghum pericarp is composed of three layers: the epicarp, mesocarp and endocarp (18). Beneath the pericarp is a testa or subcoat layer. In a study using a bird resistant sorghum (Acco 1023), a prominent testa layer was observed when compared to non-bird resistant sorghum (26).

Bird resistant sorghum has been developed to replace corn in areas where birds are a perennial threat to crop production (17,45). Bird resistant sorghum is of the genotype B_1B_2S. In the early or milk stages, the seed is unpalitable to the bird; but at maturity, brown or bird resistant grains are sometimes consumed by large flocks of birds (53).

It is believed that phenolic compounds contribute to the flavor, color, bitterness and unpalatability of bird resistant sorghum (75). The bitter flavor associated with bird resistant sorghum may result in part from the leucoanthocyanins. Investigations have reported that these polyphenolic compounds are precursors of condensed tannins (56,57). When leucoanthocyanins are present, they are found in the pericarp of grain sorghum and not in the endosperm (7,75). The tannic acid content of brown seeded sorghum ranges from 1.3 to 2.0%, compared to a range of 0.2 to 0.4% in common grain sorghum (75).

An inhibitor in Leoti sorghum was found to contain a protein denaturant capable of inhibiting a variety of enzymes. The inhibitor may be a series of oligomeric condensed tannins of the leucocyanidin group varying in degree of polymerization (70).

Alpha-amylase from the cereals, bacteria, saliva and pancreas; and β-amylase from barley were all found to be inhibited by a substance found in Leoti sorghum. When germination occurred, the inhibition disappeared. When heat was applied to the sorghum for 1 hr under pressure at various pH levels, inhibition was extremely resistant to inactivation (41).

A study conducted on the starch granule structure showed that bird-resistant sorghum (Acco 1023) and yellow type sorghum (C 42Y) had similar spherical and smooth shapes. When these grains were subjected to rumen fluid for 75 min, hydrolysis by enzyme attack occurred in a minor point attack for bird resistant type, as compared to a linear track hydrolysis on that of yellow endosperm. With longer hydrolysis (4 hr) the attack on the yellow endosperm was quite evident, whereas, little or no attack was observed on the bird resistant type. Only after an 8 hr incubation period in rumen fluid did the starch from the bird resistant type show large evidence of enzyme attack (12). In a similar in vitro experiment, bird resistant sorghum produced less gas than a non-bird resistant sorghum (58). These studies suggest that rumen microorganisms are inhibited by a substance in the bird resistant sorghum. However, it has been shown that when the inhibitor from Leoti sorghum was incorporated with rumen contents, the inhibition was inactivated rapidly (41).

When both bird resistant and non-bird resistant sorghums were steam processed and flaked, the results showed that the difference in the total gas produced in vitro was considerably less than when no heat treatment was

applied (58). However, other findings have shown that when heat and pressure were applied at various pH levels, inhibition was not inactivated (41).

Saba (58) found that when tannic acid was added to regular sorghum in an in vitro study, dry matter disappearance and gas production were greatly reduced. He also found that the addition of tannic acid to the systems of non-bird resistant sorghums was much greater than the amount which could be accounted for in bird resistant sorghum to depress gas production in equilibrium. This would then imply that tannic acid was not the only substance in bird resistant sorghum that decreased utilization of the sorghum. It has been observed that tannins of grain sorghum differ chemically from the tannic acid used in grain sorghum trials (59).

Starch Composition and Characteristics

Starch is the energy storage component in the plant unit. Most cereal grains contain approximately 75% starch (66). Starch of corn and grain sorghum is located in the endosperm. Both cereals contain a hard and soft endosperm portion. The ratio of hard to soft endosperm in normal dent corn is approximately 2:1 (85). Another area in corn where starch is located is in a thin layer just below the aleurone layer, known as the sub-aleurone or the dense peripheral endosperm. This area contains small starch granules in a thick protein matrix. Only 5% of the total starch in the endosperm is located here (29,78).

Endosperm in grain sorghum is somewhat similar to that of corn endosperm in that it also contains a sub-aleurone layer (78). However, grain sorghum contains a larger portion of hard endosperm than does corn (77).

Starch granules from cereal grains are composed mainly of carbohydrates, although they do contain small amounts of fatty acids (0.5 to 1.0%), which can effect the characteristics of the starch (66). Starch is comprised

of repeating units of D-glucose, however, the molecules in a granule are not of a homogeneous state (61,66,86).

It has been known for 100 years that starch is composed of two major types: amylose and amylopectin. Starch in corn is composed of approximately 27% amylose and 73% amylopectin (29,34,77). Depending on breeding and variety, these values can change drastically. Waxy maize, waxy sorghum or glutenous varieties can contain as much as 100% amylopectin (4,66,75,87). In amylomaize, the percentage of amylose can be as high as 80% (34,66).

The amylose portion of starch is essentially straight chained molecules which, in solution, are coiled in the form of a helix with 6 glucose units per turn of the helix (3,61). Amylopectin is a branched molecule with branching occurring on 3 to 5% of the glucose residues. The amylopectin fraction of the granule is more soluble in water and in aqueous butanol solution than the amylose portion (75).

It is believed that sucrose, the most common sugar transported in plants, is the source of glucose units used in the synthesis of the starch molecule (11). It was first believed that starch was synthesized by amylase action, and later presumed that amylose was produced by action of phosphorylase on glucose-1-phosphate, adding glucose to the nonreducing end of the polysaccharide chain. However, synthetic action of phosphorylase rarely occurs in living tissue. In 1960, a transferase was found, uridine diposphate glucose, which transfers glucose to the polysaccharide chain (13).

Some investigators believe that amylose is produced first and is the precursor for amylopectin (80). Branching in amylopectin occurs by means of α-1,6 linkages of glucose units, whereas in straight chained amylose, linkages are predominately α-1,4 bonds (61). These linkages can be determined by means of methylation accompanied with acid hydrolysis. Upon

methylation of the starch, several glucose esters are formed. The predominant ester formed is 2,3,6-trimethyl glucose, which indicates the linkage of the one and four carbons of the glucose molecules. Small amounts of 2,3,4,6-tetramethyl glucose will also be formed, indicating the end groups; and a small amount of 2,3-dimethyl glucose will be formed, defining the branched groups being linkages of the one and six carbons (24).

Little is really known about the organization of the starch granule, however, it is believed that amylose and amylopectin are associated through secondary bonding forces (66). That is, whenever the branched or linear molecules parallel one another, forces due to hydrogen bonding pull the chains together to form the crystalline bundles or micelles (11,66). The outer branches of a single branched molecule may pass through many micelles areas and, therefore, participate in several micelles setting up a three-dimensional granule (11,66).

Crystallinity in the granule produces a characteristic dark cross when viewed through a microscope equipped with crossed Nichol prisms. This cross is known as the Maltese cross (62,66). The presence of this Maltese cross, or phenomenon of birefringence, when viewed under a polorizing microscope, is used as the reference in determination of gelatinization. Gelatinization occurs as a result of the irreversible rupture of the native secondary bond forces (66). Birefringence in starch granules is also evidence that the molecules are deposited in an orderly arrangement in a starch granule (65).

Starch Gelatinization and Determination

Hale (20) reported that proper processing of grain can improve utilization of starch by ruminants. He suggests that processing improves efficency of the starch by the rumen microorganisms and/or the animal. Other researchers have shown that moist heat treatment increased digestion rate

by microorganisms (60), increased digestion of nitrogen free extract (28, 33), increased animal performance (21), increased in vitro gas production (74), increased digestibility of total digestible nutrients (28) and reduced feed requirement for gain (21). Osman (47) noted that in vitro enzymatic starch digestion was improved when grain sorghum was pressure cooked. In all of the studies mentioned above, gelatinization of starch can possibly account for some of the improved utilization and performance.

Gelatinization is a term used to indicate the changes which take place during the heating of starch in an aqueous medium (11). The native starch granule is insoluble when placed in cold water, however, a limited amount of swelling does take place. The granule will absorb 30 to 35% of its weight in water (64). When heat is applied to a starch-water medium, increased swelling of the granules will occur. Larger granules usually swell first (11). When the starch-water slurry is heated above a critical temperature, irreversible swelling and loss of birefringence will occur in some of the granules. Swelling of these granules may be several times the size of the original granule (66). When all birefringence is lost, as viewed under a polorizing microscope, the granule is considered gelatinized (11).

The changes which take place, with reference to gelatinization, are similar for all starches. However, the temperature at which these changes take place for a particular starch source may vary. Table 3 shows gelatinization temperature ranges for various starch sources. Variations in gelatinization temperatures are due to botanical species; variety; modification of the starch during isolation; granule diameter; granule density; presence of other substances in the medium; and, somewhat, by amylose content (11,75).

Changes taking place in starch due to gelatinization would include increased granular size, increased light transparency, loss of birefringence,

TABLE 3. GELATINIZATION TEMPERATURES OF VARIOUS STARCHES[a]

Starch	Midpoint C	Initiation-Completion C
Corn	67.0	62-72
Sorghum	73.5	68-78
Wheat	61.0	58-64
Tapioca:		
Brazilian	57.0	49-64.5
Siamese	68.0	62-73
Pominican	64.5	58.5-70
Potato	63.0	59.68
Waxy maize	68.0	63-72
Waxy sorghum	70.5	67.5-74
Barley	57.0	51.5-59.5
Rye	61.0	57-70
Pea (green garden)	65.0	57-70
Rice	74.5	68-78
High-amylose corn	80.0	67-212

[a]Schoch, T. J. and E. C. Maywald (65).

loss of x-ray diffraction, increased susceptibility to chemical and physical change, increased medium viscosity, increased soluble material due to leaching of polymer molecules and rupturing of some of the starch granules. If shearing action is employed or if sustained heating is used, all of the granules may rupture, which will cause a decrease in the system viscosity (66).

During the onset of gelatinization, the initial swelling of the starch granule, due to the water, brings about breaking of hydrogen bonds. This reaction is reversible simply by drying. When heat is applied to a starch-water medium, water is forced into the crystalline region of the granule and the native secondary bond forces are broken between polymer molecules; covalent linkages are not broken (66).

Gelatinization should not be considered as a loss of crystallinity, it is just that the starch granule is in a less crystalline state (66). Under certain conditions, birefringence of a granule can reappear after gelatinization, therefore, suggesting some orderly arrangement of the granule after gelatinization (39).

Heat in combination with water is not the only means of gelatinizing starch granules. Polar solvents, such as ethylenediamine or dimethylsulfoxide, gelatinize starch at room temperature. Temperatures for gelatinization in starch-water mediums can be lowered by addition of certain salts, alkali, urea or any compound that breaks hydrogen bonds (66). Gelatinization studies can be conducted and controlled with less problems by use of these compounds. Evidence shows that when gelatinization was conducted by use of chemical solutions, a gas bubble was formed at the hilum of the granule. As gelatinization progressed, the bubble increased in size, setting up a low pressure area causing the granule to collapse. The granule

swells tangentially. Therefore, the starch molecule increases in diameter and not in length (61).

Many methods of determining gelatinization have been discussed in the literature. Some methods are based on absorption (35), enzyme digestion (14,15,63,71), staining properties (32) and light transmission (6).

The absorption method is simply a measurement of water absorbed by a specific quanity of damaged starch. Water-absorption index is usually calculated in grams of gel per gram of dry substance (35). A common enzyme digestion method is one developed by Sandstedt and Mattern (63) and modified by Sung (72). This method employs the use of β-amylase and a 1 hr incubation period. Staining methods are some of the earliest techniques developed to study starch damage. The use of congo red dye is used only as a gross estimate of starch damage, and is not a quantitative measurement (32). Light transmission is normally measured as percentage transmission with regard to temperature. As temperature increases, percentage transmission increases (6).

Urea Utilization and Effects

Many experiments have been conducted showing that urea can be used successfully and utilized efficiently in cattle rations (19,38,67). However, an equal number of studies can be found discouraging the use of urea (44,46,60). The factors controlling the amount of urea which can be utilized efficiently are not well defined. The major requirement for high amounts of urea in a ruminant ration is an adequate amount of carbohydrate to supply carbon skeletons and energy for synthesis of amino acids. The most common carbohydrate sources used are starch and sugar, although several studies have shown that hemicellulose and cellulose can be used with some success (10,82).

Urea has received much publicity due to its toxic effects and unpalatability. Toxicity from high urea diets is due to the rapid release of ammonia upon hydrolysis of urea by urease (48). The pH of the rumen increases during the onset of toxicity, making the rumen ammonia lipid soluble; which allows rapid absorption of ammonia into the blood, and toxicity results (25). This rapid rate of ammonia production is, therefore, the limiting factor in urea use. Formation of urea-carbohydrate complexes have proven to decrease the rate of ammonia release (10,43,67).

The addition of a non-protein nitrogen source to a ruminant diet has stimulated cellulose digestion in vitro and has increased amylolytic and cellulolytic activity. This occurred when urea replaced soybeans as a crude protein supplement for cattle (73). It has also been observed in studies with rumen microorganisms that when urea replaced casein as a dietary nitrogen source, there was an increase in entodinia, flagellates and total bacteria. A decrease in proteolytic bacteria was found when urea replaced soybeans as a nitrogen source (73). Ojtlen (46) observed that when urea provided the only nitrogen source in ruminants diets that growth rate, feed efficiency, nitrogen retention, milk production, branched chained volatile fatty acid concentration and free blood plasma concentration of essential amino acids were all decreased.

Nitrogen retention is a large factor in utilization and efficiency of urea. In studies where urea and phosphoric acid were combined to form urea-phosphate, lower ammonia absorption was noted due to the decrease in rumen pH resulting from urea-phosphate. However, nitrogen retention was not improved over straight-fed urea (50). Tillman (73) noted that the heat treatment of casein, groundnut meal and soybean meal increased nitrogen retention in the ruminant animal. This increase in nitrogen retention was

attributed mainly to the decreased protein solubility, resulting in a reduced proteolytic activity and reduced ammonia formation. Tillman also noted that nitrogen loss, due to urinary loss, was decreased when cottonseed meal and/or soybean meal were heat treated (73). Thus, these observations would suggest that proper heat treatment can result in an increased nitrogen retention in the animal.

Urea is used as the main source of nitrogen in liquid supplements. Liquid supplements are used to supply energy, to supply nitrogen and/or protein and to act as a carrier for vitamins and minerals. Liquid supplements are used in beef wintering rations to prevent weight loss; and in dairy rations to replace soybean meal and to lower protein cost.

It has been shown that when a liquid supplement partially supplemented high corn silage rations, performance of dairy cattle equaled those supplemented with soybean meal. These supplements each contained 50% crude protein and each animal received 0.9 kg/day. However, milk production was significantly lowered on the liquid supplement diet when supplement intakes were increased to 1.8 kg/head/day (27).

Molasses is the main carbohydrate source used in liquid supplements. Molasses has been reported to cause digestive upsets and net energy decline when fed at high levels in cattle rations (37). Other findings challenged this precept and claim that molasses retains its utilization efficiency as it is increased in the ration (23,52). It has been suggested that a combination of molasses and starch as the carbohydrate source optimizes the utilization of urea in a ration (23). Williams (82) showed that gains were enhanced in sheep when fed a liquid supplement consisting of molasses, hemicellulose and urea when compared to a molasses-urea liquid supplement. The addition of fermentation solubles (fish and distillers) to liquid supplements has shown enhanced performance in cattle (27).

A typical molasses-based liquid supplement has been described by Anderson (2). Other than a carbohydrate source and a nitrogen source, liquid supplements usually contain phosphoric acid and water. The phosphoric acid is used to lower the pH and to control consumption.

In some cases, liquid supplements tend to form gels, making them inaccessible, Weber (79) has demonstrated that the tendency of gel formation can increase in liquid supplements containing phosphoric acid when both pH and temperature are increased in the mixture.

Enzymatic Digestion of Starch

Alpha-amylase attacks α-1,4 links of the starch chains, breaking the molecules into large units of dextrins. These large units can further be broken into smaller dextrins, and finally into maltose. Action on starch molecules by α-amylase is internally and totally random. Alpha-amylase cannot break 1,6 linkages, but bypasses them by breaking linkages between the branches. Therefore, this enzyme can complete digestion leaving oligosaccharides containing 1,6 and 1,3 links (61).

Beta-amylase attacks the α-1,4 linkages of starch molecules starting at the nonreducing end, cleaving off maltose units. Beta-amylase action is interrupted by 1,6 and 1,3 linkages. Complete digestion can be accomplished in pure linear amylose molecules. However, amylose contains blocking points for β-amylase action by containing a small amount of 1,6 linkages. Beta-amylase digestion also allows for the removal of the amylopectin and glycogen external branches (61).

Preservatives

The main requirement for mold-inhibiting compounds are that they are nontoxic to the animal consuming the product. Various organic and inorganic acids have been used to prevent mold growth and their undesirable effects

in high moisture grain (54,81). One of the most common organic mold-inhibiting acids used is propionic acid. It is also considered the standard acid to which other mold-inhibiting compounds are compared to.

Wiggens (81) has discussed the use of several inorganic acids that control toxic side effects in ammoniated feed supplements for ruminants. Weber (79) has reported that sulfuric acid, along with other nonphosphatic acids, can be used as components in liquid supplements to control gel formation.

Processing Techniques

Pfost (51) has described various methods of processing cereal grains that incorporate the use of heat and steam. It has also been shown that proper processing of bird resistant sorghum can increase its utilization and decrease its inhibitory effect, when compared to non-bird resistant sorghum processed under the same heat and steam treatment (58). Hale (20) has shown that proper processing can disrupt the endosperm, permitting easier enzymatic access to the starch granule. Other studies have proven that nonprotein organic matter digestion by cattle increased when grain was steam processed (8). Husted (28) processed sorghum in various ways (dry rolled, fine ground, steam processed flaked, pressure cooked flaked, water soaked and steam processed unflaked) and results indicated that the steam processed flaked or pressure cooked flaked were superior when digestibility of total digestible nutrients was used as the criterion.

Little literature was found where direct steam application was compared to indirect steam application. However, Peplinski (49) studied the effects of gelatinization of corn and sorghum grits, where direct steam was applied to the substrate, and results indicated that the direct steam effects were similar to those found where indirect steam is applied.

In Vitro Techniques

Any researcher that has been associated with feedstuff utilization by the ruminant animal has probably, at one time or another, faced the problem and selection of an *in vitro* technique. One of the major problems associated with *in vitro* techniques is that of validity.

It has been demonstrated that the morphology and end products of the microorganisms propagated and formed *in vitro* are similar to those of the intact rumen (31). Therefore, it can be assumed that the activities being measured *in vitro* are similar to those of the animal. It should be pointed out that a particular species of microorganism may be enhanced in the closed system. However, this does not make the system invalid in the qualitative measurement of the metabolic process, but the quantitative measurement may be in error (31).

In the past, *in vitro* techniques have been associated mainly with high cellulose rations. Little *in vitro* data have been accumulated in high starch diets. Johnson (30) has pointed out some of the reasons for this. First, unlike cellulose, starch incompletely digested in the rumen may be further digested later in the digestive track. Second, starch digestion is conducted at a faster rate and starch is digested by a larger number and wider species type of microorganisms than that of cellulose. Finally, the predominating species for starch digestion in the inoculum may vary from day to day.

Kumeno (36) has shown, in fermentation trials of high-energy mixed rations, where ground corn was used, a high correlation ($r=.85$) between *in vitro* dry matter digestibility and performance in the ruminant animal. High correlations in total acid production were also observed when *in vitro* results were compared to *in vivo* fermentation trials. Barr (5) has shown

that an *in vitro* protein synthesis technique, using a high speed centrifugation and a methanol extraction to remove soluble nitrogen, can predict *in vivo* utilization of urea-containing feedstuffs.

EXPERIMENTAL METHODS FOR THE INVESTIGATIONS OF LIQUID SUPPLEMENTS CONTAINING CEREAL STARCH

Introduction

The research covered deals with a liquid feed for ruminants. All analyses were conducted and designed to be of importance in the investigation of this processed feed. The tests used as guidelines in the determination of the value or characteristics of the feed were: 1. *in vitro* rumen protein synthesis, 2. Sung's method of starch damage determination, 3. ammonia nitrogen (NH_3-N) by Micro-Conway diffusion, 4. Macro-Kjeldahl nitrogen determination, 5. dry matter, 6. pH and 7. viscosity.

In Vitro Protein Synthesis Procedure

Rumen fluid was collected from a fistulated animal approximately 12 hr after feeding. Fluid was placed in a thermos to aid in the prevention of oxygen contact and to keep the fluid as warm as possible until it was brought to the laboratory. At the laboratory, the rumen fluid was filtered through four layers of cheesecloth to remove feed particles. It was then placed in a water bath set at 39 centigrade.

Feed samples (1 g) were pre-weighed in duplicate sets in 50 ml plastic centrifuge tubes. Twenty ml of warm (39 C) buffer solution (table 4) were added to each tube. Ten ml of strained rumen fluid were then added to the sample tubes. Tubes were closed with stoppers, equipped with bunsen valves, and fermented for 4 hr in a water bath set at 39 centigrade. Contents of the tubes were mixed at 30 min intervals to facilitate microbial action.

After the fermentation period, the bunsen valve caps were removed and tubes were centrifuged at 25,400 X G for 15 minutes. The supernatant was discarded. The precipitate was suspended in, and washed twice with, 25 ml

of methanol to remove soluble nitrogen. Each washing was followed by centrifugation at 25,400 X G for 15 minutes. Nitrogen determinations were made on the precipitates by a Macro-Kjeldahl method (AACC method 46-12) (1).

Rumen fluid blanks were also analyzed in the same manner as the samples, with the exception of the fermentation period. Ten ml of rumen fluid and 20 ml of buffer solution (table 4) were each added to four empty 50 ml centrifuge tubes for this analysis. Centrifugation and nitrogen determinations were run on the rumen fluid blanks, in the same manner as discussed above, for the fermented samples.

Feed blanks were prepared by adding 1 g samples and 30 ml of buffer solution (table 4) to 50 ml centrifuge tubes. Tubes were then fermented, centrifuged, washed with methanol and nitrogen determined in the same fashion as previously discussed.

Calculations for <u>in vitro</u> protein synthesized consisted of the following:

$$\text{mg protein/tube} = \text{ml acid} \times N \times 6.25 \times 14$$

$$\text{protein synthesized} = \text{mg protein in fermented sample} - \text{mg protein in rumen fluid blanks} - \text{mg protein in feed blank}$$

Protein synthesized was calculated in units of mg/g of sample.

<u>Starch Damage Determination</u>

Degree of starch damage of processed samples was determined by Sung's method (72). One g samples were incubated for 1 hr in a β-amylase solution and reducing power determined. Calculations were recorded in mg maltose per g of sample.

<u>Micro-Conway Diffusion</u>

Determination of NH_3-N in the samples was carried out by means of the Micro-Conway diffusion (9) technique. Samples were placed in the outer ring of Conway dishes along with a saturated potassium carbonate solution. A

TABLE 4. COMPOSITION OF BUFFER

Ingredient[a]	Amount (g/liter)
KH_2PO_4	4.08
$MgSO_4$	0.20
NaCl	0.50
$CaCl_2 \cdot 2H_2O$	0.05

[a]Total ingredients were made to the volume of 1 liter with water and adjusted to the pH of 6.8 by using a saturated K_2CO_3 solution.

boric acid solution was placed in the center ring of the Conway dishes. As sample and potassium carbonate were mixed, the pH of the sample increased, allowing the free ammonia to escape and to be trapped in the boric acid solution. Samples were incubated for 1.5 hours. After this period, the boric acid solutions were titrated with a standard sulfuric acid. Calculations for the amount of neutralizing acid were used to give mg NH_3-N per g of sample.

Macro-Kjeldahl Nitrogen Determination

Nitrogen contents of the samples were determined by Macro-Kjeldahl analysis. The method used was the boric acid modification, as discussed in the AACC method (46-12) (1). Samples were digested in sulfuric acid and distilled in an alkali solution (NaOH). The distillate was collected in a boric acid solution and titrated with a standard sulfuric acid solution. Percent crude protein was calculated using the following formula:

$$\% \text{ crude protein} = \frac{(\text{ml titer})\ (N)\ (14)\ (6.25)}{\text{sample weight}} \times 100$$

where: N = normality of standard weight
14 = molecular weight of nitrogen
6.25 = conversion factor of nitrogen to protein

Dry Matter Analysis

All dry matter analyses on samples were conducted using a forced air oven. Approximately 2 g samples were placed in tared drying pans and dried at 130 C for a 1 hr period. Results were recorded as percentage dry matter.

pH Analysis

All pH readings were made with a Leeds and Northrup pH Meter.[1] Readings were taken to the nearest hundredth.

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[1]Fisher Scientific Company, Pittsburgh, Pennsylvania.

Viscosity Analysis

Viscosity of the samples was estimated with a Synchro-Lectric Model LVT Viscometer.[1] The viscometer spindle speed was 30 rpm using spindle two. Results were recorded in centipoise units.

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[1]Brookfield Engineering Laboratories, Inc., Stoughton, Massachusetts.

PROCEDURES AND RESULTS

Supplement Processing Procedures

In the manufacturing of this liquid feed, raw grains were first finely ground using a 15 hp Micro-Bud[1] micro-pulverizer, equipped with an air classifier. Urea was added to the ground grain to acquire a desired ratio of starch source to urea. Water was then added to obtain a specific solids content. This slurry was then processed with a hydrothermal cooker.[2]

The cooker was equipped with a 1/4 hp Moyno Pump[3] and a Series "B" Hydro-heater.[4] Specifications on the cooker are listed in table five. The cooker worked on the principle of applying steam directly to the slurry through the hydro-heater, causing high shear cooking. During processing, steam condensed and reduced the final solids concentration by 26 percent. In the processing studies conducted on the liquid feed, the temperature ranged from 140 to 150 C and the pressure from 40 to 50 psig.

Molasses was added after the cooking process to adjust protein content. When the cooked product and molasses mixture cooled to 60 C, α-amylase, derived from Bacillus subtillis, was added at a rate of .04% of the gel's weight to lower the viscosity of the mixture. After a mixing period of 1 min, phosphoric acid was added to arrest the enzyme activity. An acid preservative was then added to prevent mold growth on the finished product.

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[1]Metals Disintegrating Co., Pittsburgh, Pennsylvania.

[2]Penick and Ford Limited, Cedar Rapids, Iowa.

[3]Robbin and Myer, Inc., Springfield, Ohio.

[4]Hydro-Thermal Corporation, Milwaukee, Wisconsin.

TABLE 5. COOKER SPECIFICATIONS

Condition	Limits
Processing temperature range	93 - 163 C
Processing pressure range[a]	0 - 100 psig
Slurry pumping rate	1.17 liter/min

[a]Temperature and pressure varied proportionally.

The investigations reported are reviewed in two sections. Each portion deals with a different aspect of the hydrothermally processed starch source-urea liquid feed. These studies were designed: 1. to determine the effects of the liquid supplement in storage using various acid preservatives and 2. to compare hydrothermal processing methods in the manufacturing of the supplement.

SECTION 1. STORAGE TRIAL

The objective of this trial was to study the effects of storage on the liquid supplement over a 2-month period. Starch sources used in this trial came from three sources: a Co-op medium maturity hybrid grain sorghum (733), a bird resistant sorghum (6-516-BR), and a feed grade yellow dent corn. The yellow dent corn sample was used as a standard prototype, whereby comparisons could be made among the various samples. In this section, the Co-op hybrid samples will be referred to as non-br sorghum, and the bird resistant sorghum samples will be referred to as br sorghum.

The tests used as guidelines for the storage trial were: 1. pH, 2. dry matter, 3. crude protein, 4. ammonia nitrogen, 5. maltose production, 6. viscosity and 7. *in vitro* rumen protein synthesis. These analyses, with the exception of the *in vitro* technique, were performed at 0, 2, 5 and 8 weeks to determine changes and/or variations in the product. The *in vitro* technique was conducted at week 8 only.

Three batches (220 kg) of liquid supplement were processed in the manner previously explained. Each batch processed was made from one of the three different starch sources discussed in this section. The gels consisted of a starch source-urea ratio of 2:1. Water was added to give a solids content of 40 percent. Processing temperature and pressure were 140 C and 40 psig, respectively. After the molasses and α-amylase were

added, phosphoric acid was added to each of the batches at a rate of .5% of the total mixture. Each batch of non-br sorghum and br sorghum supplement was then divided into 9 equal parts, making a total of 18 samples. One liquid supplement sample containing corn was also secured from its respective batch and used as a control. Various levels and blends of phosphoric, propionic, sulfuric and MBP[1] acids were then added to these samples as preservatives. Table 6 illustrates the amounts and types of acids added. Table 7 shows the percentage composition of each component in the final supplements.

Samples explained above were processed a second time in the same week as a duplicate series, making a total of 38 samples. All samples were stored at room temperature in sealed 3.8 liter plastic containers.

Results

After the 8-week trial was completed an Aardvark[2] two-way statistical analysis of variance (69) and an Econplot[3] program were used to evaluate the results of each variable. Aardvark is a computer program capable of running under two basis modes of analysis: analysis of variance, including covariance, and regression analysis. An LSD value of .05 was used to separate means that were significant. Econplot is a computer program which was used to plot the results of the storage trial. Data plotted were averages of duplicate sets.

pH

Table 8 is a listing of means of pH values throughout the storage trial. Within individual sample treatments, there were no differences

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[1]Methyl-bis propionate.

[2]Iowa State University, Ames, Iowa.

[3]Kansas State University, Manhattan, Kansas

TABLE 6. ACID LEVELS[a] FOR STORAGE TRIAL

Samples	Phosphoric %	Propionic %	Sulfuric %	MBP[b] %
Non-br sorghum				
S1&S28	1.0	0.5	---	---
S2&S29	1.0	1.0	---	---
S3&S30	1.0	2.0	---	---
S4&S31	0.5	0.5	0.5	---
S5&S32	0.5	1.0	0.5	---
S6&S33	0.5	2.0	0.5	---
S7&S34	1.0	---	---	0.5
S8&S35	1.0	---	---	1.0
S9&S36	1.0	---	---	2.0
Br sorghum				
S10&S19	1.0	0.5	---	---
S11&S20	1.0	1.0	---	---
S12&S21	1.0	2.0	---	---
S13&S22	0.5	0.5	0.5	---
S14&S23	0.5	1.0	0.5	---
S15&S24	0.5	2.0	0.5	---
S16&S25	1.0	---	---	0.5
S17&S26	1.0	---	---	1.0
S18&S27	1.0	---	---	2.0
Corn				
SC_1&SC_2	1.0	0.5	---	---

[a]Percentage acid based on total weight of formula.

[b]Methyl-bis propionate.

TABLE 7. COMPOSITION OF FORMULAS FOR SUPPLEMENTS OF STORAGE TRIAL

Component	%
Slurry	
Starch source	18.12
Urea	9.90
Water	35.31
Molasses	12.94
Acid combination[a]	1.5-3.0
Enzyme	.025
Absorbed water[b]	22.23

[a]As acid level varied from 1.5 to 3.0%, no alterations of other components were made to compensate for this percentage change.

[b]Water absorbed during processing.

TABLE 8. MEAN pH OF STARCH CONTAINING LIQUID SUPPLEMENTS DURING STORAGE

Sample	Week[a] 0	2	5	8	Row average
Non-br sorghum					
S1&S28	3.79[b]	3.82[b]	3.92[b]	3.95[b]	3.87[cd]
S2&S29	3.72[b]	3.78[b]	3.89[b]	3.79[b]	3.79[cde]
S3&S30	3.57[b]	3.68[b]	3.79[b]	3.82[b]	3.72[de]
S4&S31	3.00[b]	3.03[b]	3.13[b]	3.32[b]	3.12[f]
S5&S32	2.94[b]	2.84[b]	2.95[b]	3.23[b]	2.99[fg]
S6&S33	2.94[b]	2.85[b]	2.94[b]	3.01[b]	2.94[fg]
S7&S34	3.72[b]	3.65[b]	3.75[b]	3.44[b]	3.64[ef]
S8&S35	3.77[b]	3.85[b]	3.96[b]	3.85[b]	3.86[cd]
S9&S36	3.74[b]	3.85[b]	3.91[b]	3.79[b]	3.82[cde]
Br sorghum					
S10&S19	3.78[b]	3.82[b]	3.89[b]	3.89[b]	3.85[cd]
S11&S20	3.70[b]	3.73[b]	3.78[b]	3.93[b]	3.79[cde]
S12&S21	3.63[b]	3.66[b]	3.67[b]	3.75[b]	3.68[de]
S13&S22	2.79[b]	2.80[b]	2.85[b]	3.04[b]	2.87[g]
S14&S23	2.79[b]	2.91[b]	2.93[b]	2.93[b]	2.89[g]
S15&S24	2.89[b]	2.96[b]	2.99[b]	2.97[b]	2.95[fg]
S16&S25	3.93[b]	3.98[b]	3.95[b]	3.65[b]	3.88[cd]
S17&S26	3.91[b]	4.01[b]	3.96[b]	3.97[b]	3.96[c]
S18&S27	3.89[b]	4.00[b]	3.89[b]	3.94[b]	3.93[c]
Corn					
SC_1&SC_2	3.50[b]	3.76[b]	3.77[b]	3.76[b]	3.70[de]

[a]Values are averages of duplicate set samples. Set data of pH are located in table 1 of appendix A.

[b]Means within rows having similar superscripts are not different ($P<.05$).

[c,d,e,f,g]Values within column having similar superscripts are not different ($P<.05$).

($P<.05$) in pH from week zero to week eight. Samples treated with similar acid combinations, but in differing amounts, did not vary markedly in pH levels ($P<.05$). However, there were differences between samples treated with various acid combinations. Samples treated with .5% sulfuric acid and .5% phosphoric acid were lower ($P<.05$) in pH than those treated with 1% phosphoric acid. Propionic acid treated samples, where no sulfuric acid was added, were not different ($P<.05$) from the MBP treatments in pH. The starch source did not appear to effect the pH of the supplement. Figures 1 through 7 show the consistancy of pH over the entire 8-week period.

Dry Matter

The majority of individual treatments did not change ($P<.05$) in dry matter content over the 8-week storage trial (table 9). Samples S10&S19 and S11&S20, which did change significantly, did so within the first 2 weeks and remained stable thereafter. Means for these samples were initially low in dry matter content. These samples, however, were drawn from the same supplement batch as samples which had 34% initial dry matter content. These results would suggest that a sampling error may have occurred. Samples S10&S19 and S11&S20 show no differences with corresponding batch samples from week 2 to week 8 in dry matter content. Table 2 of appendix A also shows a trend that could suggest sampling error. Sampling error would then also explain the significant difference observed in the overall average means for weeks shown in table nine.

Treatment variation showed that, on the average, supplements containing non-br sorghum as the starch source had the higher ($P<.05$) dry matter content. However, it should be noted that water absorbed during processing can vary slightly and that these differences in dry matter contents are only borderline cases. Therefore, the differences observed may have been

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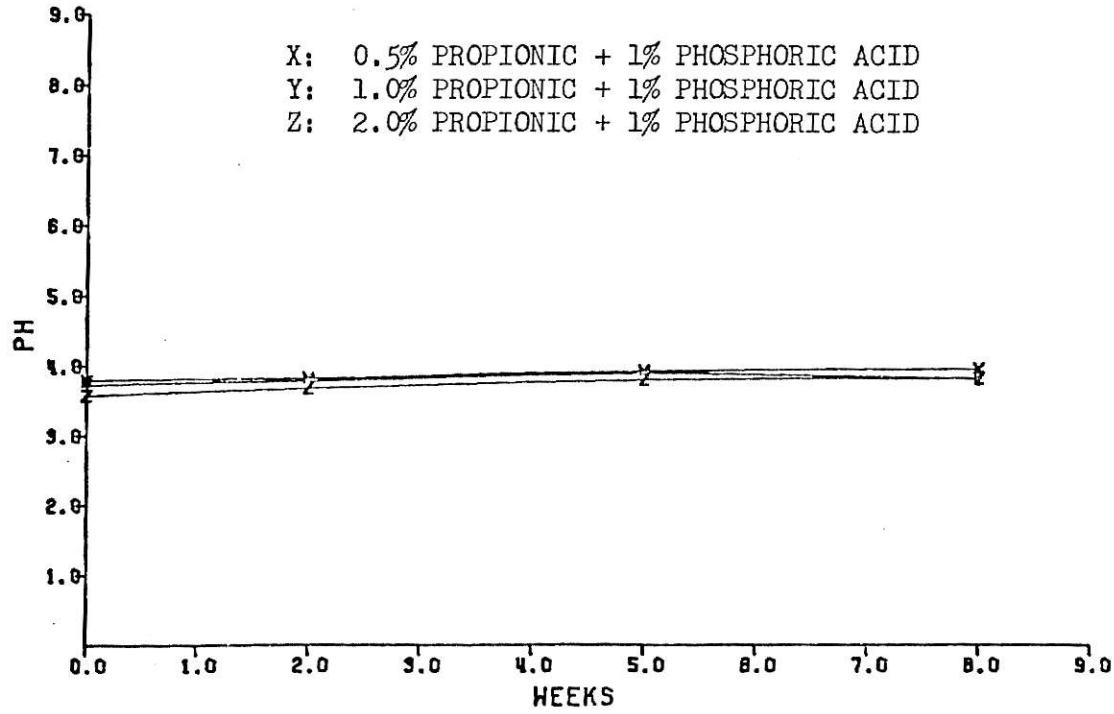

FIGURE 1. PH DURING STORAGE USING NON-BR SORGHUM AND PROPIONIC ACID

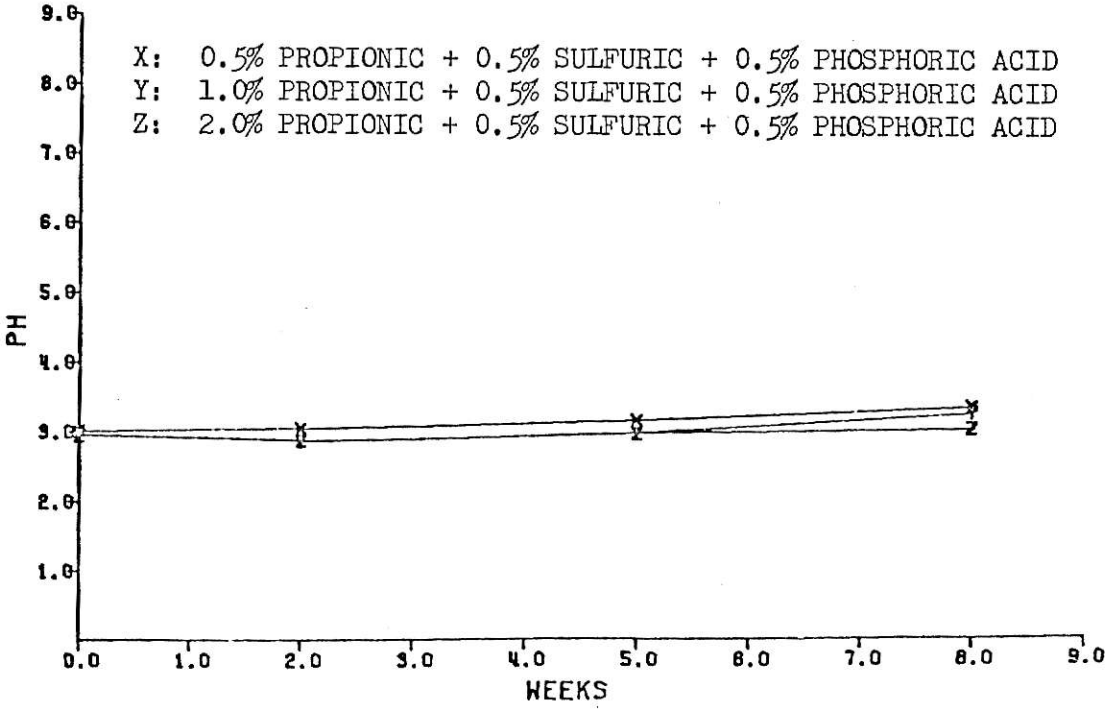

FIGURE 2. PH DURING STORAGE USING NON-BR SORGHUM, H2S04 & PROP. ACID

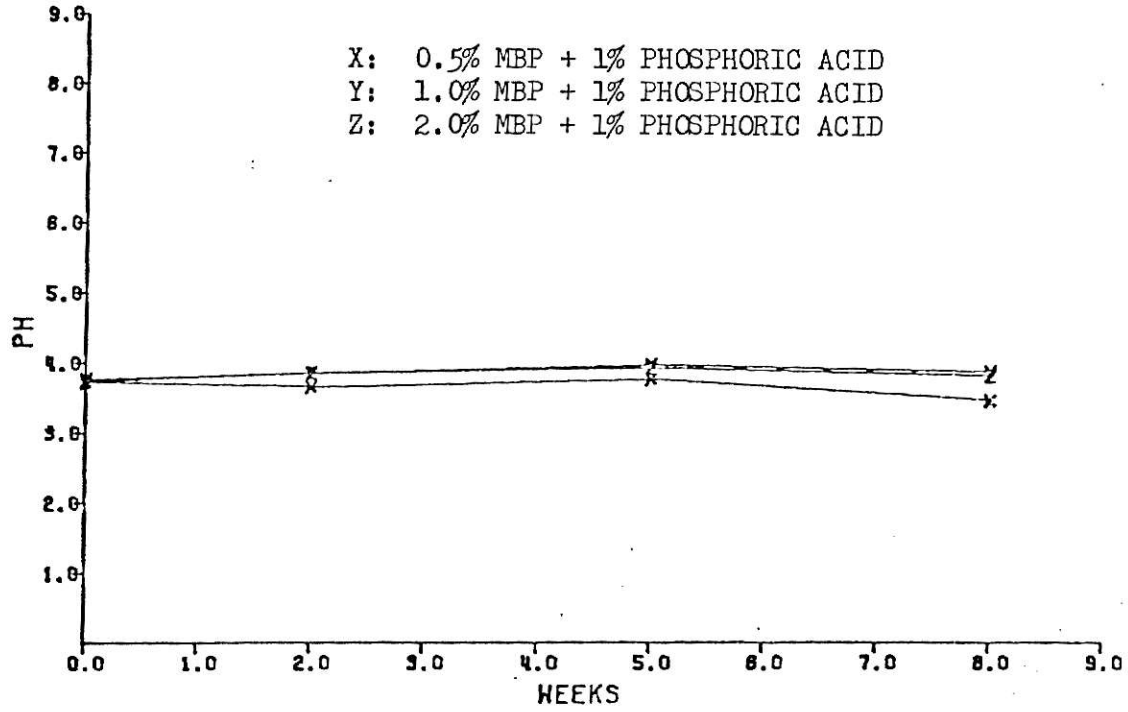

FIGURE 3. PH DURING STORAGE USING NON-BR SORGHUM AND MBP

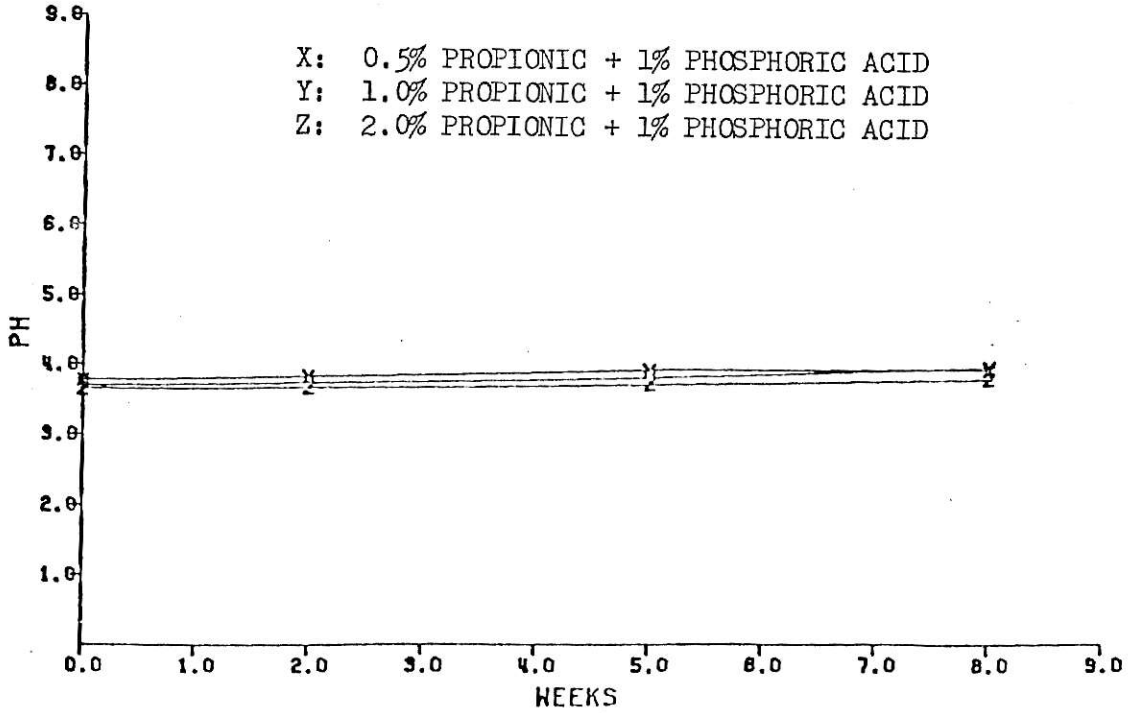

FIGURE 4. PH DURING STORAGE USING BR SORGHUM AND PROPIONIC ACID

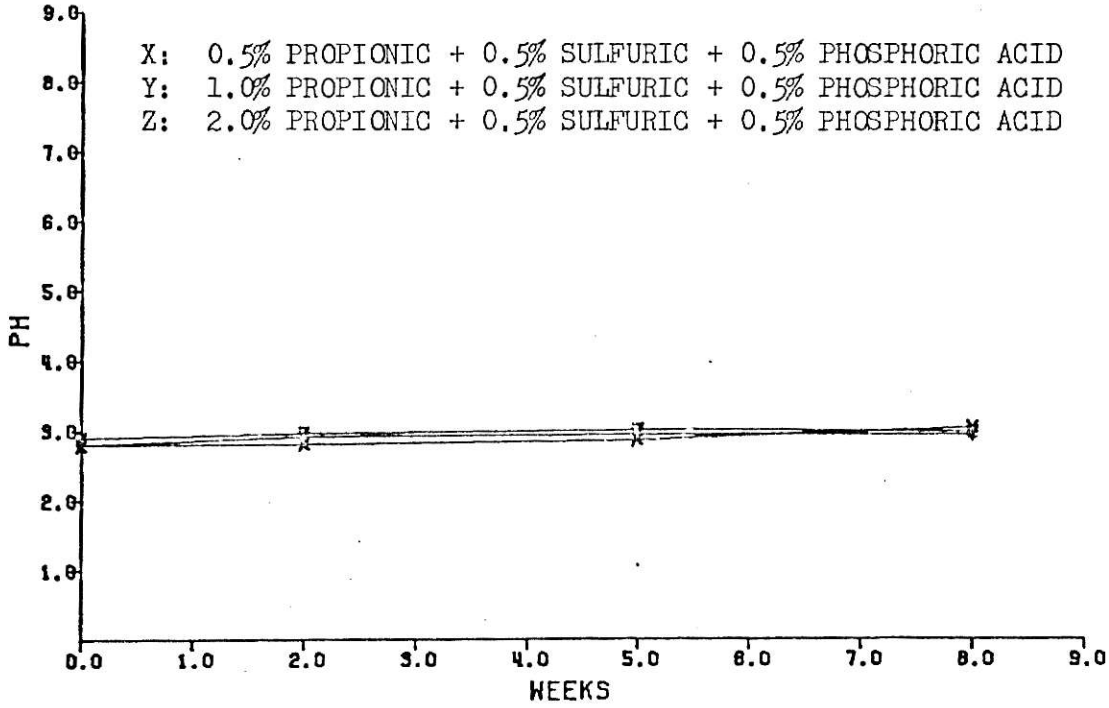

FIGURE 5. PH DURING STORAGE USING BR SORGHUM, H2S04 & PROP. ACID

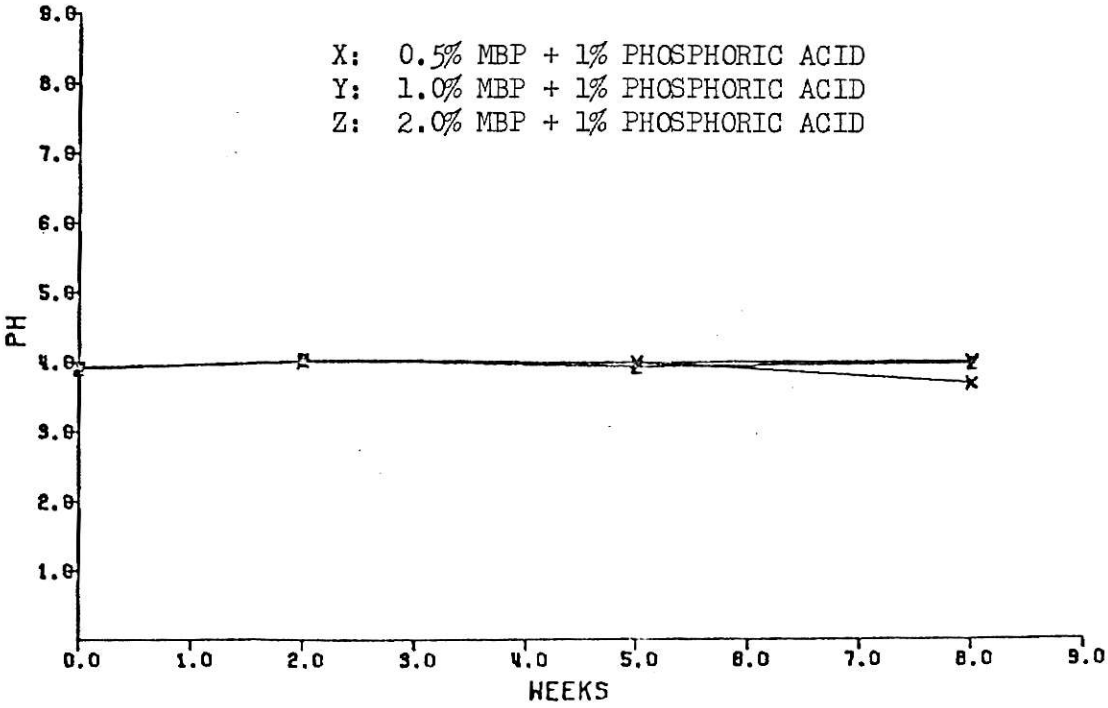

FIGURE 6. PH DURING STORAGE USING BR SORGHUM AND MBP

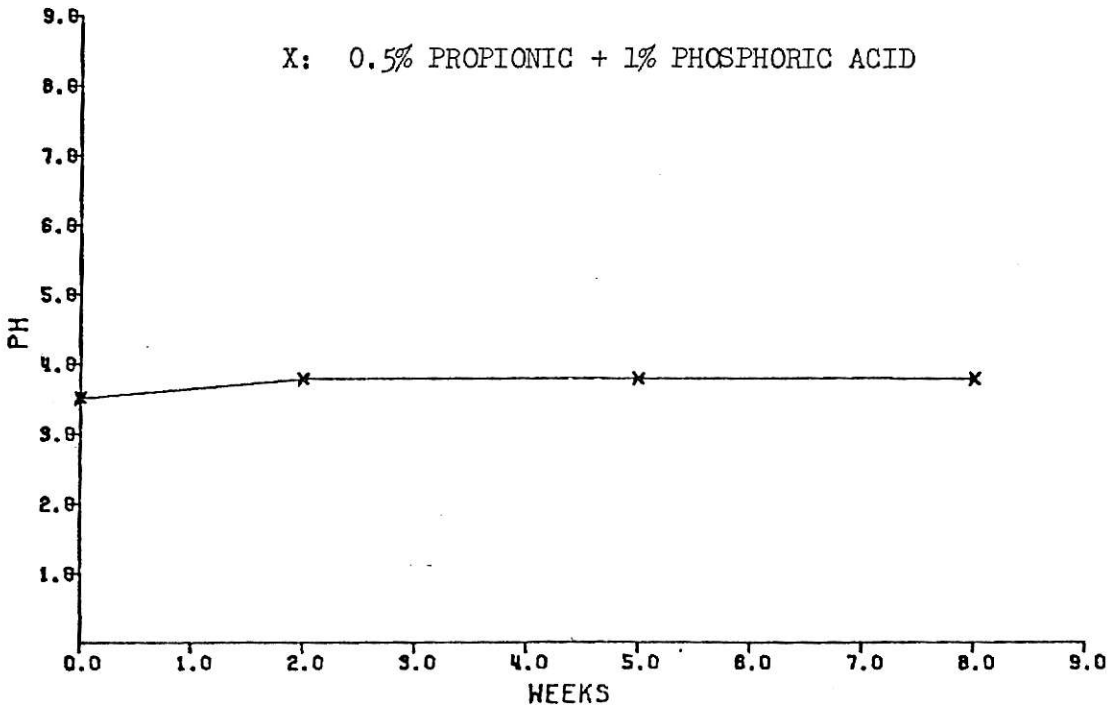

FIGURE 7. PH DURING STORAGE USING CORN AND PROPIONIC ACID

TABLE 9. MEAN DRY MATTER OF STARCH CONTAINING LIQUID SUPPLEMENTS DURING STORAGE

Sample	Week[a] 0	2	5	8	Row average
Non-br sorghum					
S1&S28	34.24^{b}	34.28^{b}	34.59^{b}	34.39^{b}	34.38^{defg}
S2&S29	34.26^{b}	34.19^{b}	34.35^{b}	34.53^{b}	34.34^{defg}
S3&S30	34.05^{b}	34.01^{b}	34.07^{b}	34.18^{b}	34.08^{efgh}
S4&S31	34.73^{b}	34.66^{b}	34.64^{b}	34.72^{b}	34.69^{d}
S5&S32	34.62^{b}	34.59^{b}	34.51^{b}	34.62^{b}	34.59^{de}
S6&S33	33.86^{b}	34.41^{b}	34.33^{b}	34.36^{b}	34.24^{defgh}
S7&S34	33.97^{b}	34.34^{b}	34.56^{b}	34.68^{b}	34.39^{defg}
S8&S35	33.84^{b}	34.31^{b}	34.77^{b}	34.69^{b}	34.40^{def}
S9&S36	34.01^{b}	34.42^{b}	34.35^{b}	34.67^{b}	34.36^{defg}
Br sorghum					
S10&S19	32.61^{b}	34.39^{c}	33.94^{c}	34.28^{c}	33.81^{hij}
S11&S20	31.64^{b}	33.94^{c}	33.85^{c}	34.32^{c}	33.44^{ij}
S12&S21	33.23^{bc}	33.79^{b}	33.45^{b}	32.28^{c}	33.19^{k}
S13&S22	34.50^{b}	34.59^{b}	34.17^{b}	34.76^{b}	34.51^{def}
S14&S23	34.36^{b}	34.37^{b}	33.98^{b}	34.42^{b}	34.28^{defgh}
S15&S24	34.07^{b}	34.09^{b}	33.83^{b}	34.24^{b}	34.06^{fgh}
S16&S25	33.71^{b}	33.85^{b}	33.83^{b}	34.14^{b}	33.88^{ghi}
S17&S26	34.03^{b}	34.16^{b}	34.56^{b}	34.29^{b}	34.28^{defgh}
S18&S27	34.13^{b}	34.27^{b}	34.35^{b}	33.89^{b}	34.16^{efgh}
Corn					
SC_1&SC_2	33.66^{b}	32.89^{bc}	32.66^{bc}	32.36^{c}	32.89^{k}
Average	33.87^{b}	34.19^{c}	34.15^{c}	34.20^{c}	

[a]Values are averages of duplicate set samples. Set data of dry matter contents are located in table 2 of appendix A. Values are reported as a percentage.

[b,c]Means within rows having similar superscripts are not different ($P<.05$).

[d,e,f,g,h,i,j,k]Values within column having similar superscripts are not different ($P<.05$).

due to processing procedures. This was also the case with the supplement containing corn as the starch source. Table 2 of appendix A shows the variation in dry matter content from batch to batch due to processing error. Acid treatment tended to have no bearing on dry matter variation. Figures 8 through 14 show the consistancy of dry matter over the 8-week period for each sample.

Protein Content

Table 10 gives the means for dry matter protein content of the various supplements. Supplements were formulated for a 30% protein content on an "as is" basis. Table 3 of appendix A gives values on an "as is" and "dry matter" basis for individual samples.

Statistical analyses showed few examples of significant differences ($P<.05$) within individual samples over the 8-week trial. Treatments S10& S19 and S11&S20 had significant differences within the first 2 weeks of the trial. This difference was probably due to the dry matter error as discussed in the section of dry matter. The average of all treatments over the 8-week period showed no change ($P<.05$) between week 0 and week 8 in protein content.

Dry matter protein content of the samples varied significantly ($P<.05$) between individual treatments. It should be noted that this variation could be due to the ingredient composition of the material processed. A slight error in urea addition can cause a large difference in protein content. Differences were grouped by starch sources, therefore, suggesting variation due to batch composition.

Preservative combinations appeared to have no effect on protein stability. Figures 15 through 21 show the dry matter protein content stability over the 8-week trial for all treatments.

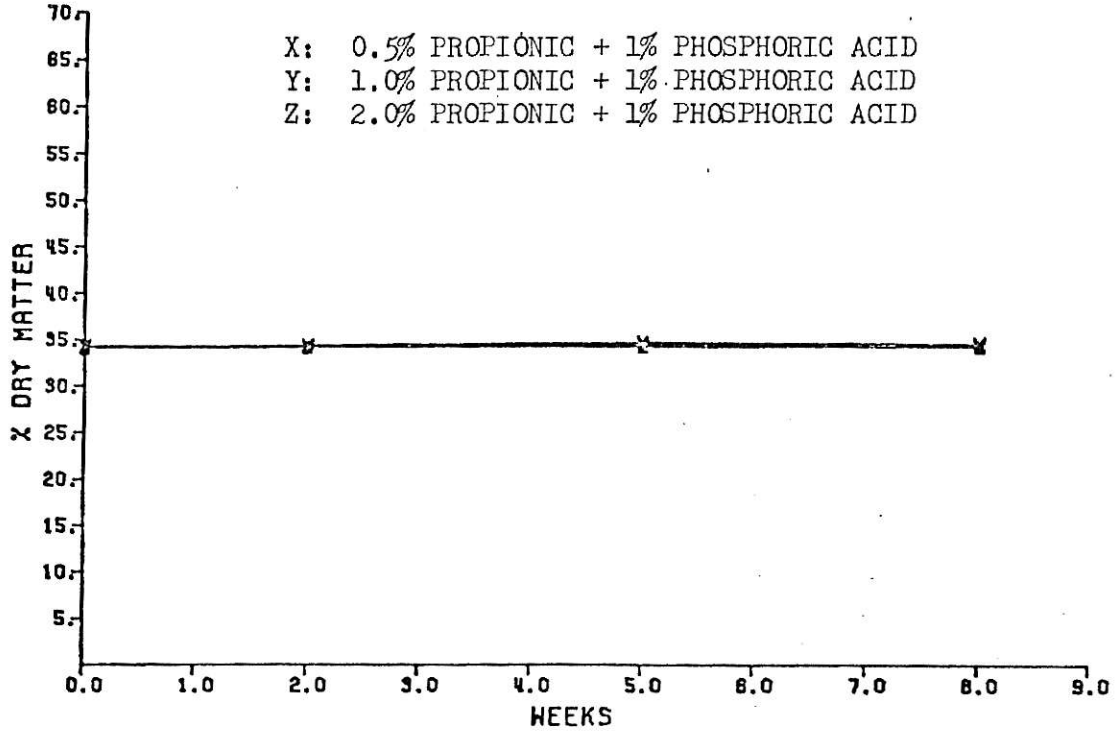

FIGURE 8. DRY MATTER DURING STORAGE USING NON-BR SORGHUM & PROP. ACID

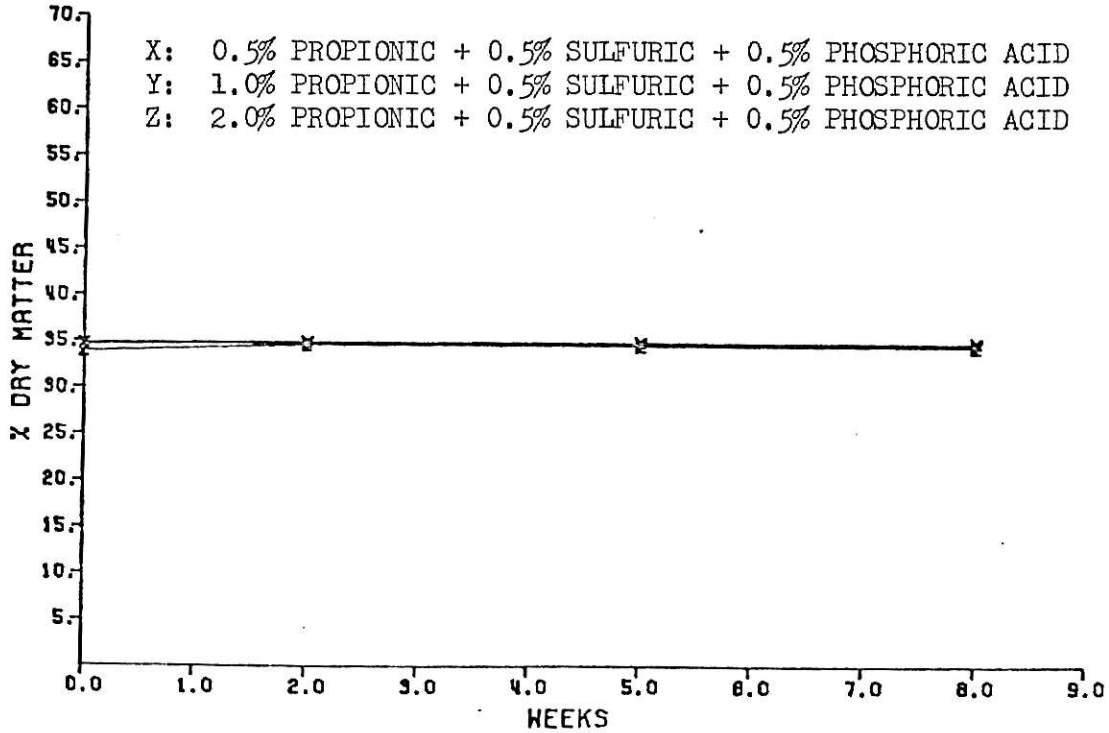

FIGURE 9. DRY MATTER DURING STORAGE USING NON-BR SORGHUM, H2S04 & PROP.

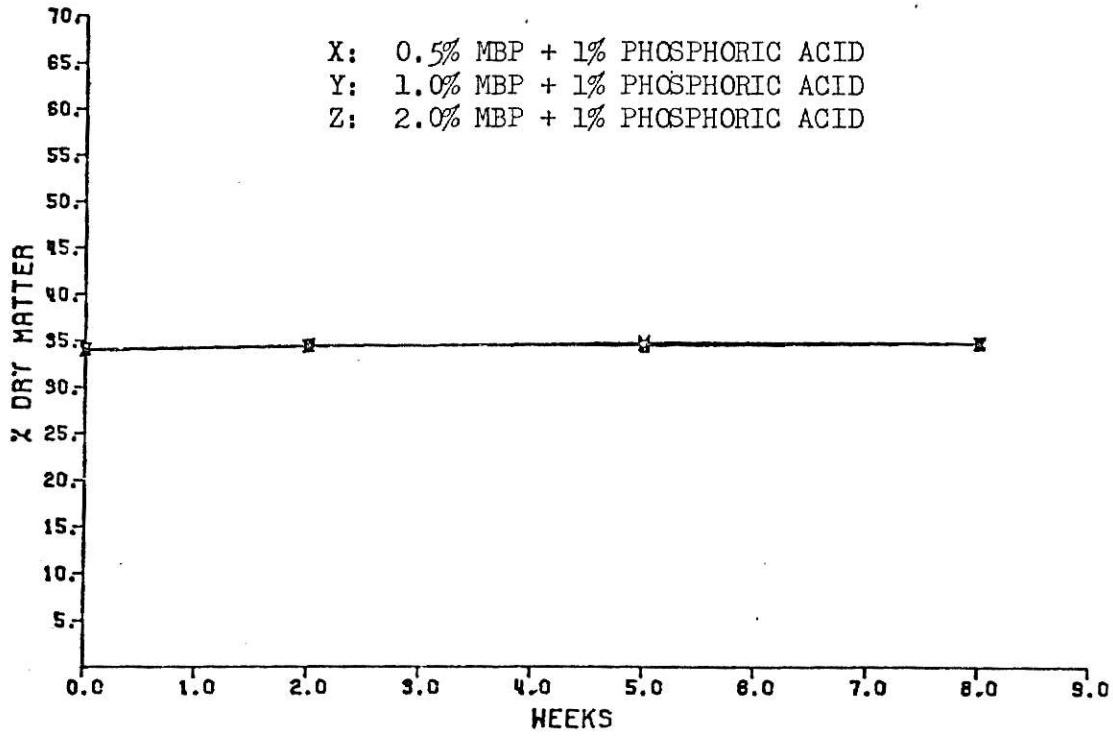

FIGURE 10. DRY MATTER DURING STORAGE USING NON-BR SORGHUM AND MBP

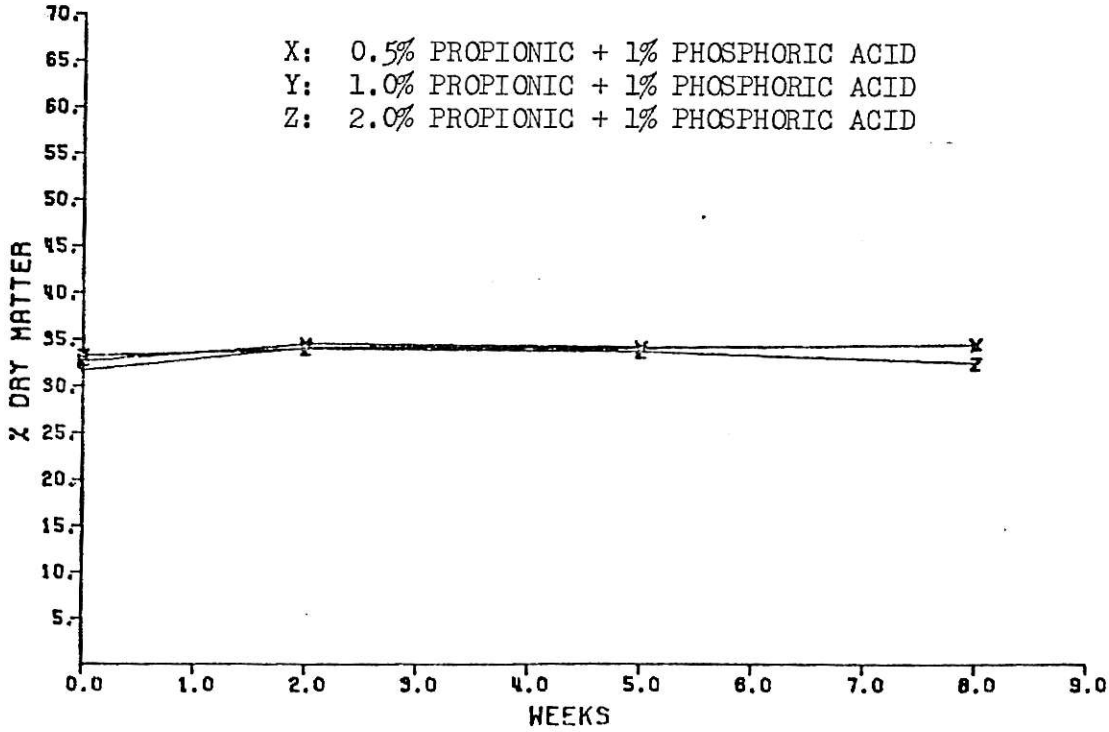

FIGURE 11. DRY MATTER DURING STORAGE USING BR SORGHUM & PROP. ACID

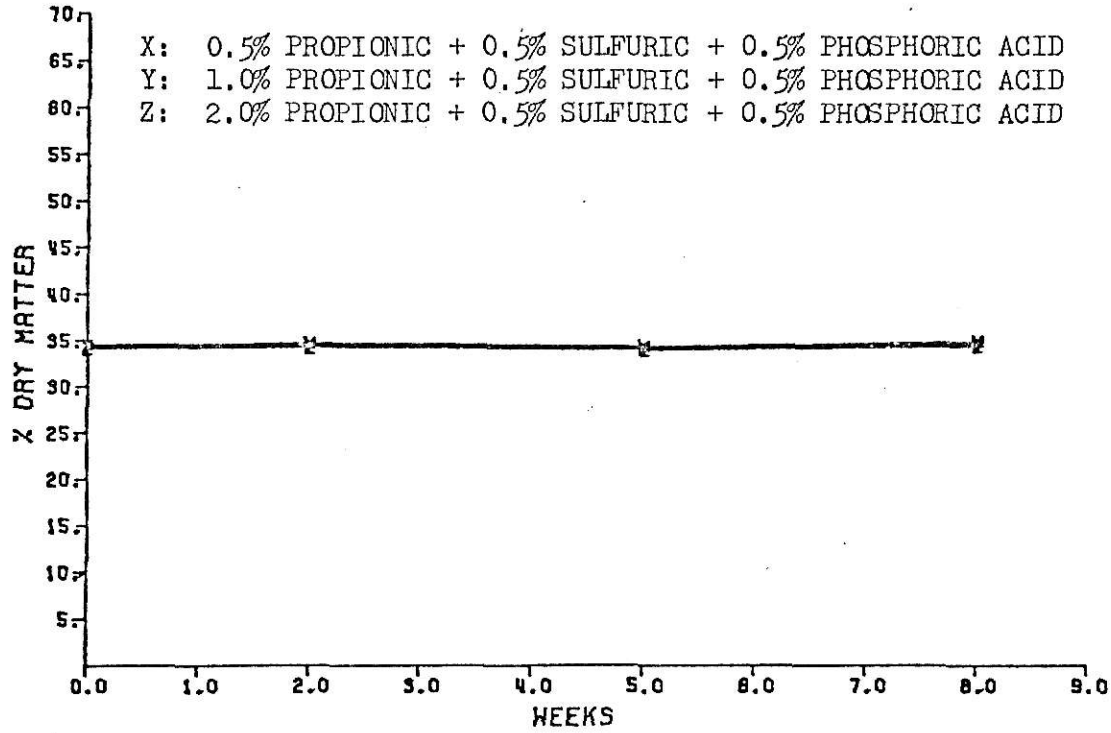

FIGURE 12. DRY MATTER DURING STORAGE USING BR SORGHUM, H2S04 & PROP.

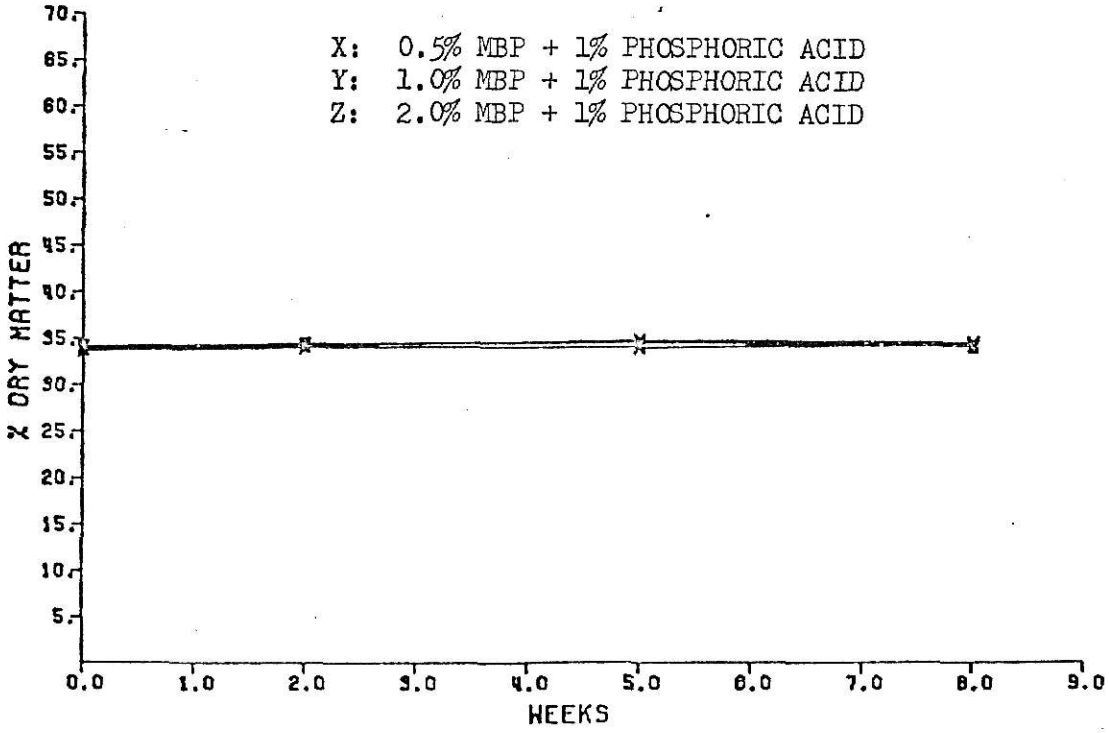

FIGURE 13. DRY MATTER DURING STORAGE USING BR-SORGHUM AND MBP

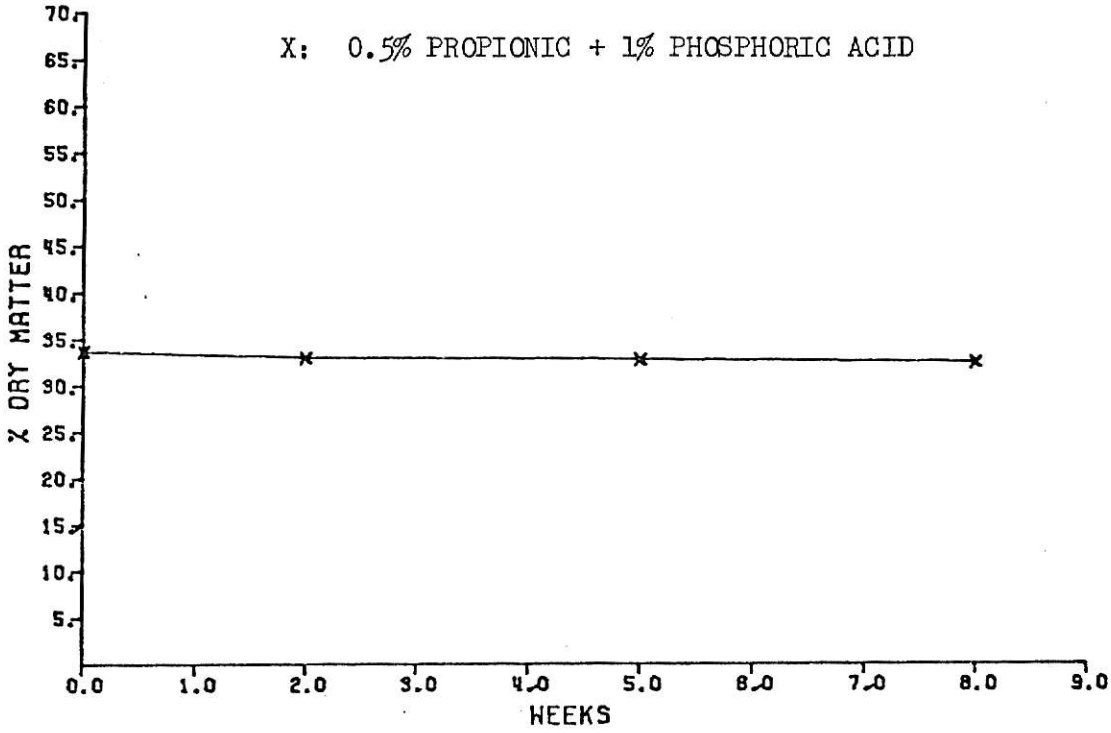

FIGURE 14. DRY MATTER DURING STORAGE USING CORN AND PROPIONIC ACID

TABLE 10. MEAN PROTEIN CONTENT OF STARCH CONTAINING LIQUID SUPPLEMENTS DURING STORAGE

Sample	Week[a] 0	2	5	8	Row average
Non-br sorghum					
S1&S28	87.06^{b}	85.53^{b}	86.89^{b}	87.32^{b}	86.70^{ghij}
S2&S29	86.83^{b}	88.56^{b}	85.45^{b}	86.32^{b}	86.79^{ghij}
S3&S30	86.77^{b}	86.52^{b}	85.98^{b}	85.91^{b}	86.29^{hij}
S4&S31	84.98^{b}	86.99^{b}	86.33^{b}	86.48^{b}	86.19^{ij}
S5&S32	86.42^{b}	86.66^{b}	86.65^{b}	86.53^{b}	86.57^{ghij}
S6&S33	87.92^{b}	85.83^{b}	86.05^{b}	86.25^{b}	86.51^{ghij}
S7&S34	88.49^{b}	87.09^{b}	87.18^{b}	86.70^{b}	87.37^{ghi}
S8&S35	88.56^{b}	87.11^{b}	86.93^{b}	86.15^{b}	87.19^{ghi}
S9&S36	86.56^{b}	85.38^{b}	86.33^{b}	85.68^{b}	85.99^{ij}
Br sorghum					
S10&S19	92.08^{b}	87.69^{c}	89.38^{bc}	88.36^{c}	89.38^{def}
S11&S20	95.96^{b}	88.59^{c}	89.58^{c}	87.44^{c}	90.39^{d}
S12&S21	90.68^{b}	88.20^{b}	89.28^{b}	91.26^{b}	89.86^{de}
S13&S22	86.79^{b}	85.82^{b}	88.74^{b}	86.93^{b}	87.07^{ghi}
S14&S23	88.23^{b}	87.17^{b}	89.11^{b}	87.87^{b}	88.09^{efg}
S15&S24	87.85^{b}	86.55^{b}	88.52^{b}	87.57^{b}	87.62^{fghi}
S16&S25	90.42^{b}	89.07^{b}	89.99^{b}	89.26^{b}	89.69^{de}
S17&S26	89.05^{b}	87.60^{b}	87.99^{b}	88.35^{b}	88.25^{efg}
S18&S27	87.56^{b}	87.83^{b}	87.95^{b}	88.94^{b}	88.07^{efgh}
Corn					
SC_1&SC_2	83.36^{b}	84.33^{bc}	86.08^{bc}	86.97^{c}	85.18^{j}
Average	88.19^{b}	86.98^{c}	87.60^{bc}	87.39^{bc}	

[a]Values are averages of duplicate set samples, recorded as a percentage on a dry basis. Set data of protein contents are located in table 3 of appendix A.

[b,c]Means within rows having similar superscripts are not different ($P<.05$).

[d,e,f,g,h,i,j]Values within column having similar superscripts are not different ($P<.05$).

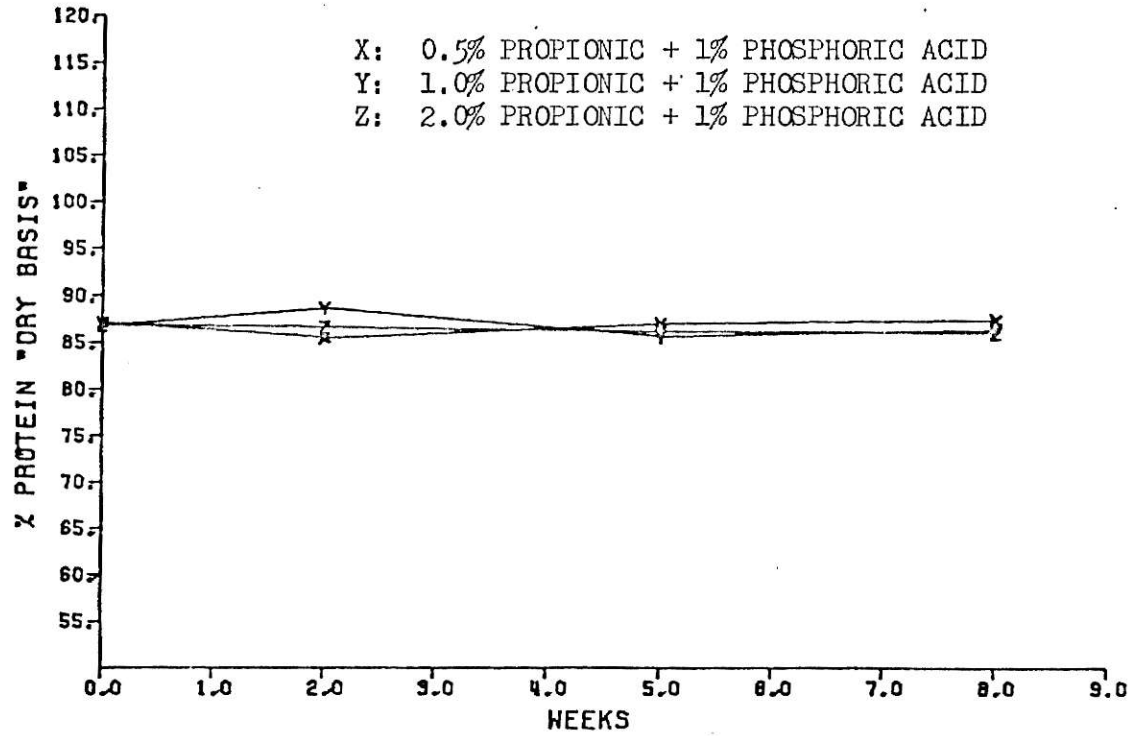

FIGURE 15. PROTEIN DURING STORAGE USING NON-BR SORGHUM & PROP. ACID

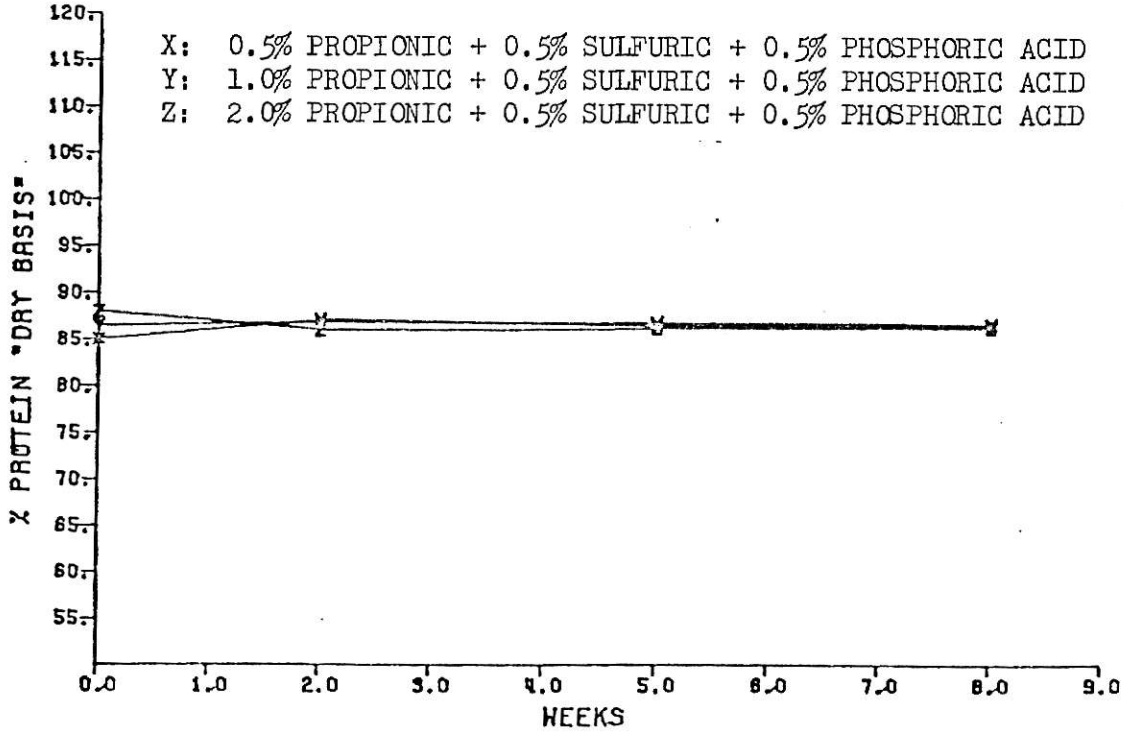

FIGURE 16. PROTEIN DURING STORAGE USING NON-BR SORGHUM, H_2SO_4 & PROP.

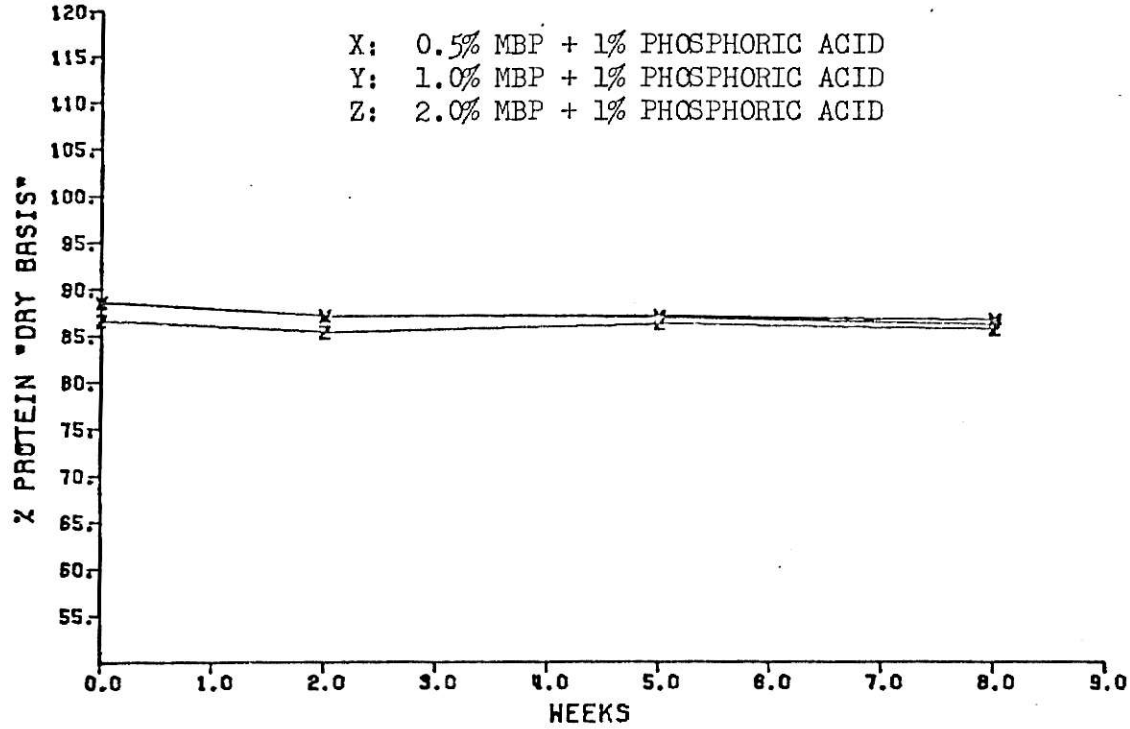

FIGURE 17. PROTEIN DURING STORAGE USING NON-BR SORGHUM AND MBP

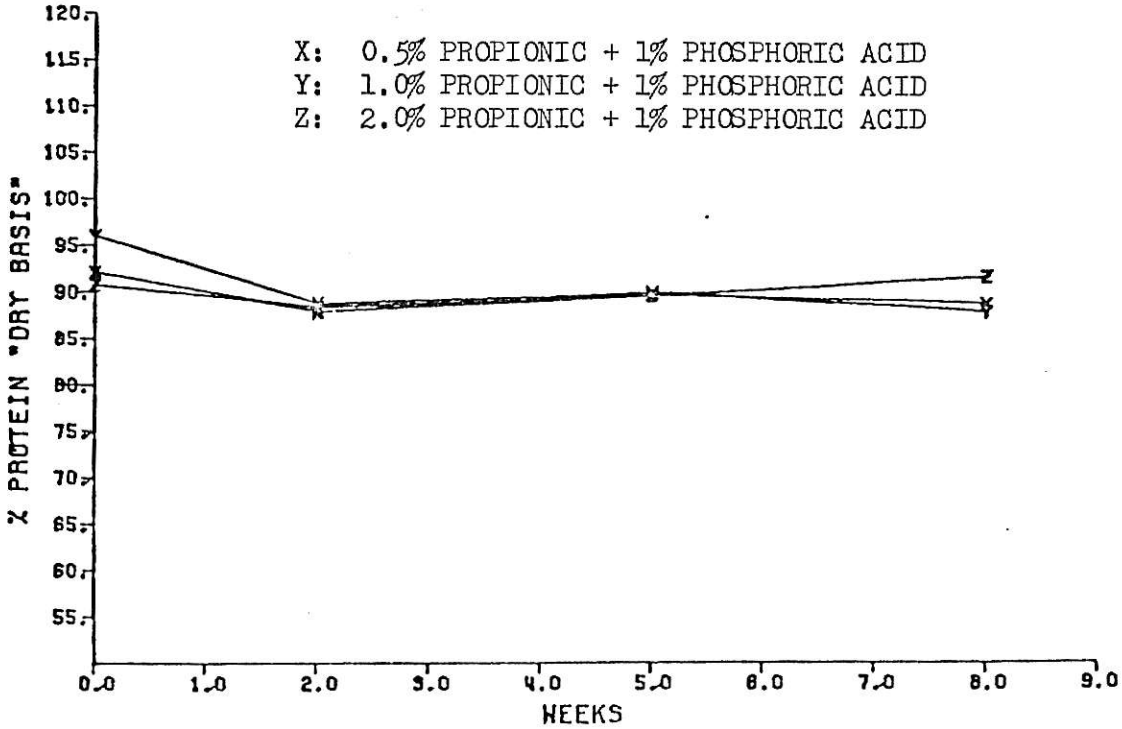

FIGURE 18. PROTEIN DURING STORAGE USING BR SORGHUM & PROP. ACID

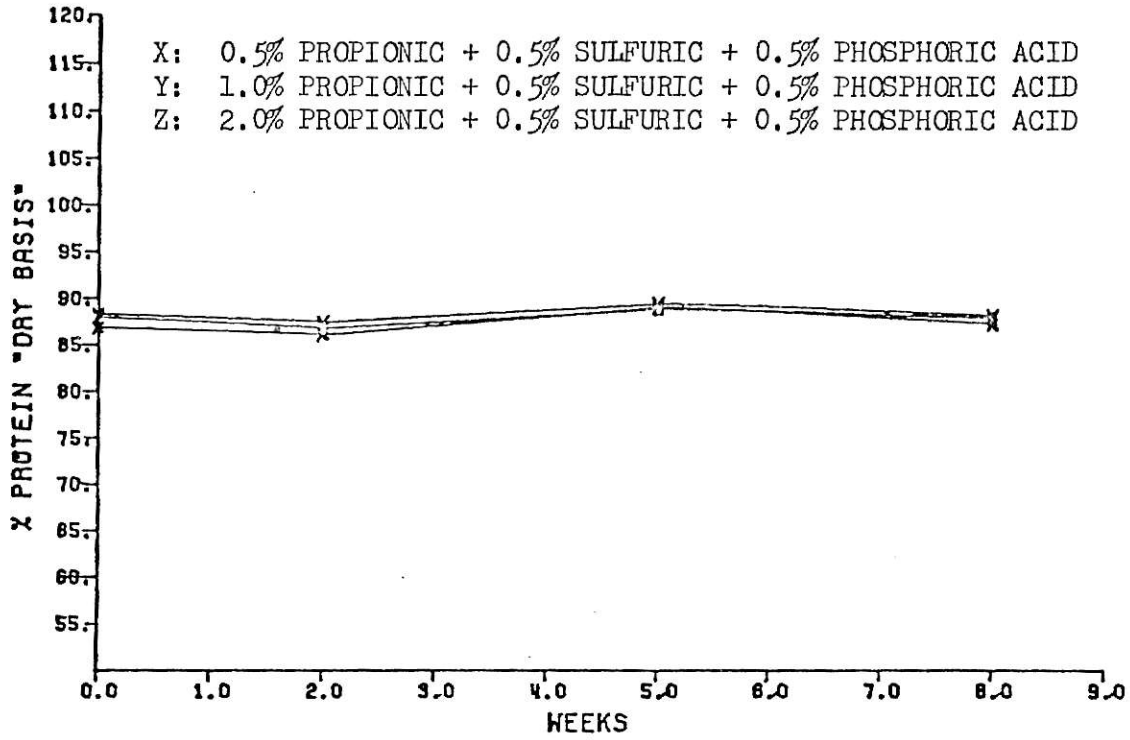

FIGURE 19. PROTEIN DURING STORAGE USING BR SORGHUM, H2SO4 & PROP.

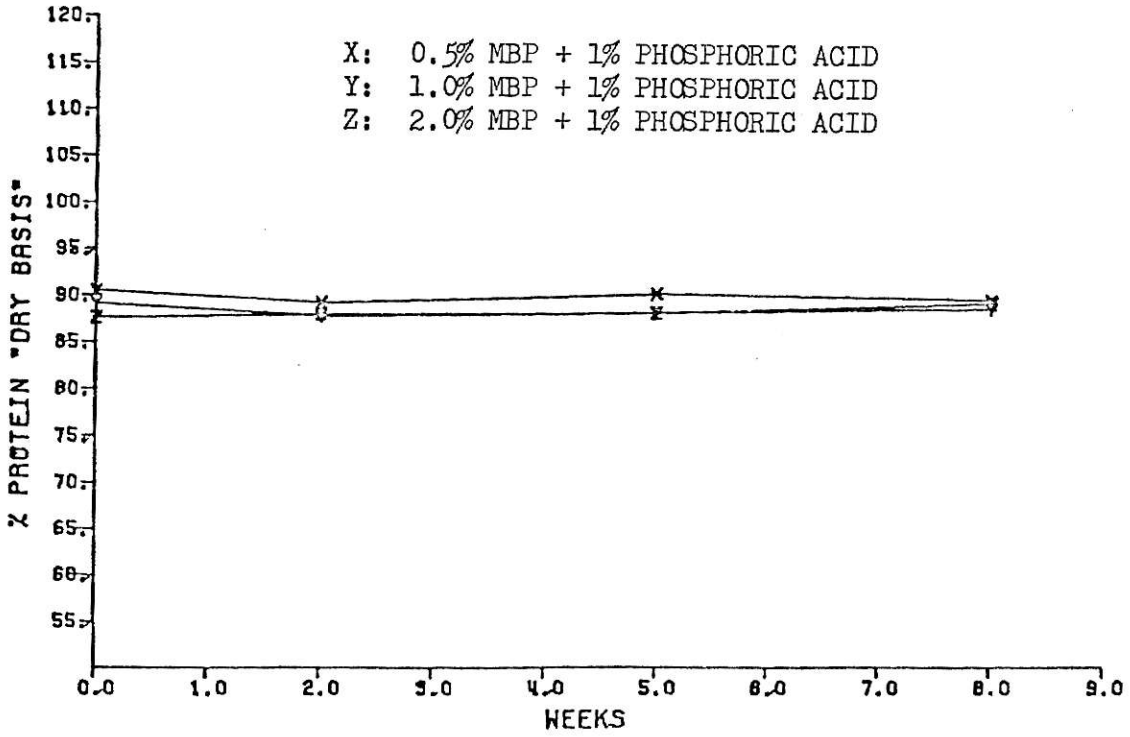

FIGURE 20. PROTEIN DURING STORAGE USING BR-SORGHUM AND MBP

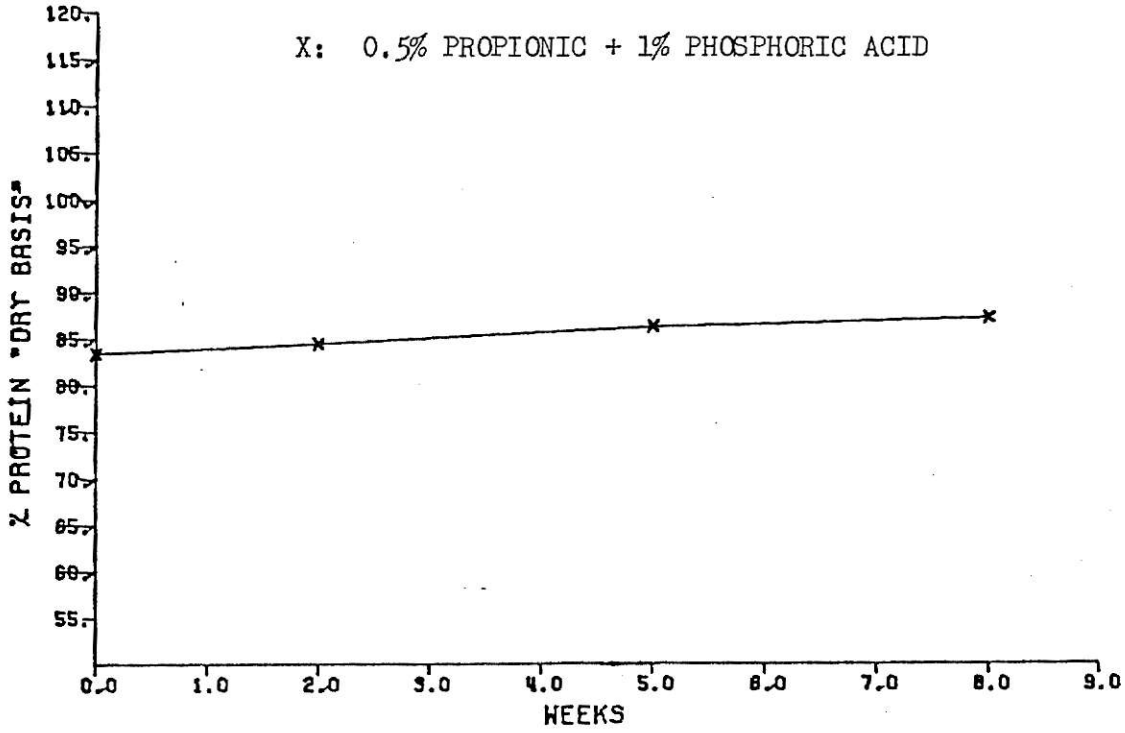

FIGURE 21. PROTEIN DURING STORAGE USING CORN AND PROPIONIC ACID

Ammonia Nitrogen

Table 11 lists the means for the NH_3-N levels in samples. No differences ($P<.05$) were noted within individual treatments over the 8-week trial, nor between the various treatments. When averages of all the treatments were analyzed, results indicated a trend for NH_3-N to increase ($P<.05$) between weeks zero and two. After the second week, no further increase was noted. Figures 22 through 28 show the levels of NH_3-N in mg/g of dry matter for individual treatments over the 8-week trial.

Maltose Equivalent

Maltose equivalents (ME) for individual treatments did not change ($P<.05$) over the 8-week period (table 12), although there were differences ($P<.05$) between the treatments. It should be noted that only 4 treatments differed significantly.

Type of acid treatment and/or starch source appeared to have no effect on ME, although the supplement containing corn as the starch source had the lowest value noted. The trend of having lower ME values for the supplement containing corn was not consistant, as will be shown in a later section. ME values were probably dependent on mixing and inactivation of α-amylase. If blending and activation time of the enzyme are not consistantly identical from batch to batch, differences in ME can result. Figures 29 through 35 illustrate the course of ME values over the 8-week trial for individual treatments.

Viscosity

Viscosity readings ranged from a high of 2600 cps (centipoise) to a low of 81 cps over the 8-week trial (table 6, appendix A). The average trend of all treatments resulted in a decrease ($P<.05$) between week 0 and week 2 (table 13). After the second week, no change ($P<.05$) was noted.

TABLE 11. MEAN AMMONIA NITROGEN CONTENT OF STARCH CONTAINING LIQUID SUPPLEMENTS DURING STORAGE

Sample	Week[a] 0	2	5	8	Row average
Non-br sorghum					
S1&S28	2.520[b]	2.844[b]	2.549[b]	2.858[b]	2.693[d]
S2&S29	2.237[b]	2.729[b]	2.574[b]	2.818[b]	2.589[d]
S3&S30	2.220[b]	2.601[b]	2.666[b]	2.656[b]	2.536[d]
S4&S31	2.268[b]	2.561[b]	2.666[b]	2.745[b]	2.560[d]
S5&S32	2.227[b]	2.605[b]	2.627[b]	2.698[b]	2.539[d]
S6&S33	2.334[b]	2.534[b]	2.578[b]	2.842[b]	2.572[d]
S7&S34	2.386[b]	2.488[b]	2.615[b]	2.659[b]	2.537[d]
S8&S35	2.098[b]	2.501[b]	2.595[b]	2.583[b]	2.445[d]
S9&S36	2.325[b]	2.432[b]	2.732[b]	2.647[b]	2.534[d]
Br Sorghum					
S10&S19	2.547[b]	2.695[b]	2.804[b]	2.842[b]	2.722[d]
S11&S20	2.519[b]	2.757[b]	2.673[b]	2.829[b]	2.695[d]
S12&S21	2.212[b]	2.651[b]	2.699[b]	2.881[b]	2.611[d]
S13&S22	2.168[b]	2.611[b]	2.697[b]	2.849[b]	2.582[d]
S14&S23	2.415[b]	2.598[b]	2.882[b]	2.824[b]	2.680[d]
S15&S24	2.333[b]	2.617[b]	2.706[b]	2.691[b]	2.587[d]
S16&S25	2.196[b]	2.652[b]	2.748[b]	2.655[b]	2.563[d]
S17&S26	2.075[b]	2.472[b]	2.739[b]	2.699[b]	2.497[d]
S18&S27	2.235[b]	2.531[b]	2.758[b]	2.638[b]	2.309[d]
Corn					
SC_1&SC_2	2.263[b]	2.299[b]	2.535[b]	2.138[b]	2.309[d]
Average	2.294[b]	2.589[c]	2.676[c]	2.714[c]	

[a]Values are averages of duplicate set samples, recorded as milligrams of ammonia nitrogen per gram of dry matter. Set data of ammonia nitrogen content are located in table 4 of appendix A.

[b,c]Means within rows having similar superscripts are not different ($P<.05$).

[d]Values within column having similar superscripts are not different ($P<.05$).

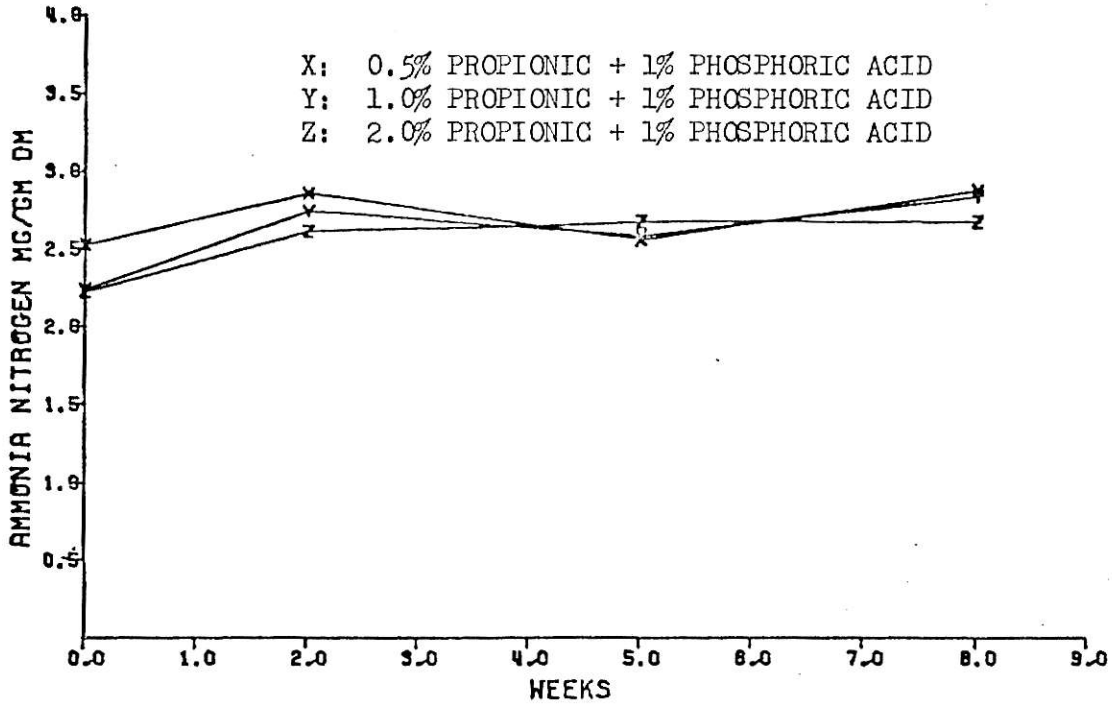

FIGURE 22. NH3-N DURING STORAGE USING NON-BR SORGHUM & PROP. ACID

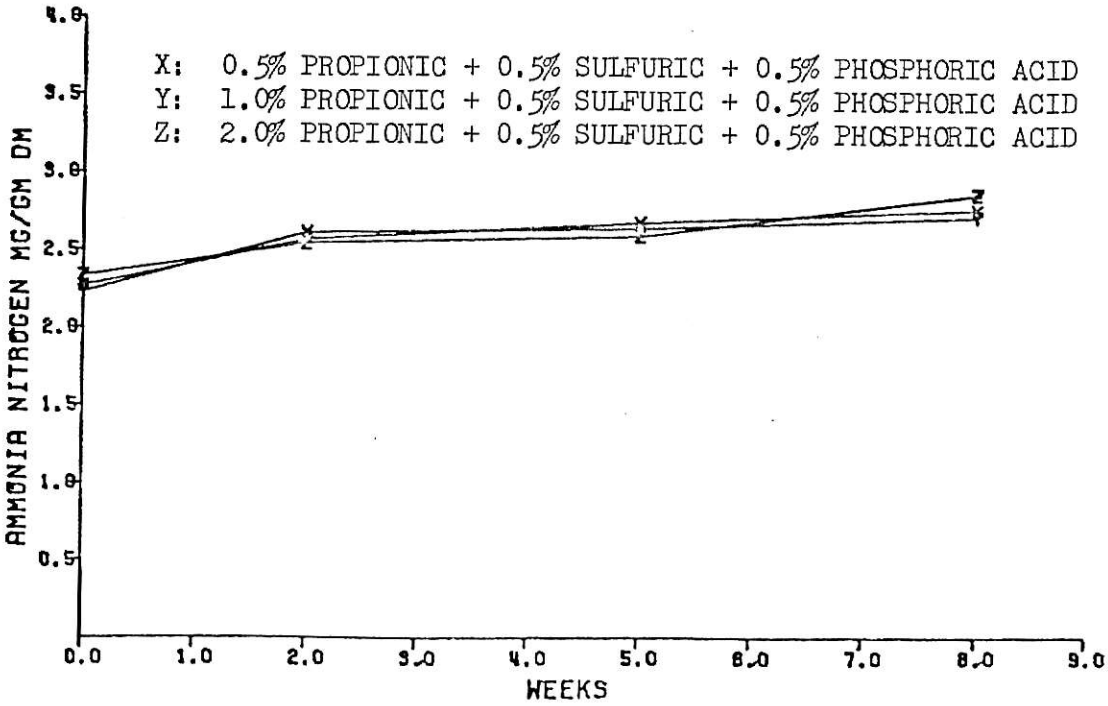

FIGURE 23. NH3-N DURING STORAGE USING NON-BR SORGHUM, H2SO4 & PROP.

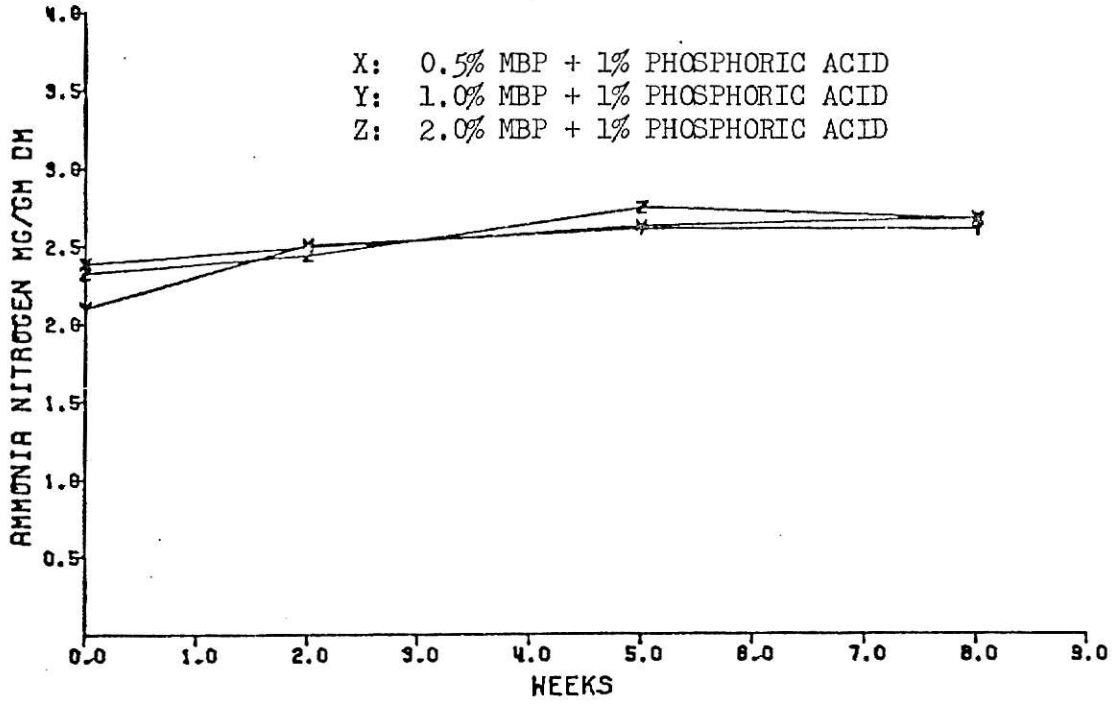

FIGURE 24. NH3-N DURING STORAGE USING NON-BR SORGHUM AND MBP

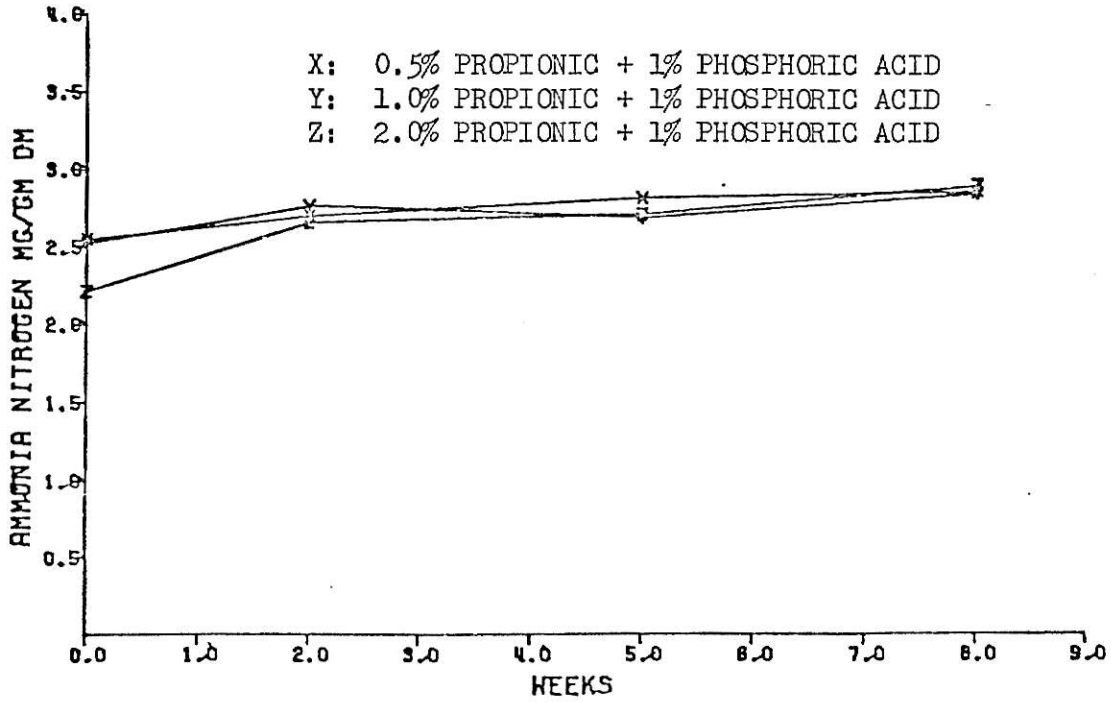

FIGURE 25. NH3-N DURING STORAGE USING BR SORGHUM & PROP. ACID

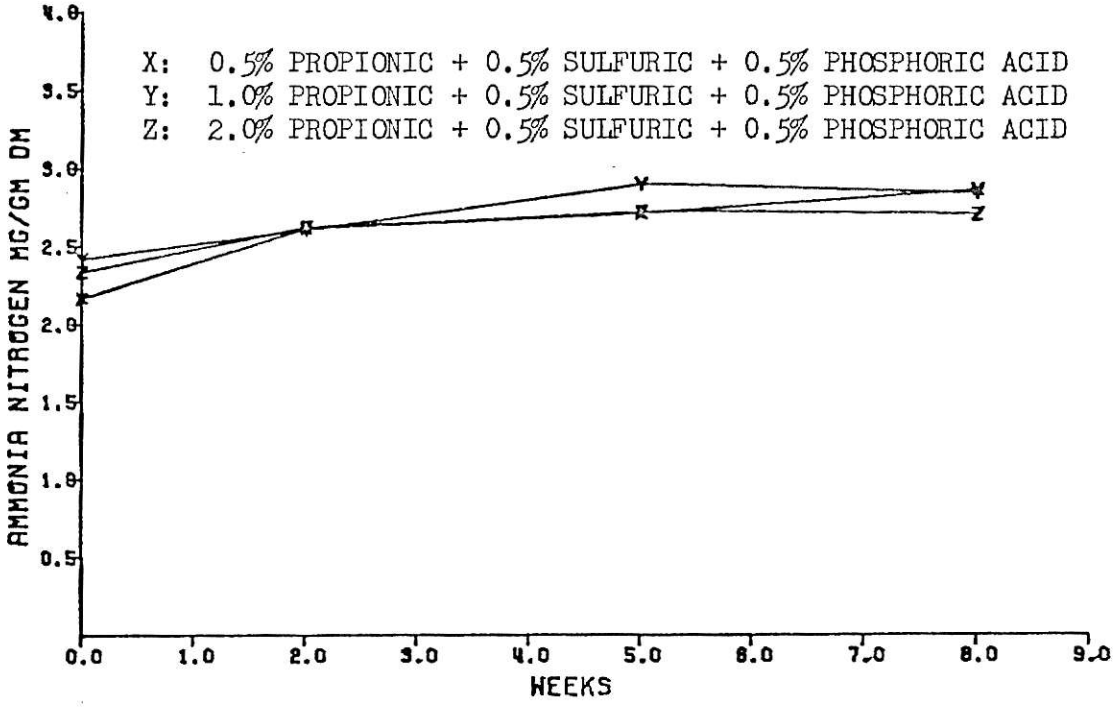

FIGURE 26. NH3-N DURING STORAGE USING BR SORGHUM, H2SO4 & PROP.

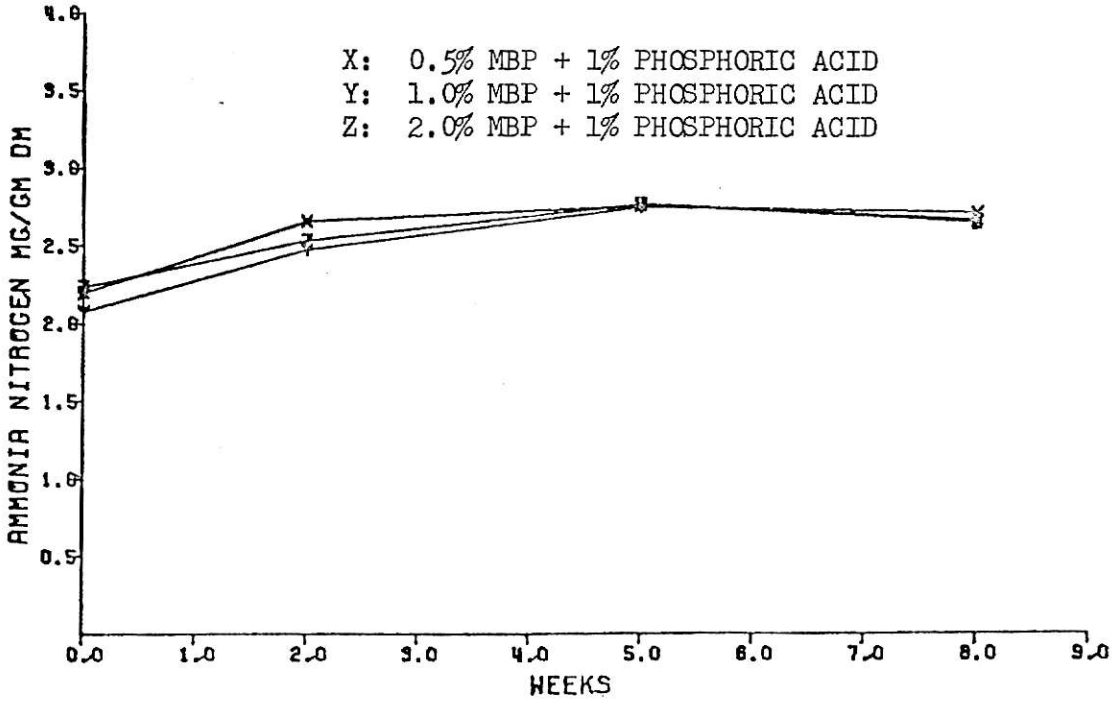

FIGURE 27. NH3-N DURING STORAGE USING BR-SORGHUM AND MBP

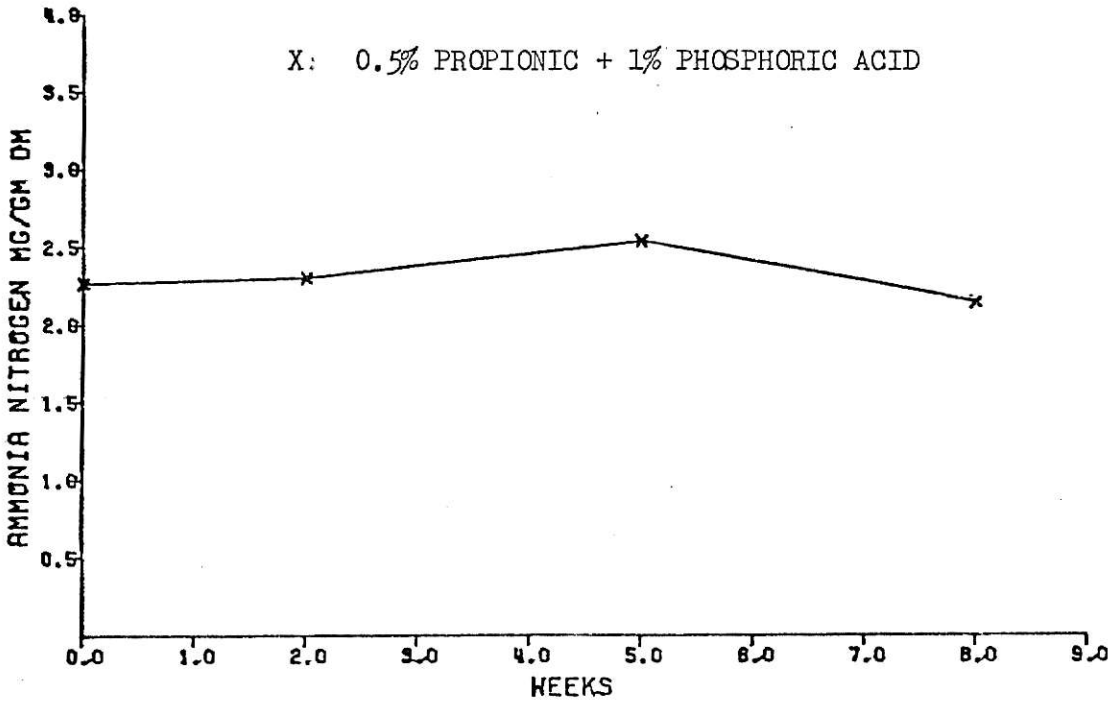

FIGURE 28. NH3-N DURING STORAGE USING CORN AND PROPIONIC ACID

TABLE 12. MEAN MALTOSE EQUIVALENTS OF STARCH CONTAINING LIQUID SUPPLEMENTS DURING STORAGE

Sample	Week[a] 0	2	5	8	Row average
Non-br sorghum					
S1&S28	234.5[b]	229.8[b]	220.4[b]	219.5[b]	226.1[defg]
S2&S29	232.5[b]	227.4[b]	222.1[b]	230.2[b]	228.1[defg]
S3&S30	230.7[b]	224.9[b]	212.4[b]	211.8[b]	219.9[fg]
S4&S31	240.8[b]	247.3[b]	246.7[b]	248.5[b]	245.8[cde]
S5&S32	240.3[b]	244.8[b]	241.1[b]	242.4[b]	242.1[cdef]
S6&S33	241.7[b]	236.8[b]	236.1[b]	239.3[b]	238.5[def]
S7&S34	234.9[b]	230.8[b]	225.0[b]	233.4[b]	231.1[defg]
S8&S35	237.4[b]	228.5[b]	223.3[b]	222.6[b]	227.9[defg]
S9&S36	233.4[b]	228.7[b]	230.3[b]	216.5[b]	227.2[defg]
Br Sorghum					
S10&S19	228.5[b]	229.5[b]	222.1[b]	212.8[b]	223.2[defg]
S11&S20	244.4[b]	221.9[b]	220.4[b]	211.2[b]	224.5[defg]
S12&S21	229.4[b]	225.6[b]	223.9[b]	214.8[b]	223.4[defg]
S13&S22	238.2[b]	256.5[b]	247.3[b]	247.4[b]	247.4[cd]
S14&S23	232.9[b]	259.0[b]	243.6[b]	230.9[b]	241.7[cdef]
S15&S24	225.9[b]	242.1[b]	244.8[b]	226.4[b]	234.8[def]
S16&S25	238.2[b]	275.5[b]	275.9[b]	268.9[b]	264.6[c]
S17&S26	231.8[b]	230.5[b]	214.9[b]	207.0[b]	221.1[efg]
S18&S27	225.4[b]	226.0[b]	214.4[b]	215.3[b]	220.3[efg]
Corn					
SC_1&SC_2	202.4[b]	207.4[b]	206.1[b]	214.9[b]	207.7[g]

[a]Values are averages of duplicate set samples recorded as milligrams of maltose per gram on a dry basis. Set data of maltose equivalents are located in table 5 of appendix A.

[b]Means within rows having similar superscripts are not different ($P<.05$).

[c,d,e,f,g]Values within column having similar superscripts are not different ($P<.05$).

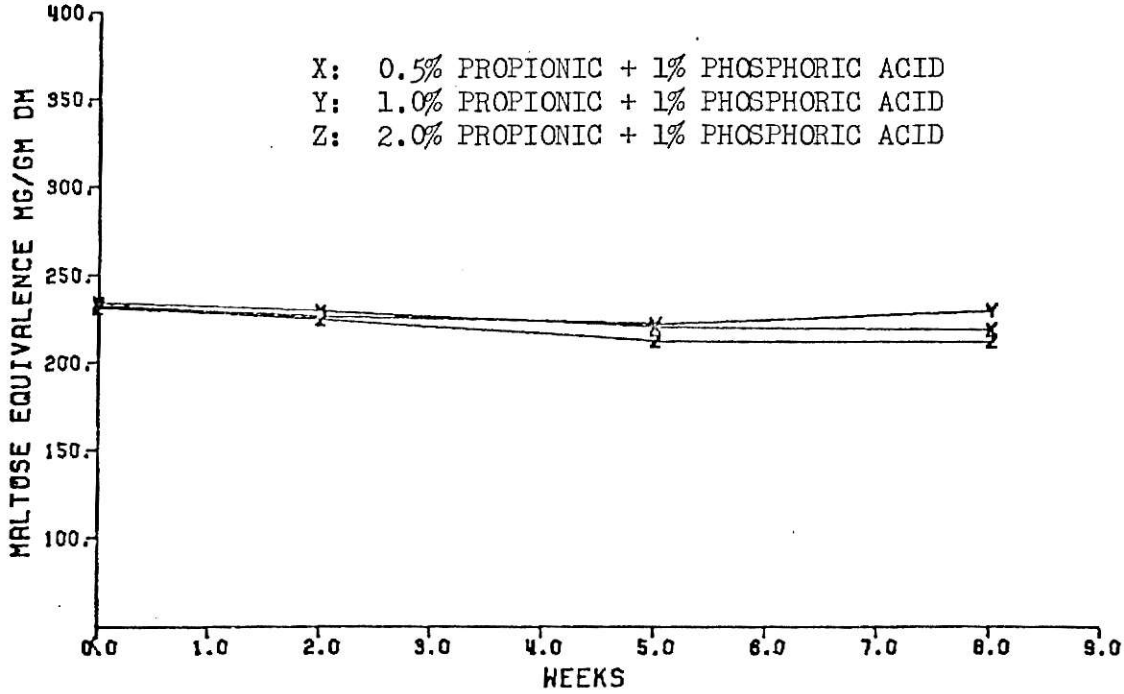

FIGURE 29. ME DURING STORAGE USING NON-BR SORGHUM AND PROPIONIC ACID

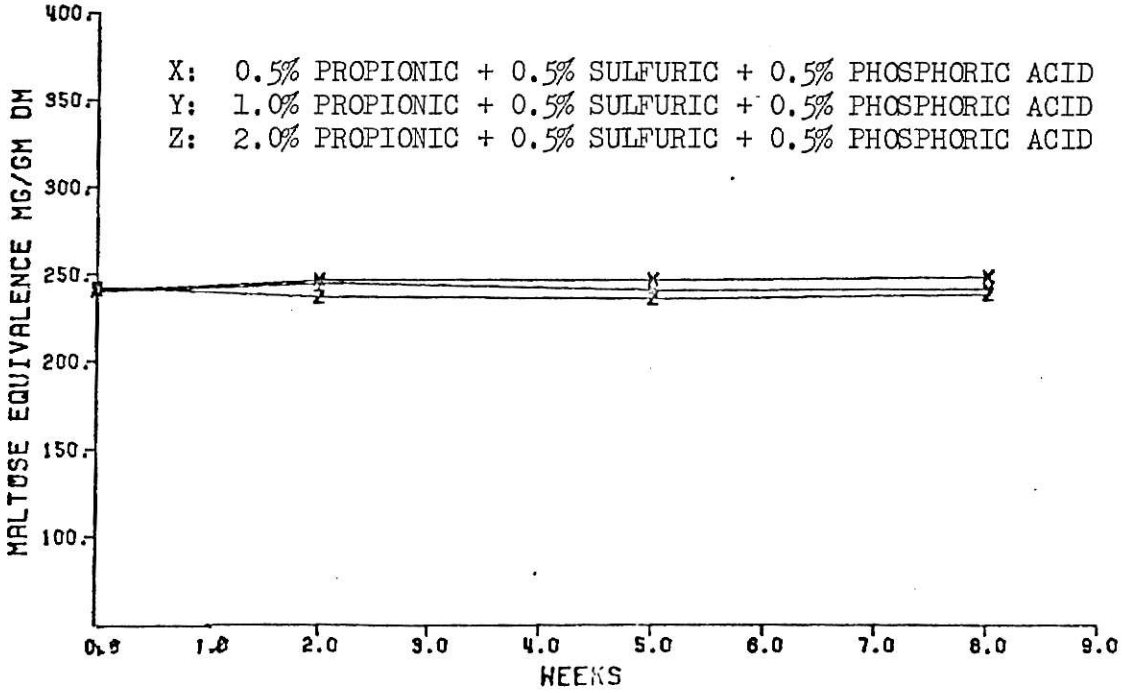

FIGURE 30. ME DURING STORAGE USING NON-BR SORGHUM, H2SO4 & PROP. ACID

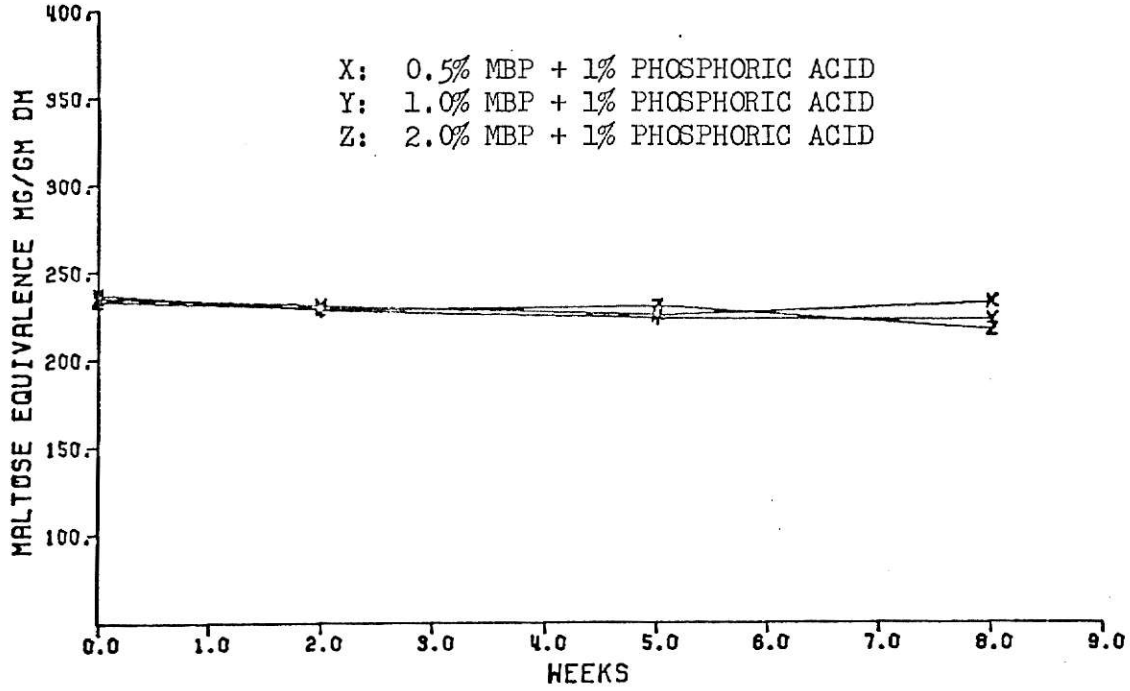

FIGURE 31. ME DURING STORAGE USING NON-BR SORGHUM AND MBP

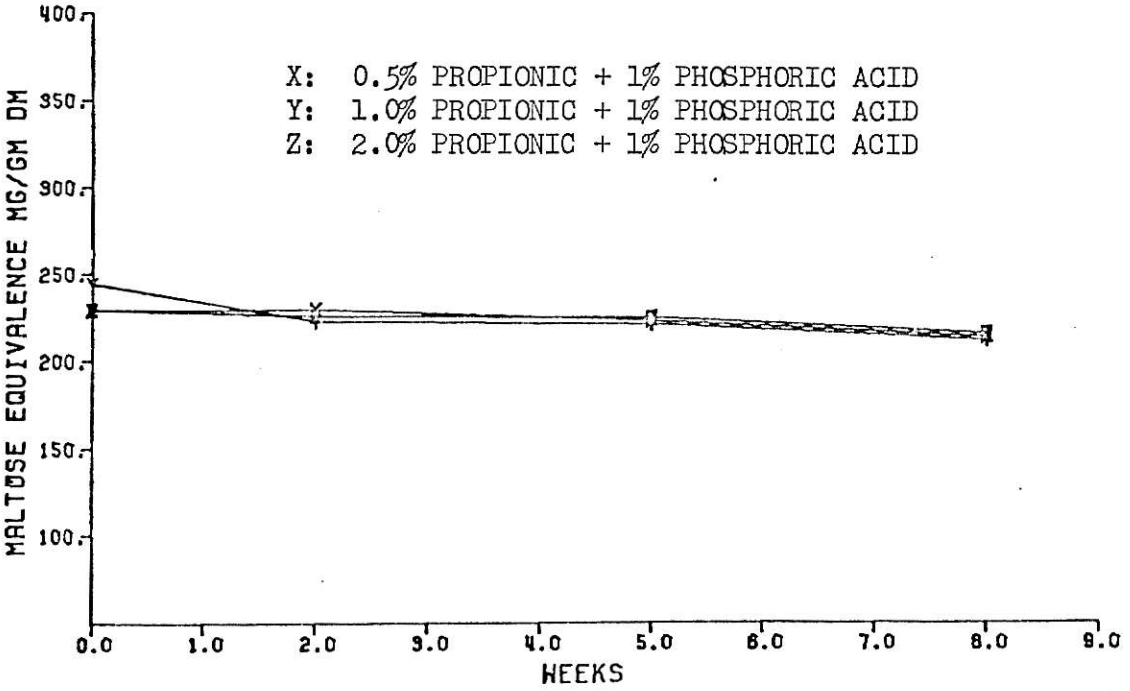

FIGURE 32. ME DURING STORAGE USING BR SORGHUM AND PROPIONIC ACID

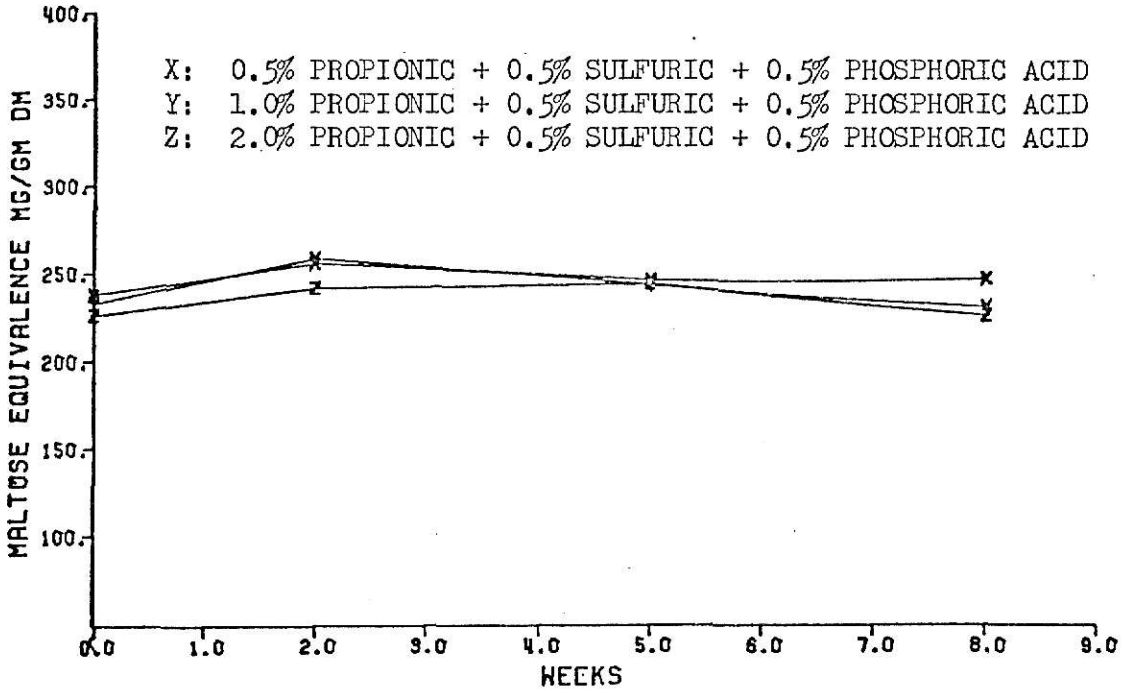

FIGURE 33. ME DURING STORAGE USING BR SORGHUM, H2SO4 & PROP. ACID

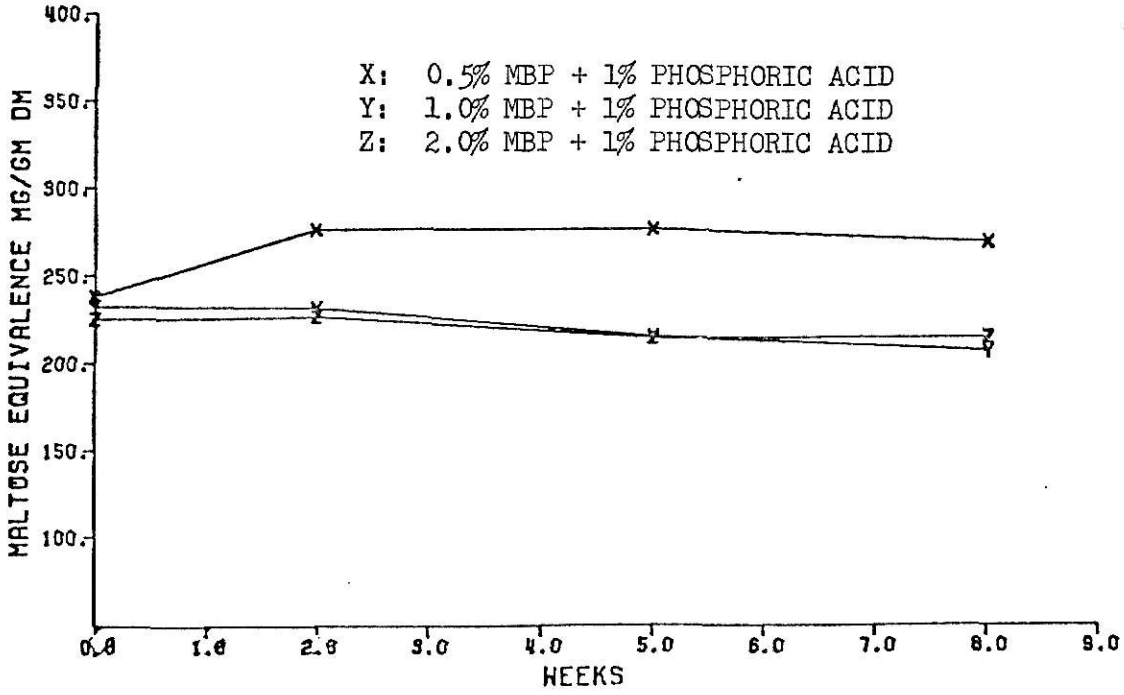

FIGURE 34. ME DURING STORAGE USING BR SORGHUM AND MBP

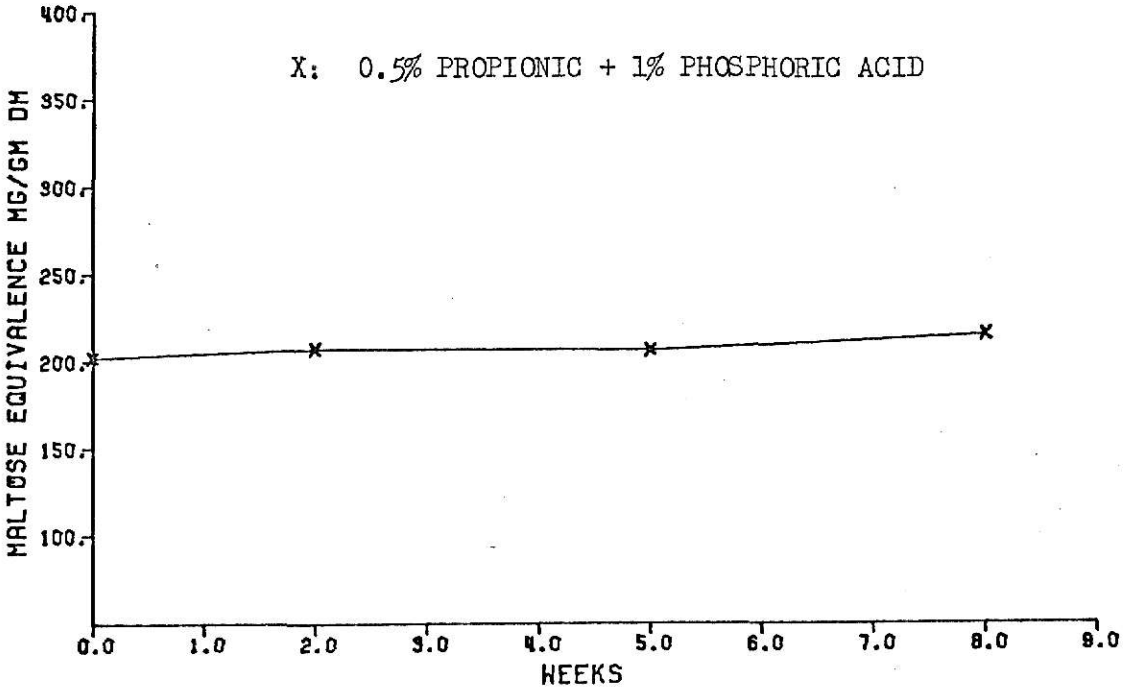

FIGURE 35. ME DURING STORAGE USING CORN AND PROPIONIC ACID

TABLE 13. MEAN VISCOSITY OF STARCH CONTAINING LIQUID SUPPLEMENTS DURING STORAGE

Sample	Week[a] 0	2	5	8	Row average
Non-br sorghum					
S1&S28	884.5^{b}	241.5^{c}	276.0^{c}	253.5^{c}	413.9^{ghi}
S2&S29	857.0^{b}	260.5^{c}	289.0^{c}	268.0^{c}	418.6^{ghi}
S3&S30	874.5^{b}	253.5^{c}	277.0^{c}	233.0^{c}	409.5^{ghi}
S4&S31	848.0^{b}	249.5^{c}	272.0^{c}	251.0^{c}	405.1^{ghi}
S5&S32	858.5^{b}	270.5^{c}	276.0^{c}	263.5^{c}	417.1^{ghi}
S6&S33	831.0^{b}	274.0^{c}	271.0^{c}	236.0^{c}	403.0^{ghi}
S7&S34	1111.5^{b}	285.0^{c}	294.0^{c}	281.5^{c}	493.0^{gh}
S8&S35	928.5^{b}	276.5^{c}	295.0^{c}	242.5^{c}	435.6^{ghi}
S9&S36	1230.5^{b}	339.0^{c}	278.0^{c}	229.5^{c}	519.3^{g}
Br sorghum					
S10&S19	622.5^{b}	183.5^{c}	174.0^{c}	170.0^{c}	287.5^{hi}
S11&S20	577.5^{b}	165.0^{b}	171.5^{b}	168.5^{b}	270.6^{hi}
S12&S21	587.0^{b}	169.0^{b}	174.5^{b}	164.5^{b}	273.8^{hi}
S13&S22	582.0^{b}	178.0^{b}	173.0^{b}	166.5^{b}	274.9^{hi}
S14&S23	599.0^{b}	161.5^{b}	177.5^{b}	168.5^{b}	276.6^{hi}
S15&S24	578.0^{b}	178.5^{b}	181.5^{b}	173.0^{b}	277.8^{hi}
S16&S25	578.0^{b}	174.0^{b}	158.5^{b}	150.5^{b}	265.3^{i}
S17&S26	647.5^{b}	176.0^{c}	177.5^{c}	161.0^{c}	290.5^{hi}
S18&S27	1076.0^{b}	252.5^{c}	202.5^{c}	127.0^{c}	414.5^{ghi}
Corn					
SC_1&SC_2	2224.0^{b}	1206.0^{c}	1112.5^{d}	389.0^{e}	1232.9^{f}
Average	868.2^{b}	278.6^{c}	275.3^{c}	215.6^{c}	

[a]Values are averages of duplicate set samples recorded in centipoise units. Set data are located in table 6 of appendix A.

^{b,c,d,e}Means within rows having similar superscripts are not different ($P<.05$).

^{f,g,h,i}Values within column having similar superscripts are not different ($P<.05$).

Several treatments containing br sorghum as the starch source showed no significant change in viscosity within the first 2 weeks of the trial. However, a downward trend was evident in each case. Figures 36 through 42 illustrate the trends in viscosity for all treatments.

No differences ($P<.05$) were noted due to acid treatment with the exceptions of samples S9&S36, S16&S25 and SC_1&SC_2. Samples S9&S36 and S16&S25 were borderline cases. Supplement sample SC_1&SC_2, which contained corn as the starch source, resulted in the highest initial viscosity value, and values decreased ($P<.05$) throughout the storage trial. This high initial viscosity was probably affected by the same factor which caused the low maltose value for this sample. If inactivation of the α-amylase was more rapid in the treatment containing corn than that in the treatment containing sorghum, viscosity would naturally be higher in the corn treatment due to the larger number and longer starch chain lengths.

In Vitro Rumen Protein Synthesis

At the beginning of the storage study, samples of each treatment were frozen. It was assumed that these samples would encounter no change in value while frozen, and therefore, could be used as controls to test the effects of storage time on *in vitro* nitrogen utilization. Comparisons were made between treatments as well as among the treatments. Due to the large number of samples, protein synthesis determinations were conducted with samples in groups with the corn containing supplement used as a common reference sample and prototype (table 7, appendix A). Milligrams of protein synthesized for each sample was converted to a percentage of the protein synthesized by the supplement containing corn.

Table 14 shows the means of duplicate samples for *in vitro* protein synthesis. Results indicated differences ($P<.05$) in 5 cases between frozen

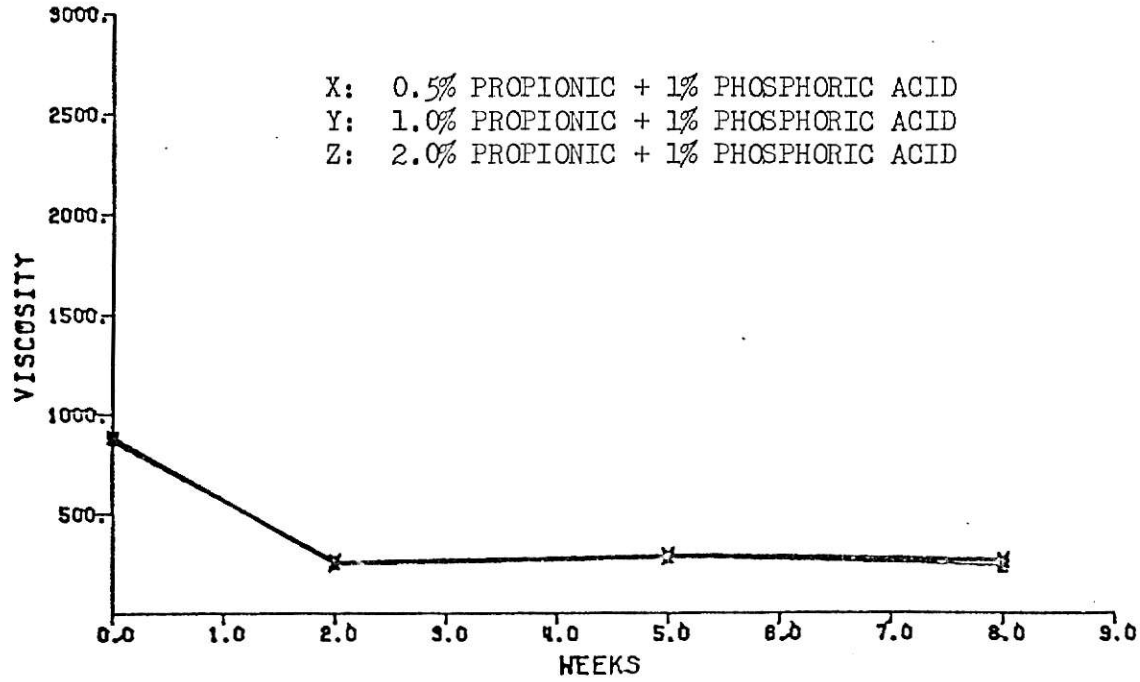

FIGURE 36. VISCOSITY DURING STORAGE USING NON-BR SORGHUM & PROP. ACID

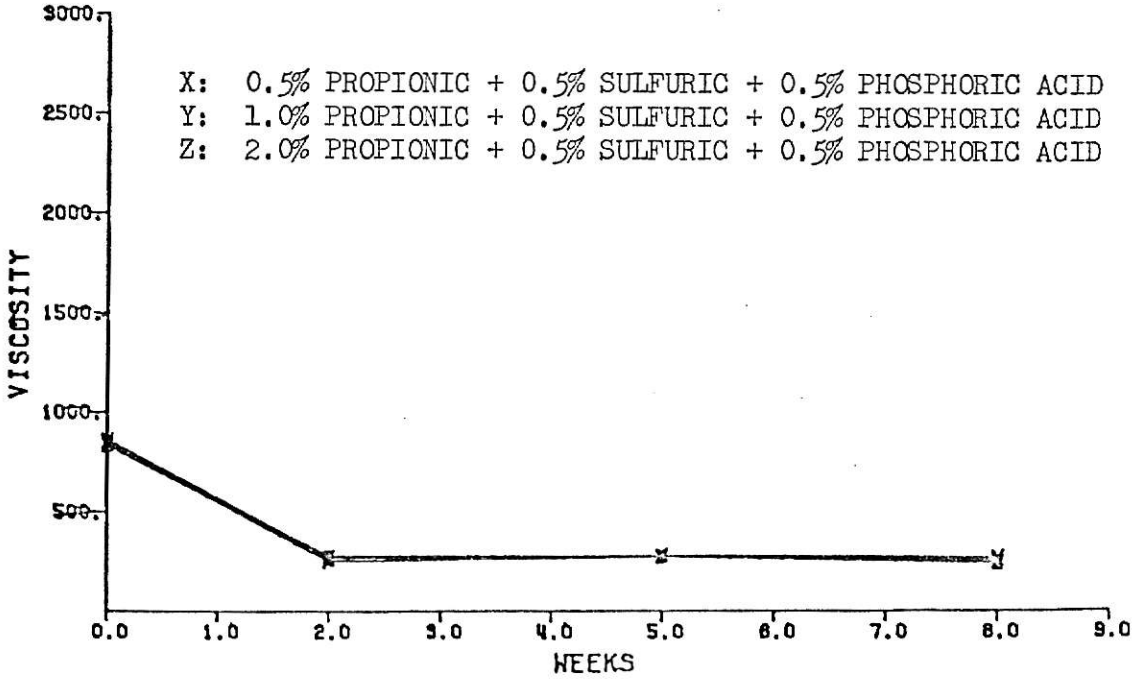

FIGURE 37. VISCOSITY DURING STORAGE USING NON-BR SORGHUM, H_2SO_4 & PROP.

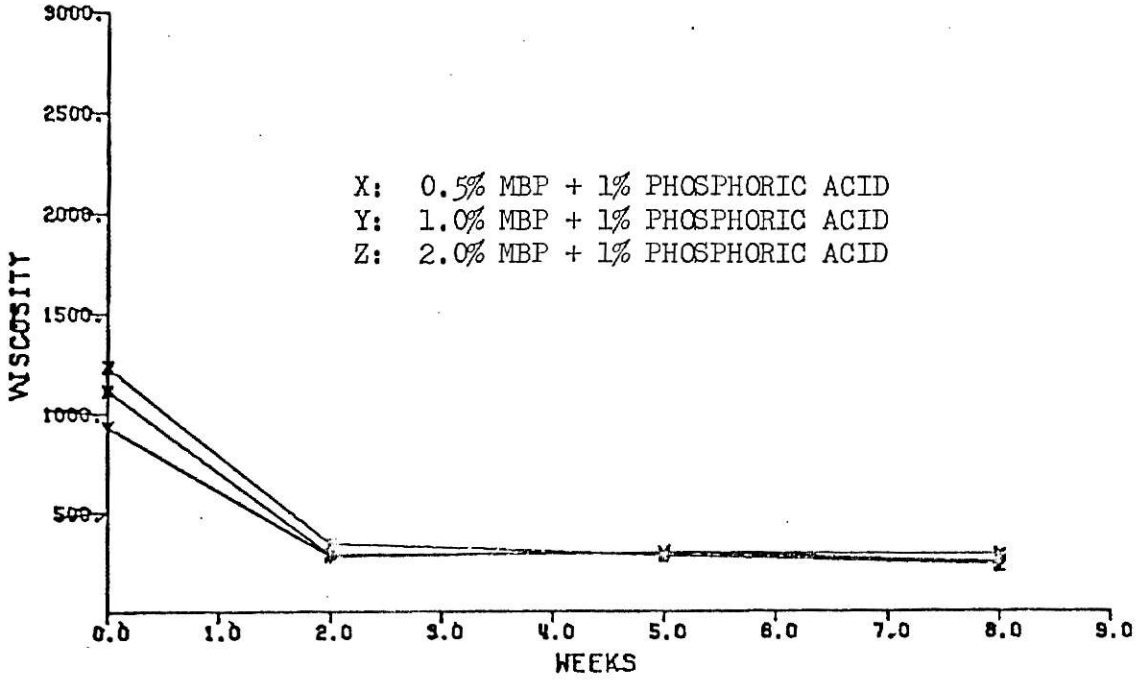

FIGURE 38. VISCOSITY DURING STORAGE USING NON-BR SORGHUM AND MBP

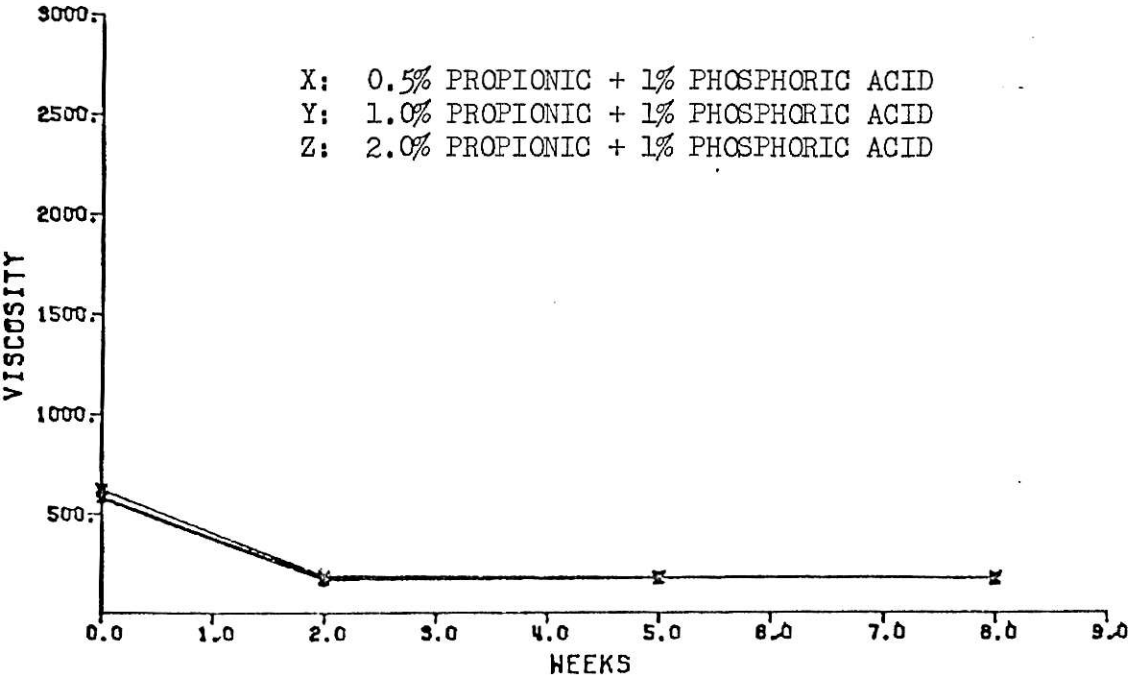

FIGURE 39. VISCOSITY DURING STORAGE USING BR SORGHUM & PROP. ACID

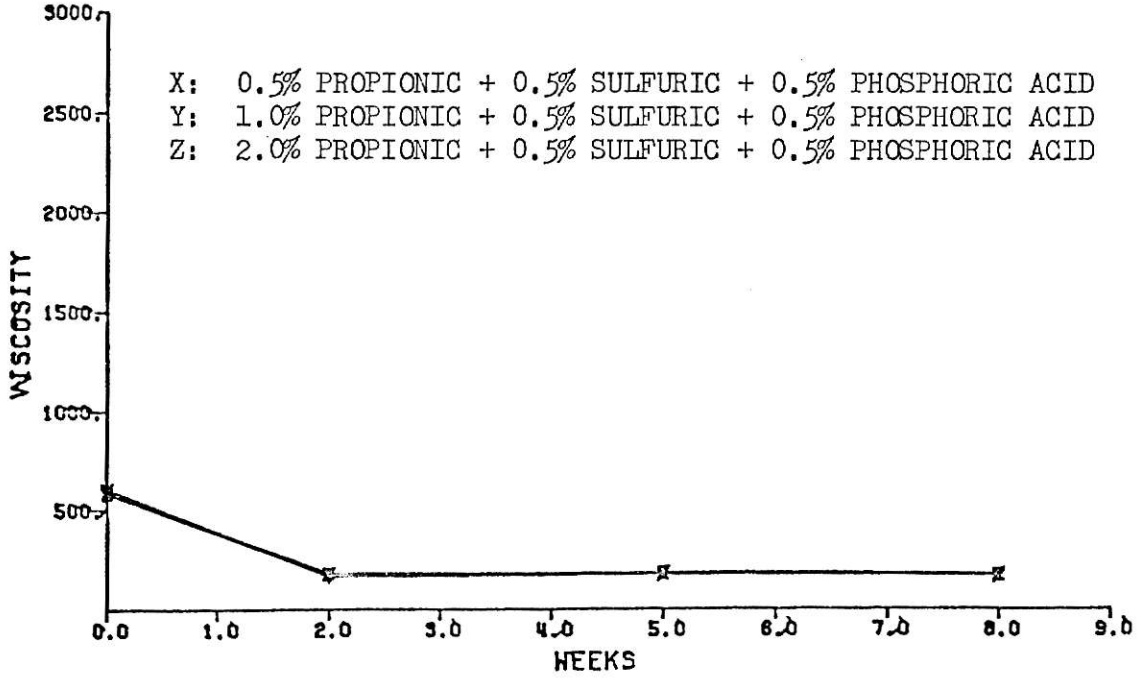

FIGURE 40. VISCOSITY DURING STORAGE USING BR SORGHUM, H2SO4 & PROP.

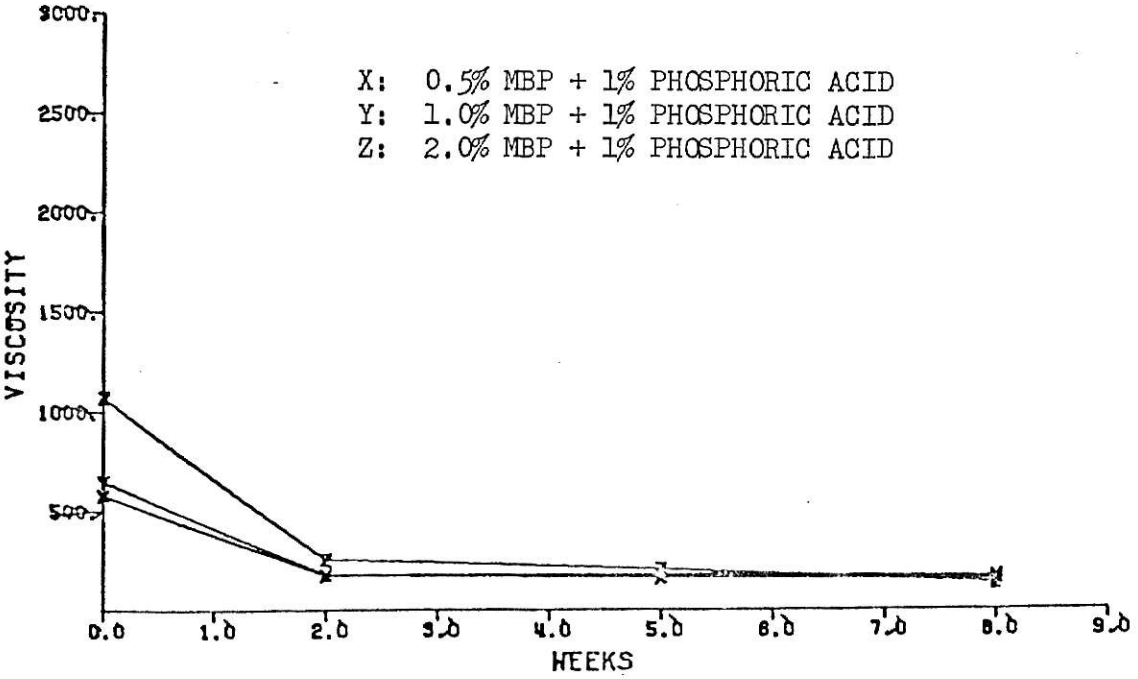

FIGURE 41. VISCOSITY DURING STORAGE USING BR-SORGHUM AND MBP

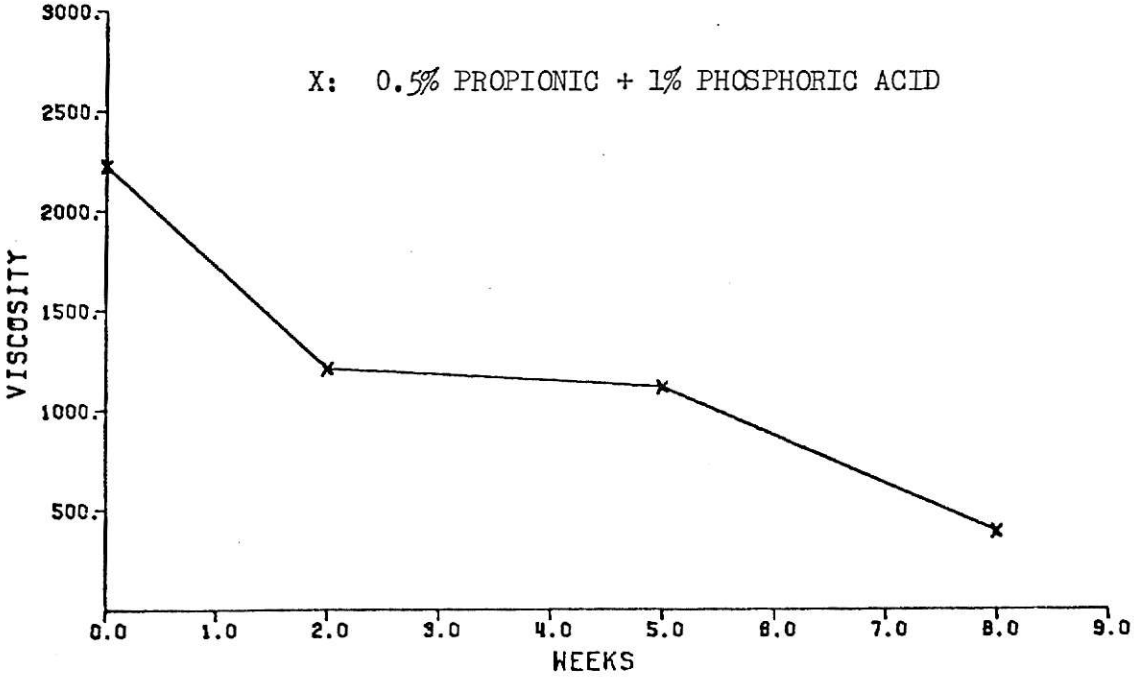

FIGURE 42. VISCOSITY DURING STORAGE USING CORN AND PROPIONIC ACID

TABLE 14. MEAN PROTEIN SYNTHESIZED[a] FROM STARCH CONTAINING LIQUID SUPPLEMENTS DURING STORAGE

Sample	Frozen samples[b]	Week 8[b]	Row average
Non-br sorghum			
S1&S28	103.0[c]	114.5[c]	108.7[e]
S2&S29	98.5[c]	123.0[c]	106.2[e]
S3&S30	76.0[c]	116.0[c]	96.0[e]
S4&S31	89.0[c]	117.0[c]	103.0[e]
S5&S32	101.0[c]	121.5[c]	111.2[e]
S6&S33	98.0[c]	105.0[c]	101.5[e]
S7&S34	98.0[c]	88.5[c]	93.2[e]
S8&S35	89.5[c]	45.0[c]	67.2[e]
S9&S36	27.0[c]	59.5[c]	43.2[e]
Br sorghum			
S10&S19	84.5[c]	183.5[d]	134.0[e]
S11&S20	77.5[c]	165.5[d]	121.5[e]
S12&S21	55.0[c]	197.5[d]	126.2[e]
S13&S22	68.5[c]	197.5[d]	133.0[e]
S14&S23	68.0[c]	189.0[d]	128.5[e]
S15&S24	92.0[c]	141.5[c]	116.7[e]
S16&S25	108.5[c]	88.0[c]	98.2[e]
S17&S26	82.5[c]	57.0[c]	69.7[e]
S18&S27	82.0[c]	55.0[c]	68.5[e]
Corn			
SC_1&SC_2	100.0[c]	100.0[c]	100.0[e]

[a]Values are reported as percentages of the corn containing sample control.

[b]Percentages are averages of duplicate set samples. Set data of protein synthesis are located in table 7 of appendix A.

[c,d]Means within rows having similar superscripts are not different ($P<.05$).

[e]Values within columns having similar superscripts are not different ($P<.05$).

samples and samples stored at room temperature. However, in each case the trend was an increase in protein synthesized, therefore, no decrease in quality had taken place in the samples. Analysis also showed there were no differences ($P<.05$) between the starch sources. Because of the wide variation in protein synthesized within a single treatment, a large statistical error mean square was encountered, resulting in no differences ($P<.05$) in protein synthesized between acid treatments. However, a trend for decreased in vitro nitrogen utilization was observed in samples where MBP levels were highest. Skoch (68) also observed similar effects with MBP when potato waste was used as a starch source for a liquid supplement.

SECTION 2. PROCESSING METHOD

The objective of this study was to determine if a starch containing liquid supplement could be produced without the direct introduction of live steam in the processing system, and exhibit similar characteristics to that of the supplement processed through the hydro-heater. Analyses performed on the supplements to determine similarity and quality of the two processing methods were: 1. crude protein, 2. maltose equivalent (ME), 3. dry matter and 4. in vitro rumen protein synthesis.

A heat exchanger was used to cook slurries at a temperature and pressure of 140 C and 40 psig, respectively. Diagrams of the heat exchanger are shown in Figures 43 and 44. Slurry was delivered to the heat exchanger by a .25 hp Moyno Pump.[1] The slurry entered the exchanger through a 1.27 cm pipe. Once in the heat exchanger the slurry passed through 112 tubes, which were .48 cm in diameter and 64.77 cm long. As the product flowed through the tubes, pressurized steam surrounded each individual tube. The

[1]Robbin and Myer, Inc., Springfield, Ohio.

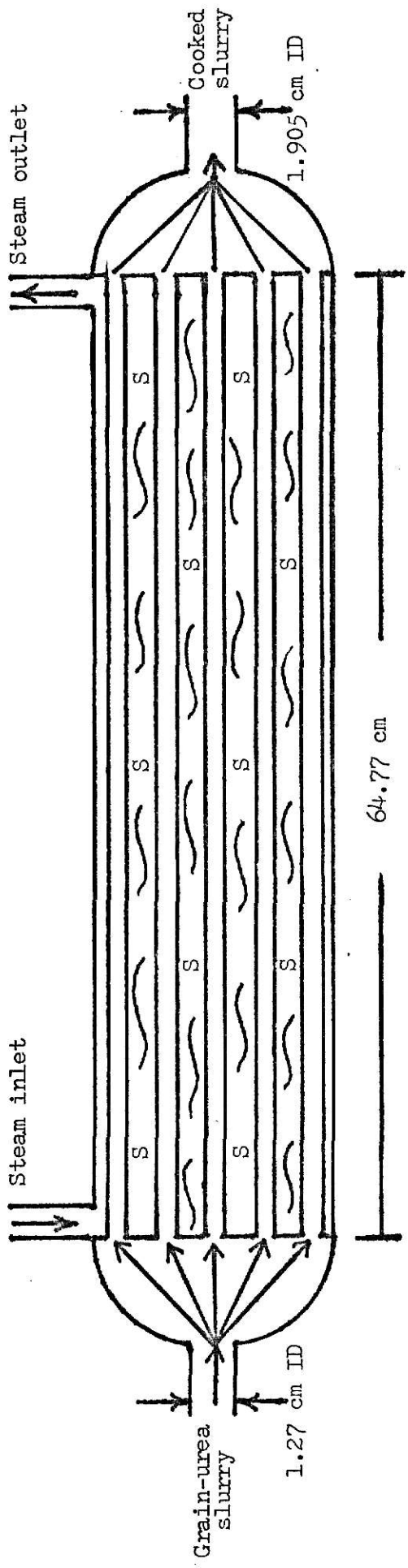

FIGURE 43. CUTAWAY SIDEVIEW OF THE HEAT EXCHANGER. ID (INSIDE DIAMETER).

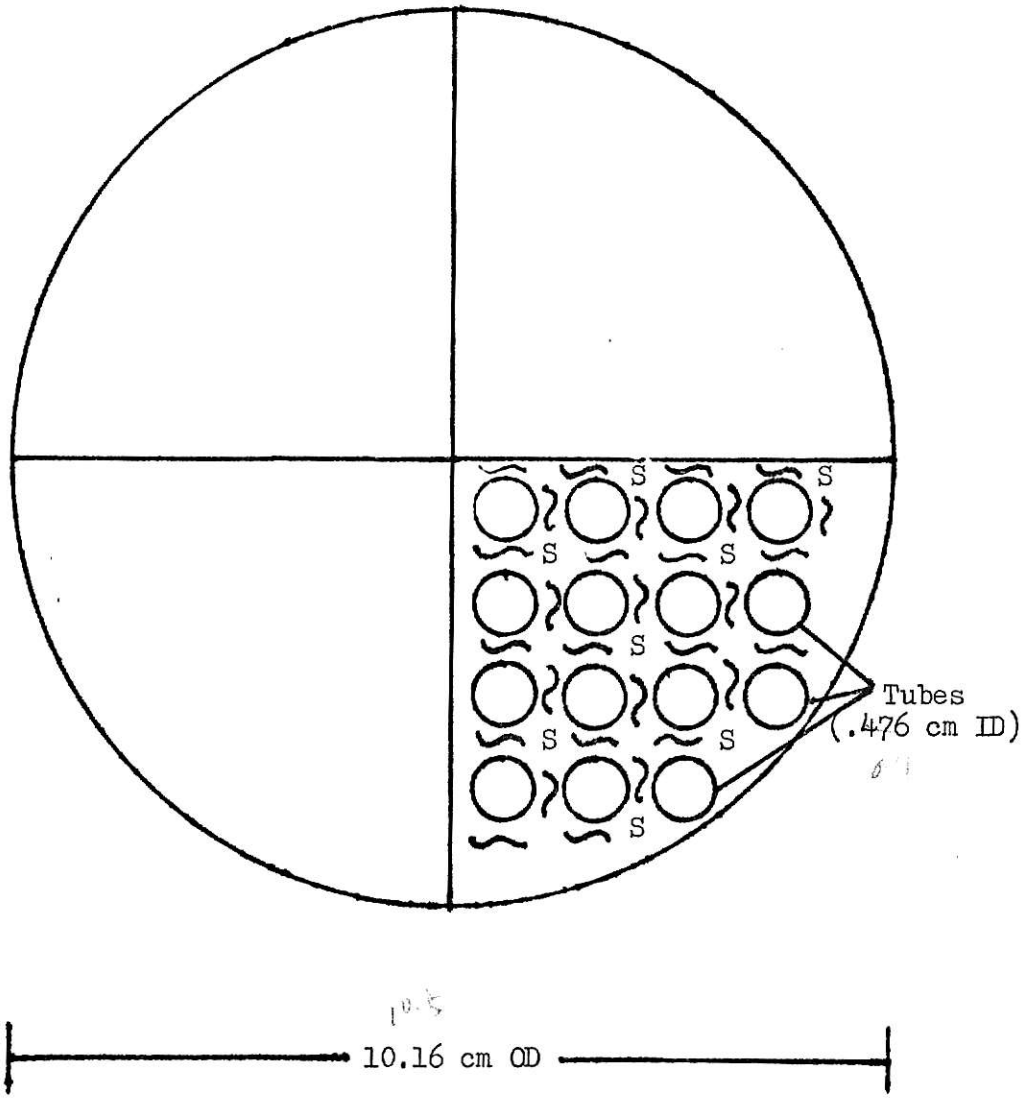

FIGURE 44. CROSS SECTION OF THE HEAT EXCHANGER.
ID (INSIDE DIAMETER) OD (OUTSIDE DIAMETER).

product was then discharged through a 1.91 cm pipe into a collecting chamber. No live steam came in contact with the slurry. From this point, the product was treated in the same manner as the product produced through the hydro-heater system.

Starch sources used in this study consisted of feed grade yellow dent corn and a Co-op hybrid sorghum (733). Raw grains were ground in the same fashion as previously discussed through a Micro-Bud[2] micro-pulverizer. Supplements were formulated for a starch source-urea ratio of 2:1 and a protein content of 30 percent. Supplement components are shown in table fifteen. The ingredients and amounts of the supplements processed through the heat exchanger were identical to that of the supplement processed through the hydro-heater, with the exception of water addition to the slurry. Additional water was added to the slurry processed with indirect steam to compensate for the water that was absorbed by the slurry processed through the hydrothermal heater. Therefore, comparisons were conducted on an equal moisture basis.

Two batches (110 kg) of supplement were produced using the hydro-heater. Each batch consisted of one of the starch sources discussed above. Samples of the processed slurries and final products were drawn and labeled as illustrated in table sixteen. Two supplements were then produced using the heat exchanger. Samples of the processed slurries and final products were drawn and labeled as shown in table sixteen. A duplicate series was then produced making a total of 8 slurry samples and 8 completed supplement samples with 2 samples per treatment.

Samples of the processed slurries were analyzed for ME analysis without the effects and/or possible interaction of additional ingredients in

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[1]Metals Disintegrating Co., Pittsburgh, Pennsylvania.

TABLE 15. FORMULAS OF HYDROTHERMALLY PROCESSED SUPPLEMENT AND HEAT EXCHANGER PROCESSED SUPPLEMENT

Ingredients	%[a]
Hydrothermally processed supplement	
Slurry	
Corn	18.12
Urea	9.90
Water	35.31
Molasses	12.96
Phosphoric acid	1.00
Propionic acid	.50
Enzyme	.02
Absorbed water[b]	22.21
Heat exchanger processed supplement	
Slurry	
Corn	18.12
Urea	9.90
Water	57.52
Molasses	12.96
Phosphoric acid	1.00
Propionic acid	.50
Enzyme	.02

[a]Percentages are on "as is" basis.

[b]Water absorbed during processing.

TABLE 16. SAMPLE IDENTIFICATION OF SUPPLEMENTS PROCESSED BY DIRECT OR INDIRECT STEAM

Starch source	Supplement portion	Processing method	Sample identification
Corn	Slurry	Hydro-heater	HHS1
Corn	Final product	Hydro-heater	HH1
Corn	Slurry	Heat exchanger	HES1
Corn	Final product	Heat exchanger	HE1
Sorghum	Slurry	Hydro-heater	HHS2
Sorghum	Final product	Hydro-heater	HH2
Sorghum	Slurry	Heat exchanger	HES2
Sorghum	Final product	Heat exchanger	HE2

the final product. Thus, results showed the actual effect the processing method had on the starch portion of the supplement.

Results

Table 17 is a listing of means for ME values and in vitro rumen protein synthesis. Analysis of processed slurries showed no differences ($P<.05$) in ME between the processing method and/or starch source. No differences ($P<.05$) in ME were found in the completed supplements sample in regards to processing method and starch source, therefore, in vitro nitrogen utilization was conducted on an equal ME basis.

In vitro rumen protein synthesis was determined on both sample sets in 3 individual experiments. This gave 6 observations per sample treatment. Data are reported as protein synthesized on a protein dry matter basis. This was done to remove effects of protein dry matter content variations. Table 18 shows protein and dry matter data for the samples. Sample HH1 was used as a control. All in vitro data were converted to a percentage of HH1, giving it a value of 100 percent. Statistical analysis of the in vitro data, using a nested mode to remove sample treatment variation from the error, resulted in no differences ($P<.05$) between sample treatment. Therefore, starch source and/or processing method appeared to have no effect on the supplement in vitro nitrogen utilization.

TABLE 17. MEAN MALTOSE EQUIVALENTS AND PROTEIN SYNTHESIZED OF STARCH CONTAINING LIQUID SUPPLEMENTS PROCESSED BY DIRECT AND INDIRECT STEAM METHODS

Sample	Maltose equivalent[a] of processed slurries	Maltose equivalent[a] of final products	Protein[b] synthesized
HHS1	169.3[c]	---	---
HES1	179.9[c]	---	---
HHS2	151.9[c]	---	---
HES2	220.9[c]	---	---
HH1	---	444.2[c]	100.0[c]
HE1	---	403.3[c]	109.8[c]
HH2	---	449.8[c]	93.2[c]
HE2	---	414.1[c]	102.2[c]

[a]Values are averages of duplicate sets.

[b]Values are results of six observations per sample treatment. Protein synthesized is a percentage of control (HH1).

[c]Means within columns having similar superscripts are not different ($P<.05$).

TABLE 18. PROTEIN AND DRY MATTER DATA OF SAMPLES PROCESSED BY DIRECT OR INDIRECT STEAM

Sample	Crude[a] protein %	Dry matter %	Protein content (dry matter basis) %
Set 1			
HH1	30.35	36.41	83.35
HE1	29.92	36.00	83.11
HH2	30.11	37.53	80.23
HE2	30.43	36.36	83.69
Set 2			
HH1	31.05	36.97	83.99
HE1	30.96	36.82	82.14
HH2	30.13	36.68	84.08
HE2	29.43	34.87	84.39

[a]Values on an "as is" basis.

SUMMARY

A hydrothermally processed liquid supplement consisting of a starch source, molasses and urea was analyzed for its storage characteristics over an 8-week period. Starch sources used consisted of feed grade yellow dent corn, grain sorghum and bird resistant sorghum. Supplements were formulated to give a grain-urea ratio of 2:1, a protein content of 30% and a dry matter content of 34 percent. Various levels and combinations of methyl-bis propionate, propionic acid, sulfuric acid and phosphoric acid were added to control pH and to prevent fungal growth. One supplement containing corn was produced and used as a prototype standard for comparisons. It was found that no one acid combination performed superior to the others over the 8-week storage trial. Variables including dry matter, pH, protein content, maltose equivalent and ammonia nitrogen showed no changes ($P<.05$) within samples. A change ($P<.05$) was noted in viscosity. Sorghum containing samples tended to decrease in viscosity during the first 2 weeks of the study, and remained stable thereafter. The corn containing supplement continued to decrease in viscosity throughout the entire 8-week trial.

Statistical analysis of *in vitro* protein synthesis showed no decrease ($P<.05$) in performance within samples. However, it was observed that increased levels of methyl-bis propionate resulted in a decrease in protein synthesized. More importantly, starch source had no significant effect ($P<.05$) on the *in vitro* performance of the liquid supplement.

A study of processing methods was conducted where direct steam application was compared to indirect steam. This investigation involved the production of the liquid supplement previously described. Grains used as

starch sources were corn and grain sorghum. Direct steam application was accomplished by using a hydro-heater system. Indirect steam processing involved the use of a heat exchanger. Results indicated that cooked gels showed no differences ($P<.05$) in maltose equivalents between processing treatments and/or starch sources. In vitro protein synthesis results indicated no differences ($P<.05$) in nitrogen utilization due to processing treatments and/or starch sources.

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APPENDIX A

TABLE 1. pH OF LIQUID SUPPLEMENTS DURING STORAGE

Sample Set 1	Week 0	2	5	8
S1	3.85	3.85	3.97	4.05
S2	3.80	3.83	3.93	3.97
S3	3.74	3.75	3.80	3.89
S4	3.21	3.16	3.20	3.49
S5	2.90	2.93	3.00	3.12
S6	2.92	2.93	2.98	3.03
S7	3.72	3.70	3.73	3.40
S8	3.95	3.90	3.98	3.85
S9	3.90	3.88	3.92	4.00
S10	3.89	3.89	3.93	3.95
S11	3.80	3.81	3.82	3.98
S12	3.71	3.76	3.76	3.81
S13	2.65	2.90	2.70	3.10
S14	2.91	2.93	2.96	3.00
S15	2.98	3.12	3.01	3.05
S16	4.51	4.44	4.24	3.90
S17	4.10	4.30	4.08	4.20
S18	4.05	4.25	4.00	4.08
SC_1	3.71	3.81	3.80	3.78
Set 2				
S19	3.68	3.76	3.86	3.84
S20	3.61	3.66	3.75	3.88
S21	3.56	3.57	3.59	3.70
S22	2.94	2.71	3.01	2.98
S23	2.68	2.90	2.91	2.86
S24	2.80	2.80	2.98	2.90
S25	3.35	3.52	3.68	3.40
S26	3.73	3.72	3.84	3.75
S27	3.74	3.77	3.79	3.80
S28	3.73	3.80	3.88	3.85
S29	3.65	3.74	3.85	3.62
S30	3.40	3.61	3.79	3.75
S31	2.80	2.91	3.06	3.16
S32	2.98	2.75	2.91	3.35
S33	2.97	2.78	2.91	3.00
S34	3.73	3.60	3.77	3.48
S35	3.59	3.80	3.95	3.85
S36	3.58	3.81	3.91	3.58
SC_2	3.30	3.72	3.75	3.75

TABLE 2. DRY MATTER[a] OF LIQUID SUPPLEMENTS DURING STORAGE

Sample Set 1	Week 0	2	5	8
S1	34.34	34.41	34.69	34.68
S2	34.50	34.42	34.39	34.76
S3	34.30	34.23	33.96	34.31
S4	35.05	34.91	34.77	34.85
S5	34.94	34.87	34.63	34.86
S6	33.58	34.67	34.25	34.38
S7	33.80	34.54	34.53	34.80
S8	33.59	34.54	34.91	34.83
S9	33.80	34.61	34.11	34.75
S10	33.38	34.15	33.80	34.47
S11	32.95	33.94	33.65	34.49
S12	32.70	33.83	33.37	34.20
S13	34.50	34.69	34.10	34.80
S14	34.49	34.51	33.96	34.27
S15	34.16	34.20	33.62	34.14
S16	33.41	33.69	33.31	33.72
S17	34.09	34.30	34.52	34.12
S18	34.11	34.15	34.09	33.30
SC_1	34.48	32.94	32.29	32.09
Set 2				
S19	31.84	34.65	34.09	34.10
S20	30.33	33.94	34.06	34.15
S21	33.76	33.76	33.53	30.37
S22	34.50	34.49	34.25	34.72
S23	34.23	34.23	34.00	34.57
S24	33.99	33.99	34.05	34.34
S25	34.01	34.01	34.36	34.56
S26	33.98	34.03	34.61	34.48
S27	34.16	34.40	34.61	34.50
S28	34.15	34.15	34.50	34.12
S29	34.03	33.97	34.31	34.31
S30	33.80	33.80	34.18	34.06
S31	34.41	34.41	34.52	34.60
S32	34.31	34.31	34.39	34.39
S33	34.15	34.15	34.41	34.35
S34	34.15	34.15	34.59	34.57
S35	34.09	34.09	34.63	34.55
S36	34.23	34.23	34.59	34.60
SC_2	32.84	32.84	33.03	32.63

[a]Values are reported in percentage.

TABLE 3. PROTEIN CONTENT[a] OF LIQUID SUPPLEMENTS DURING STORAGE

Sample Set 1	Week 0		2		5		8	
	as is	db	as is	db	as is	db	as is	db
S1	30.00	87.36	29.63	86.11	30.31	87.37	30.29	87.34
S2	30.18	87.48	30.07	87.36	30.12	87.58	30.18	86.82
S3	29.84	86.99	29.81	87.09	29.72	87.51	29.86	87.03
S4	30.52	87.08	30.53	87.45	30.30	87.14	30.35	87.09
S5	30.22	86.86	30.39	87.15	30.21	87.24	30.16	86.52
S6	30.22	89.99	29.78	85.89	29.79	86.98	29.92	87.03
S7	30.34	89.76	30.21	87.46	30.33	87.84	30.26	86.95
S8	30.51	90.83	30.40	88.01	30.85	88.37	30.12	86.48
S9	29.87	88.37	29.67	85.73	29.89	87.63	29.97	86.24
S10	29.60	88.68	29.94	87.67	30.18	89.29	30.42	88.25
S11	30.41	92.29	30.11	88.72	30.18	89.69	29.80	86.40
S12	30.06	91.93	29.66	87.67	29.75	89.15	29.43	86.05
S13	29.87	86.58	29.12	83.94	30.08	88.21	30.17	86.69
S14	30.29	87.82	29.73	86.15	30.24	89.05	30.05	87.69
S15	29.99	87.79	29.84	87.25	29.86	88.82	29.82	87.35
S16	30.33	90.78	29.99	89.02	30.26	90.84	30.13	89.35
S17	30.19	88.56	30.09	87.73	30.33	87.86	30.00	87.93
S18	28.62	83.91	29.78	87.20	30.09	88.27	30.05	90.24
SC_1	28.13	81.58	27.80	84.37	28.06	86.90	28.34	88.31
Set 2								
S19	30.40	95.48	30.39	87.71	30.50	89.47	30.17	88.48
S20	30.22	99.64	30.03	88.48	30.48	89.49	30.22	88.49
S21	30.19	89.43	29.96	88.74	29.98	89.41	29.30	96.48
S22	30.02	87.01	30.25	87.71	30.58	89.28	30.27	87.18
S23	30.34	88.64	30.19	88.19	30.32	89.18	30.44	88.05
S24	29.88	87.91	29.18	85.85	30.04	88.22	30.15	87.80
S25	30.63	90.06	30.31	89.12	30.63	89.14	30.82	89.18
S26	30.43	89.55	29.77	87.48	30.50	88.12	30.61	88.78
S27	31.16	91.22	30.43	88.46	30.33	87.63	30.24	87.65
S28	29.63	86.76	28.92	84.69	29.82	86.43	29.79	87.31
S29	29.33	86.19	30.49	89.76	28.59	83.33	29.45	85.84
S30	29.26	86.56	29.05	85.95	28.87	84.46	28.88	84.79
S31	28.52	82.88	29.78	86.54	29.52	85.52	29.71	85.87
S32	29.50	85.98	29.57	86.18	29.60	86.07	29.76	86.54
S33	29.32	85.86	29.29	85.77	29.29	85.12	29.36	85.47
S34	29.79	87.23	29.62	86.73	29.93	86.53	29.89	86.46
S35	29.42	86.30	29.39	86.21	29.61	85.50	29.65	85.82
S36	29.01	84.75	29.11	85.04	29.42	85.05	29.45	85.12
SC_2	27.96	85.14	27.68	84.29	28.16	85.26	27.94	85.63

[a]Values reported as percentage on "as is" and dry basis.

TABLE 4. AMMONIA NITROGEN CONTENT[a] OF LIQUID SUPPLEMENTS DURING STORAGE

Sample Set 1	Week 0		2		5		8	
	as is	db	as is	db	as is	db	as is	db
S1	0.964	2.806	1.080	3.139	0.958	2.763	1.111	3.205
S2	0.858	2.488	0.965	2.804	0.969	2.816	1.091	3.140
S3	0.809	2.358	0.938	2.741	0.999	2.941	1.017	2.963
S4	0.839	2.395	0.924	2.646	0.963	2.771	1.053	3.022
S5	0.799	2.289	0.994	2.851	0.968	2.796	1.063	3.050
S6	0.861	2.564	0.941	2.716	0.932	2.723	1.082	3.148
S7	0.806	2.384	0.891	2.580	1.018	2.949	1.021	2.935
S8	0.668	1.989	0.922	2.670	1.007	2.884	0.983	2.822
S9	0.899	2.660	0.901	2.604	1.057	3.100	1.000	2.878
S10	0.854	2.558	1.023	2.996	1.107	3.274	1.075	3.119
S11	0.835	2.534	1.036	3.052	1.038	3.086	1.057	3.066
S12	0.818	2.501	1.006	2.975	1.043	3.125	1.045	3.057
S13	0.845	2.448	1.006	2.899	1.065	3.122	1.114	3.201
S14	0.882	2.557	1.018	2.949	1.126	3.315	1.053	3.074
S15	0.851	2.492	0.996	2.912	1.058	3.147	1.027	3.008
S16	0.752	2.250	1.001	2.969	1.061	3.184	0.993	2.945
S17	0.762	2.236	0.934	2.722	1.092	3.162	1.049	3.076
S18	0.804	2.358	0.975	2.855	1.079	3.166	1.017	3.053
SC_1	0.783	2.272	0.788	2.393	0.877	2.715	0.779	2.428
Set 2								
S19	0.808	2.537	0.829	2.395	0.796	2.335	0.875	2.565
S20	0.760	2.506	0.836	2.462	0.770	2.261	0.886	2.593
S21	0.649	1.923	0.786	2.327	0.763	2.274	0.822	2.705
S22	0.651	1.887	0.801	2.323	0.779	2.273	0.867	2.499
S23	0.779	2.274	0.769	2.247	0.833	2.450	0.890	2.575
S24	0.739	2.174	0.789	2.322	0.772	2.266	0.816	2.376
S25	0.729	2.143	0.795	2.336	0.795	2.313	0.818	2.366
S26	0.650	1.914	0.756	2.222	0.802	2.318	0.801	2.324
S27	0.722	2.112	0.759	2.207	0.814	2.351	0.767	2.224
S28	0.763	2.234	0.871	2.549	0.806	2.337	0.857	3.205
S29	0.676	1.987	0.902	2.655	0.800	2.333	0.856	2.496
S30	0.704	2.083	0.832	2.461	0.817	2.392	0.800	2.349
S31	0.737	2.141	0.852	2.476	0.884	2.561	0.854	2.469
S32	0.743	2.165	0.809	2.360	0.845	2.458	0.808	2.347
S33	0.718	2.104	0.804	2.353	0.837	2.432	0.872	2.537
S34	0.816	2.389	0.818	2.396	0.789	2.282	0.824	2.383
S35	0.753	2.208	0.795	2.333	0.798	2.306	0.810	2.345
S36	0.681	1.990	0.774	2.261	0.818	2.365	0.836	2.417
SC_2	0.741	2.255	0.724	2.205	0.779	2.355	0.603	1.849

[a]Values are reported in mg NH_3-N per gram on "as is" and dry basis.

TABLE 5. MALTOSE EQUIVALENT[a] OF LIQUID SUPPLEMENTS DURING STORAGE

Sample Set 1	Week 0		2		5		8	
	as is	db	as is	db	as is	db	as is	db
S1	81.78	238.1	79.30	230.5	78.51	226.3	75.45	217.6
S2	81.14	235.1	79.85	231.9	75.61	219.9	83.27	239.6
S3	80.68	235.2	78.27	228.7	73.74	217.1	71.90	209.6
S4	84.00	239.7	86.57	247.9	84.98	244.4	84.37	242.1
S5	83.65	239.4	86.13	247.9	84.41	243.7	84.36	242.0
S6	82.79	246.6	83.65	241.3	82.14	239.8	83.00	241.4
S7	81.07	239.4	82.93	240.1	80.57	233.3	87.00	250.0
S8	83.03	247.2	81.28	235.3	80.31	230.1	79.00	226.8
S9	82.07	242.8	83.04	239.9	79.20	232.2	77.00	221.6
S10	82.41	246.9	84.03	246.1	79.27	234.5	79.00	229.2
S11	81.92	248.6	80.02	235.8	79.40	235.9	78.00	226.1
S12	80.90	247.4	81.05	239.6	76.26	228.5	76.00	222.2
S13	85.93	249.1	94.76	273.2	87.05	255.3	81.00	232.8
S14	83.60	242.4	101.53	294.2	86.59	254.9	84.00	245.1
S15	82.36	241.1	87.65	256.3	83.71	248.9	83.00	243.1
S16	86.80	259.8	112.89	335.1	115.00	345.2	116.00	344.0
S17	84.80	248.6	85.16	248.3	79.52	230.4	75.00	219.8
S18	79.00	231.6	84.32	246.9	79.23	232.4	77.00	231.2
SC_1	61.48	178.3	65.23	197.9	63.70	197.3	59.00	183.9
Set 2								
S19	66.94	210.2	73.80	212.9	71.49	209.7	67.00	196.5
S20	72.85	240.2	70.67	208.2	69.83	205.0	67.00	196.2
S21	71.40	211.5	71.42	211.6	73.57	219.4	63.00	201.4
S22	78.40	227.2	82.70	239.8	81.99	239.4	91.00	262.1
S23	76.50	223.5	76.67	223.9	79.02	232.4	75.00	216.7
S24	71.68	210.9	77.51	228.0	82.00	240.8	72.00	209.7
S25	73.65	216.5	73.46	216.0	71.00	206.6	67.00	194.3
S26	73.03	214.9	72.39	212.7	69.00	199.4	67.00	194.3
S27	74.89	219.2	70.58	205.2	68.00	196.5	68.79	199.4
S28	78.90	231.0	78.25	229.1	74.00	214.5	75.55	221.4
S29	78.20	229.8	75.75	222.9	77.00	224.4	75.79	220.9
S30	76.50	226.3	74.75	221.1	71.00	207.7	72.92	214.1
S31	83.24	241.9	84.88	246.7	86.00	249.1	88.21	254.9
S32	82.80	241.3	83.21	242.5	82.00	238.4	83.53	242.9
S33	80.90	236.9	79.36	232.4	80.00	232.5	81.51	237.3
S34	78.56	230.0	75.69	221.6	75.00	216.8	75.01	216.9
S35	77.61	227.7	75.62	221.8	75.00	216.6	75.45	218.4
S36	76.72	224.1	74.45	217.5	79.00	228.4	73.19	211.5
SC_2	74.40	226.6	71.25	216.9	71.00	214.9	80.24	245.9

[a]Values are reported in mg maltose per gram on "as is" and dry basis.

TABLE 6. VISCOSITY[a] OF LIQUID SUPPLEMENTS DURING STORAGE

Sample Set 1	Week 0	2	5	8
S1	685	298	326	285
S2	690	316	347	306
S3	712	309	326	251
S4	675	292	320	280
S5	665	325	321	304
S6	630	322	310	252
S7	715	338	358	348
S8	718	351	357	260
S9	933	402	314	244
S10	420	193	177	174
S11	375	166	171	166
S12	378	169	178	165
S13	380	194	167	172
S14	390	167	180	171
S15	380	196	193	181
S16	380	174	145	136
S17	455	188	184	170
S18	728	257	214	81
SC_1	1848	1850	1685	432
Set 2				
S19	824	174	171	166
S20	780	164	172	171
S21	796	169	171	164
S22	784	162	179	161
S23	808	156	175	166
S24	776	161	170	165
S25	776	174	172	165
S26	840	164	171	152
S27	1424	246	191	173
S28	1084	185	226	222
S29	1024	205	231	230
S30	1036	198	228	215
S31	1020	207	224	222
S32	1052	216	231	223
S33	1032	226	232	220
S34	1508	232	230	215
S35	1140	202	233	225
S36	1528	276	242	215
SC_2	2600	562	540	346

[a]Values are reported in centipoise units.

TABLE 7. IN VITRO PROTEIN SYNTHESIZED OF LIQUID SUPPLEMENTS DURING STORAGE

Sample-1st run non-br sorghum	Protein synthesized: Room temperature samples mg/g	% of corn treatment	Frozen samples mg/g	% of corn treatment
1	21.93	109	18.09	132
2	18.64	93	20.01	146
3	17.27	86	20.56	150
4	21.10	105	18.36	134
5	23.03	115	19.46	142
6	23.57	118	17.27	126
7	20.28	101	10.97	80
8	18.36	92	4.39	32
9	5.49	27	4.11	30
Corn 1	20.01	100	13.71	100
Sample-2nd run br sorghum				
10	17.82	108	17.72	120
11	18.64	113	19.46	131
12	13.71	83	19.74	133
13	12.88	78	17.00	115
14	11.79	72	17.90	121
15	17.55	107	17.35	117
16	23.58	143	17.35	117
17	15.35	93	8.50	57
18	17.27	105	7.13	48
Corn 1	16.45	100	14.80	100

TABLE 7. cont.

Sample-3rd run non-br sorghum	Protein synthesized: Room temperature samples mg/g	% of corn treatment	Frozen samples mg/g	% of corn treatment
28	17.00	97	16.45	97
29	15.07	86	17.00	100
30	11.52	66	13.98	82
31	12.88	73	17.00	100
32	15.35	87	17.12	101
33	13.71	78	14.26	84
34	16.73	95	16.45	97
35	15.35	87	9.87	58
36	4.66	27	6.58	89
Corn 2	17.55	100	17.00	100
Sample-4th run br sorghum				
19	10.97	61	14.25	247
20	7.67	42	11.51	200
21	4.93	27	15.07	262
22	10.68	59	16.17	280
23	11.51	64	14.80	257
24	13.98	77	9.59	166
25	13.43	74	5.48	59
26	13.16	72	3.29	57
27	10.69	59	3.57	62
Corn 2	18.09	100	5.76	100

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STUDIES ON CEREAL STARCHES AS CARBOHYDRATE SOURCES IN A UREA-CONTAINING LIQUID SUPPLEMENT

by

STEPHEN FRANCIS BINDER

B. S., Kansas State University, 1974

AN ABSTRACT OF A MASTER'S THESIS

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Liquid feed products were produced whereby molasses was added to hydrothermally processed grain-urea slurries. Grain sources consisted of micropulverized feed grade yellow dent corn, grain sorghum, and bird resistant sorghum. Liquid samples were formulated to give a grain-urea ratio of 2:1, a protein content of 30% and a dry matter content of 34%. Grain-urea slurries were processed through a cooker, equipped with a hydro-heater, at a temperature and pressure of 140 C and 40 psig, respectively. Following the cooking process, an α-amylase was added to aid in reducing slurry viscosity.

The objective of the first trial was to determine the ability of the liquid to remain stable using various combinations and levels of methyl-bis propionate, propionic acid, sulfuric acid, and phosphoric acid to control fungal bacteria and pH. Analyses of pH, dry matter content, protein content, maltose equivalence, ammonia nitrogen, and viscosity were conducted at 0, 2, 5, and 8 weeks to determine storage ability. An in vitro rumen technique was also used to evaluate the product's potential for producing microbial protein.

There were no significant changes ($P<.05$) in pH, dry matter content, protein content, maltose equivalence, and ammonia nitrogen within individual samples over the 8-week period. Comparisons between samples indicated a trend for lower pH values and higher maltose equivalence for samples containing sulfuric acid. A decrease ($P<.05$) was noted in viscosity of samples containing sorghum during the first two weeks of the trial. Following the 2nd week, the viscosity of these samples remained stable. The sample containing corn decreased in viscosity throughout the 8-week period.

At the beginning of the trial, samples of each treatment were frozen and used as controls for in vitro rumen protein synthesis evaluation. Results indicated no decrease ($P<.05$) in protein synthesized within individual samples. A trend of lower amounts of protein synthesized were noted in samples with

highest levels of methyl-bis propionate. No differences ($P<.05$) were noted among the samples containing various starch sources and with the same acid treatments.

The objective of the second trial was to compare the effects of direct steam application to indirect steam treatment in the production of the liquid feed. Direct steam application was accomplished by using a cooker equipped with a hydro-heater. Indirect steam processing was conducted by employing a heat exchanger whereby slurry passed through tubing surrounded by steam. Corn and grain sorghum samples were used as grain sources in this study. Sample formulations were identical to those used for other studies.

It was found that maltose produced by the two processing methods were not significantly different ($P<.05$). Statistical analysis of <u>in vitro</u> protein synthesis data showed no differences ($P<.05$) between processing methods and/or starch source. Findings of this study would suggest that either method could readily be adapted to in producing the liquid feed.