ENZYME ADDITION AND BLENDING VARIABLES ON NUTRITIONAL QUALITIES FOR RUMINANTS OF A LIQUID PROTEIN SUPPLEMENT CONTAINING STARCH

by

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Approved by:

[Signature]
Major Professor
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CHAPTER I

INTRODUCTION

Researchers at Kansas State University have been actively involved in the production and testing of a starch-based liquid protein supplement for ruminants. This product has been proven superior to molasses-based liquid supplements in a variety of test conditions. Interest has developed in a high protein (60%) concentrate of the starch-based product to be used as an ingredient to be blended with a variety of liquid carriers such as molasses and other ingredients. Use of the product in both a lick tank operation and as a top dress for dry feeds is possible.

This research dealt with processing variables involved in the production of a 60% protein concentrate. Of greatest concern was characterization of the variables that control enzymatic thinning for viscosity control. An attempt was made to establish optimum conditions for enzyme reaction. Viscosity was used as a predictor of product quality and starch damage.

Blending of the concentrate to a 30% crude protein product with molasses, water, lignin sulfonate, ammonium polyphosphate or fermented ammoniated condensed whey was evaluated through in vitro rumen studies.

Product viscosity and other characteristics were evaluated by animal toxicity and in vitro rumen studies.
CHAPTER II

LITERATURE REVIEW

**Liquid Supplements.** Widespread use of liquid supplements in the cattle industry is made today. Advantages of liquid supplements include ease of handling, palatable method of feeding urea, and diverse feeding applications (Wornick, 1969). Liquid supplements consisting mainly of molasses-urea blends have been compared to dry supplements. In a lactation study conducted with liquid supplement added to corn silage, performance was equal to a dry supplement (Huber et al., 1968; Van Horn et al., 1969). Since liquid supplements have not shown a consistent advantage over dry supplements, the decision to use a liquid supplement would depend on relative cost, ease of application, adaptation to individual operation and other factors (Huber, 1972).

Cane molasses has been shown to improve nitrogen utilization from urea (Hatch and Beeson, 1972). The supply of readily available carbohydrate is related to the degree of urea utilization. Starch has been shown superior to molasses in providing carbon skeletons required for ammonia to be converted to microbial protein. Studies have shown cellulose to be degraded too slowly and mono-and-disaccharides are fermented too rapidly to be well utilized (Church, 1972). Reviews of other ingredients used in liquid supplements are presented by Reddy (1976), Skoch (1976) and Binder (1976).
Starch-Based Liquid Supplement. Researchers at Kansas State University have developed a liquid supplement containing urea and hydrothermally processed carbohydrate (Reddy, 1976). The supplement contains starch and urea (ratio of 1.83:1) and water which had been processed with a hydrothermal cooker equipped with a series "B" Hydroheter®. Steam is applied directly to the slurry through the Hydroheter®, resulting in a high shear cooking. The processing temperature ranged from 140°C to 150°C. Protein content was adjusted to approximately 30% with molasses. Viscosity was lowered with a α-amylase, derived from Bacillus subtilis. Phosphoric acid was added to lower the pH and terminate the enzyme activity. Propionic acid was added to prevent microbial growth (Binder et al., 1978; Reddy, 1976).

Starch has been found superior to molasses in providing carbon skeletons for microbial protein synthesis from non-protein nitrogen (NPN) sources (Church, 1972). The starch-based liquid supplement (SBLS) was developed to improve N utilization (Binder et al., 1978).

In a feeding trial involving forty-eight steers, the starch-based liquid supplement was significantly (P < .05) better than a molasses-based liquid supplement (MBLS) or Starea², and was equal to soybean meal in weight gains and feed efficiency (Reddy, 1976). The SBLS when compared to MBLS in a lactation study involving twenty Holstein cows showed a slight advantage in milk production. A slight weight loss was recorded with the SBLS

¹Hydro-Thermal Corporation, Milwaukee, Wisconsin.
(Ibbetson, 1978a). Use of the SBLS in calf starter rations showed the SBLS to improve weight gains over urea and Starea and was comparable to soybean meal (Ibbetson, 1978b). In a wintering study involving forty-two steers, the SBLS was more efficiently utilized than the MBLS (Binder et al., 1978). A toxicity study involving four rumen fistulated adult cattle resulted in no toxic reactions in six trials with SBLS and one out of six with MBLS, when a dose of .5 g urea per 1 kg body weight was administered (Binder et al., 1978).

Starch sources from corn, grain sorghum, bird resistant sorghum and waste potato starch have been compared in vitro and no differences in protein synthesis have been established. Stability of these liquid supplements has been investigated and they have appeared stable for eight weeks (Binder, 1976; Skoch, 1976; Skoch et al., 1977).

Structure of Starch. The structure of the starch granule has been extensively reviewed by Greenwood (1964), Sandstedt (1965), Furia (1968) and French (1973). Starch is composed of chain and branched structures. Amylose consists of α-1, 4-linked glucose units, with a molecular weight of several hundred or thousand units, making up the straight chain structure. Amylopectin consists of α-1, 4-linked glucose units with branch points made of α-1, 6-links. The α-1, 6-links made up 4 to 5% of the total number of linkages in amylopectin (French, 1973).

Amylose may attain various conformations in solution, such as a helix in the presence of a complexing agent, or a random-coil. Variation in molecular configurations is responsible for "retrogradation" and "gelation" of concentrated amylose solutions. The polysaccharide molecules
may align in linear chains or in random configurations, depending on rate of cooling, and form insoluble micelles with the aid of hydrogen bonding. In solutions of amylopectin there is no tendency to retrograde (Greenwood, 1964).

The shape and size of the starch granule is dependent on plant source. The hilum is a feature present in most granules. It is centrally located and surrounded by concentric shells, thus being the nucleus around which the granule has grown. Birefringence is exhibited by starch granules when examined under a polarizing microscope. A "maltese cross" is exhibited with intersection of the cross at the hilum (Furia, 1968; French, 1973).

Gelatinization of Starch. Starch granules are insoluble in cold water. Granules exhibit swelling when placed in warm water due to the abundance of hydroxyl groups tending to absorb moisture. As the temperature is raised past a critical point, hydrogen bonds holding the granule together begin to weaken permitting the granule to swell tangentially to many times its original size. Granules lose their birefringence and the viscosity of the slurry increases. Maximum hydration is reached when molecules begin to leach out of the granules, leading to rupture and collapsing of the granule yielding to dispersion of granule fragments (Furia, 1968).

Gelatinization has been defined as the irreversible rupture of the native, secondary-bond forces in the crystalline regimes of a starch granule according to Seib (1971). Covalent linkages are not broken, only secondary-bond forces are altered. Complete loss of crystallinity does not occur, only an alteration of crystallinity (Seib, 1971).
Action of α-Amylase on Starch. Two amylolytic enzymes are capable of breaking α-1, 4-bonds found in starch. α-amylases are found in animals, plants, fungi and micro-organisms. β-amylases are found in higher plant sources (Greenwood, 1964). α-amylases are known best for their action of "dextrinizing" starch (French, 1957). Acting as an endo-enzyme, α-amylase splits α-1, 4-links in both amylose and amylopectin at different places in the interior of the molecule. Gelatinized starch is rapidly decomposed to dextrins with average chain length of 6 to 10 glucose units (Barfoed, 1976; French, 1973). The action of α-amylase is believed to be random in attack on both amylose and amylopectin molecules. Characteristics of this action are reduced viscosity, rapid loss in iodine-staining ability, and an increase in reducing power (Greenwood, 1964).

Accessibility of the starch molecules to the enzyme is the rate-determining stage of digestion of the molecule (Sandstedt and Mattern, 1960).

When corn and sorghum starch granules have been reacted with amylase and observed under a polarizing microscope, erosion and fragmentation of the granule and waxy counterparts are present. With potato starch selective-granule digestion is noted. Corn starch granules show loss of birefringence in the hilum area first due to initial enzyme attack. Since loss of birefringence also occurs first in the hilum area during gelatinization, the hilum appears most susceptible to attack. Five to ten percent of the starch granules will never show erosion. The patterns of swelling and solubility of starch in hot water is not an indication of enzyme susceptibility (Leach and Schoch, 1961).
Bacterial α-amylase generally are produced from *Bacillus subtilis* or related species. Bacterial enzymes are heat stable compared to malt enzymes. NOVO Laboratories\(^3\) manufactures a bacterial α-amylase with improved heat stability properties. Marketed as Thermamyl\(^R\), the enzyme is from a strain of *Bacillus lichenformis*. Being active within a temperature range of 95 to 100°C, the enzyme is active at 15 to 20°C higher than that of most bacterial amylase. Thermamyl produces primarily maltose, maltotriose and maltoolpentose. *B. subtilis* amylases produce mainly maltohexose, maltoolpentose and a small amount of glucose (Baroed, 1976).

**Viscosity Measurement of Starch Slurries.** Several methods of measuring viscosity have been developed for use in the starch industry. These include the Brabender Amylograph, Corn Industries Viscometer, Scott Viscometer, and the Brookfield Viscometer. The use of the latter instrument has grown due to portability and the simplicity of the procedure. Several spindles and spindle speeds are available. It is important for sample comparisons to use the same spindle number and speed to determine the calculated viscosities (Smith, 1964).

The Brookfield Viscometer has been used to measure the apparent viscosity of steam jet cooked flour paste (Ranken *et al.*, 1976), acidulated flour-water suspensions (Katenaan and Rebenthaler, 1971), and 5% starch pastes (Kite *et al.*, 1957).

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\(^3\)NOVO Laboratories, Wilton, Connecticut.
Lignin Sulfonate. Lignin sulfonates may be used in animal feeds as individually or as a combination of, the ammonium, calcium, sodium or magnesium salts of the extract spent sulfite liquor derived from the sulfite digestion of wood or of abaca. It may be used in specific quantities in animal feeds including as a surfactant in molasses not to exceed 11% of the molasses on a dry weight basis (Anon, 1976). Alkaline or base ions such as ammonium, calcium, sodium or magnesium are used for neutralization (Hathaway, 1973). Data have shown lignin sulfonate to be a good surfactant for use in molasses. At a temperature of 20 °F, molasses with a viscosity of 100,000 cps was reduced to a viscosity of 47,000 cps with the addition of 10% lignin sulfonate (Hathaway, 1973).

Palatability is not a problem with feed containing lignin sulfonate. A mixture of about one part lignin sulfonate and three parts molasses on a dry matter basis appears to be an ideal combination when used in finishing rations (Hathaway, 1973). Use of 8 and 12% lignin sulfonate on an "as is" basis did not depress daily gain in another finishing study (Chang et al., 1977). The source of lignin sulfonate has been a factor in other studies in regard to performance and feed conversion (Miron, 1975). It is thought lignin sulfonate is an aid in urea utilization (Synder, 1975). In vitro studies have shown that lignin sulfonate depresses dry matter digestibility (Chang et al., 1977).
### Table 1. Typical Analysis of Calcium Lignin Sulfonate

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>50-55</td>
</tr>
<tr>
<td>Sugars and sugar acids (dry basis)</td>
<td>20-25</td>
</tr>
<tr>
<td>Lignin sulfonate (dry basis)</td>
<td>50-60</td>
</tr>
<tr>
<td>Inorganics (ash)</td>
<td>8-13</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>-----</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.5-5.0</td>
</tr>
<tr>
<td>Sulfur</td>
<td>3-6</td>
</tr>
</tbody>
</table>


**Ammonium Polyphosphate (APP).** Ammonium polyphosphate results from the neutralization of superphosphoric acid. Minimum contents are 9% nitrogen and 13% phosphorus. APP must contain not more than 1 part Flourine (F) to 100 parts Phosphorus (P), 75 ppm of Arsenic (Ar), and 300 ppm of heavy metals reported as lead. The product is a clear, white solution if thermally processed or light green to greenish-black if wet processed (Anon., 1975). APP has the advantage of being both a source of NPN and phosphorus, but has the disadvantage of relatively low concentrations of NPN and phosphorus compared to urea and phosphoric acid (Warrick, 1969).

No differences in performance between phosphoric acid and ammonium polyphosphate have been noted (Synder, 1970; Miron 1975) as to the origin of the phosphorus source.
TABLE 2. GUARANTEED ANALYSES OF FOUR GRADES OF AMMONIUM POLYPHOSPHATE

<table>
<thead>
<tr>
<th>Grade</th>
<th>Thermal process</th>
<th>Wet process</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(11-37-0)</td>
<td>(10-34-0)</td>
</tr>
<tr>
<td>Nitrogen (N), % min</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Crude protein</td>
<td>68.75</td>
<td>62.5</td>
</tr>
<tr>
<td>Equivalent % min</td>
<td>16.0</td>
<td>14.8</td>
</tr>
<tr>
<td>Phosphorus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


**Fermented Ammoniated Condensed Whey (FACW).** FACW is a product resulting from fermentation of cheese whey with *Lactobacillus bulgaricus,* followed by treatment with anhydrous ammonia to neutralize the lactic acid formed during fermentation. Concentration of the product may range from 40 to 90% solids with the product being syrupy, salty or acid tasting and brown to reddish-brown in color (Arnott et al., 1958; Alston et al., 1973).

Early feeding trials showed that FACW was non-toxic and supported growth of six month old calves. Lactation studies showed milk, milk fat and gain to be decreased when feeding the whey product (Hazzard et al., 1958). Studies with FACW as a protein source in a beef finishing ration for 96 yearling steers showed performance essentially equal to soybean meal and superior to urea (Henderson et al., 1973; Henderson et al., 1974).

Additional studies with lactating cows fed 9 and 18% FACW showed FACW to be equal to soybean meal and urea for increasing crude protein from 10 to 14% in rations for lactating cows (Huber et al., 1975).
FACW has been tested in finishing steer rations at levels of 14.73% of total feed dry matter which resulted in slightly depressing effects. This is twice the normal feeding level of 7.35% (Crickenberger et al., 1975). FACW has been shown to be less toxic than ammonium lactate, ammonium acetate and urea (Crickenberger et al., 1977).

TABLE 3. TYPICAL ANALYSIS OF FERMENTED AMMONIATED CONDENSED WHEY

<table>
<thead>
<tr>
<th>Component</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein equivalent (CPE)</td>
<td>46%</td>
</tr>
<tr>
<td>CPE from NPN</td>
<td>38%</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>37%</td>
</tr>
<tr>
<td>Ca</td>
<td>0.28%</td>
</tr>
<tr>
<td>P</td>
<td>0.44%</td>
</tr>
<tr>
<td>Solids</td>
<td>62%</td>
</tr>
<tr>
<td>Ash</td>
<td>4.88%</td>
</tr>
<tr>
<td>pH</td>
<td>6.8%</td>
</tr>
</tbody>
</table>

*From Calor Agriculture Research, Okemos, Michigan.*
CHAPTER III

EXPERIMENTAL PROTOCOLS FOR COMPARISONS
OF LIQUID SUPPLEMENTS CONTAINING STARCH

The effects of variations in enzyme addition and blending of liquid supplements containing starch were compared by analytical laboratory analyses and rumen in vitro techniques. Following is a description of the methods used to evaluate the liquid supplements.

**Experimental Procedures.**

**Dry matter.** Using a forced air oven at 130 C, approximately 2 g samples were dried for one hour. Dry matter was calculated from the moisture values obtained.

**Crude protein.** Nitrogen content of the samples was determined by Macro-Kjeldahl analysis using the boric acid modification presented in method (46-12) (AACC, 1962). Protein equivalent was calculated by multiplying the nitrogen content by 6.25.

**pH.** All pH readings were taken with a Beckman pH meter. Readings were taken to the nearest hundredth.

**Viscosity.** Viscosity of the samples was determined using a LVT Brookfield Viscometer\(^1\). Spindle 2 at 12 rpm was used in making the determination. Results are reported in centipoise (cps).

**Starch damage.** The method described by Sandsteadt and Mattern (1960) was used to determine the degree of starch damage. Measurement of

\(^1\)Brookfield Engineering Laboratories, Inc., Stoughton, Mass.
reducing sugars is made using ferricyanide analysis. Results are reported as mg of maltose per g of sample, or as indicated.

**Free ammonia.** Micro diffusion analysis with the boric acid modification described by Conway (1957) was used to determine free ammonia (NH$_3$-N) concentrations. Free ammonia was calculated as mg NH$_3$-N per 100 ml sample.

**Protein synthesis.** An *in vitro* fermentation measure of protein synthesis according to Barr (1974) was used in evaluating the liquid supplements.

Feed samples of 1 g were weighed into 2 duplicate sets of 50 ml plastic centrifuge tubes. Phosphate buffer adjusted to pH 6.8 and warmed to 39 C was added to each set of tubes. Rumen fluid was obtained from a rumen-fistulated animal approximately 12 hr after feeding. Fluid was strained through four layers of cheesecloth into a flask, stoppered with a Bunsen valve, and placed in a 39 C waterbath. To one set of tubes containing the sample (referred to as feed blanks) 30 ml of buffer was added, the tubes evacuated with CO$_2$ and closed with a Bunsen valve. To the other set (referred to as feed samples) 20 ml of buffer and 10 ml of rumen fluid was added, the tubes evacuated with CO$_2$ and closed with a bunsen valve. In a third set of tubes (referred to as the fluid blanks) 10 ml of rumen fluid and 20 ml of buffer was added. The feed blanks and feed samples were incubated for 4 hr at 39 C being shaken every thirty minutes. Fluid blanks were not incubated. Samples were then centrifuged at 25, 400 x G for 15 min and the supernant discarded. The residue was washed twice with methanol and centrifuged after each washing to remove
soluble nitrogen. Nitrogen determinations were made on the precipitates using a Macro-Kjeldahl (AACC method 46-12) Milligrams of protein were calculated by:

\[
\text{mg protein} = \text{ml acid} \times \text{normality of acid} \times 14 \times 6.25
\]

\[
\text{mg protein synthesized} = \text{mg protein in feed samples} - \text{mg protein in feed blank} - \text{mg protein in fluid blank}
\]

Protein synthesis results are reported as milligrams of protein synthesized or as a percent of a control.

Gas Production. In vitro gas production was conducted according to el Shazly and Hungate (1965). Feed samples of 4 g were weighed in duplicate into Mojonnier bottles. Forty ml of filtered rumen fluid was added to each bottle. Eighty ml of phosphate buffer adjusted to pH 6.8 was added and the bottles were placed in a 39°C water bath. A water displacement apparatus was installed and the bottles were gently shaken. Displaced water volumes were recorded each 30 min and the bottles shaken after each reading during the 4 hr fermentation.

Percentage Urea Utilization. Using the method adapted by Barr (1974), percentage urea utilization was calculated as follows:

\[
\begin{align*}
\text{Percentage urea utilization} &= \frac{\text{Urea nitrogen from rumen fluid} + \text{urea} - \text{Ammonia nitrogen} + \text{urea nitrogen}}{\text{nitrogen from substrate}} \times 100
\end{align*}
\]

Ammonia nitrogen and urea nitrogen were measured according to method of Conway (1957).
CHAPTER IV

RELATION OF ENZYME REACTION TIME TO VISCOSITY AND STARCH DAMAGE

In the production of the liquid supplement containing starch, it is of interest to be able to predict the end product viscosity from the amount and length of reaction of α-amylase being added and from the hot (45 C) viscosity. This study was made to determine the relation of enzyme reaction time to product viscosity and starch damage. The relation of hot (45 C) to cold (22 C) viscosity was also established.

Experimental Procedure. Table 4 shows the calculated composition of the 60% protein product used in this study. Corn (grade U. S. No. 2) was ground through a Model D Fitz Mill\(^1\) equipped with a .051 mm (.020 in) screen. Ground corn-urea-water slurries were processed through a Type A\(^2\) Hydroheter to obtain 6.89 kg (15.2 lb) final product. The slurry was processed at a temperature of 149 C and a pressure of 60 psig. A Moyno\(^3\) open cavity pump, Model 2J3, was used to deliver the slurry to the Hydroheter\(^4\) at a rate of 1.7 l per minute. Samples of approximately 4.5 kg were collected from the middle of the run into a tared container. The actual sample weight was immediately determined for use in calculating the required amount of Thermamyl\(^5\) α-amylase.

\(^1\)Fitzpatrick Company, Elmhurst, Illinois.
\(^2\)Hydro-Thermal Corporation, Milwaukee, Wisconsin.
\(^3\)Robbins and Meyers, Inc., Springfield, Ohio.
\(^4\)NOVO Laboratories, Wilton, Connecticut.
TABLE 4. COMPOSITION OF 60% PRODUCT

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>22.71</td>
<td>1566.0</td>
</tr>
<tr>
<td>Urea</td>
<td>20.72</td>
<td>1429.0</td>
</tr>
<tr>
<td>H₂O</td>
<td>44.73</td>
<td>3084.0</td>
</tr>
<tr>
<td>Absorbed H₂O^a</td>
<td>8.81</td>
<td>607.8</td>
</tr>
<tr>
<td>Phosphoric acid^b</td>
<td>2.50</td>
<td>172.4</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>.53</td>
<td>36.3</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>6895.5</td>
</tr>
</tbody>
</table>

^aH₂O absorbed from steam during processing
^b65% food grade H₃PO₄.

The cooked slurry was then brought to the desired temperature by placing the container in a water bath at -12.2 °C and gently agitated for 3-4 minutes. The container with slurry was placed in a thermostatically controlled water bath to maintain a temperature of 95 °C. Thermamyl 60 α-amylase was added at a level of .12% of the dry starch by weight. Agitation throughout the reaction time was supplied by a stirrer 146 mm (5.75 in) in diameter and quartered with an angle of 17° per quarter. A Model AC-DC5 mixer operated the stirrer at 240 rpm. It was necessary to keep the stirrer well emerged in the slurry to minimize aeration and foaming of the product.

Samples of 500 g were aspirated from the reaction canister every 4 min for 28 min and samples were immediately cooled to 60 °C in an

---

5Precision Scientific Co., Chicago, Illinois.
ice-water bath within two minutes. Phosphoric acid was added at 2½% by weight of the product and mixed thoroughly for 30 seconds.

Viscosity was then measured at 45°C with a Brookfield Viscometer using Spindle 2 at 12 rpm. The product was allowed to cool to 22°C and the viscosity measured. Propionic acid was added at a .5% level by weight and mixed. Product was stored in 9 l jars with air-tight lids.

Results and Discussion. Table 5 shows mg maltose values for each sample. The regression equation \( y = 0.088 \text{ time} + 2.25 \) with \( R^2 = 0.964 \) best describes the relation of starch damage on an "as is" basis to reaction time. On a grain dry matter basis, the equation is \( y = 1.01 \text{ time} + 27.85 \) with \( R^2 = 0.962 \). In each relation, the correlation of time to starch damage was high.

**TABLE 5. COMPARISON OF STARCH DAMAGE TO LENGTH OF ENZYME REACTION TIME**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% DM</th>
<th>Mg maltose (as is)</th>
<th>Mg maltose (grain dm basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
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<tr>
<td>28</td>
<td>36.55</td>
<td>4.96</td>
<td>59.00</td>
</tr>
</tbody>
</table>

The relation of hot to cold viscosities is listed in Table 6. The regression equation \( y = 5.34 \) (hot viscosity) - 427.58 with \( R^2 = 0.969 \) described the relation of hot to cold viscosity of the product. With the high correlation, it appeared possible to predict the final viscosity
from the hot viscosity as the product was produced.

Dry matter values listed in Table 5 show an increase which is a result of moisture loss during the run. Since this occurs, it was found desirable to use a covered reaction container for further studies. Temperature of the slurry also decreased as time increased, so that an insulated container with lid was used in the following studies.

From this study, a high correlation between variables of time, starch damage, and viscosity were determined which provided the background for further study of these processing variables.

**TABLE 6. RELATION OF HOT (45 C) AND COLD (22 C) VISCOSITIES OVER TIME OF REACTION**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>45 C</th>
<th>22 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
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<td>347</td>
</tr>
<tr>
<td>28</td>
<td>130</td>
<td>309</td>
</tr>
</tbody>
</table>
CHAPTER V

OPTIMIZATION AND PREDICTABILITY OF

ENZYME ADDITION VARIABLES

Experimental Procedures. Response surface methodology was used to establish the optimum conditions for reaction of α-amylase with a hydrothermally processed corn-urea-water slurry. Fifteen samples were treated under conditions described in Table 7 for Study I and Table 8 for Study II as prescribed by response surface methodology design (Cochran & Cox, 1957; Meyers, 1971).

TABLE 7. ENZYME REACTION CONDITIONS OF SAMPLES PREPARED FOR RESPONSE SURFACE METHODOLOGY STUDY I

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Reaction temperature °C</th>
<th>Enzyme concentration (% of dry starch)</th>
<th>Reaction period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>.10</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>.10</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
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<td>16</td>
</tr>
<tr>
<td>15</td>
<td>80</td>
<td>.25</td>
<td>16</td>
</tr>
</tbody>
</table>

Samples were processed using the formula, equipment and processing conditions described in Chapter IV. Samples of approximately 4.5 kg were
collected in a tared container and the sample weight immediately determined for use in calculating the required amount of α-amylase solution. The container was a galvanized canister 203 mm (8 in) diameter and 280 mm (11 in) in height. The upper half and lid were covered with 12.7 mm (.5 in) fiberglass insulation.

The cooked slurry was adjusted to temperature, maintained at the given temperature and agitated as described in Chapter IV. A solution of 10% v/v Thermamyl® 60 α-amylase in a 15% w/v saline solution was used in the quantity calculated from the sample size and % required by design.

**TABLE 8. ENZYME REACTION CONDITIONS OF SAMPLES PREPARED FOR RESPONSE SURFACE METHODOLOGY STUDY II**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Reaction temperature °C</th>
<th>Enzyme concentration (% of dry starch)</th>
<th>Reaction period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>.10</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>92</td>
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<td>92</td>
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<tr>
<td>7</td>
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<td>.25</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>82</td>
<td>.10</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>82</td>
<td>.40</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>82</td>
<td>.10</td>
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<tr>
<td>12</td>
<td>82</td>
<td>.40</td>
<td>24</td>
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<td>13</td>
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<td>.25</td>
<td>16</td>
</tr>
<tr>
<td>14</td>
<td>82</td>
<td>.25</td>
<td>16</td>
</tr>
<tr>
<td>15</td>
<td>82</td>
<td>.25</td>
<td>16</td>
</tr>
</tbody>
</table>

After reacting with enzyme, an 800 g sample was placed in a .9 l glass jar and cooled to 60 °C in an ice water bath within two minutes.
Phosphoric acid was added at 2.5% by weight of the product and thoroughly mixed for 30 seconds.

Three hundred grams of product was placed in a 4.5 l glass jar and brought to 45°C in an ice water bath. Viscosity was measured with a Brookfield Viscometer using Spindle 2 at 12 rpm. The product was then cooled to 22°C and viscosity measured. Propionic acid was added at 0.5% by weight and mixed. Jars were stored with air-tight lids.

Of the remaining concentrate, 225 g was blended with 225 g of molasses and 0.5% propionic acid by weight. Viscosity was measured immediately after blending. This product was stored in 4.5 l jars with air-tight lids.

Viscosities were measured on the concentrate and blended samples at times of 24, 48, 72 and 168 hours. Each sample was vigorously shaken for 15 sec to simulate agitation that would be used in commercial storage before viscosity was measured.

A stability coefficient was estimated on each sample 1 wk after processing. Measurement was made of the sample and supernant depth and calculated as:

\[
\text{Stability coefficient} = \frac{\text{depth of sample} - \text{depth of supernant}}{\text{depth of sample}} \times 100
\]

Analysis and in vitro evaluation performed on the samples were as described in Chapter III.

Statistical analysis involved the use of stepwise forward and stepwise backward regression analysis to arrive at the best model for each parameter. The model involved the variables of temperature, concentration, and reaction time of enzyme addition. Using these models, contours were
plotted to aid in visual determination of the optimal set of enzyme reaction conditions. The variable held constant in each contour plot was determined to have least affect on the parameter measured by inspection of $F$ values.

**Prediction of Viscosity.** The use of the initial (45 C) viscosity was made to predict viscosities of the concentrates and blends at the various intervals measured. Regression analysis using the model $y = VC_1 * T + T$ with $VC_1$, the initial (45 C) viscosity and $T$, the enzyme reaction temperature was run on each sample presented in Table 8.

**Predictability of Models.** This study was designed to test the predictability of the models for viscosities and starch damage using the optimum conditions of 80 C, .25% of dry starch and 12 min reaction time followed by a 2 min cooling to 60 C. The study involved producing batches of 45.36 kg (100 lb) using the formula in Table 4. The reaction container was a barrel .56 m (22 in) in diameter and .81 m (32 in) in height. The mixer was constructed of 38 mm (1.5 in) steel bars with cross members of 76 mm (3 in) and 203 mm (8 in) and rotated at 40 rpm. A model 2835 P Roper\(^1\) pump powered by a 1 HP motor provided recirculation and pumping capabilities.

After each batch had been processed, the temperature was adjusted to 80 C and the enzyme solution added. The recirculation pump was run for 2 min to aid in distribution of the enzyme. After 12 min the pump was run for 2 min to aid in cooling the slurry to approximately 60 C. Two and one-half percent phosphoric acid by weight and added and mixed. Eight hundred gram samples were collected, cooled to 45 C and viscosity determined.

\(^1\) Roper, Commerce, Georgia.
Viscosities at 1 and 168 hr were taken of the concentrate and blend. Starch damage was determined.

Results and Discussion.

Study I. Viscosities are shown in Table 9. Samples 2, 6, and 8 have high viscosities and showed evidence of gelling. These samples were reacted with enzyme at 100 C. This indicated the enzyme was not reactive at 100 C as used under these conditions. Stability coefficients are given in Table 10. Samples 3 and 7 which were reacted at 60 C had low coefficients, thus indicating this temperature was not feasible. Results of determinations made on the samples are shown in Table 11. The variation in dry matter and protein can only be explained by variance in water absorbed from steam during processing.

Due to the wide range of viscosities, the data did not correlate well and another study was made with a narrower set of temperatures as shown in Table 8.
## TABLE 9. VISCOSITIES OF SAMPLES IN TABLE 7

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Concentrate</th>
<th>Time after processing (min)</th>
<th>Molasses blend</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>0 8 24 48 72 168</td>
<td>0 24 48 72 168</td>
<td></td>
</tr>
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<td>1298 2500 1088 1163 1175 1358</td>
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</tr>
<tr>
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<td>1678 775 2500++ 2500++ 2500++ 2500++</td>
<td>2500++ 2500++ 2500++ 2500++ 2500++</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>210 203 63 113 110 250</td>
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<td></td>
</tr>
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</tr>
<tr>
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</tr>
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</tr>
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</tr>
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<td>265 315 365 388 510</td>
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<td>128 200 210 63 125 140</td>
<td>165 205 158 205 215</td>
<td></td>
</tr>
</tbody>
</table>

* Gel pourable.
++ Gel non-pourable.
### TABLE 10. STABILITY COEFFICIENTS OF SAMPLES IN TABLE 7 STUDY I

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Concentrate</th>
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<td>14</td>
<td>85.7</td>
<td>96.2</td>
</tr>
<tr>
<td>15</td>
<td>66.7</td>
<td>91.5</td>
</tr>
<tr>
<td>Sample number</td>
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<td>Protein</td>
</tr>
<tr>
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<td>------------</td>
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<td>51.88</td>
</tr>
</tbody>
</table>

<sup>a</sup>Expressed on grain dry matter basis.

<sup>b</sup>% based on control with 41.58% dry matter and 60.48% protein.
Study II. Viscosities of samples are given in Table 12. Samples 1, 2, and 9 were high viscosity due to low enzyme concentration and short reaction time. Stability coefficients are shown in Table 13. Low values are due to high enzyme concentrations and long reaction time. Determinations made on samples are given in Table 14. Regression analysis information is shown in Table 15 for the best model describing each parameter measured.

Figures 1-8 present contours which were developed using the models described in Table 15.

In determination of the optimum enzyme conditions, it was desirable to minimize the reaction time, minimize the enzyme concentration and maintain the temperature between 80-90 C. The product was cooled to the desired range by steam flash immediately after hydrothermal processing.

The physical characteristics of viscosity of the concentrate after 168 hr and the stability coefficient were determined to be critical for product quality. From the comparison of viscosities of the concentrates after 168 hr in Table 12 and the stability coefficients in Table 13, a viscosity greater than 500 cps for the concentrate was necessary to maintain stability and minimize segregation. Figure 1 shows a temperature of 82 C, reaction time of 12 min and an enzyme concentration of .25% as minimum conditions for each variable to achieve a viscosity of approximately 500 centipose.
<table>
<thead>
<tr>
<th>Sample number</th>
<th>Concentrate</th>
<th>Time after processing (min)</th>
<th>Molasses blend</th>
</tr>
</thead>
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<td>163</td>
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</table>

*Gel pourable.

**Gel non-pourable.
### Table 13. Stability Coefficients of Samples in Table 8 Study II

<table>
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<th>Sample number</th>
<th>Concentrate</th>
<th>50% Molasses Blend</th>
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<sup>a</sup>Expressed on grain dry matter basis.

<sup>b</sup>% based on control with 41.58% dry matter and 60.48% protein.
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<td>T</td>
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<td>36.2048**</td>
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<tr>
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* **Significantly different from zero at the 95% and 99% level of probability, respectively.

Variables are represented as T---temperature, C---enzyme concentration, Time---time. 2 indicates variable is squared.
Figure 1. Contours of viscosity of concentrates after 168 hours as affected by enzyme concentration and time with temperature held constant at 82 C
At these conditions shown in Figure 2, the blend viscosity would be approximately 850-900 centipose. This viscosity was acceptable since no gelling problems occurred and segregation was minimal. Stability coefficient contours in Figure 3 show maximum stability at low enzyme concentrations and temperatures of 82-87°C with a reaction time of 12 minutes. Gelling would be a problem at the low enzyme concentrations. Conditions of 80°C, .25% enzyme concentration and reaction time of 12 min produced a stability coefficient greater than 85% which was acceptable.

Figure 4 shows starch damage increasing with higher enzyme concentration and longer reaction time, as would be expected. As these two variables are increased viscosity decreases rapidly and stability coefficients decrease. With respect to the above discussion on viscosity and stability, conditions of 80°C, .25 enzyme concentration and 12 min reaction time produced a starch damage value of 36 mg maltose.

Protein synthesis values in Figure 5 indicated temperatures of 70°C and 95°C to be most desirable. Temperatures in the low range were not practical from a processing design and the high range decreased the enzyme stability and activity. With a temperature of 80°C, 12 min reaction time and a .25% enzyme concentration, a value above 80 was determined to be acceptable.

Gas production was shown to be near maximum in Figure 6 with conditions of .25% enzyme concentration, 80°C, and reaction time of 12 minutes. Slightly greater values were given by increasing the temperature above the desired range.
Figure 2. Contours of viscosity of blends after 168 hours as affected by enzyme concentration and time with temperature held constant at 82°C.
Figure 3. Contours of stability coefficients as affected by temperature and enzyme concentration with reaction time held constant at 12 minutes.
Figure 4. Contours of starch damage (mg maltose on grain dry matter basis) as affected by enzyme concentration and reaction time with temperature held constant at 82 C.
Figure 5. Contours of protein synthesis as affected by temperature and time with enzyme concentration held constant at .25% of dry starch.
Figure 6. Contours of gas production as affected by temperature and enzyme concentration with time held constant at 12 minutes
Figures 7 and 8 show the hot concentrate viscosities and initial blend viscosities, respectively. These contours were used to predict the viscosity using the derived optimum conditions.

From review of the responses measured and the contours plotted from the best models, optimum conditions for the system employed in the study were determined to be a temperature of 80 °C, enzyme concentration of 25% of dry starch, and a 12 min reaction time followed by a 2 min temperature drop to 60 °C before addition of phosphoric acid to lower the pH and stop the enzyme activity.

**Prediction of Viscosity.** Regression analysis was run on viscosity data presented in Table 12 to determine the use of hot (45 °C) viscosity readings as a predictor of viscosities of concentrates and blends at latter time intervals. Table 16 shows the results of the regression analysis and information on models best describing the viscosities at a given time after processing and blending. The best model included the parameter of temperature and interaction of temperature with initial viscosity with a B value determined for each temperature level at which the enzyme had been reacted. R² values of close to 1 indicated the high correlation and predictability of viscosities at different intervals after processing from the initial hot (45 °C) viscosity of the product.
Figure 7. Contours of initial viscosity of blends as affected by temperature and enzyme concentration with time held constant at 12 minutes.
Figure 8. Contours of initial product viscosity as affected by enzyme concentration and time with temperature held constant at 82 °C.
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<th>Blend</th>
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<td>24</td>
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<td>184.25±98.90</td>
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<td>48</td>
<td>-366.66±63.76</td>
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<tr>
<td>128</td>
<td>-366.65±63.94</td>
<td>226.16±129.20</td>
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<tr>
<td>168</td>
<td>-366.65±63.94</td>
<td>24.82±147.52</td>
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<tr>
<td>24</td>
<td>-366.65±63.94</td>
<td>-184.65±228.40</td>
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<tr>
<td>48</td>
<td>-366.65±63.94</td>
<td>-19.90±313.23</td>
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**Table 16. Coefficients of Regression Equations Expressing the Relationship of Viscosity to Viscosities at Latter Intervals**
Predictability of Models. Viscosity and starch damage data are presented in Table 17. Averages are of the viscosities of the four batches and starch damage values. Expected values calculated for the optimum condition are shown for each parameter. Comparison between concentrate and expected values have an $r^2 = .73$. Differences in values are attributed to different batch size, mixing system and 5 C drop in temperature. Maltose values were higher for the larger system. Blended averages are similar to expected values.

This study illustrated the differences systems make in the product. Each system would need to be evaluated individually to determine specific predictability models.
TABLE 17. VISCOSITIES AND STARCH DAMAGE VALUES FOR
STUDY ON PREDICTABILITY OF MODELS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Viscosity (cps)</th>
<th>45 C</th>
<th>1 hr</th>
<th>1 wk</th>
<th>Mg maltose&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>450</td>
<td>48.82</td>
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<td>325</td>
<td>412.5</td>
<td>600</td>
<td>47.82</td>
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</tr>
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<td>Batch 4 Concentrate Blend</td>
<td>275</td>
<td>305</td>
<td>550</td>
<td>39.24</td>
<td></td>
</tr>
<tr>
<td>Average Concentrate Expected&lt;sup&gt;a&lt;/sup&gt;</td>
<td>272</td>
<td>326.38</td>
<td>531.25</td>
<td>44.36</td>
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<tr>
<td>Average Blend Expected&lt;sup&gt;a&lt;/sup&gt;</td>
<td>239.35</td>
<td>430.86</td>
<td>453.14</td>
<td>34.77&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Average Expected&lt;sup&gt;a&lt;/sup&gt;</td>
<td>358.35</td>
<td>876.6</td>
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<sup>a</sup>Calculated from the respective regression equations.
<sup>b</sup>Expressed on grain dry matter basis.
CHAPTER VI
RELATION OF PRODUCT VISCOSITY TO AMMONIA
TOXICITY IN VIVO AND IN VITRO EVALUATION

Ammonia toxicity has been found dependent upon processing variables in the case of Starea (Davidovich, 1977). This study was conducted to determine if differences in enzyme treatment and the resultant viscosity of the product would result in different occurrences of toxicity. In vitro methods were used to determine utilization of the products.

Experimental Procedures. Three different viscosity concentrates were produced in 34 kg (75 lb) batches from the formula in Table 18. Enzyme treatment of the three products was as presented in Table 19. The large batch mixer described in Chapter V was used as the reaction container. Each concentrate was then blended with molasses to a 32% crude protein level with a content as described in Table 20. Sufficient phosphoric acid was added to lower the pH of the samples to 3.5.

TABLE 18. COMPOSITION OF 60% PROTEIN CONCENTRATE

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<td>H₂O</td>
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<tr>
<td>Absorbed H₂O</td>
<td>9.70</td>
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<tr>
<td>H₃PO₄</td>
<td>2.50</td>
</tr>
<tr>
<td>Propionic acid</td>
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<tr>
<td><strong>Total</strong></td>
<td>100.00</td>
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*H₂O absorbed from steam during processing.*
### TABLE 19. ENZYME REACTION CONDITIONS OF CONCENTRATES

<table>
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<tr>
<th>Sample</th>
<th>Temp (C)</th>
<th>Enzyme concentrate % of dry starch</th>
<th>Time (min)</th>
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<td>80-12-8</td>
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<td>80-25-12</td>
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<td>.25</td>
<td>12</td>
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<td>80-40-24</td>
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<td>.40</td>
<td>24</td>
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*aTime of enzyme reaction at given temperature.
*bFollowed by 2 minute period to drop temperature to 60 C.

### TABLE 20. COMPOSITION OF BLENDED PRODUCT

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<td>10.64</td>
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<td>H₂O</td>
<td>21.38</td>
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<td>Absorbed H₂O</td>
<td>4.91</td>
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<tr>
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<td>H₃PO₄</td>
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<td>Propionic acid</td>
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<tr>
<td>Total</td>
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</tbody>
</table>

*aH₃PO₄ was adjusted in each sample to maintain pH 3.5.
Eight rumen fistulated adult cattle were randomly used to test the three products. The cattle were rested 1 wk between tests and fasted 16 hr preceding the test. Rumen fluid samples were collected with a syringe placed in a rubber tube extending through the cap of the rumen cannula. Blood samples were obtained from the jugular vein.

The animals were dosed with a calculated amount of the product to provide .25 g urea per 1kg body weight. Samples of blood and rumen fluid were collected at 0, 30, 60, 120 and 180 minutes. Rumen fluid was also sampled at 5 minutes.

Blood and rumen fluid ammonia-N were determined by microdiffusion (Conway, 1962).

*In vitro* methods of protein synthesis, gas production, free NH$_3$ and urea utilization were run as described in Chapter III.

**Results and Discussion.** Table 21 shows results of analysis of the three products. Differences in maltose values evident for the concentrates became less apparent when blended with molasses. A wide range in viscosities occurred as planned.

Results of the toxicity study are shown in Table 22. Rumen pH at 5 min shows the depression in pH due to the low pH of the products. Analysis by t-distribution showed no statistical difference ($P < .05$) between treatments for the variables measured. Blood NH$_3$ values did rise more rapidly for the low viscosity product. At 60 min the blood NH$_3$ values were higher for the medium and low viscosity product than for the high viscosity blend.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Dry matter</th>
<th>As is</th>
<th>Protein</th>
<th>As is</th>
<th>DM basis</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blend 80-12-8</td>
<td>52.04</td>
<td>30.57</td>
<td>58.74</td>
<td>2.69</td>
<td>14.41</td>
<td>283.55</td>
</tr>
<tr>
<td>Concentrate</td>
<td>51.46</td>
<td>30.70</td>
<td>59.66</td>
<td>5.28</td>
<td>149.10</td>
<td>289.74</td>
</tr>
<tr>
<td>Blend 80-25-12</td>
<td>52.62</td>
<td>29.47</td>
<td>56.01</td>
<td>6.07</td>
<td>165.10</td>
<td>313.59</td>
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<td></td>
<td></td>
<td>237.5</td>
</tr>
<tr>
<td>Product</td>
<td>Sample time</td>
<td>0</td>
<td>5</td>
<td>30</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td>----</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.75</td>
<td>6.94</td>
<td>7.16</td>
<td>7.25</td>
</tr>
<tr>
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<td>0.09</td>
<td>0.26</td>
<td>0.15</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Rumen NH₃</td>
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<td>15.20</td>
<td>-----</td>
<td>37.46</td>
<td>49.01</td>
<td>57.65</td>
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<tr>
<td>SE</td>
<td></td>
<td>2.85</td>
<td>-----</td>
<td>3.62</td>
<td>3.65</td>
<td>3.28</td>
</tr>
<tr>
<td>Blood NH₃</td>
<td></td>
<td>0.13</td>
<td>-----</td>
<td>0.22</td>
<td>0.29</td>
<td>0.37</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>0.01</td>
<td>-----</td>
<td>0.01</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>80-25-12</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.03</td>
<td>6.75</td>
<td>6.90</td>
<td>7.01</td>
<td>7.17</td>
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<tr>
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<td></td>
<td>0.06</td>
<td>0.08</td>
<td>0.07</td>
<td>0.06</td>
<td>0.03</td>
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<tr>
<td>Rumen NH₃</td>
<td></td>
<td>16.97</td>
<td>-----</td>
<td>40.46</td>
<td>53.67</td>
<td>56.29</td>
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<td>SE</td>
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<td>3.70</td>
<td>-----</td>
<td>3.80</td>
<td>4.62</td>
<td>3.96</td>
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<tr>
<td>Blood NH₃</td>
<td></td>
<td>0.13</td>
<td>-----</td>
<td>0.21</td>
<td>0.36</td>
<td>0.45</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>0.01</td>
<td>-----</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>80-40-24</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.27</td>
<td>7.03</td>
<td>7.17</td>
<td>7.24</td>
<td>7.32</td>
</tr>
<tr>
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<td></td>
<td>0.06</td>
<td>0.06</td>
<td>0.04</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Rumen NH₃</td>
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<td>15.36</td>
<td>-----</td>
<td>38.69</td>
<td>49.31</td>
<td>52.33</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>2.48</td>
<td>-----</td>
<td>3.65</td>
<td>3.13</td>
<td>3.48</td>
</tr>
<tr>
<td>Blood NH₃</td>
<td></td>
<td>0.15</td>
<td>-----</td>
<td>0.28</td>
<td>0.38</td>
<td>0.45</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>0.01</td>
<td>-----</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values represent the mean of eight animal observations with standard errors.
With the medium viscosity product critical rumen pH of 7.3 or greater was never reached. This product did have the lowest pH at 0 minutes. pH 7.3 or greater was only reached at 180 min for the high viscosity blend and at 120 min for the low viscosity blend.

Table 23 shows in vitro results of the three products. Analysis by t-distribution showed no significant difference between products in any analysis. This is due partly to the large standard errors that occurred. The medium viscosity, optimum product performed nearly as well as the low viscosity product in protein synthesis and minimal free NH₃ and better in gas production and percentage urea utilization.
<table>
<thead>
<tr>
<th>Product</th>
<th>Mg protein synthesized</th>
<th>Gas production</th>
<th>Free-NH₃</th>
<th>% Urea utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>80-25-8</td>
<td>17.63±3.19</td>
<td>34.03±5.61</td>
<td>133.00±4.86</td>
<td>37.21±5.31</td>
</tr>
<tr>
<td>80-25-12</td>
<td>18.96±.92</td>
<td>36.84±1.80</td>
<td>140.43±9.22</td>
<td>33.86±3.61</td>
</tr>
<tr>
<td>80-40-24</td>
<td>22.43±2.76</td>
<td>42.63±5.24</td>
<td>142.75±12.24</td>
<td>30.46±3.96</td>
</tr>
</tbody>
</table>

*Values are means ± standard error (n=3).*
CHAPTER VII

IN VITRO EVALUATION OF BLENDING A 60% CRUDE LIQUID SUPPLEMENT CONTAINING STARCH

Experimental Procedure. The production of a 60% crude protein liquid supplement containing starch required blending to a 32% crude protein product for normal free choice feeding. Molasses was used as the primary blending media. In the first study, water was substituted for molasses at various levels. The second study examined blending lignin sulfonate, ammonium polyphosphate (APP), or fermented ammoniated condensed whey (FACW) at various levels to obtain a 32% crude protein product.

Study I. The higher protein products used were of different starch:urea ratios. A 0.9:1 and 1:1 60% crude protein product was processed as described in Chapter V and blended to a 32% crude protein product using 1) molasses, 2) water, or 3) 50% molasses - 50% H₂O.

In vitro protein synthesis, as outlined in Chapter III, was determined on the blended products "as is" and with the products mixed with corn ground through a 1.59 mm (4/64 in) screen to achieve a 16% crude protein product to determine the effect of dry matter on protein synthesis.

Study II. Two 60% crude protein products of 1:1 starch-urea ratio were processed under conditions described in Chapter V. Levels of ingredients blended are given in Table 24. Samples were blended in 2.27 kg (5 lbs) lots. After blending pH was adjusted to 3.8 with 85% food grade H₃PO₄ for blends with molasses-water, lignin sulfonate or APP. Blends with FACW

1Flambeau Paper Mill, Park Falls, Wisconsin.
<table>
<thead>
<tr>
<th>Sample</th>
<th>60% crude protein product (%)</th>
<th>Molasses (%)</th>
<th>H₂O (%)</th>
<th>Lignin sulfonate (%)</th>
<th>APP (%)</th>
<th>FACW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 MW</td>
<td>50.9</td>
<td>49.1</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>2 MW</td>
<td>51.2</td>
<td>43.8</td>
<td>5.0</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>3 MW</td>
<td>51.5</td>
<td>38.5</td>
<td>10.0</td>
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<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>4 MW</td>
<td>51.7</td>
<td>33.3</td>
<td>15.0</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>5 MW</td>
<td>52.0</td>
<td>28.0</td>
<td>20.0</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>2 LS</td>
<td>51.1</td>
<td>46.4</td>
<td>-----</td>
<td>2.5</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>3 LS</td>
<td>51.2</td>
<td>43.8</td>
<td>-----</td>
<td>5.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>4 LS</td>
<td>51.3</td>
<td>41.2</td>
<td>-----</td>
<td>7.5</td>
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<td>-----</td>
</tr>
<tr>
<td>5 LS</td>
<td>51.5</td>
<td>38.5</td>
<td>-----</td>
<td>10.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>2 AP</td>
<td>48.8</td>
<td>49.2</td>
<td>-----</td>
<td>2.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>3 AP</td>
<td>46.8</td>
<td>49.2</td>
<td>-----</td>
<td>4.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>4 AP</td>
<td>44.7</td>
<td>49.3</td>
<td>-----</td>
<td>6.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>5 AP</td>
<td>42.6</td>
<td>49.4</td>
<td>-----</td>
<td>8.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>2 AW</td>
<td>44.5</td>
<td>45.5</td>
<td>-----</td>
<td>10.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>3 AW</td>
<td>38.2</td>
<td>41.8</td>
<td>-----</td>
<td>20.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>4 AW</td>
<td>31.8</td>
<td>38.2</td>
<td>-----</td>
<td>30.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>5 AW</td>
<td>25.4</td>
<td>34.6</td>
<td>-----</td>
<td>40.0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>6 AW</td>
<td>19.0</td>
<td>31.0</td>
<td>-----</td>
<td>50.0</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>
were not adjusted due to strong buffering affect of FACW. Blends with molasses-water were mixed with corn ground through a 1.59 mm screen for a resultant 16% crude protein product.

In vitro protein synthesis was run on the 16% crude protein products to determine dry matter affect of the blends. In vitro determinations of protein synthesis, gas production, free NH3-N, and percentage urea utilization, as described in Chapter III, were made on the blends containing lignin sulfonate, APP or FACW.

Results and Discussion.

Study I. Results of in vitro protein synthesis determinations on samples of molasses and water blends "as is" are shown in Table 25. Values for protein synthesis were significantly different (P<.05) for all treatments. As would be expected from the dry matter contents of the blends, protein synthesis from the blend with water was the lowest, intermediate for the concentrate itself and 50% molasses-50% H2O, and highest for molasses. No significant difference (P<.05) was present between the 0.9:1 and 1:1 starch-urea ratios.

Table 26 shows in vitro protein synthesis values of the blends mixed with corn to 16% crude protein level. Blending with molasses again produced a significantly higher value (P<.05). No significant difference (P<.05) was evident between the concentrate and 50% molasses-50% H2O blends. The value for the water blend was significantly lower (P<.05) than the other treatments. A significantly higher value resulted from the 1:1 over the 0.9:1 starch-urea ratio. This would be expected with the presence of more starch in the higher ratio.
TABLE 25. **IN VITRO PROTEIN SYNTHESIS DATA* FOR MOLASSES AND WATER BLENDS "AS IS" STUDY I**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrate</th>
<th>50% Conc.</th>
<th>50% Mol.</th>
<th>50% H₂O</th>
<th>50% Conc.</th>
<th>50% Mol.</th>
<th>50% H₂O</th>
<th>Sum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50% Conc.</td>
<td>50% Mol.</td>
<td>50% H₂O</td>
<td></td>
<td>25% Mol.</td>
<td>25% H₂O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:1 Ratios</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32.03</td>
<td>264.80</td>
<td>24.61</td>
<td>132.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47.81</td>
<td>195.74</td>
<td>24.77</td>
<td>133.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>48.79</td>
<td>293.86</td>
<td>45.03</td>
<td>134.53</td>
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<td>42.89</td>
<td>251.47</td>
<td>31.47</td>
<td>133.21</td>
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<tr>
<td>102.94</td>
<td>305.41</td>
<td>28.84</td>
<td>139.05</td>
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<td>72.51</td>
<td>222.89</td>
<td>21.35</td>
<td>105.01</td>
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<tr>
<td>87.93</td>
<td>252.87</td>
<td>29.27</td>
<td>103.02</td>
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<tr>
<td>87.89</td>
<td>260.39</td>
<td>26.49</td>
<td>115.69</td>
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</tr>
<tr>
<td>Sum</td>
<td>392.01</td>
<td>1535.57</td>
<td>173.87</td>
<td>746.7</td>
<td>2848.15</td>
<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>65.34c</td>
<td>255.93d</td>
<td>28.98e</td>
<td>124.45f</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Data reported as % DM protein basis of a 2:1 (corn:urea) 32% protein control.

*Column values with similar superscripts are not significantly different (P<.05).

**Sum values with different superscripts are significantly different (P<.05).**
TABLE 26. IN VITRO PROTEIN SYNTHESIS DATA* FOR MOLASSES AND WATER BLENDS MIXED WITH CORN TO A 16% PROTEIN LEVEL  STUDY I

| Treatments | Concentrate 50% Mol. | 50% Conc. 50% H₂O | 50% Conc. 25% Mol. | 25% H₂O | Sum | Mean
|-------------|----------------------|-------------------|-------------------|--------|-----|-----
| 8:1         | 93.46                | 117.41            | 45.64             | 95.64  |
|             | 69.60                | 116.0              | 39.93             | 88.78  |
| Ratios      | 76.81                | 100.88             | 43.00             | 98.21  | 985.36 | 82.11a |
|             | 79.96                | 111.43             | 42.86             | 94.21  |
| 1:1         | 105.43               | 128.27             | 64.12             | 105.43 |
|             | 101.91               | 112.83             | 47.12             | 72.79  |
|             | 101.82               | 105.37             | 56.60             | 83.06  | 1084.75 | 90.40b |
|             | 103.05               | 115.49             | 55.28             | 87.09  |
| Sum         | 549.03               | 680.76             | 296.41            | 543.91 | 2070.11 |
| Mean        | 91.51d               | 113.46c            | 49.40e            | 90.65d |

*Data reported as % DM protein basis of a 2:1 (corn:urea) 32% protein control, similarly blended with corn to 16% crude protein level.

a, b. Column values with different superscripts are significantly different (P<.05).

c, d, e. Row values with different superscripts are significantly different (P<.05).
From the study, it was concluded water alone is an inefficient blending medium. On an "as is" evaluation, molasses was a superior blending medium to water in terms of protein synthesis. Evidence suggested some water may be incorporated into the blends when used in conjunction with corn without depressing protein synthesis, but a ratio of 50% molasses:50% $H_2O$ appears to be beyond the upper limit.

The concentrate appeared equal to a 50% molasses:50% $H_2O$ blend when used with corn, thus suggesting use of the concentrate in a top dressing situation. Use of the 1:1 ratio appeared superior when the products are blended to a 16% crude protein level.

Study II. Molasses-$H_2O$ Blends. Table 27 shows dry matter, protein "as is," and protein dry basis for the blended samples evaluated by \textit{in vitro} techniques. \textit{In vitro} protein synthesis values are presented in Table 28 for the different molasses-$H_2O$ blends mixed with ground corn to achieve a 16% crude protein product. No significant differences ($P<.05$) were apparent even though dry matters of blended products range from 50.28% for 0% $H_2O$ to 36.88% for 20% $H_2O$ as shown in Table 27. In top dressing usage, up to 20% $H_2O$ in the blend apparently did not affect protein synthesis.

Lignin sulfonate blends. Comparison of four levels of lignin sulfonate is shown in Table 29. No significant difference ($P<.05$) in protein synthesis was shown between the 0, 2.5, 5.0, 7.5 or 10% level on either an "as is" or dry matter protein basis.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Sample ID</th>
<th>Dry matter</th>
<th>Protein</th>
<th>Protein dry basis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rep 1</td>
<td>Rep 2</td>
<td>Rep 1</td>
</tr>
<tr>
<td>H2O</td>
<td>0</td>
<td>50.28</td>
<td>51.43</td>
<td>31.07</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>46.82</td>
<td>48.86</td>
<td>30.78</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>43.79</td>
<td>44.90</td>
<td>31.23</td>
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<tr>
<td></td>
<td>15</td>
<td>41.21</td>
<td>41.96</td>
<td>32.24</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>36.88</td>
<td>38.88</td>
<td>32.59</td>
</tr>
<tr>
<td>Lignin</td>
<td>2.5</td>
<td>52.89</td>
<td>51.69</td>
<td>32.37</td>
</tr>
<tr>
<td>sulfo-</td>
<td>5.0</td>
<td>49.53</td>
<td>50.37</td>
<td>31.33</td>
</tr>
<tr>
<td>nate</td>
<td>7.5</td>
<td>48.69</td>
<td>49.76</td>
<td>31.51</td>
</tr>
<tr>
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<td>10.0</td>
<td>48.17</td>
<td>50.01</td>
<td>32.05</td>
</tr>
<tr>
<td>APP</td>
<td>2</td>
<td>50.07</td>
<td>51.24</td>
<td>31.49</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>49.94</td>
<td>51.64</td>
<td>31.03</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>50.46</td>
<td>51.78</td>
<td>31.97</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>50.19</td>
<td>51.72</td>
<td>32.42</td>
</tr>
<tr>
<td>FACW</td>
<td>10</td>
<td>49.42</td>
<td>48.86</td>
<td>30.99</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>46.81</td>
<td>52.19</td>
<td>31.77</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>50.30</td>
<td>50.04</td>
<td>32.52</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>51.04</td>
<td>50.25</td>
<td>33.60</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50.88</td>
<td>49.60</td>
<td>32.75</td>
</tr>
</tbody>
</table>

TABLE 27. DRY MATTER AND PROTEIN CONTENTS OF BLENDED SAMPLES  STUDY II
TABLE 28. *IN VITRO* PROTEIN SYNTHESIS COMPARISON OF FOUR DRY MATTER LEVELS OF A LIQUID SUPPLEMENT CONTAINING STARCH BLENDED WITH CORN TO A 16% PROTEIN LEVEL

<table>
<thead>
<tr>
<th>% Molasses-H₂O</th>
<th>Mg protein synthesized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>As is</td>
</tr>
<tr>
<td>50-0</td>
<td>17.80±1.68*</td>
</tr>
<tr>
<td>40-10</td>
<td>17.52±1.68*</td>
</tr>
<tr>
<td>30-20</td>
<td>17.90±1.29*</td>
</tr>
<tr>
<td>20-30</td>
<td>17.76±1.38*</td>
</tr>
<tr>
<td>10-40</td>
<td>17.56±1.87*</td>
</tr>
</tbody>
</table>

*Values within columns having similar superscripts are not significantly different (P < .05).*
TABLE 29. IN VITRO COMPARISON OF FOUR LEVELS OF LIGNIN SULFONATE BLENDED
IN A LIQUID SUPPLEMENT CONTAINING STARCH

<table>
<thead>
<tr>
<th>% Lignin sulfonate</th>
<th>Mg protein synthesized&lt;sup&gt;A&lt;/sup&gt; As is</th>
<th>DM prot basis</th>
<th>Gas&lt;sup&gt;B&lt;/sup&gt; production (ml H&lt;sub&gt;2&lt;/sub&gt;O displaced)</th>
<th>Free-NH&lt;sub&gt;3&lt;/sub&gt;B mg/100 ml</th>
<th>% Urea&lt;sup&gt;B&lt;/sup&gt; utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.52±1.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.44±2.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.79±2.83&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>43.50±4.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.80±3.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5</td>
<td>17.32±1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.03±1.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.19±4.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.40±5.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.79±3.24&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5.0</td>
<td>18.55±.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.97±1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.09±3.86&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>38.28±4.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.02±3.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7.5</td>
<td>17.80±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.14±1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.17±4.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>34.19±3.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.20±3.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10.0</td>
<td>17.80±1.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.61±2.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.80±6.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.43±4.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.33±3.80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>A</sup>Values are means ± standard error (n=8).

<sup>B</sup>Values are means ± standard error (n=6).

<sup>a</sup>,<sup>b</sup>Values within columns having similar superscripts are not significantly different (P<.05).
Gas production was significantly higher \( (P < .05) \) for the 2.5% level and significantly lower \( (P < .05) \) for the 10% level. No significant difference \( (P < .05) \) was apparent between the 0, 2.5, and 5% levels.

Free \( \text{NH}_3-N \) after 4 hours fermentation was not significantly different \( (P < .05) \) at any level. Levels of 2.5, 5.0, 7.5 and 10.0% resulted in significantly higher \( (P < .05) \) percent urea utilization; however, this trend was not evident in any of the other in vitro determinations. No significant difference in dry matter occurred as the percent lignin sulfonate increased as shown in Table 27. Substitution of up to 10% lignin sulfonate for molasses in the total blend appeared to have little depressing affect as tested in vitro.

**APP blends.** Table 27 shows dry matter contents not to be significantly different \( (P < .05) \) as the level of APP increases. In vitro evaluation of four levels of APP is shown in Table 30. Protein synthesis on an "as is" or dry matter protein basis was not significantly different \( (P < .05) \) at any level. Protein synthesis was lower at the 8% level. Gas production was significantly higher \( (P < .05) \) when APP was blended in the sample, but not different between the levels. Free \( \text{NH}_3-N \) after 4 hr of fermentation was significantly lower for blends containing APP, but not different between levels. Apparently APP was not broken down to \( \text{NH}_3 \) as rapidly as urea. Percent urea utilization was significantly lower \( (P < .05) \) at the 0% APP level. Possibly, since APP was not utilized as well, the urea in the samples was utilized to a greater extent.
TABLE 30. **IN VITRO COMPARISON OF FOUR LEVELS OF AMMONIUM POLYPHOSPHATE (APP) BLENDING IN A LIQUID SUPPLEMENT CONTAINING STARCH**

<table>
<thead>
<tr>
<th>APP</th>
<th>Mg protein synthesized&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Gas&lt;sup&gt;B&lt;/sup&gt; production (ml H&lt;sub&gt;2&lt;/sub&gt;O displaced)</th>
<th>Free-NH&lt;sub&gt;3&lt;/sub&gt;-N&lt;sup&gt;B&lt;/sup&gt; mg/100 ml</th>
<th>% Urea&lt;sup&gt;B&lt;/sup&gt; utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.52±1.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.44±2.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.37±2.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.30±3.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>18.25±1.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.60±1.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>120.08±6.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.42±1.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>18.62±2.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.77±2.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117.58±6.56&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>33.85±1.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>19.10±1.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.03±2.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>122.33±7.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.85±1.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>16.91±2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.36±2.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>122.93±7.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.86±1.85&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>A</sup>Values are means ± standard error (n=8).

<sup>B</sup>Values are means ± standard error (n=6).

<sup>a</sup>, <sup>b</sup>, <sup>c</sup>Values within columns having similar superscripts are not significantly different (P<.05).
Blending of up to 8% APP in liquid supplements containing starch resulted in significant differences \( (P<.05) \) in the \textit{in vitro} determinations.

\textbf{FACT blends.} No significant difference \( (P<.05) \) in dry matter occurred as FACT was blended at levels up to 50% with liquid supplements containing starch. Table 31 shows \textit{in vitro} comparison of five levels of FACT. Protein synthesis on an "as is" and dry matter protein basis was not significantly different \( (P<.05) \) for the 0 and 10% level, but was significantly higher \( (P<.05) \) than all other levels. Levels of 10 and 20% were not significantly different \( (P<.05) \) but were significantly higher than the 30, 40 and 50% levels. The 20, 30, 40 and 50% levels were significantly lower \( (P<.05) \) than the 0 and 10% levels, but were not significantly different \( (P<.05) \). Levels of 0, 10 and 20% were not significantly different \( (P<.05) \) in gas production. The 30, 40 and 50% levels were significantly lower \( (P<.05) \) than the 0% level. Free NH\(_3\)-N after four hr fermentation was significantly lower \( (P<.05) \) for all levels other than the 0% blend. The percentage urea utilization was significantly higher for all levels other than the 0% level.

From the \textit{in vitro} comparisons, it appeared levels of FACT above 10% result in decreasing performance. Thus, use of FACT as a blending medium with liquid supplement containing starch appeared to have a depressing affect on \textit{in vitro} evaluations.
TABLE 31.  **IN VITRO COMPARISON OF FIVE LEVELS OF FERMENTED AMMONIATED CONDENSED WHEY (FACW) BLENDED IN A LIQUID SUPPLEMENT CONTAINING STARCH**

<table>
<thead>
<tr>
<th>FACW</th>
<th>Mg protein synthesized(^A) (as is)</th>
<th>DM prot basis</th>
<th>Gas(^B) production (ml H₂O displaced)</th>
<th>Free-NH₃-N(^B) mg/100 ml</th>
<th>% Urea(^B) utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.52±1.66(^a)</td>
<td>29.44±2.54(^a)</td>
<td>102.79±2.83(^a)</td>
<td>43.50±4.59(^a)</td>
<td>61.80±3.46(^b)</td>
</tr>
<tr>
<td>10</td>
<td>17.08±1.88(^ab)</td>
<td>25.93±2.58(^ab)</td>
<td>107.68±5.90(^a)</td>
<td>35.55±4.41(^b)</td>
<td>65.31±4.02(^a)</td>
</tr>
<tr>
<td>20</td>
<td>14.34±1.16(^bc)</td>
<td>22.08±1.93(^bc)</td>
<td>99.75±5.04(^a)</td>
<td>35.40±3.76(^b)</td>
<td>63.97±4.15(^ab)</td>
</tr>
<tr>
<td>30</td>
<td>14.33±1.01(^bc)</td>
<td>21.60±1.50(^bc)</td>
<td>89.01±3.43(^b)</td>
<td>35.08±3.37(^b)</td>
<td>63.28±3.49(^ab)</td>
</tr>
<tr>
<td>40</td>
<td>12.66±1.36(^c)</td>
<td>18.47±1.85(^c)</td>
<td>84.06±3.54(^bc)</td>
<td>37.30±1.46(^b)</td>
<td>64.20±2.71(^ab)</td>
</tr>
<tr>
<td>50</td>
<td>12.07±1.02(^c)</td>
<td>18.32±1.54(^c)</td>
<td>76.72±3.20(^c)</td>
<td>34.90±2.91(^b)</td>
<td>64.35±3.12(^ab)</td>
</tr>
</tbody>
</table>

\(^A\)Values are means ± standard error (n=8).

\(^B\)Values are means ± standard error (n=6).

\(^a, b, c\)Values within columns having similar superscripts are not significantly different (P<.05).
CHAPTER VIII

SUMMARY AND CONCLUSIONS

Product characteristics of a hydrothermally processed liquid supplement containing starch were found to be predictable. A high correlation between length of enzyme reaction, product viscosity and starch damage was established. Predictability of final product viscosity from a hot (45°C) viscosity was highly correlated. Establishment of predictability was necessary before optimum conditions could be established.

Response surface methodology was used to establish the optimum conditions for reaction of an α-amylase solution with the hydrothermally processed corn-urea-water slurry. Variables of temperature, enzyme concentration, and time (length) of reaction were measured in terms of the parameters of viscosity, stability, starch damage, in vitro rumen protein synthesis, and in vitro rumen gas production. Use of regression analysis and contour plots was made to establish an optimum set of conditions. These were temperature of 80°C, enzyme concentration of 0.25% of dry starch in final product, and a reaction time of 12 min at 80°C followed by a 2 min cooling period to 60°C.

A high degree of correlation was established between hot and cold viscosities; however, predictability of the models was less when a larger system was used. This emphasized the dependency of the models on a given system and illustrated the need to establish
models for each system used in processing.

Comparison of three products of different viscosity was made with respect to ammonia toxicity and \textit{in vitro} measurements. No significant differences ($P<.05$) were found in the probable incidence of ammonia toxicity or \textit{in vitro} evaluations between the three products. The product resulting from the optimum conditions appeared to be most feasible in terms of viscosity, stability and \textit{in vitro} performance.

A 60\% crude protein concentrate was blended to a 32\% crude protein liquid supplement containing starch with molasses-water, lignin sulfonate, APP, or FACW. No significant difference ($P<.05$) in protein synthesis occurred when the molasses-water blends were mixed with finely ground corn to a 16\% crude protein product. Thus in a top dressing application, up to 20\% $H_2O$ in the blend apparently did not affect protein synthesis.

Substitution of up to 10\% lignin sulfonate for molasses in the total blend appeared to have no significant effect ($P<.05$) on the supplement as tested \textit{in vitro}.

Blending of up to 8\% APP in the total liquid supplement resulted in no significant difference ($P<.05$) in protein synthesis. Free $NH_3-N$ was significantly ($P<.05$) lower, gas production was significantly higher ($P<.05$), and urea utilization was significantly higher ($P<.05$) for the APP blends. This may be due to slower breakdown of APP compared to urea by the rumen micro-organisms.
Blends with FACW resulted in significantly lower (P<.05) protein synthesis, gas production, and free NH$_3$-N. Urea utilization was significantly greater (P<.05) for the FACW blends. This would suggest the nitrogen sources in FACW are not broken down as rapidly nor utilized *in vitro* as efficiently as urea. The higher levels of lactic acid in FACW may not provide as fermentable substrate as starch nor the carbon skeletons or energy for good protein synthesis.
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ENZYME ADDITION AND BLENDING VARIABLES ON NUTRITIONAL QUALITIES FOR RUMINANTS OF A LIQUID PROTEIN SUPPLEMENT CONTAINING STARCH

by

KEITH ELLIS PIKE

B. S., Kansas State University, 1977

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1978
Starch-based liquid protein supplements have been shown superior to molasses-based liquid supplements in both in vitro and in vivo evaluations. Interest has developed in a high protein (60%) starch-based liquid concentrate which could be blended to a 32% crude protein product. The concentrate was produced by processing a 1:1 corn-urea-slurry through a Hydro-heterR at 149 C and 60 psig. Viscosity of the cooked slurry was reduced by addition of an α-amylase solution.

Product characteristics of the hydrothermally processed supplement was found to be predictable. A high correlation between length of enzyme reaction, product viscosity, and starch damage was established. Predictability of final product viscosity from a hot (45 C) viscosity was highly correlated.

Response surface methodology was used to establish the optimum conditions for reaction of an α-amylase solution with the hydrothermally processed corn-urea-water slurry. Variables of temperature, enzyme concentration, and reaction time were measured in terms of viscosity, stability, starch damage, rumen in vitro protein synthesis, and rumen in vitro gas production. Use of regression analysis in contour plots was made to establish an optimum set of conditions. These are temperature of 80 C, enzyme concentration of .25% of dry starch in final product, and a reaction time of 12 min followed by a 2 min cooling period to 60 C before acid addition.

A high degree of correlation was established between hot and cold viscosity, however, predictability of the models was less when the larger
system was used. This emphasized the dependency of the models on a given system and illustrated the need to establish models for each system used in processing. Comparison of three products of different viscosity was made with respect to ammonia toxicity and in vitro rumen measurements. No significant differences ($P<.05$) were found in the probable incidence of ammonia toxicity for in vitro evaluation between the three products. The product resulting from the optimum conditions appeared to be most feasible in terms of viscosity, stability, and in vitro performance.

Effects of blending the 60% crude protein concentrate to a 32% crude protein product with the various levels of molasses, water, lignin sulfonate, ammonium polyphosphate (APP), or fermented ammoniated condensed whey (FACW) were evaluated through in vitro rumen studies.

The concentrate was blended with molasses-water combinations to attain 0, 5, 10, 15, and 20% water in the blend. No significant difference ($P<.05$) in rumen in vitro protein synthesis occurred when molasses-water blends were mixed with finely ground corn to a 16% crude protein product. Thus in a top dressing application, up to 20% water in the blend apparently did not effect protein synthesis.

Lignin sulfonate was substituted at levels of 0, 2.5, 5, 7.5, or 10% in the total blend for molasses. No significant effects ($P<.05$) were observed in the in vitro evaluations.

Levels of 0, 2, 4, 6, or 8% APP were added to the total supplement. No significant difference ($P<.05$) in protein synthesis resulted. Free $\text{NH}_3-N$ was significantly lower ($P<.05$), gas production was significantly higher ($P<.05$), and urea utilization was significantly higher ($P<.05$)
for the APP blend. This may be due to a slower breakdown of APP compared to urea by rumen microorganisms.

FACW was substituted at levels of 0, 10, 20, 30, 40, or 50%. Significantly lower (P<.05) protein synthesis, gas production, and free NH$_3$-N resulted from blending FACW into the supplement. Urea utilization was significantly greater (P<.05) for the FACW blends. This would suggest that the nitrogen forces in FACW were not broken down as rapidly nor utilized in vitro as efficiently as urea. The higher levels of lactic acid in FACW may not provide as fermentable substrate as starch nor the carbon skeletons or energy for good protein synthesis.