EFFECT OF ADDED FAT ON EXTRUSION PROCESSED CORN-UREA MIXTURES (STAREA)

by

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TO MY PARENTS

They furnish shade to others
while standing in the sun themselves

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INTRODUCTION

Ruminants have the unique ability to convert nonprotein nitrogen (NPN) to protein. Increasing competition from man and other nonruminants for the available protein is forcing the ruminants to utilize more NPN. The most economical source of NPN is urea.

There are several basic problems concerning use of urea and other NPN compounds by ruminants. Perhaps, the most significant of these is efficiency of utilization of NPN in the rumen. A great deal of interest has been shown in the past several years by the feed industry and others in new methods of processing to obtain products of higher digestibility and better efficiency. An example of this is the extrusion-cooked mixture of grain and urea (Starea®) developed at Kansas State University. Starea makes energy available to the rumen microorganisms at a rate similar to that at which urea releases ammonia so the microorganisms are simultaneously provided with the main components for microbial protein synthesis.

Fat has become more common as a feed ingredient due to changes in energy cost. Fat has also proved beneficial in processing Stareas of high urea contents. Feedlot and metabolism studies (13, 90) showed that rate of gain was consistently depressed by simultaneously adding fat and urea. Digestibility of crude protein was also reduced. The purpose of the research presented in this thesis was to evaluate the effect of added fat on extrusion processed corn-urea mixtures (Starea).

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REVIEW OF LITERATURE

PROCESSING GRAINS FOR NUTRITIONAL BENEFIT

Physical preparation of cereal grains for livestock has been practiced by feed producers and stockmen for more than 75 years. Grinding, crushing, rolling or soaking have been used to improve palatability and/or utilization of certain grains (31). Results varied with the grain species being tested. Riggs (67) stated that rolling or crushing had little advantage over grinding.

In the past 15 years there has been a rapid increase in feed processing which involves some type of wet or dry heat treatment or 'predigestion' of cereal grains for livestock feed. Grinding, cracking or rolling has apparently become inadequate to produce the gain and efficiency required (30). However, these are still important secondary operations to most newer processing techniques.

During processing, some physical and chemical changes are known to occur in the structure of the starch endosperm, germ, bran layer and the protein rich aleurone layer (64). The aleurone layer surrounds and protects the endosperm cells. These endosperm cells contain starch granules. Heat and moisture are the principal swelling and dehydrating agents of starch during processing (59).

Some of the latest methods of processing are: pulverization, steam pelleting, steam pressure and extrusion cooking, steam flaking, explosion cooking, dry heat roasting, micronizing and popping.

In recent years, extrusion cooking which has existed for quite some time in the plastic and food industry, has been adapted to the feed manufacturing industry. Extrusion cooking uses a combination of moisture, temperature, high pressure and mechanical friction to accomplish in seconds the same physical and chemical changes in grains that normally would take several minutes, hours

or even days to accomplish by other methods.

There are several advantages of the extrusion process: (a) the starch portion of the feed may be fully gelatinized for maximum digestibility and availability, (b) growth inhibitors in oilseeds and pulses are effectively inactivated whereas the heat-labile amino acids and other nutrients are preserved (60, 80), (c) large volume production may be achieved on a continuous basis at a relatively low cost, (d) products with good color and flavor, high nutritive value and oxidative stability can be produced by extrusion cooking (81). Extrusion cooking is a flexible system in that a variety of conditions may result in the desired product. Excellent vitamin retention was observed when encapsulated vitamins were used (60, 80).

Extrusion processes used in food and feed industry can be classified into three general types. They are: cold forming, low-pressure cooking and forming and high-pressure cooking and forming. The cold forming process is a low-pressure extrusion of usually soft masses and is a mixing and shaping process. The low-pressure cooking and forming involves ingredients which are moistened, mixed and cooked and characteristically has a final product temperature at the die that is less than 100 C. The high-pressure cooking and forming is characterized by sufficient heat applied to the raw materials to cook and completely gelatinize the starch. This process is also called short time-high temperature (ST-HT) extrusion. The temperature used in this process ranges from 100 C to as high as 250 C, depending on the ultimate use of the product (25, 58).

The basic principles of plasticating and gelatinizing extrusion are the same, i.e., the conversion of a solid material to a fluid state by the application of heat, followed by extrusion through a die to form a product of predetermined geometric and physical characteristics (19). In ST-HT extrusion, which is the type most predominantly used by the feed industry, the raw mater-

ials are ground, mixed and preconditioned to a selected temperature and moisture level. The preconditioned material is delivered to the extruder by a feeder, which may be a simple hopper and vibratory feeder or screw feeder to move material into the inlet of the extruder screw. The screw rotates in a barrel which has a hardened liner. The material enters the feed section of the screw and is thoroughly mixed and then transported to the second or transition section of the screw. The temperature is increased and compression is initiated in this section. At the end of this section the material is altered into a thermoplastic viscous material (58). The third section is the melting section. Its function is to homogenize the materials and force them through the die at a constant pressure (70). In this section the material is cooked and further mechanically worked to a fluid, semi-plastic material at high pressure (58). When extrudate emerges from the die, some moisture flashes off as steam. This sudden release of pressure causes the product to expand and become cellular and porous.

Since extrusion cooking is capable of producing total starch damage, new products have been developed. One such product is Starea (6). Starea is a mixture of finely ground cereal grain and urea which is processed by extrusion cooking. Research revealed a lack of toxicity when diets were supplemented with NPN in the form of Starea rather than urea.

The use of urea in ruminants has been restricted by poor conversion of urea nitrogen to microbial protein, by potential toxicity, by low palatibility and by processing and storage problems related to its hygroscopicity (18). Starea reduces these problems and in some cases eliminates completely all of the problems.

A limiting factor concerning the use of urea is its potential toxicity. The rumen microflora have an efficient urease enzyme system capable of rapid

urea degradation. The resulting ammonia is released into the rumen fluid for utilization in synthesis of microbial protein (21). If synthesis does not keep pace with ammonia production, excess ammonia results and is absorbed into the venous blood surrounding the rumen. The liver converts most of this ammonia to urea and it is eliminated in the urine. If blood ammonia is in excess, ammonia toxicity and death are possible results (6).

A study using expanded grain as a highly available energy source resulted in significantly lower rumen ammonia concentration and more efficient mocrobial protein synthesis (21). In another study by the same workers, they found that feeding extruded grain and finely ground alfalfa resulted in higher milk yield and more protein and lactose in the milk when compared to cracked grain and chopped hay (20).

During extrusion, high temperature, high pressure and shearing action occurs in the extruder. It was suspected that some of the carbohydrate in the sample might be degraded or changed by the extrusion process. A comparision of the mono- and oligo-saccharides in the raw ingredients and that in the extruded product showed significant increases in fructose, glucose and melibose and a slight increase in maltose, maltotriose and maltotetrose. It was suggested that the 1, 2 glycosidic bonds of sucrose and raffinose and the 1, 4 glycosidic bonds of mono- and oligo-saccharides are broken by the combination of high temperatures, high pressures and severe shearing during the extrusion process.(19).

The above factors are responsible for the success of Starea. The starch and nitrogen of Starea are presented to the rumen microflora in a highly available form compatible with urea degradation and protein synthesis (38, 86). The hydrolysis products of starch not only provide the required energy but are also sources of carbon skeletons needed for amino acid synthesis.

In toxicity studies using identical twins, Starea was compared to grain plus urea at various levels (6). The extruded grain urea product was significantly less toxic than the same mixture unprocessed.

When the extrusion cooked Starea was compared with soybean meal or urea as a protein supplement for lactating cows, it was nearly equal to soybean meal and superior to urea (38).

In rumen metabolism studies, Starea was compared with cracked grain sorghum plus urea, fine ground sorghum grain plus urea and expanded sorghum grain and urea (86). All diets were formulated on an isonitrogeneous basis. Rumen fluid was analyzed for ammonia content by a micro-diffusion method. By the second day of the trial the rumen ammonia content of animals fed Starea was significantly less ($P < .01$) than that of those fed other rations. By the seventh day of the trial, the animals fed Starea, expanded grain and finely ground grain rations were significantly different ($P < .01$) from those fed the control ration, but among the experimental rations, rumen ammonia content was the lowest for the Starea ration.

In <u>in vitro</u> studies, the rumen inoculum from urea adapted animals were incubated with Starea (34%, 39% and 44% protein equivalent), expanded corn plus urea and ground corn plus urea (39). Bacterial residues were analyzed for amino acids and protein and the fluid was analyzed for <u>in vitro</u> ammonia nitrogen. Results indicated that Starea supplements improved NPN (urea) utilization more than expanded corn plus urea but not significantly so ($P < .05$). All substrates containing extruded grain had significantly higher protein synthesis and lower ammonia nitrogen levels than the ground corn plus urea substrates.

GRAIN COMPOSITION

Before discussing the changes that occur within the physical structure of

cereal grains, the native structure and components of the cereal should be well understood.

The two most widely used grains in the United States for livestock feeding are corn and sorghum grain. Of these corn is ranked first (8).

The starch portion of corn grain contributes to its high energy value. On the average, 71% of the kernel of ordinary dent corn is comprised of starch (95). This starch, on the average, is comprised of 27% amylose and 73% amylopectin. Some breeds of corn (waxy maize) have been produced with starch comprised of 100% amylopectin while others contain as much as 50% amylose in the starch (92).

Fiber is also a source of energy, particularly for the ruminant. The pericarp of corn, which contributes the bulk of the fiber fraction, is about 40% cellulose and 40% pentoglycan (98). These two components are found primarily in the cell walls of the pericarp and other fibrous tissue. The lignin portion accounts for the remainder of the fiber fraction.

A small percentage of free sugars are found in the mature corn kernel. Of approximately 2.5% free sugars available, about one-half to three-fourths is sucrose with the remainder being D-glucose and D-fructose.

Approximately 3 to 4% of the kernel is comprised of oils, glycerides and other ether extractable materials (7). The mixture is complex and variable. However, it should be noted that the lipid content is thought to measurably affect processing characteristics during extrusion.

Corn protein is a mixture of several distinct protein types: salt-soluble globulins, alcohol-soluble prolamin, zein and the alkali-soluble protein glutelin (14). The average percentages of the total protein are: globulin-25, zein-48, glutelin-25 and insoluble 2.

Grain sorghum is the second largest grain crop utilized for livestock feeds.

About 95% of the total annual production is used for feeding purposes with the remainder used in wet and dry milling operations (95). The important feature of sorghum grain is that in periods of drought, the plant becomes practically dormant allowing it to withstand this stress much better than corn. For this reason sorghum grain has become important in the more arid areas of the United States.

The morphological structure of sorghum grain is almost identical to that of corn with respect to the germ and endosperm. The pericarp or fibrous portion of grain sorghum is similar in structure to that of corn except that it is covered with a thick layer of wax and includes many very small starch granules in the mesocarp layer (22, 72).

Sorghum grain differs from corn, in that it has a lower fat content but slightly higher protein and starch content. It also seems more variable in protein content than does corn. This has caused problems in feed formulations (55).

Sorghum grain also differs from corn in that its protein is much less soluble in normal extraction solvents (79). Five samples of sorghum grain were extracted to fractionate the protein. The average contents were as follows: albumin-3.8%, globulins-4.0%, prolamines-5.8%, glutilins-17.7% and insoluble protein residue-59.1%. The insoluble residue for high lysine corn in the same study contained 10.6% of the total protein. This value compared favorably with normal dent corn.

The starch of grain sorghum is very similar to that of corn except that average granule diameter is a little larger than that of corn starch: 15 μm for milo starch compared to 9.2 μm for corn starch on the same basis (75). The amylose and amylopectin content is similar for both grains.

STRUCTURE OF STARCH GRANULES

Starch granules from different sources show much variation in shape, average size and other superficial physical characteristics. Starch granules from the white potato are among the largest; those from rice and buckwheat are the smallest in cereal starches. Generally, the size of starch granules is expressed as the length of the longest axis in microns. They vary in size from 2 μm to 150 μm (46).

When viewed under a polarized microscope, sound starch granules show a characteristic birefringence pattern which demonstrates the crystallinity of starch granules (96). The amount of crystalline material in starch granules has been roughly estimated at about 50 to 60% (71). The crystallinity of starch has been classified as A, B, C and V patterns by X-ray diffraction with wheat starch observed to be A-type (84).

Intact starch granules contain 10 to 17% moisture under normal atmospheric conditions. About 8 to 11% of water bound by starch is held more tenaciously than that bound at higher moisture levels. This amount of water corresponds closely to that required to form a starch monohydrate (48). The starch granule exhibits some reversible swelling when they are exposed to a water saturated atmosphere, the average increase in granule diameter is as follows: corn 9.1%, potato 12.7%, tapioca 28.4% and waxy corn 22.7% (34).

Starch granules from cereal grains are composed almost entirely of carbohydrate. However, they contain .5 to 1.0% fatty acids which may affect some of their properties (76). The carbohydrate portion of a starch granule is made up of linear and branched glucose polymers known as amylose and amylopectin respectively. These linear and branched components of starch granules are associated by hydrogen bonding either directly or through water hydrate bridges to form radially oriented micellar network. The overall strength of the mi-

cellar network, which is dependent on the degree of association and the molecular arrangement, controls some preperties of starch granules (34).

Most common cereals of the non-waxy type contain 25% amylose. The amylose stains bright blue with iodine and has an X-ray diagram consistent with a helical structure in its crystalline complexes. In contrast to amylose, amylopectin possesses branches, stains red violet with iodine and shows an amorphous structure in X-ray analysis (99). The branching occurs by means of the alpha (1→6) linkages. The extent of branching varies among the starches from a relatively low degree as in potato to a high degree as in sago (65). The amylopectin component comprises about 75 to 80% of most starches with some genetic species possessing essentially 100% amylopectin and others as little as 20% (92).

ENZYMATIC DEGRADATION OF STARCH

Enzymes which are capable of catalyzing the hydrolysis of starch are widely distributed in nature and are generally classified into four groups according to their action patterns: alpha-amylase, beta-amylase, gluco-amylase and oligo-saccharide hydrolase. The hydrolysis of starch and oligo-saccharides by enzymes involves the addition of the elements of water to the D-glucosidic bond (62).

Beta-amylase is a plant enzyme which hydrolyses alpha-(1→4) D-glucosidic linkages and stepwise degrades the amylose and amylopectin to maltose and high molecular weight limit dextrin (62, 94). The enzyme attacks amylose and amylopectin from their non-reducing ends and releases the beta-maltose (66). Beta-amylase does not attack the alpha (1→6) branch linkages of amylopectin and glycogen, so that action ceases when these branch points are approached and high molecular weight limit dextrins remain (62, 66).

GELATINIZATION OF STARCH

Characteristics of starch gelatinization:

The term starch gelatinization, most frequently refers to the sequence of changes which occur when starch is heated in an aqueous medium or treated with chemical reagents (26). This sequence of changes are due to the fact that heat and chemical reagents weaken the micellar network within the granules (48). Starch gelatinization is believed to begin in this accessible and weak micellar area of the granule where the bonding is the weakest.

Different workers have defined gelatinization differently. Earlier workers tried to relate it to changes in starch viscosity, swelling power and solubility which occur at a much higher temperature than those encountered in the process of gelatinization (23). The loss of birefringence is widely accepted as an indication of gelatinization (48). A recent definition of gelatinization by Seib (76) seems to be a better explanation of this phenomenon. To quote Seib, "Gelatinization is the irreversible rupture of the native, secondary bond forces in the crystalline regions of a starch granule." The word 'native' is used only to indicate that gelatinization makes the granules much less crystalline but not amorphous. The gelatinized granules show a new X-ray diffraction pattern (Type-V) (35).

Apart from water, starch also gelatinizes in several polar organic solvents such as dimethyl sulfoxide (DMSO) (23) and ethylene diamine (76). Starch can be solublized in inorganic solvents such as liquid ammonia and cold alkali (89), but the changes that take place in starch during gelatinization in aqueous systems are of prime importance to us.

As stated earlier when starch granules are exposed to a water saturated atmosphere at room temperature, the granules undergo some reversible swelling

showing the limited elastic nature of the intermicellar network. They absorb about 40 to 50% water (on a weight basis) and this process is exothermic. The extent of swelling (9 to 30%) (48), the amount of water held (40 to 50%) (23) and the heats of hydration (23) vary between different botanical types of starches. These variations are attributed to differences in the granular structure of the different starches. According to Ullman (91) cold water is absorbed by the starch granule in one or more of the following ways: (a) water of crystallization, (b) absorbed water and (c) interstitial water. Another explanation is that water is firmly bound as hydrate until free hydroxyl groups are available in the starch molecule for binding (45).

The micellar network of granular starch is freely accessible to the entry of water and of most liquids (76). A starch-in-water suspension, when subjected to heating, absorbs a small amount of water without losing its birefringence until it reaches a crucial temperature (76). At this point some granules swell rapidly and irreversibly, losing their birefringence characteristics and the process is known as gelatinization. In gelatinisation, water or a polar solvent in the presence of added heat (or chemical reagent) destroys the native crystalline structure by breaking the bonding forces (76). Gelatinization is therefore an endothermic process (23). As the temperature of a starch in aqueous media is increased, more and more granules undergo the changes described above, until all the granules are gelatinized completely. The entire process of gelatinization takes place over a range of temperature (about 10 C) known as gelatinization temperature range, which varies in different starches. Gelatinization starts in the region of the granule where the associative forces are the weakest (amorphous region). The strength of the associative bonds in this region varies among the different granules belonging to the same botanical type (48). This is why gelatinization takes place over a range rather than a

single temperature. It is also observed that larger granules loose their birefringence at a lower temperature than smaller granules (23).

Agents that can break hydrogen bonds such as salts, alkali and urea were reported to accelerate the process of gelatinization (76), whereas, others retard gelatinization by acting as desolvating agents (48). Starch granules continue to swell as the temperature is increased beyond the gelatinization temperature. Simultaneously soluble materials leach out of the granule and some of the granules rupture completely. By this process the viscosity and soluble materials in the aqueous phase increases (76). Prolonged boiling or mechanical shear will ultimately rupture all the granules. Granules of wheat starch were found to retain their identity until a temperature of 95 C and on further heating in an autoclave to 105 C the identity of granules was completely lost (24). The swelling power, viscosity and pasting properties are very important characteristics.

Determination of Starch Gelatinization:

The intact starch granule differs from gelatinized starch in many respects such as viscosity, solubility, water absorption, swelling power, paste clarity, X-ray diffraction pattern, birefringence, susceptibility to staining materials and enzymatic reaction. Based on some of these properties a number of different methods for measuring degree of gelatinization have been developed. These methods may be conveniently divided into three groups:

(A) Those involving the use of microscope, including direct observation of swelling of starch granules (24), staining techniques (44) and loss of birefringence (48, 75).

(B) Those involving physical measurement on the starch paste such as swelling power (49), solubility (49), viscosity (73), paste clarity (73), X-ray diffraction pattern (23), proton magnetic resonance (41) and differential scanning calorimetry (85).

(C) Those involving the enzymatic digestion by one or more of amylases such as beta-amylase (88), gluco-amylase (78) and a mixture of alpha- and beta-amylase (61).

Among the methods, the loss of birefringence and enzymatic digestion are the most sensitive and accurate and thus widely used. The physical measurements for starch gelatinization are often dependent on a variety of different factors and the results are difficult to interpret (23). Generally the loss of birefringence is good for determining the gelatinization temperature and also for detecting the initial gelatinization of starch (48). The quantitative determination of starch gelatinization based on the loss of birefringence is laborious and subject to large sampling errors (97). Moreover, the birefringence method cannot be used for cooked heterogeneous samples (76).

The methods based on enzymatic digestion on the other hand, are commonly used and are a good quantitative measure of the degree of gelatinization. It is believed that this enzymatic approach has the added advantage of simulating in vivo digestibility of starch (76). All enzymatic procedures are based on the experimental finding that gelatinized starch granules are more susceptible to attack by amylases than intact starch granules (87).

EFFECT OF CARBOHYDRATE ON UREA UTILIZATION

It has been established that urea-nitrogen is utilized by rumen microorganisms to synthesize microbial protein. This microbial protein is utilized postruminally by the host animal. The mechanism of urea utilization is not completely understood. Briggs (15), Loosli and McDonald (51) and Helmer and Bartley (37) have reviewed the literature on urea as a protein replacer for ruminants and the progress made in increasing the efficiency of urea utilization.

Efficiency of urea utilization is affected by several factors such as (a) nature and level of protein in the ration (17, 40, 54, 63); (b) animal

adaptation to urea (53, 74); (c) level of sulfur (28, 83); (d) cobalt (12) and probably other minerals in the ration; (e) antibiotics (33); (f) diethyl stilbesterol (11); (g) fat (13, 90); (h) amino acid supplementation (27, 29); (i) treatment of urea (42, 93); (j) feed processing (5, 6) and amount and nature of carbohydrates. So, it may be generalized that any factor which affects the rumen microbial activity or dry matter digestibility may affect the efficiency of urea utilization.

Carbohydrates serve as a source of energy and carbon skeletons in converting ammonia into microbial protein (37). Volatile fatty acids (VFA) derived from carbohydrates lower rumen pH. This prevents rapid absorption of ammonia through the ruminal wall thus reducing the possibility of toxicity (5). These VFA also provide carbon skeletons to increase the conversion of ammonia into microbial protein. To obtain maximum protein synthesis in the rumen, the rate of ammonia release should parallel the availability of energy. For this reason, carbohydrates from various sources differ widely in their ability to promote protein synthesis in the rumen. Cellulose is least effective while starch is most effective and sugars and molasses are intermediate (4).

True protein of rumen digesta increased and ammonia-nitrogen (NH_3-N) decreased when starch was added to a ration of hay and urea (56). Greater protein synthesis was obtained when rumen fluid with or without added urea was incubated with starch than with several sugars, glycerol or lactic acid (63). McDonald (52) noted a rapid reduction in ammonia level of rumen contents when starch was added to the rumen of sheep fed a diet containing casein, suggesting that starch provided the energy needed by microorganisms to utilize ammonia.

Significantly higher total protein and lower ammonia and urea nitrogen in rumen digesta and higher growth rates of dairy heifers were reported when starch replaced half the molasses in a ration composed of hay, molasses and urea (57).

Steers retained more nitrogen when a hay-urea ration was supplemented with corn, barley, milo or dehydrated sweet potatoes than with cane molasses (27).

Belasco (10) compared several carbohydrates as energy sources for in vitro urea utilization by rumen microorganisms. Starch was superior to cellulose while xylan and pectin were intermediate. Further, as starch input was increased, urea utilization also increased and ammonia level decreased. Rumen ammonia concentration was more effectively reduced by starch than by glucose or xylan, while cellulose had very little effect (50). Level of readily fermentable carbohydrates in the ration significantly influenced the retention of absorbed nitrogen in lambs (53). In these experiments retention of absorbed nitrogen increased by approximately two percentage units per 100 kcal of readily fermentable carbohydrate.

PROCESSED STARCH-UREA SUPPLEMENTS

Various approaches have been used to increase ruminal conversion of urea to microbial protein. One approach was to slow down the rate of conversion of urea to ammonia either by inhibiting urease activity (1) or by treating urea to make it less susceptible to urease (42, 93). These procedures generally reduced ruminal ammonia concentration but did not increase urea utilization or protein synthesis significantly.

Helmer and Bartley (37) reported that "Since the majority of rumen bacteria prefer amoonia to amino acids, perhaps they should be permitted to produce ammonia from urea but encouraged to utilize the ammonia more efficiently." Since a reduction in the rate of ammonia formation would reduce the danger of toxicity, but would not improve nitrogen utilization greatly, Smith (82) suggested controlling urea toxicity by ensuring an adequate matched supply of available energy. This approach was used by Bartley et al.(6).

It is known that steam processing, autoclaving in the presence of moisture

and cooking makes grain starch more digestible. Energy release from cooked starch is therefore closer to the rate of ammonia release from urea. This permits a more efficient utilization of ammonia by rumen microorganisms.

Rumen bacterial protein had higher levels of essential amino acids when the substrate was soybean meal or Starea rather than ground grain and urea (39).

Bartley and Deyoe (5) reviewed research on Starea. In the manufacture of Starea, starch was nearly completely gelatinized and urea converted into an amorphous form (9). These two changes appear to be essential to maximize nitrogen retention. Non-crystalline urea was thought to be less susceptible to urease activity (9), releasing ammonia more slowly than crystalline urea. When equal quantities of isonitrogenous products were incubated with rumen fluid in vitro, significantly lower NH_3-N and higher bacterial protein were obtained with Starea than with ground corn plus urea. Expanded corn with urea added separately was intermediate (39). Several workers (3, 77) reported lower rumen and blood ammonia levels in Starea fed animals when compared with grain-urea fed animals. Being highly fermentable, gelatinized starch reduces rumen pH and absorption of ammonia into blood (5). This reduces toxicity and increases opportunities for microbial protein synthesis.

Helmer (36) and Barr (3) reported higher in vitro microbial protein synthesis when the substrate was Starea instead of grain-urea mixtures. Starea feeding resulted in greater quantity and concentration of bacterial and protozoal nitrogen and lower NH_3-N level in rumen contents when compared with a cracked grain plus urea ration (86).

Starea was superior to grain-urea rations and nearly equal to natural protein in terms of platability and feed intake (38, 77), feed efficiency (77), milk production (38) and weight gain (77).

EFFECT OF ADDED FAT ON RUMEN FERMENTATION

It has been stated earlier that the presence of fat affects the efficiency of urea utilization. Various workers (13, 16, 43, 90) studied the effect of fat and urea in rations and concluded that the rate of gain was consistently depressed by simultaneous addition of fat and urea. The addition of fat in urea containing rations reduced digestibility of dry matter, energy, NFE as well as crude protein digestibility. However, the depression in both gains and feed efficiency were less with pelleted rations than with meal rations (90).

Early work (16, 32, 68) showed that the infusion of lipid into the rumen brought about a decrease in total concentration of VFA (production of acetate decreased whereas the production of both propionate and butyrate increased), but had a variable effect on ammonia concentration in the rumen. This was confirmed by Kirk et al.(47).

Robertson and Hawke (68) showed that there was an increase in ammonia concentration with the addition of linseed oil to the ration. Furthermore, pH and total VFA concentration showed an inverse relationship. In another study by the same workers (69), incubation of 1 and 2 g of linseed or whale oil with liquor supplemented with ryegrass juice resulted in a slight reduction in fermentation as measured by gas production compared with incubation without lipid. The ammonia concentration was also higher in samples with lipid.

Thompson et al. (90) compared 2 rations, one with urea and the other with urea and fat. They concluded that steers fed the urea ration had higher concentrations of VFA, lower percentage of acetate, greater percentage of propionate, more total nitrogen, more protein, more NPN and more non-ammonia NPN per unit of ruminal fluid than steers fed fat and urea rations.

EXPERIMENT I

The objective of this experiment was to determine the effect of added fat on extrusion processed corn-urea mixtures (Starea). The various raw ingredients, viz., corn (U.S. grade #2), feed grade urea and animal fat were obtained commercially by the Department of Grain Science and Industry, Kansas State University.

MATERIALS AND METHODS

The corn-urea mixtures used in this experiment were prepared by finely grinding corn to pass a 1.59 mm hammer mill[a] screen, mixing urea with the ground corn to produce the desired protein equivalence, adding fat to the desired added fat level and processing the mixture through a Wenger X-25 Continuous Cooker Extruder[b]. The mixture of corn, urea and fat was hand fed continuously to the extruder at the the conditioning chamber. The material was advanced from the conditioning chamber to the first head of the extruder by a variable speed feeder screw and a high speed continuous paddle type conveyor. The composition of the various samples (having different protein equivalences and different levels of added fat) studied in this experiment are presented in table 1. The processing conditions used during extrusion are presented in table 2. Temperatures in the vicinity of 150 C were maintained at the die head to ensure complete gelatinization of the starch component of the mixtures. This was obtained by controlling the steam into and out of the jackets surrounding the barrel. High pressure

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[a]25 h.p. Sprout-Waldron Tear-Drop Screen hammer mill was used. Sprout-Waldron Co., Muncy, Pa.

[b]Wenger Manufacturing, Sabetha, Kansas.

TABLE 1. COMPOSITION AND ANALYSIS OF EXPERIMENTAL SAMPLES OF EXTRUDED CORN-UREA MIXTURES (STAREA)

	Sample number					
	1-1	1-2	1-3	2-1	2-2	2-3
Protein Equivalent, % (expected, dry basis)	88.00	88.00	88.00	154.00	154.00	154.00
Composition						
Corn, ground, %	71.83	69.76	67.70	49.77	47.71	45.64
Urea, %	26.17	26.24	26.30	48.23	48.29	48.36
Added Fat, %	2.00	4.00	6.00	2.00	4.00	6.00
Analysis*						
Dry matter, %						
Trial A	92.34	92.54	92.79	92.66	93.59	93.42
Trial B	92.28	91.83	90.51	92.32	93.56	91.94
Trial C	90.73	91.29	90.66	90.62	90.04	90.66
Protein Equivalent, % (dry basis)						
Trial A	90.33	87.91	86.18	153.28	150.17	148.66
Trial B	88.72	90.33	91.19	158.38	156.72	151.86
Trial C	91.18	90.70	90.04	154.86	156.16	152.03

*Determined by A.O.A.C. methods (2)

TABLE 2. CONDITIONS OF PROCESSING CORN-UREA MIXTURES (STAREA)[a]

Sample Number[b]	Feeder setting	Dry feed rate[c] kg/hr	Steam on feeder[d]	Water added[e] kg/hr	Temperature at die[f] C	Motor load %
1-1						
Trial A	2.25	150	90	10.10	154	35
Trial B	2.25	150	90	10.10	154	35
Trial C	2.25	150	180	10.10	149	32
1-2						
Trial A	2.25	150	0	10.10	154	30
Trial B	3.00	194	90	6.86	149	25
Trial C	3.00	194	180	6.86	146	25
1-3						
Trial A	2.25	150	90	2.40	157	<25
Trial B	3-2.25	194-150	90	3.97	149	<25
Trial C	3.00	194	180	3.97	146	25
2-1						
Trial A	2.25	150	90	0	152	<25
Trial B	2.25	150	90	0	152	<25
Trial C	2.25	150	90	0	152	<25
2-2						
Trial A	2.25	150	90	0	152	<25
Trial B	2.25	150	90	0	154	<25
Trial C	2.25	150	90	0	149	<25
2-3						
Trial A	2.25	150	90	0	152	<25
Trial B	2.25	150	90	0	154	<25
Trial C	2.25	150	90	0	148	<25

[a]All samples were processed on a Wenger X-25 cooker extruder (Wenger Manufacturing, Sabetha, Kansas) with 5 heads, 2 die spacers, (each die spacer 12.25 mm thick) a die with four 4.75 mm diameter holes. Die thickness = 10 mm.

[b]For sample identification refer to table 1.

[c]From calibration curve using fine ground corn.

[d]Rotation of the steam valve in degrees.

[e]From calibration chart supplied with Rotometer.

[f]From calibration curve for temperature recorder.

was maintained on the material in the barrel by using a die with four 4.75 mm diameter holes. The cooked and thoroughly mixed material was extruded through these die openings to obtain an expanded and gelatinized product. The expanded product was cut to convenient size by adjusting the speed of the variable speed knife. Samples were collected when processing had stabilized and when a unifrom product was being extruded. The samples were air dried for 24 hr and then ground in a laboratory mill equipped with a U.S. #40 screen.

The above procedure was followed for all samples. Three replicate trials were made for each sample condition, each trial on a different day to compensate for day to day variations. With minor exceptions, identical processing conditions were maintained for identical samples during the three trials.

Analytical Techniques

Moisture: Moisture was determined by drying the samples in a forced air oven for 1 hr at 130 ± 1 C (2). Moisture of each sample was determined in duplicate and the results reported as a percentage of the dry matter.

Protein Equivalent (PE): PE of samples was determined using the boric acid modification of the Macro-Kjeldahl method (2). The percentage nitrogen was converted to PE using a factor of 6.25. Determinations were made in duplicate and results reported as percentage PE (dry basis).

Starch Damage: Starch damage of samples were determined by Sung's method (88) modified for more rapid analysis. Approximately one gram of sample material was incubated for 60 minutes in 5 ml of enzyme solution (5 ml containing 60 mg of beta-amylase) and 41 ml (pH 4.6-4.8) of acetate buffer at 40 C in a thermostatically controlled water bath. Reducing

sugars produced by enzyme activity were determined using a standard ferricyanide method and results were reported as mg maltose per gram sample. All determinations were made in duplicate. Starch damage was corrected to a 100% dry grain base by dividing the maltose value by the percentage dry grain in the particular sample.

In vitro protein synthesis: The procedure used was the one described by Barr (3).

Step 1. Collection of rumen fluid

Rumen fluid was collected from a rumen-fistulated Angus steer fed a urea containing diet. Collection was made approximately 12 hr after feeding. The rumen fluid was collected in a thermos-flask to keep it warm. The fluid was filtered through four layers of cheese cloth into a flask which was closed with a Bunsen valve and kept in a warm water bath (39 C) until used.

Step 2. Preparation of feed samples

Samples were first balanced isonitrogenously such that a sample of 44% PE had a sample weight of .8 g, then raw corn (ground through a 1.59 mm hammer mill screen) was added to bring the sample weight to .8 g. Samples were weighed in duplicate into 50 ml plastic centrifuge tubes. Twenty milliliters of warm (39 C) mineral buffer (pH 6.8) was added to each tube.

Step 3. Fermentation

Ten milliliters of the strained rumen fluid was added to each of the centrifuge tubes. The tubes were closed with Bunsen valves and incubated in a thermostatically controlled water bath at 39 C for 4 hr. The tubes were shaken every 30 minutes to keep the sample and bacteria in suspension in the buffer.

Step 4. Centrifugation

At the end of the 4 hr fermentation period, the Bunsen caps were removed and the tubes were centrifuged at 15,000 rpm for 15 minutes. The supernatant was discarded and the precipitate resuspended in 25 ml of methanol. This was centrifuged at the same rpm for 15 minutes. The supernatant was discarded, the precipitate was again resuspended in another 25 ml of methanol and centrifuged at the same speed for the same length of time. The supernatant was discarded and the residue retained for protein determination.

Step 5. Kjeldahl determination

The protein content of the precipitate obtained at the end of step 4 was determined using the boric acid modification of the Macro-Kjeldahl method (2).

Step 6. Rumen fluid blank

Ten milliliters of rumen fluid was taken in each of two centrifuge tubes. Twenty milliliters of buffer solution was added and steps 4 and 5 were performed without incubation.

Step 7. Feed blanks

Samples were prepared as described in step 2 and 30 ml of buffer was added instead of 20 ml. Steps 3, 4 and 5 were performed on the contents of these tubes, except that no rumen fluid was added.

Step 8. Calculations

mg protein per tube = ml acid x normality of acid x 6.25 x 14

mg protein synthesized = mg protein in fermented sample - mg protein in rumen fluid blank - mg protein in feed blank.

Step 9. Results

Results were reported as percentage of the control. The control was

TABLE 3A. EFFECT OF ADDED FAT AND PROTEIN EQUIVALENT (PE) ON STARCH DAMAGE[1] OF EXTRUDED CORN-UREA MIXTURES (STAREA)

	Starch damage					
	88.00 % PE			154.00 % PE		
	Added Fat %			Added Fat %		
	2	4	6	2	4	6
Trial A	219.39	220.72	207.06	222.10	208.13	208.38
Trial B	237.74	216.86	195.00	238.62	240.58	227.37
Trial C	229.92	248.37	210.32	210.11	218.95	216.62
Average	229.02	228.65	204.13	223.61	222.55	217.46

[1]Starch damage determined by beta-amylase hydrolysis method (88). Starch damage expressed im mg maltose/g grain dry matter.

TABLE 3B. EFFECT OF ADDED FAT AND PROTEIN EQUIVALENT (PE) ON STARCH DAMAGE[1] OF EXTRUDED CORN-UREA MIXTURES (STAREA)
(MEAN VALUES)

	Sample treatment	Starch damage[2]
Protein Equivalent		
	88.00 %	220.60
	154.00 %	221.21
Added fat level		
	2.00 %	226.31
	4.00 %	225.60
	6.00 %	210.79

[1]Same as in table 3A.

[2]Each value was the average of all samples having the same protein equivalent or the same level of added fat.

a commercial sample of Starea (FMC-15)[a] which had a PE of 72.15% (as is basis).

pH measurement: Samples were prepared in the same way as described in step 2 of *in vitro* protein synthesis. Step 3 of the *in vitro* protein synthesis was performed on these samples. At the end of the 4 hr fermentation period the Bunsen caps were removed and the pH of the contents of each tube was measured using a Leeds and Northup pH meter.

RESULTS AND DISCUSSION

The dry matter and PE of the extruded samples are presented in table 1. These values are very close to the expected values

The starch damage values of the extruded samples as determined by the beta-amylase hydrolysis method are presented in tables 3A and 3B. The results indicate that the effect of PE on starch damage was not significant ($P < .05$). The differences due to the level of added fat were not statistically significant, but the starch damage values consistently decreased as the level of added fat increased. These differences were greatest between the 4% and 6% added fat levels. At higher levels, the added fat was probably acting as a barrier and reducing the contact between the enzyme and substrate. From the above results it would seem that the differences between 2% and 4% added fat would be minimal.

The *in vitro* protein synthesis data, expressed as a percentage of the control sample are presented in tables 4A and 4B. There was a significant ($P < .05$) effect of the PE on this measurement. Higher PE resulted in lower *in vitro* protein synthesis.

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[a] FarMarCo, Hutchinson, Kansas.

TABLE 4A. EFFECT OF ADDED FAT AND PROTEIN EQUIVALENT (PE) ON *IN VITRO* PROTEIN SYNTHESIS[1] OF EXTRUDED CORN-UREA MIXTURES (STAREA)

	Protein synthesized					
	88.00 % PE			154.00 % PE		
	Added Fat %			Added Fat %		
	2	4	6	2	4	6
Trial A	74.18	75.29	70.22	57.31	58.46	46.06
Trial B	92.75	83.02	79.60	69.50	65.70	60.00
Trial C	90.73	97.36	82.79	66.88	66.88	65.58
Average	85.89	85.22	77.54	64.56	63.68	57.21

[1] *In vitro* protein synthesis was determined by the procedure of Barr (3). Values were expressed as a percentage of control. In all cases the control was a mixture of .4879 g of FarMarCo Starea FMC-15 and .3121 g ground corn. All samples were first balanced on equal nitrogen basis and ground corn was added to bring the sample weight to .8 g.

TABLE 4B. EFFECT OF ADDED FAT AND PROTEIN EQUIVALENT (PE) ON IN VITRO PROTEIN SYNTHESIS[1] OF EXTRUDED CORN-UREA MIXTURES (STAREA)

(MEAN VALUES)

	Sample treatment	Protein synthesized[2]
Protein Equivalent		
	88.00 %	82.88[a]
	154.00 %	61.82[b]
Added Fat level		
	2.00 %	75.22
	4.00 %	74.45
	6.00 %	67.37

[1] Same as in table 4A.

[2] Each value was the average of all samples having the same protein equivalent or the same level of added fat.

[a, b] Differences due to protein equivalent were significant ($P < .05$).

TABLE 5A. EFFECT OF ADDED FAT AND PROTEIN EQUIVALENT (PE) ON PH[1] OF IN VITRO FERMENTATION SAMPLES FOR EXTRUDED CORN-UREA MIXTURES (STAREA)

	pH					
	88.00 % PE			154.00 % PE		
	Added Fat %			Added Fat %		
Trial A	6.60	6.65	6.65	6.90	6.90	6.95
Trial B	6.65	6.60	6.65	6.85	6.83	7.05
Trial C	6.60	6.65	6.70	6.85	6.90	7.00
Average	6.62	6.63	6.67	6.87	6.88	7.00

[1]pH of the sample determined at the end of 4 hr of incubation at 39 C with 10 ml of rumen fluid and 20 ml of mineral buffer. pH was measured using a Leeds and Northup pH meter. For sample preparation refer to table 4A.

TABLE 5B. EFFECT OF ADDED FAT AND PROTEIN EQUIVALENT (PE) ON PH[1] OF IN VITRO FERMENTATION SAMPLES FOR EXTRUDED CORN-UREA MIXTURES (STAREA) (MEAN VALUES)

	Sample treatment	pH[2]
Protein Equivalent		
	88.00 %	6.64[a]
	154.00 %	6.92[b]
Added Fat level		
	2.00 %	6.75[c]
	4.00 %	6.76[c]
	6.00 %	6.84[d]

[1]Same as in table 5A.

[2]Each value was the average of all samples having the same PE or the same level of added fat.

[a, b]Differences due to PE were significant ($P < .05$).

[c, d]Figures caaying different superscripts were significant ($P < .05$).

The gross energy (calculated from ingredient composition) contents of samples 1-1, 1-2, 1-3, 2-1, 2-2 and 2-3 were 24.36, 25.07, 25.28, 23.31, 23.58 and 23.23 kcal/g of urea respectively. Since the energy to urea ratios were nearly the same for all samples it could be concluded that differences noticed were not due to the energy level but due to one or both of the following: (a) the ratio of cooked to uncooked corn and or (b) the level of added fat. Samples having lower PE had a higher proportion of cooked corn as substrate and resulted in significantly higher protein synthesis indicating that processed corn was superior to ground corn in incorporating urea nitrogen into microbial protein. This was also reported by Helmer (36).

Among the samples having the same PE, samples with higher proportions of added fat resulted in consistently lower protein synthesis. The differences between 2% and 4% added fat were less pronounced that those between the 4% and 6% added fat levels.

The pH values of the fermented samples are presented in tables 5A and 5B. The differences in pH values of samples having different PE were significant ($P < .05$). This probably could be attributed to higher free NH_3-N levels in samples having higher PE. The average pH of samples having 6% added fat were significantly higher ($P < .05$) than those of samples having 2% and 4% added fat. Various workers (16, 32, 68, 69) have indicated that the addition of fat to ruminant rations resulted in higher levels of NH_3-N and lower VFA concentration in the rumen fluid. This could explain the higher pH in those samples with higher amounts of added fat.

In brief, the results indicate that starch damage was not significantly ($P < .05$) affected by PE. As PE increased, <u>in vitro</u> protein synthesis decreased and pH of fermented samples increased because of the higher ammonia concentrations. As the level of added fat increased, starch damage consis-

tently decreased but not significantly ($P < .05$). Protein synthesis decreased with higher fat level[5] and pH of the fermented samples increased because of lower VFA and higher ammonia concentration.

EXPERIMENT II

The objective of this experiment was to evaluate the effect of the level of urea in Starea at a constant fat level on protein synthesis and starch damage. Preliminary trials showed that it was difficult to extrude high urea formulations. It was thus decided to include 2% added fat in all samples processed for this study. The results of the previous experiment indicated minimal defferences between 2% and 4% added fat. However, to keep the effect of fat in this study to a minimum, the level of 2% was chosen. Fat, when used in extrusion cooking reduces the friction between the screw and barrel of the extruder and aids processing. The raw ingredients needed for this study were obtained commercially by the Department of Grain Science and Industry, Kansas State University. Six levels of PE were selected: 44%, 66%, 88%, 110%, 132% and 154% (dry basis).

MATERIALS AND METHODS

The corn-urea mixtures used in this experiment were prepared by finely grinding corn to pass a 1.59 mm hammer mill screen, mixing urea with the ground corn to produce the desired protein equvalence, adding 2 kg of fat to 98 kg of corn-urea mixture and processing the mixture in a Wenger X-25 Cooker Extruder. The mixture of corn, urea and fat was hand fed to the extruder at the conditioning chamber. The compositions of the various samples (having different PE) studied in this experiment are presented in table 6. Temperatures in the vicinity of 157 C were maintained at the die head to ensure gelatinization of the starch component of the mixture. This was done by controlling the steam in and out of the steam jackets surrounding the barrel. High pressure was maintained on the material by using four small openings at the die (diameter of each hole = 4.75 mm). The throughly mixed and cooked material was extruded through these open-

TABLE 6. COMPOSITION AND ANALYSIS OF EXPERIMENTAL SAMPLES OF EXTRUDED CORN-UREA MIXTURES (STAREA)

	Sample number					
	1	2	3	4	5	6
Protein Equivalent, % (expected, dry basis)	44.00	66.00	88.00	110.00	132.00	154.00
Composition						
Corn, ground, %	86.54	79.18	71.83	64.48	57.12	49.77
Urea, %	11.46	18.82	26.17	33.52	40.88	48.23
Added Fat, %	2.00	2.00	2.00	2.00	2.00	2.00
Analysis*						
Dry matter, %						
Trial A	98.87	97.82	97.50	96.65	95.32	95.61
Trial B	95.17	94.33	93.97	92.97	92.15	95.53
Protein Equivalent, % (dry basis)						
Trial A	46.27	67.69	85.38	113.20	136.76	158.72
Trial B	45.36	68.09	85.90	110.30	133.65	154.16

* Determined by A.O.A.C. methods (2)

TABLE 7. CONDITIONS OF PROCESSING CORN-UREA MIXTURES (STAREA)[a]

Sample Number[b]	Feeder setting	Dry feed rate[c] kg/hr	Steam on feeder[d]	Water added[e] kg/hr	Temperature at die[f] C	Motor load %
1						
Trial A	2.25	150	45	24.77	157	40
Trial B	2.25	150	90	24.77	154	40
2						
Trial A	2.25	150	45	8.41	157	40
Trial B	2.25	150	90	8.41	157	40
3						
Trial A	2.00	135	45	8.41	160	35
Trial B	2.25	150	90	8.41	160	35
4						
Trial A	2.00	135	45	8.41	160	25
Trial B	2.25	150	45	8.41	160	25
5						
Trial A	2.25	150	90	2.40	160	<25
Trial B	2.25	150	90	2.40	157	<25
6						
Trial A	2.00	135	45	0	154	<25
Trial B	2.25	150	45	0	157	<25

[a]All samples were processed on a Wenger X-25 cooker-extruder (Wenger Manufacturing, Sabetha, Kansas) with 5 heads, 2 die spacers (each spacer 12.25 mm thick), die with four 4.75 mm diameter holes. Die thickness = 10 mm.

[b]For sample identification refer to table 6.

[c]From calibration curve using fine ground corn.

[d]Rotation of the steam valve in degrees.

[e]From calibration chart supplied with Rotometer.

[f]From calibration curve for temperature recorder.

ings and cut to a convenient size by adjusting the speed of the variable speed knife. Samples were collected when processing conditions were stable and a uniform product was being extruded. The samples were air dried for 24 hr and then ground in a laboratory mill equipped with a U.S. #40 screen.

The above procedure was followed for all samples. Two duplicate trials were made for each level of PE. Each trial was conducted on a different day to compensate for day to day variations. Identical processisng conditions, with minor exceptions, were maintained during both the trials for samples having the same level of PE. The processing conditions during processing are presented in table 7.

Analytical Techniques

Moisture, PE and starch damage were determined by procedures described in the Materials and Methods section of the previous experiment. All samples were run in duplicate and the average values are presented in tables 6 and 8.

In vitro protein synthesis was by the Barr (3) procedure. Runs were made in duplicate on each sample of each trial. In the first run the sample preparation was the same as in Experiment I, viz., all samples were first balanced isonitrogenously such that a sample of 44% PE had a sample weight of .8 g. Then ground corn was added to each sample to bring the sample weight to .8g. Step by step description of the rest of the procedure has been explained under the heading 'in vitro protein synthesis' in the Materials and Methods section of Experiment I. Values for protein synthesized were expressed as a percentage of the control. The control sample was the same one that was used in Experiment I.

In the second run, samples at three levels of PE (44%, 88% and 154%) were used. Here instead of ground corn, extruded corn (270 mg of maltose

per gram grain) was added to bring the sample weight to .8g.

In the third run, the samples including the control were first balanced isonitrogenously but instead of adding ground corn, a mixture of ground alfalfa hay and ground corn (alfalfa hay : ground corn :: 9 : 1) was added to bring the sample weight to .8 g.

The reason for making these three runs was to simulate conditions of a low roughage diet and a high roughage diet (first and third runs respectively) and to evaluate the effectiveness of the Stareas (of different PE) under these two conditions. The second run was made to determine the effect of added extruded corn on protein synthesis.

RESULTS AND DISCUSSION

The dry matter and PE of the extruded samples are presented in table 6. These values are very close to the expected values.

The starch damage values of the extruded samples as determined by the beta-amylase hydrolysis method are presented in table 8. The results indicated that though PE had no significant effect ($P < .05$) on the starch damage there was a decrease in starch damage values as the PE of the sample increased. The differences were more apparent between samples 1 and 2 and 2 and 3. Samples 3, 4, 5 and 6 had nearly equal values for starch damage with 4 being a little higher than the rest. This may be attributed to experimental variation. These results support the results obtained in the previous experiment.

The in vitro protein synthesis data for the low roughage diets are presented in table 9. There were significant ($P < .05$) differences due to level of PE for this measurement. Samples having Starea of higher PE resulted in lower protein synthesis. Here again, as in Experiment I, the energy to urea ratios were nearly the same for all samples. Thus the

TABLE 8. EFFECT OF PROTEIN EQUIVALENT (PE) ON STARCH DAMAGE[1] OF EXTRUDED CORN-UREA MIXTURES (STAREA)

	Starch damage					
	PE %					
	44.00	66.00	88.00	110.00	132.00	154.00
Trial A	231.60	208.50	187.70	222.50	172.50	203.90
Trial B	240.60	222.60	214.90	203.20	230.10	197.80
Average	236.10	215.55	201.30	212.85	201.10	200.85

[1] Starch damage determined by beta-amylase hydrolysis method (88). Starch damage expressed in mg maltose/g grain dry matter.

TABLE 9. EFFECT OF PROTEIN EQUIVALENT (PE) ON *IN VITRO* PROTEIN SYNTHESIS[1] OF EXTRUDED CORN-UREA MIXTURES (STAREA)

	Protein synthesized					
	PE %					
	44.00	66.00	88.00	110.00	132.00	154.00
Trial A	91.10	69.30	49.51	71.28	57.43	56.42
Trial B	96.21	77.36	72.64	56.62	58.48	62.27
Average	93.66[a]	73.33[a,b]	61.08[b]	63.95[b]	57.96[b]	59.35[b]

[1] *In vitro* protein synthesis was determined by the procedure of Barr (3). Values were expressed as a percent of control. In all cases the control was a mixture of .4787 g of FarMarCo Starea FMC-15 and .3213 g ground corn. All samples were balanced first on equal nitrogen (as is) basis and then ground corn was added to bring the sample weight to .8 g.

[a,b] Figures sharing a common superscript were not significantly different ($P < .05$)

TABLE 10. EFFECT OF PROTEIN EQUIVALENT (PE) ON *IN VITRO* PROTEIN SYNTHESIS[1] OF EXTRUDED CORN-UREA MIXTURES (STAREA)

	Protein synthesized		
	PE %		
	44.00	88.00	154.00
Trial A	96.26	93.29	103.74
Trial B	90.32	105.24	101.50
Average	93.29	99.27	102.62

[1] *In vitro* protein synthesis was determined by the procedure of Barr (3). Values were expressed as percentage of the control. In all cases the control was a mixture of .4787 g of FarMarCo Starea FMC-15 and .3213 g of extruded corn (starch damage value = 270.00 mg maltose/g grain as is). All samples were balanced first on equal nitrogen basis and then extruded corn was added to make the sample weigh .8g.

The differences in the average values were not significant. LSD = 17.56.

TABLE 11. EFFECT OF PROTEIN EQUIVALENT (PE) ON *IN VITRO* PROTEIN SYNTHESIS[1] OF EXTRUDED CORN-UREA MIXTURES (STAREA)

	Protein synthesized					
	PE %					
	44.00	66.00	88.00	110.00	132.00	154.00
Trial A	75.67	80.02	67.82	62.61	43.49	60.01
Trial B	81.73	64.36	73.06	56.51	53.05	62.43
Average	78.70[a]	72.19[a,b]	70.44[a,b]	59.56[b,c]	48.27[c]	61.22[b,c]

[1] *In vitro* protein synthesis was determined by the procedure of Barr (3). Values were expressed as a percentage of the control. In all cases the control was a mixture of .4787 g of FarMarCo Starea FMC-15 and .3213 g ground hay (ground hay made up of 9 parts of alfalfa hay and 1 part of ground corn).

[a,b,c] Figures sharing a common superscript were not significantly different ($P < .05$)

same conclusions may be drawn i.e., differences in protein synthesis were not due to the energy level but due to the source of energy. In other words, due to the ratio of extruded corn to ground corn. Samples using Starea of lower PE had more extruded corn than those using Starea of higher PE. Helmer (36) has shown that extruded corn is more efficient in incorporating urea nitrogen into microbial protein than ground corn. Thus the results obtained were not unexpected.

Results supporting the above are presented in table 10. When samples had extruded corn added instead of ground corn, the protein synthesized was nearly equal in all cases.

In vitro protein synthesis data for the high roughage diet are presented in table 11. The energy (calculated from ingredient composition) content of samples 1, 2, 3, 4, 5 and 6 were 18.57, 14.17, 11.80, 11.40, 9.81 and 10.25 kcal/g of urea respectively. Since the energy to urea ratios decreased with the use of Starea of higher PE a decrease in protein synthesis was expected. In vitro protein synthesis values and the corresponding energy contents were positively correlated (r =.85) indicating that the differences noticed were mainly due to the energy content of the samples.

SUMMARY

Experiment I. The effect of added fat on extrusion processed corn-urea mixtures was studied by processing Starea-88% protein equivalence(PE) and Starea 154% PE with 2%, 4% and 6% added fat. Samples were processed in a Wenger X-25 Cooker Extruder under conditions favoring good starch gelatinization. It was found that the level of PE of the sample had no significant effect on the degree of starch damage. However, the level of PE had significant ($P < .05$) effects on *in vitro* protein synthesis and on pH of fermented samples. Higher PE resulted in lower protein synthesis and higher pH. Energy-urea ratios for all samples (during protein synthesis and pH measurement) were nearly the same, but samples using Starea of higher PE had lower proportion of extruded corn than did samples using Starea of lower PE. This would suggest that cooked corn was more efficient in incorporating urea-nitrogen into microbial protein. Furthermore, pH values indicated that there might be higher levels of free NH_3-N in samples using Starea of higher PE. It may be concluded that lower amounts of cooked corn and higher levels of free NH_3-N were probable reasons for decreased *in vitro* protein synthesis in samples using Starea of higher PE.

It was found that as the level of added fat increased, starch damage values decreased but not significantly so ($P < .05$). This would suggest that perhaps, at higher levels, the fat was acting as a barrier and preventing contact of the enzyme with the substrate. The level of added fat had no significant effect on the protein synthesis. But, among samples using Starea of same PE, higher levels of added fat resulted in consistently lower *in vitro* protein synthesis.

The average pH of samples having 6% added fat were significantly ($P < .05$) higher than those samples having 2% or 4% added fat. Various workers have

shown that the addition of fat to ruminant rations resulted in higher levels of ammonia and lower volatile fatty acid (VFA) concentrations. This could explain the higher pH in the samples having 6% added fat. Samples using Starea of the same PE but having higher amounts of added fat had lesser amounts of extruded corn. This could probably explain the lower protein synthesis.

Experiment II. The effect of the level of urea in Starea was evaluated in this study. Samples at 6 levels of PE: 44%, 66%, 88%, 110%, 132% and 154% (dry basis) were processed (all samples had 2% added fat for easier processing) under conditions favoring good starch gelatinization, in a Wenger X-25 Cooker-Extruder.

The level of PE had no significant effect ($P < .05$) on starch damage but starch damage values decreased with higher PE.

Ground corn or a mixture of ground alfalfa hay and ground corn were used with Starea to simulate low roughage and high roughage diets respectively. In vitro protein synthesis using these diets as substrates showed that the PE level of the Starea used had a significant effect ($P < .05$) on the protein synthesized. The decrease in protein synthesis in samples using Starea of higher PE (in the low roughage diet) could be attributed to the fact that they had lesser amounts of extruded corn than did samples having Starea of lower PE. This was supported by nearly equal protein synthesis obtained when samples were first balanced isonitrogenously and extruded corn was added instead of ground corn to make up the sample weight to .8g.

The decrease in protein synthesis in samples using Starea of higher PE (in the high roughage diets) may be attributed to the energy content of the samples. Protein synthesis values and energy contents were positively

correlated (r =.85). The energy content of samples using Starea of higher PE was lower than those using Starea of lower PE. In addition to the decreased energy content in samples using Starea of higher PE they also had lesser amounts of extruded corn.

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EFFECT OF ADDED FAT ON EXTRUSION PROCESSED CORN-UREA MIXTURES (STAREA)

by

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ABSTRACT

Increasing competition from man and other nonruminants for the available natural protein has forced ruminants to utilize more and more NPN. To increase the efficiency of urea utilization new methods of processing have been developed. An example of this is the extrusion processed corn-urea mixture (Starea).

Fat has become a common ingredient in animal diets because of changes in energy costs. Fat has also proved beneficial in processing Stareas of high urea contents. Feedlot and metabolism studies have shown that the rate of gain and digestibility of crude protein were consistently depressed by the simultaneous addition of fat and urea.

This study was made (1) to evaluate the effect of added fat on extrusion processed corn-urea mixtures (Starea) and (2) to evaluate the effect of the level of urea in Starea on <u>in vitro</u> protein synthesis and starch damage.

In Experiment 1, Starea at 2 levels of protein equivalance (PE): 88% and 154% (dry basis) and 2%, 4% and 6% added fat were studied. Results showed that the level of PE had no significant effect on starch damage, but did significantly ($P < .05$) affect <u>in vitro</u> protein synthesis and pH of fermented samples. The level of added fat had no significant effect on starch damage values, but increased levels of added fat produced an apparent decrease in starch damage values. Higher fat levels resulted in decreased protein synthesis but not significantly so ($P < .05$). Possible explanations are: at higher levels of fat, the fat may be acting as a barrier between the enzyme and substrate, thus resulting in lower amounts of maltose production (measure of starch damage). Samples having higher PE had smaller amounts of cooked corn than did samples of lower PE. This could explain the lower protein synthesis. At higher levels of fat, there was higher ammonia concentration and lower

volatile fatty acid (VFA) concentration resulting in higher pH values.

In Experiment II, Starea at 6 levels of PE: 44%, 66%, 88%, 110%, 132% and 154% (dry basis) were extruded (all with 2% added fat for easier processing). Results showed the level of PE had no significant effect ($P < .05$) on starch damage values. In vitro protein synthesis was performed using mixtures of ground corn and Starea or ground hay, ground corn and Starea as substrates. The first mixture represented a low roughage diet and the latter a high roughage diet. Protein synthesis values were significantly ($P < .05$) affacted by the level of PE of the Starea used irrespective of the substrate. In the low roughage diet, since samples using Starea of higher PE had lesser amounts of cooked corn than did the other samples, the efficiency of incorporating urea-nitrogen would decrease, i.e., a decrease in protein synthesis. This was supported by the data that nearly equal protein synthesis was obtained from all samples when extruded corn was added instead of ground corn to make up the substrate. In the high roughage diet, the protein synthesis values and the energy content of the samples were positively correlated ($r=.85$), i.e., samples having Starea of higher PE had lower energy.