EFFECT OF AN EXPANSION PROCESSED MIXTURE OF GRAIN AND UREA (STAREA) ON NITROGEN UTILIZATION IN THE RUMEN OF CATTLE AND ON UREA TOXICITY

by 45

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INTRODUCTION

In nonruminant animals, the nitrogen requirements are met by the ingestion of proteins that are broken down in the stomach and small intestine, and absorbed as amino acids. The situation in ruminant animals differs markedly in that a large part of the ingested proteins are subjected to the activities of the rumen microbial population before passing onto the abomasum and small intestine. This function of the rumen is advantageous because inferior proteins and nonprotein nitrogenous (NPN) compounds can be converted into presumable high quality microbial proteins.

Urea is the primary NPN compound in use at the present time. There are several problems associated with the use of urea. These include inefficient utilization because of rapid production and rapid absorption of ammonia from the rumen, production of ammonia toxicity, poor palatability, and segregation of urea in the mixed feed.

It has been suggested that the utilization of urea may be improved by: 1) vitamin-mineral supplementation, 2) addition of some natural protein, 3) coating of urea to slow release of ammonia, 4) providing readily available sources of energy.

Great advances have been made in methods of processing grain. Certain of these methods have improved the digestibility of the carbohydrate fraction of grain thus creating a more readily available energy source.

The study reported here was designed to evaluate the effect of different methods of grain processing on the utilization of urea by cattle.

REVIEW OF LITERATURE

Urea. As early as 1891 Zuntz suggested that rumen bacteria could utilize NPN. Twenty years later, Armsby (1911) stated that NPN of vegetable substances appears to be converted to protein by the microorganisms of the digestive tract. By means of its conversion to bacterial protein, the NPN compounds of feed may serve indirectly for maintenance and also as a source of protein for synthesis of milk and probably for growth in rations deficient in protein.

Voltz (1919) showed the value of amides in ruminant nutrition and confirmed that urea was capable of replacing digestible protein in the metabolism of adult ruminants. Hansen (1922) stated that urea might be of practical value in feed rich in carbohydrates but poor in protein. Klein et al. (1936) found that when sheep were fed a ration of straw, molasses, and starch, they were first in nitrogen equilibrium and later in positive nitrogen balance. When amide nitrogen was added to the ration, there was more true protein in the feces than in the feed. They concluded that the bacteria in the rumen were necessary for the conversion of this amide nitrogen to protein.

Hart et al. (1939) demonstrated that when urea nitrogen constituted 43% of the nitrogen of the ration, the growth of dairy heifers was but slightly less than that secured with a ration containing 66% of its nitrogen as casein.

Harris and Mitchell (1941) conducted nitrogen balance studies with sheep and found considerable variation among individual sheep in the conversion of urea. Also, the efficiency of urea conversion depended on the level of intake: small quantities were used more effectively than

large amounts. By use of metabolism tests, Harris et al. (1943) supported the theory that protein is synthesized in the paunch of ruminants. The rumen contents of steers fed a low protein ration with urea contained 15.71% true protein whereas the contents of steers fed the low protein ration without urea contained 9.62% true protein.

Loosli et al. (1949) found that sheep and goats could maintain growth on a ration containing urea as the only source of nitrogen. The rumen material contained 9 to 20 times more of the amino acids than the diet fed. Watson et al. (1949) studied body protein formation with N^{15} labeled urea and observed that the nitrogen of urea was utilized by sheep for the formation of body proteins. Land and Virtanen (1959) reported 17 to 25% of the N^{15} in $N^{15}H_4^+$ salts was incorporated into milk protein and no more than 40% into the milk and tissue protein combined.

Ward et al. (1955) concluded from trials with lactating cows that urea and soybean meal were equal under the conditions of their experiment. Thompson et al. (1952) reported that urea and cottonseed meal were of comparable value for milk production under the conditions of their experiment. In a recent report by Virtanen (1966) requirements for maintenance and milk production (4217 kg) were met when all of the nitrogen in purified rations was furnished by urea and ammonium salts. Huber et al. (1967) observed that urea could supply 11% of the total dietary nitrogen without depressing milk production. Other workers (Archibald 1943; Lassiter et al., 1958) have fed concentrate rations containing 45 to 70% of their nitrogen as urea without depressing milk production. Reid (1953) in an extensive review concluded that urea can be used for milk production,

growth and fattening of cattle.

Rumen Ammonia Production and Utilization. McDonald (1952) demonstrated that rumen ammonia-N was absorbed directly from the rumen of sheep and could amount to as much as 4 to 5 g per day. Later, Lewis et al. (1957) showed that larger quantities could be absorbed from the rumen. The absence of ammonia-N in peripheral blood indicates that most of the absorbed ammonia-N is removed by the liver and converted to urea. The urea can then be excreted, recycled to the rumen through saliva or rumen epithelium transfer, or may be used by some body tissue as a nitrogen source.

A large concentration of ammonia in the rumen depends on the nature and level of the protein or NPN source and the amount and type of carbohydrate available. Considerable losses of ammonia-N from the rumen contents occur when the amount of nitrogen in the diet is high (Kay, 1963). The greatest losses occur when only small amounts of digestible carbohydrates are present for protein synthesis (Phillipson et al., 1961) or when the protein is particularly soluble. The feeding of large quantities of NPN such as urea give rise to high rumen ammonia-N levels as shown by numerous in vivo and in vitro investigations. Annison (1956) and Lewis (1962) found that certain proteins varied in the amount of ammonia-N produced. Chalmers et al. (1964) observed that when heated groundnut meal was fed to ruminants, the protein was less soluble and produced less ammonia-N than when unheated groundnut meal was fed. Chalmers et al. (1964) concluded that decreased solubility occurred because of increased heating of the protein and this in turn influenced the rate of conversion of protein to ammonia-N. These workers also

demonstrated that ammonia-N concentration can be correlated with nitrogen retention in the animal as measured by nitrogen balance techniques.

Early *in vitro* work by Pearson and Smith (1943) showed that microbial protein synthesis took place in the presence of carbohydrate and that starch was more valuable than other carbohydrates in promoting this synthesis. The need for sufficient amounts of readily available carbohydrates for utilization of ammonia has been repeatedly demonstrated both *in vivo* and *in vitro*. Lewis and McDonald (1958) in a study on the effect of adding different carbohydrates to a ration of hay and casein, found that the level of rumen ammonia was more effectively reduced by starch and levan than by glucose or xylan. The effect of cellulose was slight. The changes in ammonia concentration occurring in the rumen liquor of sheep fed different rations to which NH_4Cl had been added were studied employing short time *in vitro* incubations under CO_2 (Phillipson *et al.*, 1962). Decreases in concentration usually occurred in rumen liquor of sheep fed large quantities of flaked corn. The decreases were small when fodder beets or hay were fed. These workers concluded that the presence of soluble carbohydrate is necessary for the assimilation of ammonia-N. Chou and Walker (1964) found that rice, corn or potato (raw and cooked) produced lower concentrations of rumen ammonia than alfalfa hay or wheat.

Barth (1962) demonstrated in sheep that increasing the quantity of readily available starch from 1200 kcal per day to 1900 kcal resulted in a linear increase of approximately 60% in nitrogen utilization. McLaren (1961) concluded that the improvement in nitrogen utilization

which results from supplying readily available ration carbohydrates is due to increased microbial protein synthesis. This may be related to the availability of an excess of carbohydrates or their intermediates to the microorganisms at the time of ammonia-N release from urea or protein.

Phillipson et al. (1962) in studies on ammonia-N assimilation by bacteria, found that a decrease in ammonia during incubation of rumen fluid accompanied by an increase in compounds precipitated by trichloroacetic acid. When N^{15} labeled NH_4Cl was added, the isotope was found concentrated in the precipitate and a substantial decrease in ammonia occurred. Starch and glucose fermenting bacteria isolated from the rumen liquor were found to assimilate ammonia. These organisms were more numerous in the rumen liquor of sheep fed flaked corn than in the liquor of those fed hay and groundnut meal.

Rumen ammonia level as a criterion for measuring the value of protein was proposed by Annison et al. (1954). According to Chalmers (1961) ammonia-N concentration in rumen liquor at any one time is a function of many factors acting simultaneously. Concentration, however, does not indicate total ammonia production, the rate of production, or the rate of absorption. A high ammonia concentration indicates a greater potential for deamination than the ability (potential) of the microorganisms to utilize ammonia for protein synthesis. Lewis and McDonald (1958) conclude from work with carbohydrate and protein supplements that the best utilization of protein is probably obtained when carbohydrate is present and fermented at a rapid enough rate to stimulate microbial synthesis.

Absorption of Ammonia and Rumen pH. Coombe and Tribe (1958) and Coombe et al. (1960) in studies on ammonia toxicity in sheep presented evidence to support the idea of interrelations between rumen ammonia, rumen pH and ammonia toxicity. These workers observed that when rumen pH rose above 7.3 toxicity usually occurred. However at pH levels of less than 7.0, high rumen ammonia levels were not associated with toxicity. These workers postulated that rumen pH has some effect on the rate of uptake of ammonium compounds by ruminal blood. They concluded that as the pH rises from 7 to 8, the rate of absorption of ammonia will also rise.

The absorption of ammonia from the rumen was first demonstrated by McDonald (1948). Lewis (1957) later demonstrated there was a curvilinear portal blood relationship between ammonia and rumen ammonia content. Gärtner (1961) found a greater absorption of ammonia from the rumen at higher rumen ammonia levels and considered the process to be diffusion. Hogan (1961) found water flux across the rumen wall to have no effect on ammonia absorption. His data showed ammonia absorption to be dependent on the concentration gradient at pH 6.5, but no absorption of ammonia occurred at pH 4.5. Gärtner (1963) presented data that showed a reduction in ammonia absorption with a decrease in rumen pH.

Bloomfield et al. (1963) observed that when rumen pH was below 6.45, ammonia absorption was nil, but when rumen pH exceeded 7.55, ammonia absorption ranged from 7 to 26 mM per L per hour. Bloomfield et al. (1966) observed that as urea is hydrolyzed by microorganisms to ammonia and carbon dioxide, rumen pH is elevated, resulting in more

lipid-permeable ammonia which is rapidly absorbed from the rumen. These workers observed that the buffering capacity of rumen fluid is greater on the acid side than the alkaline side.

Feed Processing. Wallis and Olson (1941) showed that the feed value of hard grains, with smooth glossy seed coats, is increased more by grinding than that of soft grains having rougher hulls. Atkeson and Beck (1942) using normal rations containing Atlas sorgo grain showed a recovery of grain in the feces of 41% when fed whole, 3.7% when coarsely ground and 1.3% when finely ground.

Wilbur (1953) reported that a medium to finely ground corn and oats mixture was superior for milk production to the same grains fed whole, coarsely ground or pulverized. Wisconsin workers (1931) concluded from feeding trials with lactating dairy cows that barley ground to a granular condition was superior to finely ground or whole barley. Darnell and Copeland (1936) found that when a mixture of corn, oats, milo, and barley was fed to dairy cows in the ground state, it was consumed in larger quantities and resulted in greater production than when fed in a mixture containing 50 to 75% of whole grain.

Colovos et al. (1951), working with dairy heifers, observed a T.D.N. value of 63.0 for a coarse grain mixture as compared with a T.D.N. value of 68.6 for the same grain mixture when it was ground through a 3/32 inch screen. In further studies with lactating cows Colovos et al. (1953) observed the following T.D.N. values for different feed textures: coarse, 63.5%; pelleted, 65.8%; and fine, 67.9%.

The expansion process for gelatinizing cereal grains is similar to the process used in preparing pet food. According to Williams and Baer

(1964) the expansion of cereal grains involves heat, pressure and high-shear mixing treatment while introducing and blending with super-heated water for a short time. The material at an elevated temperature becomes a dough-like mass which is passed through an extrusion device so that a sudden decrease in pressure when exposed to the atmosphere allows the super-heated water to vaporize immediately. This allows the material to swell or expand up to five times its original volume. The final drying of the product is at high heat for a short time.

A distinction must be made between cooking and expansion (Williams and Baer, 1964). Cooking is the general term describing conditions of temperature, time and pressure used to change physical and chemical characteristics. Expansion is the actual swelling of the material which depends on the pressure differences before and after extrusion and the plasticity of the material. The conditions causing expansion are also sufficient to insure cooking of the material. Cooking is important in that it converts the cereal grain into a sticky paste which is plastic and can be inflated. Williams and Baer (1964) emphasize that expansion should be defined as a method of treatment in which a material is subjected to high mechanical pressure in the presence of a super-heated liquid, followed by a sudden release of pressure causing the material to swell.

During expansion, cereal grains undergo pronounced changes, including gelatinization of starch, formation of torrefaction dextrin and denaturation of protein. In the expansion process starch becomes 100% gelatinized. The starch granules lose their granular structure and spread out in a loosely packed homogeneous mass, indicating partial destruction of molecular bonding.

According to French (1950) the breakdown of the structure of the starch granule on heating in water occurs in three distinct phases. During the first phase, water is slowly taken up and limited swelling occurs. The granule retains its characteristic appearance and bifringence. Within a small range of temperature, at approximately 65 C, the second phase starts where the granule suddenly swells, increases many times in size, and takes up a large quantity of moisture and rapidly loses its bifringence. A small amount of starch is made soluble. During the third phase of swelling, which takes place with increasing temperature, the granule becomes almost a formless sac, and the most soluble part of the starch is leached out. The hydrogen bonds of the starch are thought to be broken with hot water during the gelatinization process (hydration) which is initiated by the dissociated water molecules (Caesar, 1950). The starch so processed becomes water soluble.

The cooking which precedes expansion has been found to provide sufficient heat to denature protein. The denaturation of protein affects the secondary bonds which hold the molecule in definite arrangement with respect to amino acid structure. When the secondary bonds are ruptured the molecule loses its distinctive physical arrangement (Williams and Baer, 1964).

Williams and Baer (1964) suggested that expansion processing of cereal grains may improve the digestibility of carbohydrate and protein. Hale (1965) observed that steam flaking milo improved digestibility of dry matter, nitrogen-free extract and energy when compared with untreated milo fed to beef cattle. For heifers, Haenlin et al. (1962) found that digestion coefficients were highest for rations containing expanded corn

and soybeans as compared with pelleted corn and soybeans. Protein digestibility was not affected. Shaw et al. (1960) found crude protein digestibility increased markedly (12%) when beef animals were fed finely ground hay and cooked corn as compared with long hay and corn grain. Most workers report that fiber digestibility is lowered when the concentrate portion of the ration is increased. The resultant depression in fiber digestibility from feeding rations of finely ground hay and high concentrate appears to be caused by rapid passage through the digestive tract and decreased activity of cellulolytic bacteria at lower rumen pH's resulting from altered rumen fermentation. The improved utilization of heated or steamed cereal grains also seems to be due to changes in rumen fermentation (Shaw, 1961).

Weight Gain. Grinding and pelleting of a forage can improve animal performance in terms of weight gain (Beardsley, 1964). Hale (1965) found that steam processing milo improved weight gains 10% and reduced feed requirements 5% when compared with rolled milo. In a similar comparison by Hale (1965) for steam processed barley, weight gains and feed intake were increased but feed requirements were unchanged. Shaw et al. (1960) compared the effects of feeding rations resulting in rumen acetic to propionic acid ratios of 4.2:1 or 1.1:1. The two different rations were fed to two groups of steers. A ration of finely ground alfalfa hay and highly cooked starch concentrates (a low acetic to propionic ratio ration) increased body weight gain 22%, efficiency of gain 18%, and protein digestibility about 12% compared with a ration of untreated hay and concentrates.

Colenbrander et al. (1967) observed that cows fed expanded sorghum

grain (gelatinized) and ground alfalfa hay produced more milk, milk protein, and lactose than cows fed cracked grain and unground hay. However, cows receiving cracked sorghum grain produced more fat-corrected milk due to a higher milk fat content. Feed protein was more efficiently converted to milk protein by cows fed expanded grain.

Lactic Acid. Lactic acid is an intermediary in the breakdown of feed carbohydrates to volatile fatty acids. Lactic acid is not normally present in large quantity in the rumen and will only accumulate when large amounts of readily fermentable carbohydrates are ingested by the animal. This accumulation occurs when the production of lactic acid exceeds its rate of utilization.

Phillipson (1942) first observed lactic acid accumulation in the rumen of sheep fed mangolds. Phillipson (1952) later reported lactic acid concentrations of up to 72 mM per liter with a pH of 4.5 to 4.9 in the rumen contents of sheep fed flaked corn. Only trace amounts of lactic acid, usually less than 1 mg per 100 ml of rumen fluid, were found in the rumen contents of animals feed ration containing smaller amounts of flaked corn (Balch and Rowland, 1957). However when large amounts of flaked corn were fed, lactic acid concentration ranged from 95 to 270 mg per 100 ml rumen fluid. These researchers observed that lactic acid disappeared rapidly from the rumen after 2 hr. Briggs et al. (1957) found significant but transitory accumulations of lactic acid when sheep were fed rations containing large quantities of wheat and additional amounts of finely divided wheat starch or molasses and starch. Lactic acid levels of 124 and 169 mM per liter were observed for rations containing wheat plus starch and starch respectively.

Waldo and Schultz (1956) observed that feeding of hay had little effect on lactic acid production or concentration in the rumen. However, the addition of grain to the ration increased lactic acid production and caused peak production to occur earlier. Feeding silage gave the highest lactic acid concentration but this appeared to be from introduction into the rumen rather than production within the rumen. These workers, observed the following peak concentrations per 100 ml rumen fluid: hay, 21.8 mg; silage, 209 mg; grain, 57.7 mg.

Helmer et al. (1965) fed alfalfa hay ad libitum and very low levels of sorghum grain to cattle and observed rumen lactic acid levels of 0.51 µmoles per ml to 1.76 µmoles per ml. Wilson and Woods (1966, 1967) fed varying levels of gelatinized corn to cattle and observed increased rumen lactic concentrations as the level of gelatinized corn in the ration was increased.

Synthesis of Microbial Protein. The synthesis of microbial protein in the rumen has been established. Mills et al. (1942) fed urea to ruminants and showed that the amount of protein in the rumen increased after feeding. These workers fed timothy hay, starch and urea and observed that as the rumen ammonia level fell, there was a concomitant rise in the protein level of rumen contents. Pearson and Smith (1943) demonstrated the conversion of NPN to protein. Wegner et al. (1940) presented evidence, through in vitro work, that the NPN was converted to protein by means of bacteria in the rumen. These workers also noted that a decrease in ammonia-N can be accounted for by an increase in protein-N.

Loosli et al. (1949) demonstrated the synthesis of the 10 amino acids, essential for rat growth, in the rumen of sheep and goats fed rations containing urea as the sole (97%) source of nitrogen. Duncan et al. (1953) presented evidence for the synthesis of amino acids from ammonia-N by rumen microorganisms in vivo. Degradation of protein furnished a constant supply of peptides, amino acids, and ammonia-N to the rumen microorganisms. Though the actual importance of each what is not fully known, the main source of nitrogen for microbial synthesis appears to be ammonia-N (Burroughs et al., 1951; McDonald, 1952).

Bryant and Robinson (1962) using C^{14}-labeled protein hydrolysates, showed that bacteria fixing large amounts of ammonia-N must utilize carbohydrates or constituents other than amino acids for cell carbon. Labeled CO_2 was incorporated into the protein of mixed rumen organisms, (Otagaki et al., 1955).

Pearson and Smith (1943) using in vitro systems have estimated the daily protein synthesis in the rumen to be 72 to 450 g. Agrawala et al. (1952) in working with purified rations, where urea was the sole nitrogen source fed to 400 to 560 lb calves, observed the synthesis of 33 to 109 g of "true" protein (trichloroacetic acid precipitate). This true protein was assumed to be due to the biosynthetic activity of rumen microorganisms. Ferber and Winogradowa-Federowa (1929) state that about 3 kg of protozoa can be found in the normal sheep's rumen.

Weller et al. (1958) observed that 100 g rumen contents from sheep on forage rations contained about 148 mg of nitrogen. The distribution of nitrogen observed was: plant-N, 26%; bacterial-N, 46%; protozoal-N,

21%; and soluble-N, 7%. Blackburn and Hobson (1960) fed sheep casein of varying solubility, and their results on the distribution of nitrogen in the rumen are shown in table 1.

Table 1. Separation of rumen microbial and soluble nitrogen.

Casein-N fed	Bacterial-N	Protozoal-N	Total Microbial-N	Soluble-N
(g)	---------- mg % ----------			
20.2	24.9	81.6	106.5	41.5
20.6	-	-	124.0	55.9
24.5	36.5	41.7	78.3	67.2

Meyer et al. (1967) studied rumen protozoal and bacterial nitrogen in the rumen of cattle fed expanded sorghum grain. They observed that a decrease in rumen ammonia was correlated with a sizable increase in bacterial protein but only a small increase in protozoal protein. The quality of rumen microbial protein, determined by several methods, has been shown to be high.

Johnson et al. (1944) presented data that rumen protozoa contained 54.75% crude protein and had a biological value of 68. Rumen bacteria examined by the same workers was found to contain 58.81% crude protein and have a biological value of 66. Smith and Baker (1944) presented the following analytical data on rumen microbes: moisture, 0.5%; protein, 36.3%; polysaccharides, 46.6%; lipoid matter, 9.5%; and ash, 6.2%.

McNaught et al. (1950) studied the nutritive value of dried rumen bacteria and observed a biological value of 88.3 and a chemical composition of: crude protein, 44.4%; carbohydrate, 40.3%; lipid, 3.1%;

fiber, 0.3%; and ash, 7.1%. McNaught et al. (1954) in further studies were able to collect on the average 2.5 g of dry bacteria and 0.5 g of dry protozoa from a liter of rumen liquid. The composition and biological value of rumen bacteria and protozoa are shown in table 2.

Table 2. Proximate composition of rumen bacteria and protozoa.

Fraction	Rumen bacteria	Rumen protozoa
	(%)	(%)
Moisture	11.2	8.2
Protein	41.8	26.5
Polysaccharides	32.0	62.1
Ash	6.7	2.3
Lipid	2.4	1.4
Fiber	2.2	1.8
Biological value	81	80

Meyer et al. (1967) studied the amino acid composition of bacterial and protozoal hydrolysates collected from animals fed different rations. The amino acid composition of the bacteria was quite similar regardless of the ration, animal or amount of nitrogen fed. The same was true for the protozoa collected. Other workers, (Weller, 1957; Purser and Buechler, 1966) also found this to be true with sheep and with pure cultures of rumen microorganisms. The amino acid composition of bacteria and protozoa collected by these three groups of workers was quite similar despite the different rations, animals, environment and experimental technique used.

Rumen pH. The hydrogen ion concentration of rumen digesta is subject to considerable variation resulting from many factors with volatile fatty acid and lactic acid concentration playing a major role.

Olson (1941) showed that rumen digesta becomes more alkaline when exposed to air. Smith (1941), using an extended electrode, made *in vivo* determinations of rumen pH through the rumen fistula of cows. He observed that *in vivo* pH values of rumen digesta were lower than *in vitro* pH values of digesta from the same animal. The effect of volatile fatty acid (VFA) concentrations on rumen pH was demonstrated by Phillipson, (1942). As VFA accumulated to the extent of 57 to 162 mM per liter, the rumen pH values ranged from 7.03 to 5.80. Pursor and Moir (1959) infused 7, 35, and 70 g of glucose into the rumen of sheep maintained on an adequate diet. Ruminal pH fell 2 to 4 hr after infusion from a mean of pH 5.66 without added glucose, to 5.40 at the 70 g infusion level. The decrease in pH values was related to the increase in VFA concentration. Briggs *et al.* (1957) fed a wide range of rations to sheep and observed no accumulation of lactic acid in the rumen. In these instances, rumen pH followed VFA concentration closely and inversely. Only on rations containing high levels of soluble carbohydrates or starch did lactic acid accumulate. Lactic acid levels above 20 mM per liter were associated with rumen pH values below 5.0 when higher levels of lactic acid were encountered, rumen pH never fell below 4.35. When lactic acid producing rations were fed, pH was closely related to rumen concentration of VFA or lactic acid or both. Agrawala *et al.* (1953) reported pH values as low as 4.3 on purified diets containing 67% corn starch. According to Kay (1963) most of the pH values for rumen contents normally range between 5.5 to 7.3. Balch and Rowland (1957) observed changes in the pH of rumen contents of dairy cattle as they were fed a variety of rations. These workers

observed that pH values varied inversely with VFA concentration. The lowest pH values and the greatest range in pH were found for high energy rations containing low roughage. The pH and VFA relationship was slightly altered when rumen ammonia production was high. Briggs et al. (1957) found that the addition of casein to high starch rations had a modifying effect on lowering pH when the concentration of VFA was greater than 200 mM per liter. Bath and Rook (1963) found that pH fell as total VFA concentration increased after feeding. Balch and Rowland (1957) observed lowered pH with increased VFA concentration. The lowest pH values and highest lactic acid concentrations were observed when flaked corn was fed.

Variation in VFA as Related to Time of Sampling. Bath and Rook (1963) observed that pH falls as total VFA increases after feeding and that there is a decrease in molar percentage of acetic acid and a corresponding increase in other acids. Similar changes were found by Gray and Pilgrim (1951) for sheep fed wheaten and alfalfa hays. Reid et al. (1957) concluded from studies of diurnal VFA concentration in sheep fed various rations that it was not valid to compare individual ratios of VFA determined samples taken at random when the same or different rations were fed. Shaw (1961) reported small changes in molar proportions of individual acids during the feeding cycle and considered that a single sample drawn at any time after feeding is representative of the VFA status of the animal. The findings of Stewart et al. (1958) supported those of Shaw (1961) in that diurnal changes in molar proportion were only slight. However, the work of Annison (1954) and El-Shazly (1952) showed that the molar proportions

of the individual acids varied with the time after feeding that the rumen sample was taken.

Role of Higher and Branched Chain VFA. The extent to which the various rumen microorganisms can synthesize individual amino acids is incompletely known, but information is available for a few. Allison and Bryant (1963) and Allison et al. (1959; 1962 a,b) showed that valine was synthesized by Ruminococcus flavefaciens from isobutyrate and leucine from isovalerate. The branched-chain acids were required nutrients. Some strains of Ruminococcus albus require isobutyrate, isovalerate, and 2-methylbutyrate (Hungate, 1963a).

Isovalerate is incorporated in toto into leucine (Allison et al., 1962a). In addition to serving as a precursor of leucine, isovalerate is synthesized into the terminal portions of some long-chain fatty acids in ruminococci.

Burroughs et al. (1950) found that the addition of autoclaved rumen fluid to an in vitro fermentation system would enhance the digestion of cellulose. Bentley et al. (1954, 1955) reported that the cellulolytic factor of rumen fluid was present in the volatile acid fraction and that valeric, caproic, isobutyric, and isovaleric acids stimulated cellulose digestion in in vitro systems. Bryant and Doetsch (1954, 1955) reported that a branched-chain volatile acid which could be either isobutyric, isovaleric or DL-α-methyl-n-butyric acid and a straight chain acid of 5 to 8 carbons was required for the growth of Bacteroides succinogenes, a cellulolytic bacteria found in the rumen. Cline et al. (1958) observed that, when valeric acid was added to an

in vitro system (urea as nitrogen source) the concentration of this acid decreased at the same time that there was an increase in cellulose digestion and an increase in the content of trichloroacetic acid-insoluble nitrogen. The reason suggested by these workers was that as the rate of microbial growth increased, the rate of valeric acid synthesis decreased or the rate of utilization of valeric acid increased. Stewart *et al.* (1958) showed that when urea was added to the ration of steers, the concentration of valeric acid in the rumen fluid decreased.

Cline *et al.* (1966) reported that isobutyric and isovaleric acids were present in minimal quantities in rumen liquor of lambs fed purified diets devoid of intact protein. Nitrogen digestibility and retention were improved by adding a mixture of isobutyrate, isovalerate, and valerate to a purified diet containing 39% cellulose, 37% corn starch, and urea as the sole nitrogen source. The addition of the acids also had the effect of reducing rumen ammonia-N levels.

Rumen VFA Metabolism. The rumen VFA have been intensively studied since the discovery (Phillipson and McAnally, 1942; Barcroft *et al.*, 1944) that these main hydrolytic products of carbohydrate digestion by rumen microorganisms can be absorbed from the rumen. Subsequent findings have shown that VFA serve as an important energy source for the ruminant (Blaxter, 1962). The predominant organic acids in the rumen are acetic, propionic, and butyric with smaller amounts of isobutyric, valeric, isovaleric and 2-methyl butyric (Annison, 1954). Numerous studies have been conducted on the influence of ration on proportions and concentrations of VFA and their relation to animal per-

formance in terms of milk production, rate of gain and feed utilization.

Rations that influence rumen VFA and specifically the acetic to propionic ratio are: low-roughage high-grain rations, rations in which the cereal grains have been altered by cooking or expansion, or those in which the physical fibrousness has been reduced by grinding. The ratio of acetic to propionic is usually highest when only long roughage is fed (Ensor et al., 1951; Chou and Walker, 1964a). As the particle size is reduced by grinding, the ratio of acetic to propionic is reduced (Balch and Rowland, 1957; Ensor et al., 1959; Balch, 1960). Proportional changes are greatest on rations which contain large amounts of heated starchy feeds such as flaked corn (Phillipson, 1952; Shaw et al., 1959: Ensor, 1959). Feeding a combination of finely ground roughage and cooked concentrates resulted in a narrow ratio of acetic acid to propionic acid (Ensor et al., 1959; Shaw et al., 1960: Shaw, 1961). Colenbrander et al. (1967) fed a 1:1 ratio of hay to grain to lactating cows and observed that expansion processing of the grain reduced the acetic to propionic acid ratio.

Recent advances in technology and analytical techniques have allowed researchers to separate the higher branched and straight-chain fatty acids from the lower straight-chain acids. Data on the effect of ration on the molar proportions of rumen VFA are found in table 3.

Total Rumen VFA Concentration. The total concentration of VFA in the rumen at any one time is a function of intake, time elapsed after feeding, frequency of feeding, and type of ration. William and Christian (1956) using sheep, observed a decline in VFA from 97 to 71 mM per liter as daily intake was reduced from 1000 to 400 g.

Bath and Rook (1963) obtained similar results with non-lactating cows when varying daily dry matter intake of hay or hay and concentrate. Bath and Rook (1963) noted that VFA concentration peaked between 2 and 6 hr after feeding. Balch et al. (1955) using a low-hay and high-concentrate ration found concentrations of VFA varied markedly in relation to time of feeding. The VFA reached the highest concentration 4 to 6 hr after feeding and were lowest at feeding time. Briggs et al. (1957) have also demonstrated diurnal variation in VFA concentration in sheep on different feeding regimes. For roughage alone, VFA peaked at 4 hr and slowly decreased. When different rations containing concentrates and by-products were fed, maximum VFA values occurred between 2 and 8 hr after feeding.

Phillipson (1942) demonstrated that the level of VFA found in the rumen was related directly to the nature of the ration. Kay (1963) observed the following concentrations of VFA in rumen contents of animals fed different rations: a) 150 mM per liter for grazing or rations high in concentrates, b) 100 to 150 mM per liter for hay and silage rations, and c) usually less than 100 mM per liter for rations containing large amounts of corn. Colenbrander (1966) observed that the expansion processing of grain contributed to higher VFA concentration as compared with that resulting from cracked sorghum grain. Data presented by various research workers are summarized in table 3.

Urea (Ammonia) Toxicity. An increased use of urea as a NPN source for ruminants has increased the interest in research directed towards the problem of urea (ammonia) toxicity.

Clark et al. (1951) studied urea toxicity in sheep and concluded

Table 3. Ration effects on rumen VFA concentration (μmoles/ml) and ratio.

		Volatile Fatty Acid							
			Molar Proportion						
Ration	Species	Total concentration	C_2	C_3	C_4	IC_4	C_5	IC_5	2-metyl butyric	Source
Concentrate, casein. hay	sheep	160.0	53.1	21.7	16.6	2.7	3.3	3.6		El-Shazly (1952)
Dried grass	sheep	135.0	63.5	26.0	11.9	0.5	1.5	0.8		El-Shazly (1952)
Frozen grass	sheep	138.0	55.2	24.3	12.5	1.2	1.6	1.3		El-Shazly (1952)
Silage	sheep	145.0	66.6	23.6	10.7	1.5	2.3	2.7		El-Shazly (1952)
Groundnut, maize, hay	sheep	142.0	44	32	17.4	1.9	2.6	1.4	0.7	Annison (1954)
Casein, maize, hay	sheep	87.0	70	18	7.3	1.3	1.3	1.1	1.0	Annison (1954)
Maize, hay	sheep	89.0	56	28	13.3	0.4	1.0	1.0	0.3	Annison (1954)
Hay-expanded sorghum	cow	107.4	70.5	16.5	10.3		1.5	1.2		Colenbrander (1966)
Hay-cracked sorghum	cow	92.2	71.5	16.2	8.6		1.5	2.1		Colenbrander (1966)
Corn-hay-SBM Ration										
Mixed loose	sheep	107.8	56.5	20.6	18.7	1.4	1.3	1.5		Rhodes & Woods (1962)
Pelleted ration-fine grind corn	sheep	109.3	45.3	33.2	17.0	0.5	3.8	0.1		Rhodes & Woods (1962)
Heated at 100°C	sheep	75.1	44.4	39.8	12.0	0.5	2.9	0.3		Woods & Luther (1962)
Autoclaved 15% H_2O	sheep	73.6	45.0	37.9	13.0	0.2	3.7	0.1		Woods & Luther (1962)
Pelleted then reground	sheep	95.6	39.9	44.2	11.1	0.2	4.4	0.0		Woods & Luther (1962)
Hay-grain	cow	121.1	65.9	16.7	13.4		4.0			Stewart et al. (1958)
Hay-grain-urea	cow	126.7	67.2	16.7	13.1		3.0			Stewart et al. (1958)

that it was largely influenced by the type of diet that was provided in combination with urea. They reported that death could be caused by as little as 10 g of urea administered as a drench to a poorly fed sheep. Sheep fed lucerne hay tolerated larger quantities of urea than those fed poor quality hay. Toxicity was thought to be the result of increased ammonia levels and an alkaline pH. They also felt organic acids might help prevent toxicity. The symptoms of toxicity occurred 30 to 60 min. after ingestion of urea and included bloat, severe tetany, prostration, labored breathing and regurgitation of rumen contents. The front legs of the animals became stiff with the claws abducted. Post mortem examination indicated acute circulatory collapse. Toxicity would result from urea ingestion of 18 g of urea per hundred pounds of body weight.

Dinning et al. (1948) in work with 500 lb steers observed toxicity symptoms of ataxia, severe tetany, labored respiration and excessive salivation when 114, 272 or 490 g of urea were administered in a drench. A quantity of 57 g of urea did not cause toxicity. Toxic symptoms were first observed when blood ammonia levels reached 2 mg per 100 mls of blood and death occurred when blood ammonia levels of 4 to 7 mg per 100 mls were reached.

Davis and Roberts (1959) studied urea toxicity in cattle when urea was administered by three methods: drench, gelatin capsules, and feed. A single feed or drench of 14 to 20 g of urea per hundred pounds of body weight precipitated an acute toxicity whereas larger quantities could be incorporated into feed safely. Adaptation to high levels of urea was rapidly lost when urea is removed from the diet. Their work

also showed that starving the animals for short periods (24 to 48 hr) decreased the levels of urea which could be safely fed as a single feeding. Toxic symptoms observed by these workers included uneasiness, muscle and skin tremors, excessive salivation, labored breathing, incoordination, tetany and death. When blood ammonia levels exceeded 4.0 mg per 100 ml of blood, no animals survived. When acetic acid as a 5% solution was given before severe tetany developed, most animals recovered.

Repp et al. (1955) observed an association between blood ammonia and dose level of NPN compound administered orally. No clinical symptoms of toxicity were observed until the blood ammonia-N level rose to about 1 mg per 100 ml of blood. In their work with sheep, 35 to 40 g of urea per hundred pounds of body weight caused toxic symptoms.

Gallup et al. (1953) observed that cattle could consume 545 to 530 g of urea (100 g urea per 100 lb live weight) in a 1 to 5 molasses urea mixture daily without exhibiting toxic symptoms. However, toxicity was observed when urea was fed to starved or fasted animals, to animals not previously fed urea containing feeds, and when urea containing feeds were rapidly consumed. These researchers demonstrated that larger amounts of urea can be fed in the presence of carbohydrate feed than in its absence.

Russell et al. (1962) studied the relative effects of urea and diammonium phosphate (DAP) on sheep and steers. Their experimental procedure consisted of withholding feed 18 hr prior to the administration by drench of urea or DAP. They observed that as little as 15 g of urea per hundred pounds live weight was toxic. They also observed a close

relationship between blood ammonia levels and toxicity. These workers studied the effect of urea and DAP on rumen pH of sheep (table 4).

Table 4. The effect of urea and DAP on rumen pH.

Treatment	No. of sheep	pH of rumen fluid		
		0	30 min	60 min
Urea (15 to 20 g)	8	6.8	8.1	8.3
DAP (33 to 44 g)	8	6.8	7.2	7.2

These workers observed that the average maximum increase in blood NH_3-N was 42 mcg per 100 mls per unit of urea and 17.6 mcg per unit of DAP.

Oltjen et al. (1963), in working with sheep, observed that as little as 30 g of urea per 100 lb live weight could cause death. These workers also followed rumen pH and venous blood ammonia levels (table 5).

Table 5. The effect of urea and DAP on rumen pH and blood ammonia.

Treatment	Level gm/100 lbs	No. of animal	No. died	Rumen pH min after drenching				Blood ammonia min after drenching mmoles/liter			
				0	15	30	60	0	15	30	60
Urea	20	3	1	6.84	7.81	7.50	7.50	0.06	0.41	0.22	0.26
Urea	25	2	2	6.55	8.40	a	a	0.05	0.82	a	a
DAP[b]	55	2	0	6.92	7.31	7.19	7.08	0.06	0.13	0.17	0.19

a animal died
b 55 gm DAP equal to 25 gm urea

When the sheep exhibited toxic symptoms within 15 min after drenching, the blood ammonia was 0.41 mmoles/liter. However, these animals recovered and were able to stand when the blood ammonia dropped to 0.22 mmoles/liter.

Lewis (1960) in his studies on ammonia toxicity in the ruminant observed several relationships between blood and ruminal ammonia and pH

levels. Toxicity was observed when rumen ammonia levels exceeded 100 mmoles per liter and when blood ammonia exceeded 0.4 mg per 100 mls of jugular vein blood. When rumen pH exceeded 8.0 the blood ammonia concentration increased greatly. Also, Lewis observed a direct relationship between rumen ammonia and blood ammonia concentration.

Coombe and Tribe (1958) found that by mixing urea with hay, sheep would consume up to 100 g of urea per day. Death resulted when 25 g of urea were directly introduced into the rumen. They also noted that when high levels of urea were fed, rumen pH would rise to nearly 8, causing inhibition of rumination and rumen movements.

Coombe et al.(1960) in further studies on urea toxicity in sheep postulated and supported the idea of interrelationships among rumen ammonia, rumen pH and ammonia toxicity. These workers observed that large amounts of urea mixed with feedstuffs could be consumed by sheep without ill effect but when small amounts were administered as a drench toxicity would often occur.

However, Coombe and his co-workers did not feel that rumen ammonia levels by themselves could explain differences in animals reactions in that rumen ammonia nitrogen levels in excess of 100 mg per 100 ml did not cause toxicity. It was evident from their work that rumen pH is also important in so far as toxicity is concerned. These workers noted when rumen pH rose above 7.3 that signs of toxicity were observed. They hypothesized that rumen pH has some effect on the rate of uptake of ammonia by ruminal vein blood. They felt that as the pH rises from 7 to 8 the rate of absorption of ammonia will also rise and that the chances of toxicity occurring might be reduced if the rumen pH could be maintained below 7. During one of their trials rumen ammonia nitrogen

levels of 132 mg per 100 mls and rumen pH of 6.9 to 7.0 were observed and symptoms of ammonia toxicity were not evident.

Trial I. Effect of Sorghum Grain Processing on Urea Utilization as Measured by Rumen Ammonia Concentration.

Experimental Procedure

The effect of processing sorghum grain on the utilization of urea fed to two sets of identical twins weighing 440 to 460 kg was studied. The trial consisted of two 4 week periods. Four different methods of processing sorghum grain were tested. Each ration contained 5% urea. The same batch of sorghum grain was used for all four rations.

The first ration (cracked) was produced by cold rolling (cracking) the sorghum grain and adding 5% urea and mixing. Problems of maintaining a uniform mix led to remixing the ration and dividing it into daily individual portions for feeding.

The second ration (fine grind) consisted of grinding the grain finely (to produce a flour) through a Miag Multimat experimental mill. A dry sieve analysis of the ground grain (Feed Production Handbook 1961) gave a Fineness Modulus of 1.155 and Modulus of Uniformity of 0:2:8. The sieve analysis of the grain is shown in table 6.

Table 6. Sieve analysis of finely ground sorghum grain.

Screen	Size of opening	Material remaining on screen
	(μ)	(%)
4	4699	
8	2362	
14	1191	2.0
28	589	14.2
48	294	11.6
100	147	41.7
150	104	23.3
200	73	7.2

The finely ground sorghum grain was then mixed with 5% urea and pelleted through a 0.76 cm die.

The third ration (expanded) consisted of expanded sorghum grain. The grain was ground through a 0.16 cm hammer-mill screen and processed by a Wenger[1] X-50 continuous extruder cooker. The grain was heated in the conditioning chamber and pressure cone for about 5 min where moisture was raised to 20 to 30%. The temperature at the extruder cone was 149 C. After expansion the grain was dried, cold rolled, and mixed with 5% urea.

The fourth ration (Starea) also consisted of sorghum grain. This ration differed from the third ration in that 5% urea was added to the ground sorghum grain before it was processed by a Wenger X-50 continuous extruder cooker.

The urea content and degree of gelatinization of the four rations are presented in table 7.

Table 7. Average urea content and degree of gelatinization[a] of grain rations.

Ration	Urea		Gelatinization
	(%)	(range)	(%)
Cracked	4.06	2.97-4.96	0
Fine grind	5.09	4.36-5.88	10
Expanded	4.36	3.71-5.04	100
Starea	4.81	4.46-5.11	100

a Gelatinization determined by microscopic examination.

The two sets of rumen fistulated identical twin cattle (04-05, 18-19) were fed daily a total of 3.6 kg grain ration and 3.6 kg alfalfa hay in two equal feedings. The ration fed each animal was changed weekly. The experimental design is shown in table 8.

[1]Wenger Manufacturing Company, Sabetha, Kansas.

Table 8. Experimental design.

Days	Twin pair 04	05	Twin pair 18	19
	Ration			
1- 7	C[a]	FG[b]	E[c]	S[d]
8-14	FG	E	S	C
15-21	E	S	C	FG
22-28	S	C	FG	E
29-35	C	FG	E	S
36-42	FG	E	S	C
43-49	E	S	C	FG
50-56	S	C	FG	E

a cracked
b fine grind pelleted
c expanded
d Starea

Due to the difficulty experienced in getting the twins to eat the cracked, finely ground and expanded rations, the grain ration was placed in the rumen via the fistula at both feedings. The twins were fed the long alfalfa hay.

Rumen fluid samples for ammonia determinations were obtained before feeding (prefeed) and at 1, 2, 3, 4, 6, and 8 hr after the morning feeding at the end of each 7 day feeding period. Samples were also obtained at the beginning of the second day after the rations were changed. These samples were obtained before and at 1, 2, 3, and 4 hr after feeding.

The rumen fistulas of all animals were fitted with sampling tubes which permitted withdrawal of a representative sample of rumen fluid without opeining the fistula cap or disturbing the rumen contents. These sampling tubes were fitted on one end with a perforated metal tube to strain out large particles of rumen digesta. The metal tube was positioned in the ventral sac of the rumen about 30 cm below the fistula opening.

The other end of the sampling tube extended outside the rumen through a hole in the fistula cap, and when not in use was kept closed with a screw clamp. A stainless steel syringe was attached to the sampling tube to withdraw rumen fluid. Ammonia was determined by the method of Conway (1957).

Results and Discussion

Due to the different levels of urea intake with the different rations, the average rumen ammonia concentration (samples at 1, 2, 3, 4, 5, and 8 hr) was adjusted to the level of urea intake per feeding (table 9). The lowest rumen ammonia levels were observed when Starea was fed. The cracked ration produced the highest rumen ammonia values. The rumen ammonia concentration resulting from feeding the fine grind was just slightly lower than that observed when the cracked ration was fed, whereas the concentration resulting from the expanded ration was slightly higher than that observed from Starea.

The diurnal rumen ammonia levels produced by the four rations are shown in appendix table 14 and graphically presented in figures 1 and 2.

Table 9. Ration effects on rumen ammonia concentration.

Grain treatment	Daily intake per feeding	Ammonia concn	Adjusted[1] ammonia concn
	(g)		mg/100 ml
Cracked	73.9	39.8	49.8
Fine grind pellet	92.4	38.5	38.5
Expanded	79.4	32.0	37.1
Starea	87.5	31.9	33.8

1 Since urea intake varied among treatments, ammonia concentration was adjusted to a common intake of 92.49.

The diurnal picture is similar for both sets of twins in that the highest rumen ammonia concentrations were observed when the cracked ration was fed followed in order by the fine grind, expanded, and Starea. The diurnal pattern for twin pair 04-05 was slightly different to that of 18-19. Both animals of twin pair 04-05 were slower eaters taking approximately 30 min longer to consume their hay than 18-19. While grain was given via the rumen fistula, perhaps a difference in rate of ingestion of hay nitrogen would account for the slight difference in shapes of the ammonia concentration curves for the two sets of twins.

It has been suggested that a lowered rumen ammonia concentration after feeding is indicative of improved nitrogen utilization (Annison et al., 1954). Consequently the greatest nitrogen utilization should occur with the Starea ration followed in order by the expanded, fine grind, and cracked rations. The effect of the processed rations on rumen ammonia concentration was rapid and was observed in the sample taken the second day after the rations were changed. McLaren (1961) concluded that improvements in nitrogen utilization result from supplying readily available ration carbohydrates. Apparently the processed rations tested here provided carbohydrate that was metabolized in a manner commensurate with good nitrogen utilization.

It is well known that urea is an unpalatable feed ingredient. The Starea ration was the only ration readily consumed. Palatability problems were encountered with the other three rations.

Urea in rations containing ground ingredients usually segregates. It is apparent from the data in table 13 that considerable variation existed in nitrogen contents of the cracked and expanded rations. This suggests some segregation of urea in these rations. The most uniform

ration was the Starea ration.

From these data it appears that expansion processing of a mixture of grain and urea yields a product that reduced the problems of segregation, palatability, and nitrogen utilization associated with mixtures of grain and urea.

Trial II. The Effect of Starea on Urea Utilization as Measured by Rumen Fermentation and Microbial Synthesis.

This trial was conducted to examine further the effect of expansion processing of grain and urea (Starea) on urea utilization.

Experimental Procedure

Two sets of identical twin cattle (04-05, 18-19) weighing 440 to 460 kg were used. The trial consisted of two 4 week periods. Two rations consisting of sorghum grain with 5% urea were fed with alfalfa hay.

The control ration was cracked (dry rolled) sorghum grain to which 5% urea was added. This ration was pelleted (0.636 cm die) to prevent segregation of the urea. The ration contained an average of 5.16% urea and under microscopic examination showed no gelatinization.

The experimental ration (Starea) was prepared by finely grinding sorghum grain through a 0.16 cm hammer-mill screen, mixing urea (5%) with the ground sorghum grain, and processing the mixture through a Wenger X-50 continuous extruder cooker. The grain was heated in the conditioning chamber and pressure cone for about 5 min where the moisture content was raised to 20 to 30%. The temperature at the extruder cone was 149 C. After expansion the grain was dried and crumblized. The

Starea contained an average of 5.51% urea and by microscopic examination was found to be 100% gelatinized.

The two sets of identical twin cattle were fed daily 3.6 kg of the grain mixture and 3.6 kg of alfalfa hay in two equal feedings. One member of each twin pair received one ration for 4 weeks and then the second ration for the second 4 weeks.

Changes in rumen ammonia, pH, lactic acid, VFA, and extracellular water volume were studied. Microbial synthesis was studied by following changes in the nitrogen content of the rumen bacterial, protozoal and cell-free fractions of rumen fluid. These fractions were ashed to determine their organic matter content.

Rumen samples were obtained with a sampling tube and stainless steel syringe. They were obtained at weekly intervals immediately before and at 1, 2, 3, 4, 6, and 8 hr after the morning feeding.

Two sets of samples were obtained each time. The sample for pH and ammonia was held under mineral oil and the sample for lactic acid and VFA was immediately frozen. Duplicate determinations of rumen ammonia were completed by the method of Conway (1957). The rumen fluid was tested with a Leeds and Northrup potentiometer to determine pH. The samples were stirred and well mixed before pH readings were made. The meter's electrodes were washed with distilled H_2O and then wiped off again between each sample.

Rumen lactic acid was determined by the colorimetric method described by Baker and Summerson (1941). Rumen VFA was determined by two methods. Total VFA was determined by the steam distillation method of Fina and Sincher (1959). Rumen VFA were determined by using the gas - liquid

chromatographic method of Rumsey (1963).

Polyethylene glycol (PEG), with an average molecular weight of 4000, has been used as an inert marker for determining rumen water volume. PEG was added to the rumen as an aqueous solution before and at 1, 2, 3, 4, 6, 8 hr following the morning feeding. The amount of PEG in the rumen was determined both before and after a known amount of PEG was added to the rumen. Fifty g of PEG contained in a beaker of water was poured in the rumen. The rumen contents were vigorously hand stirred for 5 min. Approximately 500 g of rumen contents was removed from the rumen for PEG analysis and microbial protein. The concentration of PEG in the samples was determined by the method described by Hyden (1955a). The volume of rumen water was calculated from the change in concentration due to a known amount of added PEG.

The bacterial separation was based on the method of Blackburn and Hobson (1960d) and the modification of Colenbrander (1967). The protozoal separation was by the method of Colenbrander (1967) which was a modification of the methods of Oxford, 1951; Heald and Oxford, 1953; Abou Akkada and Howard, 1960; and Eadie, 1963.

The method was as follows: Rumen fluid (400 to 500 ml) was collected in Erlenmeyer flasks and immediately strained through two layers of cheesecloth to remove the coarse debris.

For bacterial and cell-free separation, approximately 150 ml of well-mixed rumen fluid was placed in a 200 ml centrifuge bottle to which was added 5 mcg of aureomycin per ml of rumen fluid to inhibit bacterial growth. The sample was centrifuged at 114 G for 5 to 10 min. The supernatant was decanted and saved and the precipitate discarded. Centrifugation was repeated if any debris remained in the supernatant. This

step removed protozoa as well as debris. Two 40 ml aliquots of supernatant in 50 ml plastic tubes were centrifuged at 19,000 G and at 0 to 5 C for 20 min in a super centrifuge. When the supernatant appeared to contain slime-producing bacteria, 30,000 G was used. The bacteria appeared as a gray-green precipitate at the bottom of the tube. The supernatant, consisting of the cell-free extract, was decanted and frozen until analyzed for nitrogen. The bacterial precipitate was resuspended to 40 ml in acetate buffer (table 10), centrifuged, and supernatant discarded. The previous step was repeated a second time. The two bacterial aliquots were pooled and made up to the original 80 ml volume with distilled water. The samples were then frozen and stored until analyzed for nitrogen content.

For protozoal separation, 100 ml of well-mixed rumen fluid was placed in a 175 ml separatory funnel to which was added 50 ml of acetate buffer and 5 mcg of aureomycin per ml of rumen fluid. The separatory funnel was placed in an incubator maintained at 38 to 41 C. Lower temperatures were found to cause poor separation. During incubation protozoa settled to the bottom and the remaining debris moved toward the surface. Complete separation of protozoa was difficult and depended on the respiration and concentration of protozoa under anaerobic conditions. After 1 hr of incubation the precipitated protozoa were released into a 60 ml separatory funnel, also in the incubator, containing 20 ml of acetate buffer. The previous step was repeated at 2 and 3 hr intervals during which the precipitated protozoa were released into the same separatory funnel. The protozoal fraction was incubated for an additional 1 hr after which the protozoal were transferred to a 50 ml centrifuge tube at 114 G

for 5 to 10 min, decanted, and the supernatant discarded. The previous step was repeated twice after which the protozoa were made up to original volume with distilled water and stored in a freezer until nitrogen was determined.

Table 10. Composition of acetate buffer.[a]

Reagent	Quantity[b]
	(g)
NaCl	5.00
$NaC_2H_3O_2$	2.15
KH_2PO_4	0.35
K_2HPO_4	1.00
$MgSO_4$	0.12

a Eadie (1963)
b Quantity of reagent in 1 liter made up to volume with distilled water

The microbial fractions were analyzed for nitrogen using the method described by Conway (1957). For the organic (volatile) matter determination, aliquots of the different fractions were first dried and then ashed at 600 C for 12 hr in a muffle furnace.

Results

The Starea ration lowered rumen ammonia concentration compared with the cracked grain ration (figure 3). The ratio of post-feeding rumen ammonia to morning feeding intake of urea (g) was 0.362 for the cracked ration and 0.303 for the Starea ration. The Starea ration consistently caused a lower rumen pH (figure 4). Rumen lactic acid varied between 3.55 to 9.46 µg per ml but the Starea ration resulted in slightly higher average levels (6.66 µg per ml) as compared with 4.71 µg per ml for the cracked grain. These data are presented graphically in figure 5.

The rumen VFA concentration usually peaked between 3 and 6 hr post feeding. Only small differences were observed in the molar proportions of acetic, propionic and butyric acids between the two rations. The average concentration of VFA as determined by steam distillation was 106.9 mM per liter for the Starea ration and 98.3 for the cracked sorghum grain ration. The gas-liquid chromatographic method showed the same relationship and differences in total concentration and molar proportion of isobutyric, isovaleric and valeric acid (table 11).

Table 11. Average rumen VFA of twins fed Starea or cracked grain.

Ration	Total VFA	Molar proportion					
		C_2	C_3	IC_4	C_4	IC_5	C_5
	(mM/L)	(%)					
Starea	103.5	75.7	13.0	0.4	8.2	1.5	1.2
Cracked	87.2	73.7	14.7	0.7	7.2	2.1	1.5

It is apparent that the Starea ration not only lowered the molar proportions of IC_4, C_5, and IC_5 acids but also lowered the concentration of these acids. The PEG determination of rumen extracellular water showed that when the twins were fed Starea they usually had a greater rumen fill than when fed the cracked sorghum grain. The diurnal values are presented in figure 6.

The total microbial nitrogen values were highest when the animals were fed the Starea ration and lowest when fed the control ration of cracked sorghum grain. The results were similar for both the protozoal and bacterial fractions though there was proportionally a greater difference between the two rations in the bacterial than in the protozoal fraction. The organic matter contained in these fractions followed the

same patterns as did the microbial nitrogen. The cell-free fraction of rumen fluid collected from the animals on Starea was higher than when they were fed the control ration of cracked sorghum. When Starea was fed, the organic matter contained in the cell-free fraction was higher than when the control ration was fed. The data are presented in graphical form in figures 7 to 13.

Discussion

Rumen ammonia concentration was lowered by feeding Starea, as compared to cracked sorghum, and was essentially identical to the observations collected during Trial I. The increased microbial protein (bacterial and protozoa) observed when the twin cattle were fed Starea is apparently correlated with the decrease in rumen ammonia concentration. This increase in microbial protein synthesis may result because Starea releases ammonia and energy at a rate favoring synthesis.

The lactic acid concentration of the rumen contents was higher when Starea was fed. Phillipson (1942) and Wilson and Woods (1966, 1967) observed a higher lactic acid concentration when animals were fed flaked corn or gelatinized corn.

The feeding of Starea brought about an increased rumen VFA concentration. Colenbrander (1966) observed an increased VFA concentration when expanded grain was fed.

The increased lactic acid and VFA concentration was related to a decreased rumen pH. Briggs et al. (1957) and Balch and Rowland (1957) observed an inverse relation between lactic acid and VFA concentration and rumen pH.

A lowered concentration and proportion of isobutyric, valeric and

isovaleric acid was observed in those animals fed the Starea ration as compared to those fed the cracked grain ration.

Allison (1959, 1962) and Hungate (1966) observed that the branched chain acids were required nutrients for certain rumen bacteria. Stewart et al. (1958) showed that when urea was added to the ration of steers, the concentration of valeric acid decreased. Cline et al. (1966) observed an increase in nitrogen digestibility and retention when a mixture of isobutyrate, isovalerate and valerate was added to a purified diet containing urea.

In the study reported here a decrease in the IC_4, IC_5, and C_5 acids found in the rumen of animals fed Starea, compared with the control cracked grain ration, was related to an increase in microbial protein synthesis.

Trial III. Urea Toxicity.

The problem of urea (ammonia) toxicity was studied. Observations were made on the comparative toxic levels of urea in a Starea mixture and in a rolled sorghum grain-urea mixture.

Experimental Procedure

Two pairs of mature, fistulated identical twin cattle were used. One pair consisted of Holstein females weighing 680 kg and the other pair of Shorthorn steers weighing 500 kg. The Holsteins had not been fed urea for 250 days previous to the trial while the Shorthorns had been fed 180 to 200 g of urea daily up to 10 days previous to the trial. All animals were fed 2.8 kg of rolled sorghum grain and 2.8 kg of alfalfa hay daily. The animals were weighed at the initiation of each phase of

the trial and the feed mixture was then fed according to weight.

On the day of the trial, all animals were fed 1.4 kg of sorghum grain and 1.4 kg of alfalfa hay at 8:00 a.m. and then fed at 1:00 p.m. 1.4 kg hay and grain containing 20% urea. After the first day, due to poor acceptability of the grain containing urea the Starea or grain containing urea was placed in the rumen via the rumen fistula in amounts to supply the quantities of urea shown in table 12. Alfalfa hay (1.8 kg per animal) was fed at the time the grain was administered to help promote normal actions resulting from ingestion of feed.

Rumen fluid samples for ammonia determinations were obtained before feeding and at 30 min intervals post feeding. Rumen samples were drawn with a stainless steel syringe from a sampling tube located permanently in the rumen.

Results

The Holstein twins (14-15) who had not been on urea for 250 days previous to the trial appeared to be more susceptible to ammonia toxicity than did the Shorthorn steers (04-05) which had been adapted to urea (table 12). The Holsteins exhibited toxic symptoms when they were fed 30 g of urea (control ration) per 50 kg body weight. However, this level of urea in the Starea ration did not produce toxic symptoms.

The steers (04-05) which had been adapted to urea were less sensitive to high levels of urea. It required 54 g of urea per 50 kg weight to produce toxic symptoms. As was the case with the Holsteins, urea in combination with rolled sorghum grain was toxic, whereas greater amounts of urea in the Starea ration were not. In one phase of the trial a steer was given 616 g of urea in the form of Starea but toxicity did not occur.

Table 12. Effect of urea on rumen ammonia concentration and toxicity.

Twin pair	Ration	Days from start of trial	Urea intake		Rumen ammonia					Toxic reaction[d]
			Per 50 kg body wt.	Total /animal	Time after administering urea (min)					
					Pre-fed	30	60	90	120	
			(g)	(g)	------- (mg/100 ml rumen fluid) ------					
14[a]	Starea	0	22	284	5	38	65	74	104	-
15	Control	0	13	185	6	42	59	79	75	-
14	Starea	2	34	427	8	58	150	177	193	-
15	Control	2	31	434	6	105				+ recovered
14	Control	14	30	379	6	97	131	128	161	+ died
15	Starea	14	31	418	34	62	97	131	138	-
04[b]	Starea	0	22	207	5	21	35	45	58	-
05	Control	0	13	134	9	39	66	66	63	-
04	Starea	2	32	319	11	31	66	85	87	-
05	Control	2	30	317	23	63	100	118	108	-
04	Control	7	39	401	17	90	106	111	116	-
05	Starea	7	42	443	39	44	60	55	50	-
04	Control	11	54	551	43	143				+ recovered
05	Starea	11	59	616	64	90	151	182	216	-
04	Starea	24	55	541	16	145	160	161		+ slight, recovered
05	Control	24	54	581	19	94	115	155	145	+ died

a 14 and 15 (Holstein cows) had not received urea for more than 250 days preceding the trial
b 04 and 05 (Shorthorn steers) had not received urea for 10 days preceding the trial
c 1.8 kg of alfalfa hay fed with Starea or control ration
d + positive, - negative

Toxicity developed in animal 15 on the second day while she was receiving the control ration. This animal responded favorably when given 18 liters of 5% acetic acid via the rumen fistula. Similarly animal 04 (control) was treated on the eleventh day and responded. However, animals 14(control - fourteenth day) and 05 (control - twenty-fifth day) were treated with acetic but failed to respond. Perhaps this failure was due to a delay in administering acetic acid after first signs of toxicity were observed. To be effective it would appear that acetic acid should be administered within 30 min of the first signs of toxicity. The symptoms of toxicity observed were: dullness, muscle tremors, eyes rolling back into the head, excessive urination, excessive salivation, muscular incoordination, labored breathing, and at the approach of the terminal stage - prostration. In the death of animal 14, it was observed that in the time preceding death, the venous circulatory system was in a state of collapse.

Discussion

The results of this experiment indicate that urea contained in a Starea feed mixture is not so toxic to animals as is urea mixed with rolled sorghum grain. An adaptation to high levels of urea was also illustrated because animals which had recently been fed urea ingested almost twice as much urea before toxicity occurred as was ingested by non-adapted animals.

The findings of this experiment were in accord with the findings of Clark et al. (1951) Coombe and Tribe (1958) and Coombe et al. (1960) in that rumen ammonia levels are not necessarily a good indicator of toxicity.

The work reported here on rumen fermentation measurements showed elevated lactic acid levels and lowered pH values with the Starea feed mixture as compared with urea and rolled grain.

This work supported the hypothesis that lowered pH in the rumen will slow absorption of ammonia from the rumen. The high rumen ammonia level of 216 mg ammonia per 100 ml rumen fluid observed in one animal suggests that ammonia can build up in the rumen without producing toxicity.

SUMMARY

The feeding of processed grain (fine grind-pellet, or expanded) with urea or Starea to cattle lowered free ammonia in the rumen more than did feeding urea and unprocessed grain. The lowered rumen ammonia concentration suggests greater nitrogen conservation because microbial protein synthesis increased when the Starea ration was fed.

The Starea ration was more palatable than mixtures of unprocessed grain and urea. The urea contained in the Starea ration did not segregate out as it did in the mixtures of unprocessed grain and urea.

The Starea ration was less toxic than equivalent quantities of grain and urea.

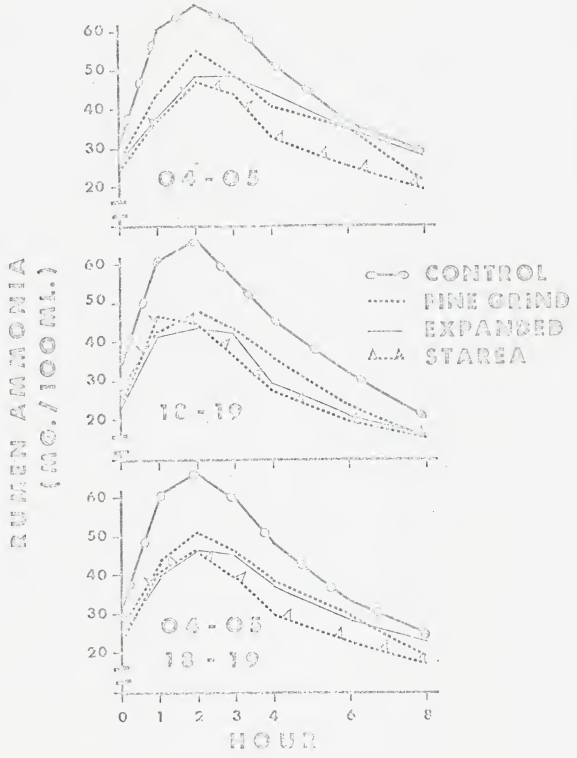

Figure 1. Effect of ration on rumen ammonia concentration of twin pairs 04-05 and 16-19. (Trial 2)

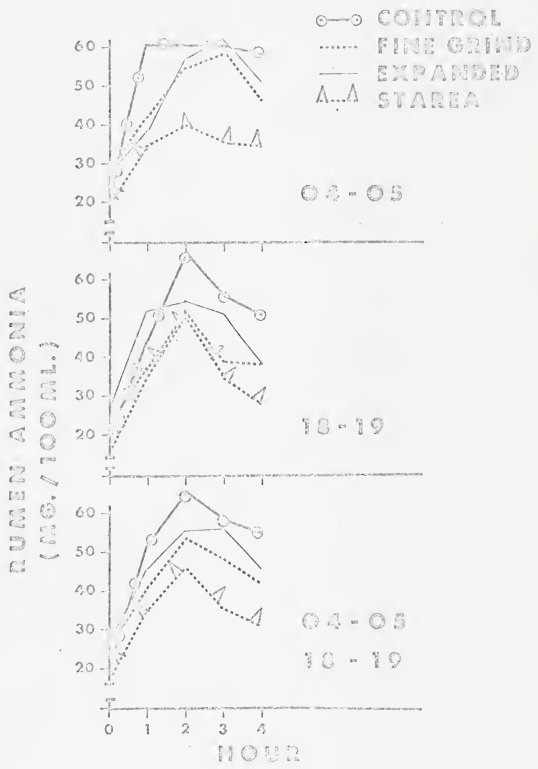

Figure 2. Effect of ration on ammonia concentration of rumen fluid obtained (48 hours after ration changes) from twin pairs 04-05 and 18-19. (Trial I)

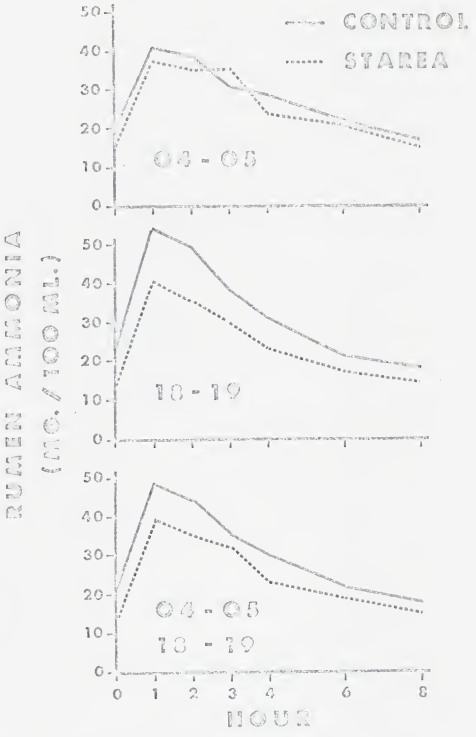

Figure 3. Rumen ammonia concentration of twins (04-05 and 18-19) fed hay with Starea or cracked grain plus urea. (Trial II)

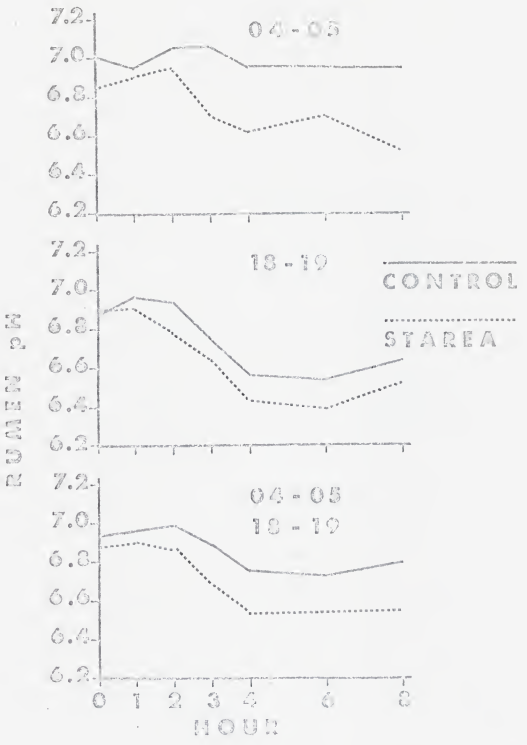

Figure 4. Rumen pH of twins (04-05 and 18-19) fed hay with Starea or cracked grain plus urea.

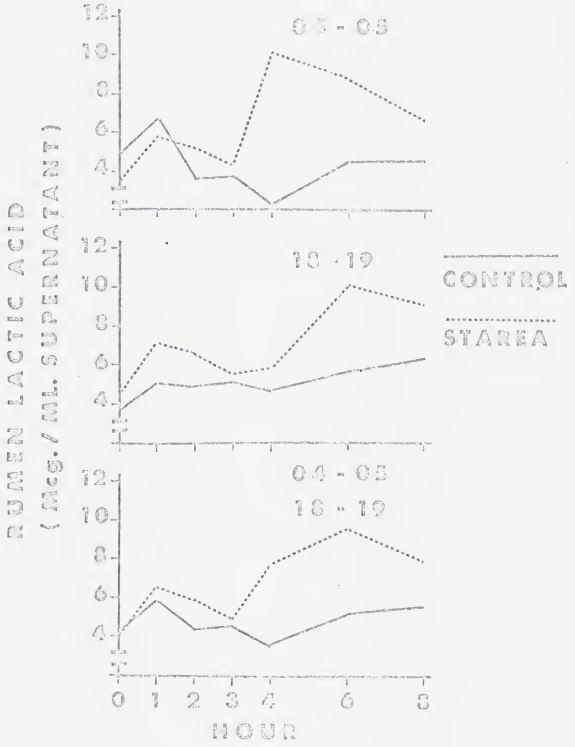

Figure 5. Rumen lactic acid concentration of twins (04-05 and 18-19) fed hay with Starea or cracked grain plus urea.

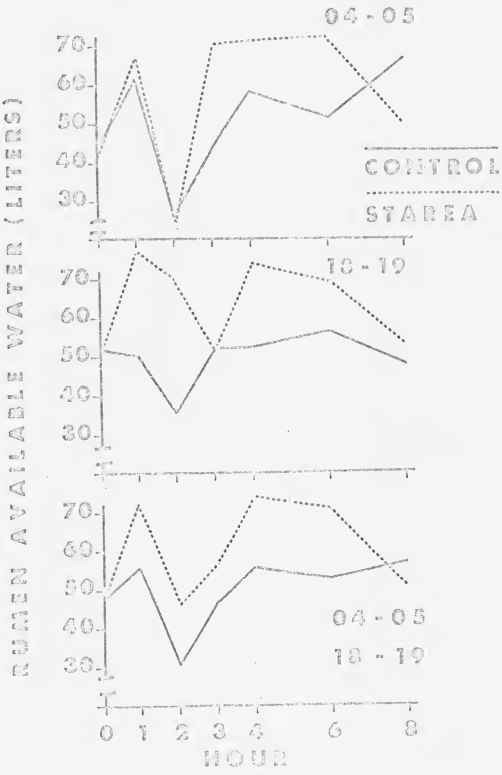

Figure 6. Rumen extracellular water volume of twins (04-05 and 18-19) fed hay with Starea or cracked grain plus urea.

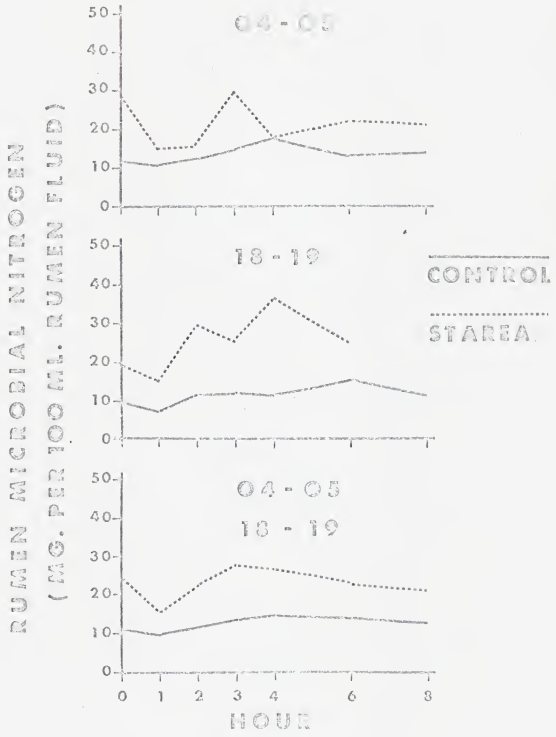

Figure 7. Total rumen microbial nitrogen concentration of twins (04-05 and 18-19) fed hay with Starea or cracked grain plus urea.

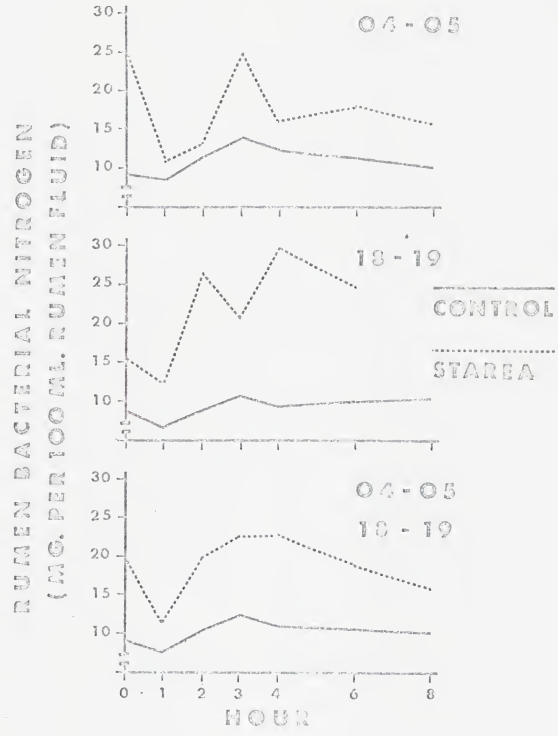

Figure 8. Rumen bacterial nitrogen concentration of twins (04-05 and 18-19) fed hay with Starea or cracked grain plus urea.

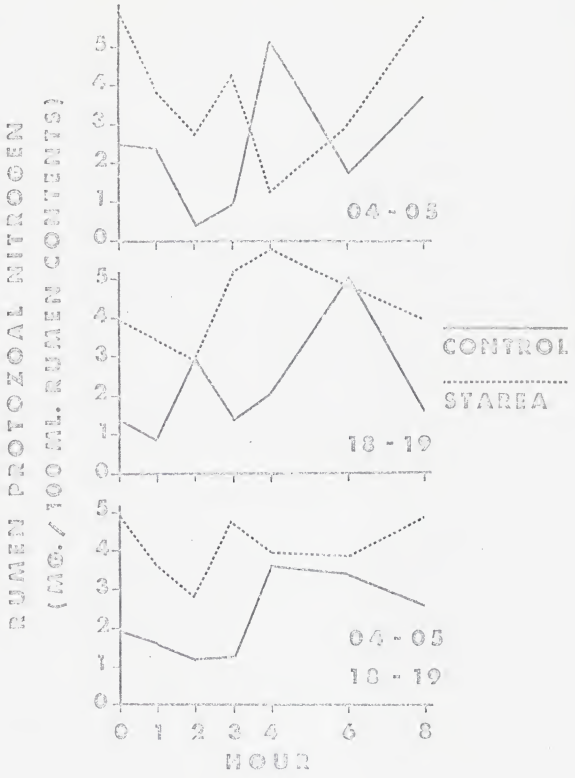

Figure 9. Rumen protozoal nitrogen concentration of twins (04-05 and 18-19) fed hay with Starea or cracked grain plus urea.

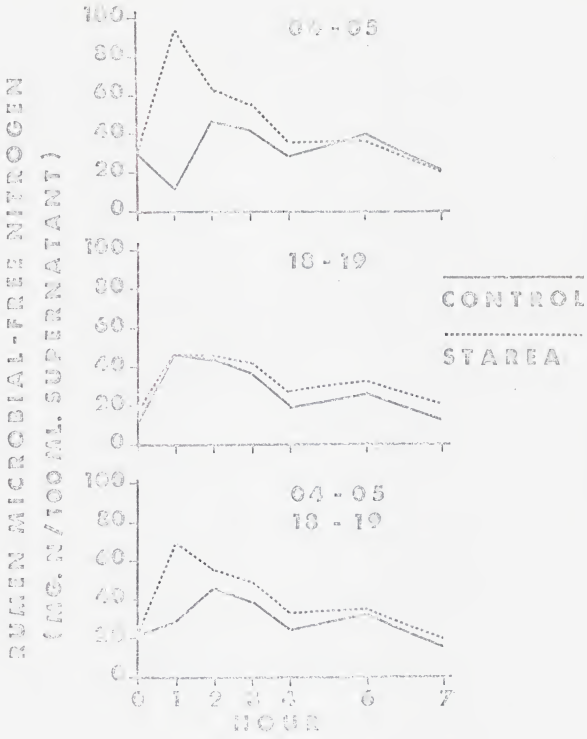

Figure 10. Nitrogen concentration of the microbial-free fraction of rumen fluid from 1 pig (04-05 and 16-19) fed hay with Starea or control gruel by tube [illegible]

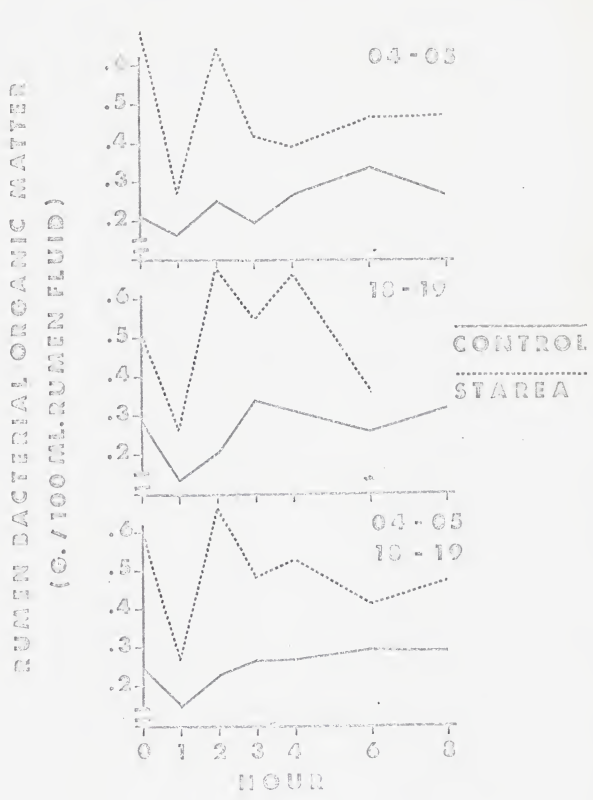

Figure 11. Rumen bacterial organic matter concentration of twins (04-05 and 10-19) fed hay with Starea or cracked grain plus urea.

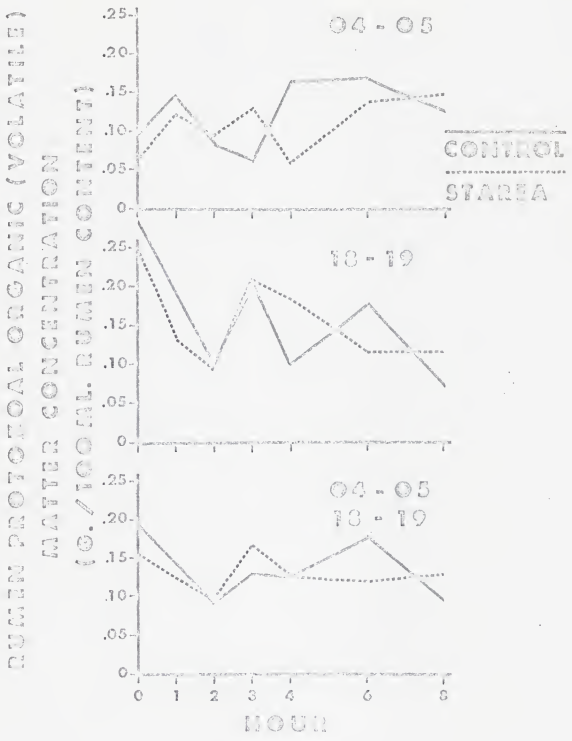

Figure 12. Rumen protozoal organic matter concentration of twins (04-05 and 18-19) fed hay + 6% starch or cracked grain plus urea.

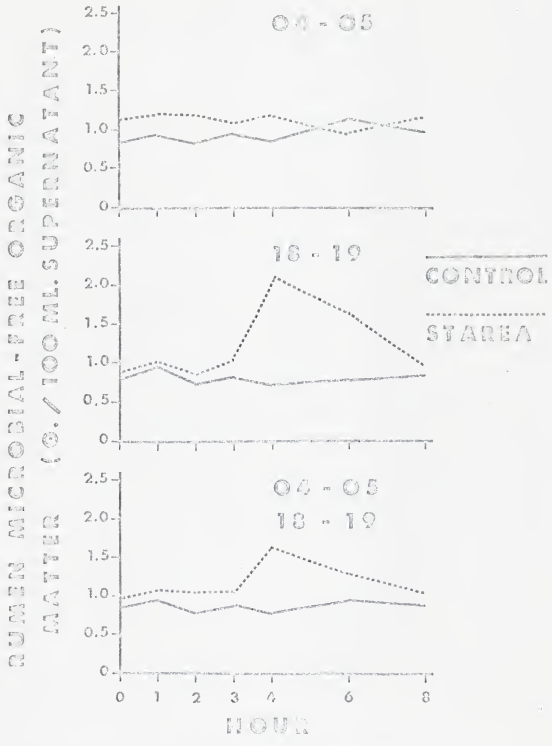

Figure 13. Organic matter concentration of the microbial-free fraction of rumen fluid from twins (04-05 and 18-19) fed hay with Starea or cracked grain plus urea.

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APPENDIX

Table 13. Nitrogen, protein equivalent (PE), non-protein nitrogen (NPN), and urea content of rations.

Ration	Day fed	Total nitrogen	Total PE (Nx6.25)	NPN	PE of NPN	Urea
		(%)				
			Trial I			
Alfalfa hay (Composite)		3.53	22.06	-	-	-
Cracked	7	3.38	21.13	1.48	9,25	3.19
"	14	3.79	23.69	1.92	12.00	4.14
"	21	3.49	21.81	1.38	8.63	2.97
"	28	4.08	25.50	2.25	14.06	4.85
"	35	3.58	22.38	1.68	10.50	3.62
"	42	3.77	23.56	1.84	11.50	3.97
"	49	4.09	25.56	2.21	13.84	4.76
"	56	4.18	26.13	2.30	14.38	4.96
Fine grind	7	4.50	28.13	2.73	17.06	5.88
"	14	4.40	27.50	2.67	16.69	5.75
"	21	4.48	28.00	2.69	16.81	5.80
"	28	4.45	27.81	2.61	16.25	5.62
"	35	3.79	23.81	1.92	12.00	4.14
"	42	3.98	24.88	2.12	13.25	4.56
"	49	3.96	24.75	2.15	13.44	4.63
"	56	3.87	24.19	2.02	12.63	4.36
Expanded	7	3.90	24.38	1.97	12.31	4.25
"	14	3.42	21.38	1.78	11.13	3.84
"	21	3.61	22.56	1.77	11.06	3.81
"	28	3.56	22.25	1.72	10.75	3.71
"	35	4.03	25.19	2.16	13.50	4.65
"	42	4.22	26.38	2.34	14.63	5.04
"	49	4.03	25.19	2.17	13.56	4.68
"	56	4.18	26.13	2.27	14.19	4.89
Starea	7	4.05	25.31	2.37	14.81	5.11
"	14	4.00	25.00	2.18	13.63	4.70
"	21	4.13	25.81	2.24	14.00	4.83
"	28	3.87	24.19	2.07	12.94	4.46
"	35	4.02	25.13	2.13	13.31	4.59
"	42	4.15	25.94	2.24	14.00	4.83
"	49	4.12	25.75	2.24	14.00	4.83
"	56	4.25	26.56	2.39	14.94	5.15

Table 13. (cont.)

Ration	Day fed	Total nitrogen	Total PE (Nx6.25)	NPN	PE of NPN	Urea
				(%)		
			Trial II			
Alfalfa hay (Composite)		3.35	20.94	0.33	2.06	0.71
Starea	7	4.56	28.50	2.56	16.00	5.52
"	14	4.47	27.94	2.56	16.00	5.52
"	28	4.48	28.00	2.56	16.00	5.52
"	35	4.48	28.00	2.55	15.94	5.50
"	42	4.49	28.06	2.59	16.19	5.58
"	49	4.48	28.00	2.55	15.94	5.50
"	56	4.41	27.56	2.53	15.81	5.45
Cracked	7	4.08	25.50	2.31	14.44	4.98
"	14	4.14	25.88	2.30	14.38	4.96
"	28	4.23	26.44	2.43	15.19	5.24
"	35	4.22	26.38	2.40	15.00	5.17
"	42	4.13	25.81	2.42	15.13	5.22
"	49	4.33	27.06	2.46	15.38	5.30
"	56	4.24	26.50	2.44	15.25	5.26

Table 14. Effect of ration on rumen ammonia concentration (Trial I).

Rumen ammonia concentration (mg/100 ml rumen fluid)

Time of sampling after feeding (hr)	Days 7	14	21	28	35	42	49	56	Days 7	14	21	28	35	42	49	56
	Ration C	FG	E	S	C	FG	E	S	Ration FG	E	S	C	FG	E	S	C
	Animal 04								Animal 05							
Pre	17.1	24.1	15.7	17.1	28.4	29.6	18.9	28.4	27.4	20.4	19.1	24.1	28.4	36.5	31.5	28.7
1	55.4	47.6	25.8	25.2	52.3	47.9	27.1	44.1	30.8	32.2	40.9	53.8	52.3	46.6	32.8	34.6
2	59.4	42.0	38.1	37.0	63.0	53.6	27.7	53.5	48.7	59.9	62.7	58.2	77.2	44.2	30.2	35.9
3	54.6	35.6	37.0	48.7	52.3	50.4	20.2	54.8	51.8	58.3	32.5	45.4	42.8	53.5	32.8	52.3
4	38.1	39.2	30.5	37.5	49.8	52.9	25.8	36.5	40.9	50.4	29.9	29.7	31.5	48.8	22.4	49.7
6	25.5	24.9	24.6	21.3	38.4	63.0	31.5	30.9	27.7	31.1	23.0	21.3	31.5	36.5	21.4	33.4
8	22.4	12.3	24.6	18.5	30.2	36.5	25.2	25.2	21.3	27.2	15.1	15.1	17.0	26.1	17.3	27.7

	Days 7	14	21	28	35	42	49	56	Days 7	14	21	28	35	42	49	56
	Ration E	S	C	FG	E	S	C	FG	Ration S	C	FG	E	S	C	FG	E
	Animal 18								Animal 19							
Pre	18.2	18.2	33.6	21.3	17.6	17.0	37.8	40.3	17.9	26.3	37.8	19.0	32.8	13.9	12.0	23.9
1	47.0	37.2	50.4	53.2	21.4	62.4	54.8	43.2	43.7	45.9	49.8	42.6	41.0	47.3	27.1	35.3
2	40.3	44.2	57.7	48.7	34.1	61.7	59.5	46.6	35.6	48.2	73.4	39.2	30.2	51.7	26.1	37.2
3	34.7	32.8	39.8	32.5	34.2	45.4	50.4	48.2	25.2	45.3	55.2	47.0	32.1	47.3	40.3	34.0
4	24.6	21.9	40.9	32.3	23.3	37.2	30.9	39.1	24.6	37.5	45.9	23.8	21.4	38.4	29.9	31.5
6	13.4	18.5	20.7	20.2	19.5	31.5	27.4	27.1	14.0	24.9	20.2	20.2	12.6	25.8	27.7	20.2
8	10.6	14.8	15.7	15.1	11.3	20.2	17.6	20.2	12.9	17.9	13.4	10.1	12.6	15.1	11.7	23.6

Table 14 (cont.)

Rumen ammonia concentration (mg/100 ml rumen fluid)

Time of sampling after feeding (hr)	Days 9	16	23	30	37	44	51	58	Days 9	16	23	30	37	44	51	58
	Ration FG	E	S	C	Ration FG	E	S	C	Ration E	S	C	FG	Ration E	S	C	FG
	Animal 04								Animal 05							
Pre	23.2	21.8	14.6	23.5	36.5	15.4	21.7	13.2	24.4	16.2	19.0	25.8	34.7	24.9	22.4	30.2
1	30.8	28.6	17.4	57.1	47.3	32.4	52.6	41.0	26.3	31.9	51.2	59.4	46.0	30.2	47.9	32.8
2	49.8	-	29.1	63.6	54.2	42.2	46.6	29.0	42.8	38.1	51.5	75.6	64.9	38.1	53.6	44.1
3	61.6	47.0	33.0	55.2	57.3	47.9	42.8	41.6	43.1	38.1	41.2	67.2	82.5	23.6	59.9	50.4
4	37.6	48.2	46.5	47.6	61.2	29.6	36.5	46.6	32.8	25.8	33.6	40.3	74.8	23.0	65.5	45.4
	Days 9	16	23	30	37	44	51	58	Days 9	16	23	30	37	44	51	58
	Ration S	C	FG	E	Ration S	C	FG	E	Ration C	FG	E	S	Ration C	FG	E	S
	Animal 18								Animal 19							
Pre	11.8	21.3	18.2	28.6	25.8	12.9	33.1	19.2	13.4	22.4	23.8	21.0	16.4	20.5	24.6	10.4
1	15.4	35.3	37.8	56.0	49.8	23.9	37.2	41.0	27.7	38.1	46.2	50.1	56.7	39.7	39.1	22.1
2	47.3	48.7	56.0	42.8	54.2	38.1	44.7	54.2	70.8	49.0	53.2	53.8	81.6	60.1	40.3	46.0
3	25.8	24.9	41.7	42.8	47.3	33.1	41.6	46.0	55.4	39.5	41.4	28.3	68.0	36.5	48.5	33.4
4	18.2	22.4	37.0	17.1	44.1	43.2	52.3	38.4	40.3	30.8	38.1	24.9	61.8	35.9	39.7	21.2

Table 15. Effect of ration on rumen ammonia concentration (Trial II).

Rumen ammonia concentration (mg/100 ml rumen fluid)

Time of sampling after feeding (hr)	Days								Days							
	7	14	21	28	35	42	49	56	7	14	21	28	35	42	49	56
	------- Control-------				------ Starea ------				----- Starea ------				------ Control-------			
	---------- Animal 04 ----------								---------- Animal 05----------							
Pre	24.5	18.1	-	-	13.3	13.8	17.6	11.3	32.0	13.0	-	-	17.6	17.9	22.2	25.5
1	24.2	33.4	-	-	40.4	49.3	57.7	34.3	43.8	20.7	-	-	34.6	62.9	46.1	51.2
2	23.4	49.5	-	-	39.2	41.1	57.7	31.7	29.4	34.6	-	-	33.2	30.6	57.7	42.4
3	32.3	40.3	-	-	38.4	44.1	57.7	18.2	41.8	33.1	-	-	22.8	28.6	43.3	23.6
4	42.6	39.7	-	-	32.0	30.3	26.2	15.0	25.9	28.8	-	-	19.0	21.1	37.8	16.7
6	31.7	23.9	-	-	38.7	32.3	21.9	14.4	16.1	19.3	-	-	12.4	19.6	26.5	22.5
8	23.6	14.4	-	-	15.3	28.8	17.3	10.7	16.4	14.4	-	-	14.7	19.9	21.6	15.7
	---------- Animal 18 ----------								---------- Animal 19 ----------							
Pre	28.8	36.6	-	41.5	14.7	13.8	13.8	11.9	13.8	14.1	-	19.0	16.7	18.2	20.2	12.8
1	58.5	45.2	-	51.6	44.7	27.7	47.6	62.7	49.0	31.7	-	51.5	54.2	42.1	71.5	72.1
2	52.4	42.6	-	44.1	48.0	31.2	34.0	47.3	41.8	32.5	-	43.1	60.0	55.7	54.8	50.2
3	39.5	35.7	-	36.8	32.6	34.0	35.2	23.7	36.7	28.8	-	41.5	45.0	46.7	46.1	29.7
4	33.4	36.2	-	26.3	26.0	25.4	31.7	21.1	26.2	25.6	-	25.0	32.3	35.5	33.2	30.0
6	17.3	18.1	-	23.5	13.0	22.2	26.0	16.0	20.1	17.5	-	18.6	22.5	26.5	25.4	20.8
8	16.4	13.2	-	17.3	13.0	19.6	21.6	11.7	17.9	12.1	-	16.0	18.2	25.7	21.6	15.3

Table 16. Effect of ration on diurnal rumen pH (Trial II).

Time of sampling after feeding (hr)	Days 7	14	21	28	35	42	49	56	Days 7	14	21	28	35	42	49	56
	-------Control-------				------- Starea -------				------- Starea ------				------- Ration -------			
	---------- Animal 04 ----------								---------- Animal 05 ----------							
Pre	7.10	7.00	-	-	6.65	6.57	6.90	7.35	7.00	6.55	-	-	6.75	7.03	6.90	7.20
1	7.00	6.90	-	-	6.95	6.70	6.95	7.20	6.90	6.65	-	-	6.83	7.00	7.05	6.93
2	7.25	7.05	-	-	7.00	6.97	6.80	7.25	6.90	6.75	-	-	6.90	7.00	7.05	7.03
3	7.20	7.20	-	-	6.93	6.63	6.40	7.15	6.75	6.35	-	-	6.85	7.03	7.00	7.03
4	7.40	7.05	-	-	6.87	6.57	6.65	6.90	6.50	6.25	-	-	6.65	7.10	6.85	6.70
6	7.15	6.75	-	-	6.87	6.40	6.80	6.90	6.50	6.70	-	-	6.90	7.13	6.95	6.91
8	7.00	6.90	-	-	6.95	6.27	6.40	7.20	6.40	6.20	-	-	7.00	7.10	6.90	6.90
	---------- Animal 18 ----------								---------- Animal 19 ----------							
Pre	6.90	6.70	-	7.30	6.85	6.83	6.85	7.03	6.85	6.70	-	7.00	6.90	6.73	6.70	7.00
1	7.00	6.90	-	7.07	6.95	7.00	6.80	7.13	6.75	6.80	-	6.93	7.00	6.85	6.80	7.10
2	6.95	6.95	-	7.03	6.80	6.77	6.65	6.97	6.70	6.80	-	6.84	7.00	6.83	6.75	7.10
3	6.80	6.70	-	6.80	6.60	6.50	6.50	6.80	6.65	6.85	-	6.67	6.90	6.65	6.50	6.90
4	6.50	6.60	-	6.73	6.40	6.47	6.45	6.43	6.35	6.50	-	6.50	6.60	6.50	6.40	6.63
6	6.60	6.40	-	6.63	6.50	6.45	6.40	6.40	6.45	6.30	-	6.33	6.60	6.53	6.40	6.65
8	6.60	6.65	-	6.70	7.00	6.50	6.40	6.63	6.30	6.40	-	6.50	6.90	6.60	6.45	6.60

Table 17. Effect of ration on diurnal rumen lactic acid concentration (Trial II).

Rumen lactic acid µg/ml

Time of sampling after feeding (hr)	Days								Days							
	7	14	21	28	35	42	49	56	7	14	21	28	35	42	49	56
	Control				Starea				Starea				Control			
	Animal 04								Animal 05							
Pre	6.7	1.5	-	-	1.4	1.9	2.1	2.8	7.8	4.1	-	-	1.7	7.1	8.4	3.9
1	12.0	4.8	-	-	1.6	12.0	5.9	2.2	6.8	6.1	-	-	5.8	5.8	7.7	4.2
2	4.8	4.8	-	-	1.4	17.0	2.0	2.4	5.0	3.4	-	-	1.8	4.8	1.3	4.4
3	3.8	7.4	-	-	1.7	7.2	2.9	1.6	3.2	9.3	-	-	2.3	5.5	0.8	2.3
4	2.0	2.0	-	-	1.9	11.2	3.0	1.0	10.6	32.4	-	-	2.5	2.3	1.3	3.7
6	4.3	4.2	-	-	2.0	16.4	2.9	3.8	15.7	12.0	-	-	1.5	7.2	2.7	6.7
8	5.2	8.4	-	-	9.1	11.8	3.6	3.4	5.8	6.1	-	-	2.0	5.0	2.8	3.9
	Animal 18								Animal 19							
Pre	8.3	1.6	-	6.5	1.6	3.0	4.3	2.7	9.9	2.1	-	7.6	1.0	3.9	2.7	1.7
1	15.9	3.2	-	2.2	2.1	5.9	3.8	3.3	23.0	4.7	-	7.0	2.0	3.9	1.5	6.5
2	2.9	3.7	-	9.4	1.6	17.0	2.8	3.5	4.5	6.2	-	11.0	1.6	12.5	1.3	2.8
3	1.2	12.7	-	6.8	2.1	6.5	0.8	1.6	1.8	22.5	-	3.5	3.9	8.7	0.7	1.3
4	14.2	5.3	-	1.4	2.5	5.9	1.1	1.9	3.0	25.1	-	2.0	1.6	8.6	0.1	1.2
6	2.8	11.4	-	1.9	6.5	4.0	32.1	2.4	11.0	11.3	-	2.9	2.2	3.6	9.6	8.5
8	19.0	7.7	-	3.1	2.0	6.9	28.8	2.1	7.9	15.7	-	3.2	1.5	5.7	3.7	3.8

Table 18. Effect of ration on rumen volatile fatty acids (Trial II).

Time of sampling after feeding (hr)	Rumen volatile fatty acid concentration (meq/liter)															
	C_2	C_3	IC_4	C_4	IC_5	C_5	Total	Steam distill	C_2	C_3	IC_4	C_4	IC_5	C_5	Total	Steam distill
	Animal 18								Animal 19							
	Starea								Control							
Pre	59.3	7.3	0.2	7.0	1.4	1.3	76.5	64.5	66.2	13.9	0.8	8.7	2.3	1.4	93.3	86.1
1	77.5	15.1	0.5	9.1	1.6	1.0	104.8	62.1	64.0	13.4	0.3	4.3	1.2	2.1	85.3	106.3
2	95.9	16.9	0.6	10.0	1.7	1.7	126.6	83.4	60.4	14.3	0.6	6.0	1.3	1.1	83.7	107.4
3	114.6	22.3	0.8	12.8	1.4	2.3	154.2	276.8	74.2	13.2	0.4	7.1	1.5	1.5	97.9	115.1
4	94.5	20.7	0.3	12.5	1.4	2.0	131.4	157.1	67.7	15.4	0.5	8.4	1.6	1.7	95.3	107.6
6	93.9	16.6	0.3	10.1	1.7	1.5	126.8	106.7	61.7	15.2	0.2	8.0	1.3	1.2	87.6	95.4
8	96.6	16.6	0.3	10.1	1.7	1.5	126.8	106.7	58.2	12.7	0	7.5	1.4	1.4	81.2	99.8
Avg total	90.3	16.7	0.4	10.5	1.6	1.6	121.1	129.0	64.6	14.0	0.4	7.1	1.5	1.5	89.1	102.5
Molar %	74.6	13.8	0.3	8.7	1.3	1.3			72.5	15.7	0.5	7.9	1.7	1.7		
	Control								Starea							
Pre	53.6	10.4	0.8	6.1	2.2	1.0	74.1	61.6	53.8	8.1	0.4	6.6	1.6	0.9	71.4	83.3
1	51.9	10.9	0.5	4.6	2.2	1.2	71.3	71.3	71.3	13.4	0.5	7.1	2.0	0.8	95.1	103.0
2	46.4	10.8	0.3	4.2	1.4	1.1	64.2	135.6	46.0	9.4	0.1	4.7	0.9	1.0	62.1	106.2
3								56.6								87.3
4								69.9								73.5
6								81.9								93.5
8								82.6								115.3
Avg total	50.6	10.7	0.5	5.0	1.9	1.1	69.8	79.9	57.3	10.3	0.3	6.1	1.5	0.9	76.4	94.6
Molar %	72.5	15.3	0.7	7.2	2.7	1.6			75.0	13.5	0.4	8.0	2.0	1.2		

Table 18. (cont.)

Rumen volatile fatty acid concentration (meq/liter)

Time of sampling after feeding (hr)	C_2	C_3	IC_4	C_4	IC_5	C_5	Total	Steam distill	C_2	C_3	IC_4	C_4	IC_5	C_5	Total	Steam distill
	Animal 04								Animal 05							
	Starea								Control							
Pre	54.7	9.5	0.5	7.2	1.8	0.6	74.3	87.7	58.1	9.7	1.3	5.6	2.5	0.8	78.0	97.6
1	69.2	11.1	0.4	5.7	1.3	0.8	88.4	91.4	76.0	15.8	1.3	3.2	2.4	1.5	100.1	105.4
2	62.6	10.4	0.4	6.0	1.4	1.2	81.9	84.8	81.4	14.2	1.2	3.4	1.7	1.6	103.5	117.7
3	82.1	11.3	0.5	6.6	1.0	1.0	102.5	84.0	94.2	15.6	1.4	7.6	2.7	1.9	123.4	121.3
4	168.2	24.6	0.2	17.5	2.6	2.6	215.7	240.8	88.8	14.4	0.9	5.1	1.9	1.5	111.9	123.5
6	100.4	15.9	0.4	11.0	1.8	1.1	130.6	63.9	77.4	13.9	1.4	4.0	1.6	1.0	99.2	112.2
8	74.7	12.2	0.4	8.2	1.4	0.9	97.7	110.2	67.0	12.0	1.1	3.6	1.9	0.8	86.4	110.0
Ave total	87.4	13.6	0.4	8.9	1.6	1.2	113.1	97.0	77.6	13.7	1.2	6.8	2.1	1.3	102.7	112.5
Molar %	77.3	12.0	0.4	7.9	1.4	1.0			75.6	13.3	1.2	6.6	2.0	1.3		

Table 19. Effect of ration on rumen microbial nitrogen concentration (Trial II).

Rumen microbial nitrogen (mg N/100 ml rumen fluid)

Time of sampling after feeding (hr)	Control			Starea			Starea			Control		
	Proto-zoa	Bac-teria	Cell free	Proto-zoa	Bac-teria	Cell free	Proto-zoa	Bac-teria	Cell free	Proto-zoa	Bac-teria	Cell free
	Animal 04						Animal 05					
Pre	0.33	8.93	35.90	8.44	18.99	22.08	3.48	28.67	28.95	4.48	9.29	22.10
1	-	-	-	3.81	10.92	93.73	-	-	-	2.33	8.32	11.55
2	-	-	-	2.74	12.97	62.80	-	-	-	0.43	11.56	46.20
3	1.34	19.76	59.43	6.64	19.67	54.33	1.92	30.36	56.63	0.58	8.29	26.15
4	-	-	-	1.18	16.01	35.65	-	-	-	5.18	12.31	28.90
6	1.31	8.93	47.23	3.82	12.45	22.00	2.08	23.76	52.43	2.25	13.68	31.25
8	-	-	-	5.74	15.86	21.10	-	-	-	3.65	10.04	20.13
	Animal 18						Animal 19					
Pre	0.81	8.61	15.43	6.87	15.15	16.13	0.97	15.19	15.05	1.97	8.42	15.43
1	-	-	-	3.43	12.39	45.10	-	-	-	0.88	6.63	46.80
2	-	-	-	2.87	26.80	46.15	-	-	-	2.96	8.98	44.35
3	1.69	10.55	42.08	6.29	20.26	39.10	4.01	20.98	46.93	1.19	10.72	30.63
4	-	-	-	6.72	29.46	28.65	-	-	-	2.06	9.69	19.38
6	5.36	10.09	23.78	6.38	23.47	36.98	3.23	15.89	29.18	4.78	10.14	27.05
8	-	-	-	3.98	-	20.55	-	-	-	1.66	10.08	12.30

Table 20. Effect of ration on organic (volatile) matter in rumen fluid microbial extract (Trial II).

Organic matter in microbial extract (grams per 100 ml rumen fluid)

Time of sampling after feeding (hr)	Control			Starea			Starea			Control		
	Proto-zoa	Bac-teria	Cell free	Proto-zoa	Bac-teria	Cell free	Proto-zoa	Bac-teria	Cell free	Proto-zoa	Bac-teria	Cell free
	Animal 04						Animal 05					
Pre	.067	.224	1.154	.080	.673	1.130	.050	.696	1.117	.115	.208	.593
1	-	-	-	.123	.267	1.173	-	-	-	.148	.166	.929
2	-	-	-	.091	.644	1.185	-	-	-	.083	.250	.840
3	.074	.192	.910	.196	.354	1.113	.062	.477	1.079	.054	.190	.927
4	-	-	-	.061	.389	1.172	-	-	-	.162	.269	.858
6	.062	.333	1.054	.103	.381	1.037	.171	.548	.818	.278	.333	1.205
8	-	-	-	.146	.473	1.135	-	-	-	.122	.269	.946
	Animal 18						Animal 19					
Pre	.390	.212	.825	.254	.418	.577	.234	.591	1.112	.185	.368	.763
1	-	-	-	.130	.263	.983	-	-	-	-	.132	.939
2	-	-	-	.094	.676	.846	-	-	-	.102	.201	.750
3	.174	.406	.754	.162	.543	1.066	.254	.556	.952	.227	.270	.908
4	-	-	-	.186	.662	2.070	-	-	-	.100	-	.701
6	.192	.194	.611	.152	.474	2.128	.077	.259	1.190	.166	.327	.904
8	-	-	-	.116	-	.929	-	-	-	.073	.317	.812

Table 21. Polyethylene glycol determination of extracellular water (Trial II).

Time of sampling after feeding (hr)	Control	Starea	Starea	Control
	Animal 04		Animal 05	
Pre	32.3	47.6	36.2	54.1
1	-	66.7	-	60.6
2	-	23.5	-	25.5
3	31.8	69.4	-	54.1
4	-	-	-	58.8
6	51.3	60.6	83.3	50.0
8	-	48.8	-	66.7
	Animal 18		Animal 19	
Pre	42.6	57.1	44.4	60.6
1	-	76.9	-	50.0
2	-	69.7	-	35.4
3	46.5	48.8	52.6	56.6
4	-	74.1	-	52.1
6	54.9	90.9	47.6	57.1
8	-	52.6	-	47.6

EFFECT OF AN EXPANSION PROCESSED MIXTURE OF GRAIN AND UREA (STAREA) ON NITROGEN UTILIZATION IN THE RUMEN OF CATTLE AND ON UREA TOXICITY

by

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A study was conducted with identical twin cattle to determine the effect of several feed processing treatments on the utilization of urea. The experimental rations consisted of: (1) a mixture of cracked grain plus urea, (2) a mixture of a pelleted mixture of finely ground grain plus urea, (3) a mixture of expansion processed grain plus urea and (4) Starea. Starea was produced by mixing finely ground grain with urea. The mixture was processed by passing it through a cooker-extruder under moisture, pressure and temperature conditions that caused the starch to gelatinize completely. All rations contained 5% urea and the grain was sorghum.

In Trial I, two sets of identical twins were fed the four experimental rations in combination with an equal amount of alfalfa hay. The finely ground pelleted grain and the expanded grain lowered rumen ammonia levels compared with the control cracked grain. Rumen ammonia levels were reduced most with the Starea ration. Starea was eaten more readily than the other three rations.

In Trial II, Starea and the cracked grain (control) were fed to the two sets of identical twin cattle. Starea lowered the rumen ammonia concentration more than did the control ration. The lower rumen ammonia concentration by Starea was accompanied by higher rumen microbial protein nitrogen, lower rumen pH, higher rumen lactic acid concentration, and lower concentration and molar proportion of isobutyric, valeric and isovaleric acids.

In Trial III, the problem of urea (ammonia) toxicity was studied. Two pairs of mature fistulated identical twin cattle were used. One pair

of twins had not been fed urea for 250 days preceding the experiment, the other pair had been fed 180 to 200 g of urea daily up to 10 days before the trial. Urea was not fed during the trial except on the sampling days when large amounts of urea were fed. It required at least 30 g of urea per 50 kg of body weight to produce toxicity in the twin pair which had not been fed urea 250 days preceding the trial. Fifty g of urea per 50 kg of body weight was required to produce toxicity in the twin pair fed urea to within 10 days of the start of the trial. The data show that Starea is less toxic than equivalent quantities of urea mixed with unprocessed grain.

Feeding Starea to cattle lowered free ammonia in the rumen more than did feeding urea and unprocessed grain. The lowered ammonia concentration suggests nitrogen conservation because microbial protein synthesis increased when Starea was fed. The synthesized protein apparently is available to the host. Starea was more palatable than mixtures of untreated grain and urea. Starea was less toxic than equivalent quantities of grain and urea.