EFFECT OF RATIONS FED DONOR COWS ON IN VITRO PROTEIN SYNTHESIS FROM STAREA OR GRAIN PLUS UREA SUBSTRATES.

by

THOMAS BURT AVERY JR

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Approved by:

Eric E. Bartley
Major Professor

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INTRODUCTION

As the demands made on the resources of this world are increased by an evergrowing human and animal population, the ability of ruminant animals to convert feedstuffs which are poorly utilized by monogastrics into high quality protein becomes increasingly important.

The efficiency of this process is a primary concern. Starch, more specifically cooked starch, has been shown to increase urea utilization. Starea, an extrusion-cooked mixture of grain and urea, has been shown to increase urea utilization. Other endeavors to increase urea utilization include the adsorbtion of urea on diatomaceous earth and coating this with molasses.

In vitro techniques for evaluating the efficiency of non-protein nitrogen (NPN) utilization are advantageous for several reasons. Many samples, such as new NPN compounds, different treatments, or quality evaluation for manufacturing batches of products such as Starea, can be evaluated without the expense, time and effort required for *in vivo* trials.

This study is divided into three experiments. The first evaluates a mixture of urea, diatomaceous earth, and molasses by *in vitro* comparisons with Starea or grain plus urea substrates. The second and third experiments attempt to define some of the influences which inoculum donor rations may have on *in vitro* fermentations. Adaptation of donor animals to urea or Starea is investigated in Experiment III using *in vitro* fermentations.

REVIEW OF LITERATURE

Metabolism of Urea

Utilization of urea by ruminants is only indirect. The microbial population of the rumen must metabolize urea to quality protein which then may be used by the animal for production or maintenance. Knowledge of the pathways used by the rumen microbes to break down urea and build proteins is still incomplete.

Hydrolysis. Rumen fluid possesses urease activity. Brent and Richardson (1967) indicated that rumen urease is intracellular. Pearson and Smith (1943) found that urease activity in rumen fluid was greatest between pH 7 and 9 and was not dependent on anaerobic conditions. Rumen urease is very active, hence large concentrations are not needed. Many bacteria possess urease activity according to Hungate (1966). Farlin et al. (1968) infused either ^{14}C-labeled urea or sodium bicarbonate into the rumen and found that the carbon of urea did not equilibrate with the carbon dioxide pool. They suggested that urea need not be hydrolyzed to carbon dioxide and ammonia for utilization. This is contrary to the popular opinion which is that urea is hydrolized and ammonia is the preferred form of nitrogen for bacterial protein synthesis (Helmer and Bartley, 1971).

Ammonia absorption. Ammonia may escape the rumen before being utilized for protein synthesis. McDonald (1948) first recognized this and Lewis (1957) demonstrated a curvilinear relationship between portal vein and rumen ammonia concentrations. Passage of ammonia from the rumen is largely dependent on rumen pH. Bloomfield et al. (1963) showed that absorption was insignificant when rumen pH was below 6.45, but when rumen

pH reached 7.55, ammonia absorption ranged from 7 to 26 mM per liter per hour. Gartner (1963) also demonstrated this same relationship.

Urea Excretion and Recycling. Ammonia absorbed from the rumen is delivered to the liver and is converted to urea. Urine is the major route for urea loss; however, urea is not totally excreted. Houpt (1959) injected urea intravenously into sheep and failed to recover 52% of the injected urea in the urine or body fluids. The same author replaced the rumen contents with saline and found 5.2 mmoles urea nitrogen per hour returned to the rumen. Of this amount 0.3 mmole per hour was contributed by saliva. Bailey and Balch (1961) found that the urea content of mixed saliva was about 65% of plasma urea concentration, which ranged from 4-19 mg urea N per 100 ml in cattle. Somers (1961) also found a significant correlation between concentrations of urea in the plasma and saliva of sheep. Blood urea levels were influenced by nitrogen intake of the sheep.

Ammonia Utilization by Rumen Microbes. The synthesis of bacterial protein using ammonia nitrogen is well accepted even though the pathways for this synthesis remain undefined. Bryant and Robinson (1962), Dehority (1963) and Hungate (1966) indicate that ammonia is essential for growth of some rumen bacteria and stimulates growth in other strains. Using a criterion of either decreased ammonia concentration or increased protein levels, Wegner et al. (1941), Harris et al. (1943), Duncan et al. (1952) and Phillipson et al. (1962) demonstrated ammonia utilization by rumen bacteria. Pearson and Smith (1943) added toxic substances to fermentations and demonstrated a decrease in protein synthesis. Arthur et al. (1967) found pH values within physiological range had little effect on ammonia uptake in an *in vitro* system. Hoshino et al. (1966) showed the

presence of NAP- and NADP-linked glutamic acid dehydrogenase and suggested that the initial step in fixing ammonia is an amination reaction. Isotope studies by Watson et al. (1949) using ^{15}N and Land and Virtanen (1959) using an ammonium salt labeled with ^{15}N, showed that urea and ammonium nitrogen can be incorporated into body and milk proteins respectively.

Quality of Microbial Protein. Microbial protein plays a large part in the protein nutrition of ruminants. This is discussed by Weller et al. (1962) who found that 80% of the nitrogen passing into the omasum of sheep was microbial nitrogen. Bacterial protein is of high quality and is nearly equal to casein in biological value. Amino acid analysis of rumen protozoa by Weller (1957) and Meyer et al. (1967) indicated that protozoal protein may be of higher biological value than bacterial protein.

PROBLEMS OF UREA UTILIZATION

Toxicity. Ingestion of excess urea produces a toxic syndrome. Symptoms include uneasiness, dullness, muscle and skin tremors, excessive salivation, frequent urination and defecation, rapid respiration, incoordination, stiffening of the front legs, tetany and death [Word et al. (1969), Coombe et al. (1960), and Dinning et al. (1948)]. Urea toxicity is predisposed by sudden intake as by drench (Dinning et al., 1948), if the diet is deficient in carbohydrate (Clark et al., 1951), if the animals have been fasted (Davis and Roberts, 1959), or if the animals are not adapted to urea (Stiles et al., 1960). There is no consensus as to rumen ammonia concentrations needed to produce toxicity. Symptoms of toxicity correlate better with blood ammonia concentrations than blood urea or rumen ammonia values (Helmer and Bartley, 1971).

Palatability. Depressed concentrate intake was attributed to 2.2 or 2.7% urea in the concentrate mixture by Van Horn et al. (1967). Loosli and Warner (1958) and Holter et al. (1968) indicate that consumption of rations containing urea was slower than control rations not containing urea. The large amount of concentrate needed for high producing milk cows accents the need for palatable rations.

METHODS OF IMPROVING UREA UTILIZATION

Most efforts to improve the microbial utilization of urea nitrogen have involved efforts to minimize ammonia losses. Attempts to reduce the rate of urea hydrolysis and/or increase the ability of rumen microbes to utilize ammonia nitrogen have been the major areas of study.

Adaptation. Some researchers including Owen et al. (1943), King et al. (1965) and Campbell et al. (1963) have indicated that animal performance improves with increasing time urea containing rations are fed. The possibility and mechanism of adaptation has been investigated by several workers. Schaadt et al. (1966) found evidence of adaptation to urea by lambs during a 72 day metabolism trial. Nitrogen balance and percentage biological value increased during the trial period. An earlier study by Johnson and McClure (1964) had failed to demonstrate adaptation to urea. Multiple regression analysis by Smith et al. (1959 and 1960) of observations from 63 lambs fed a high urea ration strongly indicated increased urea nitrogen utilization. Nitrogen retention was significantly improved by approximately two percentage units with each consecutive 10 day feeding period up to 50 days. Similar findings were made by McLaren et al. (1965) who found that absorbed nitrogen retention was

improved three percentage units with each 10-day period of urea feeding. Ammonia utilization by rumen microbes in vitro was significantly greater when inoculum was obtained from lambs fed a diet of 3.5% urea compared to inoculum from lambs fed control diet containing no urea. This same study by Caffrey et al. (1967a) indicated that maximum in vivo adjustments were reached in 19 days, but in vitro the criteria adjustments were complete in 13 days. Blood urea and urine excretion concentrations were measured following intravenous injections of urea or saline. Urea recycling was not believed to be involved in urea adaptation. Other studies by Caffrey et al. (1967b) substantiate these findings. Helmer (1969) using inoculum from urea-fed or roughage-fed donors found increased utilization of either Starea or grain plus urea substrates using inoculum from urea-fed donors compared to non-urea-fed donors.

Diethylstilbestrol (DES) has been shown to affect nitrogen retention through direct action at the tissue level (McLaren et al., 1960 and Karr et al., 1965). DES also shortens the time required for maximum utilization of urea. Welch et al. (1957) found that 35 days of urea intake was needed for maximum utilization of urea, but when DES was fed this period was reduced to 10 days.

Inhibiting Urease Activity. Some investigators have attempted to control excess rumen ammonia concentration by limiting urea hydrolysis through inhibition of urease activity.

Barbituric acid was added to rations by Harbers et al. (1962). In vivo findings indicated decreased cellulose digestion and depressed feed utilization and body growth by lambs. Brent and Richardson (1967) found that rumen microbes must be homogenized before S-2 carboxyethal 3-thiosulfopropionate would inhibit urease activity of rumen fluid in vitro.

Acetohydroxamic acid (AHA) added to rumen fluid at the rate of 10 ug per ml decreased ammonia production 50% and when added at a 200 ug per ml level almost completely blocked urea hydrolysis (Brent and Adepoju, 1967). Much higher levels were required for in vivo decreases in ammonia production. Loper et al. (1967a,b) also used in vivo and in vitro techniques to screen several urease inhibitors. Copper sulfate, neomycin sulfate, and bacitracin-MC were most effective in vitro; however, when fed to steers receiving 50 g urea per day, none reduced rumen ammonia concentrations. Neomycin sulfate did reduce rumen ammonia values in steers fed 100 g urea per day. Failure to improve urea utilization was also noted by Clifford et al. (1968) when they included barbituric acid, copper, or nitrate ion in urea containing diets.

A more promising method of limiting urease activity appears to be by immunization. Jackbean urease is used to produce the immunologic response. Glimp and Tillman (1965) reported that urease immunity was produced and that rate of gain and feed efficiency were increased in three out of four trials. After intraruminal infusion of a urea-dextrose solution immunized animals were found to have lower plasma ammonia concentrations and higher plasma urea levels than controls. Ruminal, jugular, and cecal vein blood was sampled. Similar in vivo findings were made by Sidhu et al. (1968), except plasma urea concentrations were not affected by urease immunity in this study.

Physical Protection. Coating urea with less soluble substances has been one method of delaying hydrolysis. Johnson et al. (1962) investigated 20 different fat or waxy coatings, several of which did delay ammonia production in vivo. In vitro results failed to establish that urea was better utilized. Patents for urea coatings are reviewed by Gutcho (1970).

Belasco (U.S. Patent 3,295,984) added clay-like materials to urea which were thought to absorb ammonia after urea hydrolysis. Martin et al. (1969) investigated 2% sodium bentonite added to urea containing high roughage rations and indicated that nitrogen and phosphorus utilization were increased.

Use of Other Nonprotein Nitrogen Compounds. Many ammonia containing substances other than urea have been studied. Helmer and Bartley (1971) have reviewed many of these investigations. Most animals used for *in vivo* or *in vitro* tests were not fed (adapted) rations containing the compound being studied. Problems associated with these NPN compounds include unpalatability, too rapid hydrolysis or insufficient hydrolysis, and toxicity. Biuret is the most popular NPN compound other than urea. Results of sheep and cattle performance trials indicate that biuret is better utilized by sheep than cattle. An area of general agreement among biuret researchers is that the utilization of biuret becomes more efficient with time. There is no concensus on the length of time needed for maximum utilization, but it is longer than the adjustment period for urea. Welch et al. (1957) and Tomlin (1967) report periods of 35 days and 21 days for maximum utilization of biuret by lambs. Oltjen et al. (1969) report maximum urea utilization in 7 days and maximum biuret utilization in 21 days. *In vitro* studies have shown that an animal adapted to urea is not adapted to biuret. Oltjen et al. (1968) and Johnson and McClure (1964) agree that urea adapted rumen microbes were unable to hydrolyze biuret to ammonia. Farlin et al. (1968) conducted *in vitro* studies of biuret metabolism. When inoculum from urea-fed lambs was used, up to 21% of the ^{14}C from biuret was recovered as $^{14}CO_2$. This indicates that biuret can be hydrolyzed without an adaptation period. When biuret was substituted

for urea in donor rations, ^{14}C recovery declined for nine days then increased by day 17 so that ^{14}C recovery was greater for biuret-fed donor lambs. Recovery was nearly equal after 51 days from either urea-fed or biuret-fed donors. Studies by Farlin et al. (1968) showed that 95% of intravenously injected biuret ^{14}C was recovered in the urine, 2.5% in the rumen, and less than 2% was recovered as expired $^{14}CO_2$. These results further discredit the theory that adaptation occurs in body tissues. This same study by Farlin et al. (1968) compared urea and biuret metabolism in the rumen. Specific activity of expired $^{14}CO_2$ from urea was reached in 80 min, biuret required 175 min after intraruminal administration. Specific activity of $^{14}CO_2$ from biuret remained high for several hours, while that of urea dropped sharply from the maximum value. Because it is apparently slowly hydrolyzed, biuret is less toxic than urea (Hatfield et al., 1959).

The use of diammonium phosphate (DAP) and ammoniated molasses as ruminant nitrogen sources has also been investigated and is reviewed by Helmer and Bartley (1971).

Supplements to Urea. Sulfur deficiency can limit urea utilization when purified diets are used (Thomas et al., 1951). Most common feedstuffs are adequate sources of sulfur (Conrad et al., 1967). Many researchers have failed to demonstrate improved nitrogen utilization from sulfur supplementation of practical rations (Davis et al., 1954). Some benefits have been demonstrated by adding either inorganic or organic sulfur to purified rations which contain small amounts of natural protein (Starks et al., 1954).

Protein levels in the ration influence urea nitrogen utilization. In vitro studies indicate that the solubility of added protein is an

important factor. Wegner et al. (1940) reported that as the concentration of casein increased, urea utilization decreased. Belasco (1954), Burroughs (1951a), Pearson and Smith (1943) and McNaught and Smith (1947) suggested that less soluble proteins would yield less ammonia to compete with urea ammonia and that this competition is the mechanism of decreased urea nitrogen utilization. Protein may aid urea utilization by contributing minerals and energy.

Embry et al. (1957) added fat to urea containing rations and improved nitrogen retention and ration digestibility. Subsequent studies by Bradley et al. (1966) and Thompson et al. (1967) have indicated depressed rate of gain and reduced urea utilization when fat and urea were added to rations.

Carbohydrate. The role of carbohydrates in urea nitrogen utilization is to supply energy and carbon skeletons for protein synthesis. Differences between carbohydrates in their ability to perform this task are significant. Aries et al. (1951) found that any energy source, capable of being digested, aided in urea utilization. Starch was added to an *in vitro* system by Hunt et al. (1954) and urea utilization improved. The studies of Belasco (1955 and 1956) compared several carbohydrate sources. Starch improved urea utilization better than cellulose, xylan or pectin. Urea utilization increased as starch was added in larger amounts. Mills et al. (1944), Bell et al. (1953) and Oltjen and Putnam (1966) agree that starch was superior to soluble sugars such as molasses or glucose in increasing nitrogen retention, and protein levels of rumen contents. Using *in vitro* methods, Barr (1974) evaluated the sugars of hemicellulose extracts for effectiveness in promoting urea nitrogen utilization and found that combinations of soluble carbohydrates occurring in hemicellulose extracts

were equal to or surpassed starch in their ability to increase urea nitrogen utilization.

Molasses has been added to urea containing rations to increase palatability and nitrogen utilization despite data by many researchers which indicate that molasses is poorly utilized to increase microbial protein synthesis (Bohman et al., 1954; Rupel et al., 1943; Potter et al., 1968 and Kercher and Paules, 1967).

Mills et al. (1942) fed hay with urea and demonstrated delayed hydrolysis of urea. Dehydrated alfalfa meal has been found to increase urea utilization by Nelson et al. (1957) and Karr et al. (1965). Conrad and Hibbs (1966 and 1968) indicated that a pelleted combination of urea and dehydrated alfalfa meal (Dehy-100) was an effective source of supplemental nitrogen for high producing cows.

Processing Starch. Boiling starch greatly improved urea utilization compared to raw starch in studies by McNaught (1951). Many have demonstrated that cooking starch has improved its digestibility and made its energy more available for microbial synthesis (Salsbury et al., 1960; Osman et al., 1966; Husted et al., 1968; Theurer et al., 1967; Hale et al., 1965 and Buchanan and Smith, 1968).

Feeding expanded grain (95-100% gelatinized) reduced rumen ammonia levels when compared to unprocessed grain, Colenbrander et al. (1967 and 1968). Meyer et al. (1967) using a mixture of expanded grain plus ground alfalfa hay found rumen ammonia levels only 50% of those values obtained when cracked grain was fed with ground alfalfa. Stiles et al. (1969) investigated a product formed by processing a mixture of finely ground grain and urea through a cooker-extruder under moisture, pressure, and temperature conditions that caused the starch to gelatinize completely

(Starea). Starea was found to be less toxic and more palatable than equal amounts of unprocessed grain plus urea. Stiles et al. (1970), Helmer (1969) and Helmer et al. (1970a and 1970b) reported that Starea reduced ammonia nitrogen levels and increased bacterial protein when compared to unprocessed mixtures of grain plus urea. Also, Starea fed cows consumed more concentrate mixture and produced more milk than cows fed the same grain mixture supplemented with urea.

Muhrer et al. (1968) indicated that a similar product created by heating starch and urea under pressure to 170 C resulted in better nitrogen utilization than from unprocessed urea. They reported that under these conditions urea was converted to ammonia and cyanic acid; these compounds then combined with the starch to form a starch carbamate.

EXPERIMENT I.

Introduction

Optimum conversion of urea to bacterial protein in the rumen requires a carbohydrate source to be available when the urea is hydrolyzed to ammonia. Previous work by Helmer et al. (1970) has shown increased utilization of urea when grain and urea were mixed and processed with a cooker-extruder. The gelatinized starch appears to be better used for bacterial protein synthesis than unprocessed grain starch. The increased utilization is due in part to the cooked starch being metabolized by the rumen bacterial at the same time that the urea is hydrolyzed to ammonia and available for protein synthesis.

Adsorbing urea on diatomaceous earth and coating the mixture with molasses was considered as a method of increasing urea utilization. It

was hypothesized that adsorbed urea on diatomaceous earth would slow the release of urea to ammonia in the rumen and molasses would supply energy and carbon skeletons necessary for the conversion of ammonia to microbial protein. The product was compared with extrusion processed grain and urea (Starea) and unprocessed grain and urea.

Experimental Procedure

Three substrates containing urea and formulated to provide 44% crude protein (CP) were compared *in vitro*. The substrates were an extrusion processed mixture of corn and urea (Starea), an unprocessed mixture of corn and urea, and a mixture of urea (40%), diatomaceous earth (24%), and molasses (36%). The mixture of urea, diatomaceous earth (UDM), and molasses was prepared by adsorbing a solution of urea on diatomaceous earth and coating the mixture with molasses. The mixture was diluted to 44% CP by adding ground corn. The substrates containing 44% CP were compared. Also, they were diluted with ground corn to give 16% CP and then compared. All substrates were finely ground through a 40-mesh screen using an 20.3 cm Christy-Norris laboratory mill.

Rumen fluid inoculum was obtained from a rumen-fistulated, grade Jersey cow (No. 34), fed twice daily 0.91 kg alfalfa hay and 1.82 kg of a grain mixture composed of 8% cracked corn, 27% cracked sorghum grain, 22% dehydrated alfalfa pellets, 30% Starea (44% CP), 11% soybean meal, 1% salt, and 1% dicalcium phosphate. Rumen fluid was collected from the ventral sac of the rumen just prior to the morning feeding, and strained through 4 layers of cheesecloth.

Trichloroacetic acid (TCA) precipitable protein was determined similar to the method of Helmer (1969). Fifty ml rumen fluid was placed in a

250-ml fermentation bottle with 4 g substrate and 60 ml of a mineral-buffer solution (McDougall, 1948) wormed to 39 C. The head-space was flushed with CO_2 for 15 sec and the bottles were capped with Bunsen valves. Bottles were swirled hourly during the 4 hr incubation at 39 C.

Following fermentation, the bottles were placed in ice water for 5 min. Samples were then centrifuged at 114 x g for 5 min. Eighty ml of supernatant was filtered through glass wool into clean 250-ml bottles. Twenty ml of 64.5% TCA was added and allowed to stand for 24 hr. Samples were again centrifuged at 114 x g for 5 min and the supernatant discarded. The precipitate was transferred to tared sintered glass crucibles using acetone to wash the precipitate. A slight vacuum aided in filtration. Crucibles were dried in 100 C oven for at least 1 hr and weighed. A blank containing inoculum and buffer was treated in a like manner to ascertain the TCA soluble protein in the inoculum. Also a blank containing feed and buffer was used to find the TCA precipitable soluble protein in the substrate. The total of these blanks was subtracted from the substrate-inoculum fermentation total.

Ammonia nitrogen determinations were made, using the same fermentation samples. At the end of the 4 hr incubation period, 1 ml samples were removed and ammonia N assayed by the Conway microdiffusion technique (1957). A blank containing inoculum and buffer was also titrated and subtracted from the sample values.

Results and Discussion

The ammonia nitrogen concentration for the 16% CP substrates was lowest for Starea followed by corn plus urea and UDM (Table 1). The difference between the values for Starea and UDM were significant ($P<.05$).

Table 1. Utilization of nitrogen in Starea, corn plus urea, or urea plus diatomaceous earth plus molasses (UDM) as measured *in vitro* by ammonia concentration and TCA precipitable bacterial protein.

Substrate	Ammonia nitrogen[1] (mg/100 ml)			TCA precipitable protein[1] (mg/100 ml)		
	16% CP	44% CP	Avg	16% CP	44% CF	Avg
Starea	46.7a	112.0a	79.4a	65.5a	77.0c	71.3a
Corn + urea	48.4a,b	116.6a	82.5a	63.2a	34.6d	48.9a,b
UDM	51.2b	100.7a	75.9a	33.3a	19.6d	26.4b
Avg	48.8e	109.8f		54.0e	43.7e	

[1]Each value is an average of five determinations made on five different days and conducted in duplicate.

[a,b]Values within columns sharing a common letter do not differ significantly (P>.10).

[c,d]Values within columns sharing a common letter do not differ significantly (P>.05).

[e,f]Mean values between columns sharing a common letter do not differ significantly (P>.05).

No significant differences were found in the ammonia N values of the 44% CP substrates.

The average ammonia N concentration of the 16% CP substrates was significantly less than that of the 44% CP substrates ($P<.01$).

No significant differences were found in the TCA precipitable protein produced by fermentation of the three 16% CP substrates; however, the value for the UDM substrate was considerably lower than that of Starea or corn plus urea. Starea (44% CP) produced more TCA precipitable protein than either corn plus urea (44% CP) or UDM (44% CP) ($P<.01$).

The 16% CP substrates yielded more TCA precipitable protein than did the 44% CP substrates, but this difference was not statistically significant ($P>.10$).

The increase in protein synthesis observed for Starea over an unprocessed mixture of corn plus urea agrees with the results of Helmer et al. (1970) and Barr (1974). However, Helmer et al. (1970) found more protein synthesized with the 16% CP than with 44% CP substrates, and that the 44% CP substrate magnified the difference between Starea and corn plus urea. Adsorbing urea on diatomaceous earth and coating it with molasses lowered the concentration of ammonia nitrogen, but did not increase the quantity of protein synthesized. The efficiency of utilization of urea in the rumen is related, in part, to the rate of release of ammonia from urea. If ammonia is released too rapidly, considerable quantities will be absorbed from the rumen and be unavailable for microbial protein synthesis. However, if the release of ammonia from urea is too slow, there may be insufficient nitrogen available for maximum protein synthesis. It would appear from the results of this experiment that the urea adsorbed on diatomaceous earth and coated with molasses released insufficient nitrogen

for maximum protein synthesis. The processing of grain and urea through a cooker-extruder appears to provide a carbohydrate source which is available and can be utilized at the same time that the urea is being hydrolyzed. This is essential for optimum nitrogen utilization in the production of bacterial protein.

EXPERIMENT II.

Introduction

In vivo utilization of urea improves with time as cattle become adapted to urea containing rations. This experiment was designed to determine if urea adaptation by the inoculum donor influenced urea utilization *in vitro*.

Experimental Procedure

Sorghum Starea (40% CP) was compared with a mixture of urea and sorghum grain (40% CP) utilizing the *in vitro* technique described in Experiment I. Ammonia N and TCA precipitable protein were measured. Twin rumen-fistulated Holstein cows were used as inoculum sources. Both cows initially received 0.91 kg alfalfa hay and 7.26 kg of a concentrate mixture daily. The concentrate mixture was composed of 69% sorghum grain, 29% soybean meal, 1% dicalcium phosphate and 1% salt. One cow (no. 19) remained on this ration for the entire experiment. The other cow (no. 18) was adapted to urea by gradually replacing the soybean meal with urea over a period of 14 days until the ration was 94% sorghum grain, 4% urea, 1% dicalcium phosphate, and 1% salt. This mixture was isonitrogenous with the soybean meal supplemented ration and was fed in equal amounts. Inoculum

was removed from the ventral sac of the rumen via the rumen fistula approximately 12 hr post feeding. In vitro determinations were made every fourth day. The values in Table 2 are the average of the 4 determinations made in duplicate after cow 18 was fed the 4% urea concentrate mixture.

Results and Discussion

Starea substrates produced more TCA precipitable protein than did the mixture of grain plus urea ($P<.05$) (Table 2). Ammonia N concentration was lower for the Starea substrates than the grain plus urea substrates, 97.9 vs 112.5, but not significantly so. This is consistent with the results of Experiment I and those of Helmer (1969).

Fermentations using inoculum from the soybean meal-fed cow (19) yielded less TCA precipitable protein ($P<.10$) than those from the urea-fed cow (Table 2). Ammonia N concentrations were higher for the in vitro fermentations using inoculum from the soybean meal-fed cow (19) and approached significance at the $P=.10$ level. It would appear that adapting the donor animal to urea improves nitrogen utilization in vitro for both Starea and corn plus urea substrates. The enchancement is greater for Starea than for corn plus urea.

Helmer (1969) reported lower TCA precipitable protein values for two Starea substrates when the inoculum donors were fed all roughage rations compared to all concentrate rations containing urea. In vitro ammonia N values were lower when donors were fed all roughage rations, but this was attributed to lack of urease activity in the inoculum.

Table 2. *In vitro* utilization of Starea or grain plus urea substrates using inoculum from either urea-fed (18) or soybean meal-fed (19) donors.

Substrate	Ammonia nitrogen[1] (mg/100 ml)			TCA precipitable protein[1] (mg/100 ml)		
	Urea-fed(18)	SBM-fed(19)	Avg	Urea-fed(18)	SBM-fed(19)	Avg
Starea	89.8	106.0	97.9	65.3	39.8	52.6] a
Grain + urea	101.4	123.5	112.5	29.1	24.2	26.7] a
Average	95.6	114.8		47.2	32.0	
				b		

[1]Each value is an average of four determinations made on four different days and conducted in duplicate.

[a]Significantly different at ($P<.05$).

[b]Significantly different at ($P<.10$).

EXPERIMENT III.

Introduction

Results of Experiment II indicated that a urea adapted inoculum influenced *in vitro* bacterial protein synthesis. This experiment attempted to define further the influence of the inoculum donor ration on *in vitro* nitrogen utilization. Rations for the donor animals were supplemented with Starea or grain plus urea. Substrates for *in vitro* fermentation were Starea or grain plus urea. In this way substrate versus inoculum donor interactions could be determined.

Experimental Procedure

In vitro fermentations and measurements followed the same procedure as Experiment I. Four fistulated Jersey cows were used as inoculum donors. All were fed 3.64 kg daily of a concentrate mixture [sorghum grain (67%), soybean meal (31%), salt (1%), and dicalcium phosphate (1%)] and 1.82 kg alfalfa hay for a period of 8 days. Rumen fluid was obtained for *in vitro* fermentations with either sorghum Starea or sorghum grain plus urea three times as shown in Table 3. At the end of the preliminary period, two cows were fed 3.64 kg daily of a concentrate mixture of sorghum (94%), urea (4%), dicalcium phosphate (1%), and salt (1%). Also, 1.82 kg of alfalfa hay was fed daily. Concentrate mixture remaining after 30 min was administered via the rumen fistula. A concentrate mixture of sorghum (67%), Starea (31%), dicalcium phosphate (1%), and slat (1%) was fed to the remaining cows. The cows were fed daily 3.64 kg concentrate mixture and 1.82 kg alfalfa hay. Rumen fluid was obtained for *in vitro* fermentations twice weekly. All four cows were placed on the soybean ration for 3 weeks, and then changed to either urea or Starea supplemented rations. The two

Table 3. In vitro utilization of Starea or grain plus urea substrates using Starea-fed or urea-fed donor animals for inoculum.

	Ammonia nitrogen (mg/100 ml)				TCA precipitable protein (mg/100 ml)			
	Substrate				Substrate			
Day	Starea	Urea	Starea	Urea	Starea	Urea	Starea	Urea
Pre	Soybean meal-fed				Soybean meal-fed			
1	51.9	64.0	58.3	78.3	31.2	- .7	32.2	11.1
4	39.6	48.8	58.1	65.9	32.0	1.7	34.8	4.8
8	59.4	59.6	74.9	79.9	35.8	5.4	45.0	7.7
Avg	50.3a	57.5c	63.8a	74.7a	33.0e	2.1g	37.3a	7.9i
Exp	Starea-fed		Urea-fed		Starea-fed		Urea-fed	
11	32.2	40.3	43.9	51.6	20.3	2.5	44.4	5.4
15	45.5	57.9	58.5	62.5	28.7	3.3	43.6	7.1
18	38.5	46.1	38.2	47.4	24.0	12.6	59.4	14.5
22	37.7	51.6	54.1	58.9	19.4	4.6	33.4	1.1
25	32.1	42.2	49.5	60.3	11.6	8.7	72.9	17.8
29	38.6	49.3	38.9	47.1	32.6	22.6	53.3	8.5
32	10.8	37.4	47.7	60.9	36.8	16.7	61.6	15.3
Avg	33.8b	46.4d	47.3b	55.5b	24.8f	10.1h	52.7b	10.0j
	Starea-fed donor		Urea-fed donor		Starea-fed donor		Urea-fed donor	
Avg	40.1k		51.41		17.4m		31.3n	
	Starea substrate		Urea substrate		Starea substrate		Urea substrate	
Avg	40.5k		51.01		38.7o		10.0p	

[1]Each value is an average of one determination made in duplicate on four different cows on one day.

[a,b]Values within columns sharing a common letter do not differ significantly ($P>.01$).

[c,d]Values within columns sharing a common letter do not differ significantly ($P>.06$).

[e,f]Values within columns sharing a common letter do not differ significantly ($P>.09$).

[g,h]Values within columns sharing a common letter do not differ significantly ($P>.10$).

[i,j]Values within columns sharing a common letter do not differ significantly ($P>.50$).

[k,l]Values between columns sharing a common letter do not differ significantly ($P>.05$).

[m,n]Values between columns sharing a common letter do not differ significantly ($P>.10$).

[o,p]Values between columns sharing a common letter do not differ significantly ($P>.01$).

cows receiving the Starea supplement were given the urea ration and the two receiving urea supplement for the first half received Starea. All rations were isonitrogenous. Rumen fluid for in vitro fermentation with either Starea (44% CP) or sorghum grain urea (44% CP) substrates was obtained twice weekly. Three samples were fermented while the donor cows were on soybean meal supplementation and 7 samples were fermented while the cows were supplemented with urea or Starea.

Results and Discussion

Starea substrate fermentations resulted in lower ammonia nitrogen concentrations than grain plus urea substrates ($P<.05$). TCA precipitable protein was significantly lower ($P<.01$) for grain plus urea substrates than for Starea substrates. This agrees with the results of Helmer et al. (1970) and Barr (1974).

Average values obtained when donors were fed soybean meal were different from those obtained when donors were fed urea or Starea (Table 3). Ammonia nitrogen values were lower ($P<.01$) for the Starea-fed, Starea substrate; urea-fed, Starea substrate; and urea-fed, urea substrate than for the same substrates tested during the preliminary period when the cattle received soybean meal. The Starea-fed, urea substrate average value was lower ($P<.06$) than the average value obtained during the soybean meal feeding period. The trend of increased nitrogen utilization is also apparent when measured by TCA precipitable protein. Starea-fed, urea substrate; urea-fed, Starea substrate; and urea-fed, urea substrate averages were higher than the same substrate averages obtained when inoculum was obtained from soybean meal-fed donors ($P<.10$, $P<.01$ and not significantly different, respectively). The Starea-fed, Starea substrate

value was lower ($P<.09$) than the average value obtained from fermentations using soybean meal-fed donors. Barr (1974) reported that a polysaccharide layer rich in bacteria was removed during the low speed centrifugation used to remove feed particles remaining after fermentation. This layer was evident only with substrates containing gelatinized starch. This can account for the loss of considerable bacterial protein and is a possible explanation for the decreased TCA precipitate found in this treatment.

This same phenomenon may explain the lower TCA precipitable protein values found for fermentations using Starea-fed donor inoculum compared with urea-fed donor inoculum ($P<.10$). Ammonia nitrogen values for Starea-fed donors were lower ($P<.05$) than those from urea-fed donors.

CONCLUSIONS

1. More bacterial protein is synthesized from Starea than from mixtures of grain plus urea.

2. Adsorbing urea on diatomaceous earth and coating the mixture with molasses slows down the release of ammonia, but reduces microbial protein synthesis. Apparently the urea is so well adsorbed on the diatomaceous earth that ammonia is not available for microbial protein synthesis.

3. Inoculum from urea-fed donors synthesize more protein from urea containing substrates than inoculum from soybean meal-fed donors.

SUMMARY

Experiment I. In vitro values of TCA precipitable protein and

ammonia nitrogen indicate that the NPN of Starea was better utilized than ground grain plus urea or a mixture of urea, diatomaceous earth and molasses. The three substrates were compared at 16% CP and 44% CP equivalents. Substrates containing 16% CP produced lower ammonia nitrogen and slightly higher TCA precipitable protein values than those containing 44% CP. Differences between substrates were magnified at the 44% CP level.

Experiment II. Inoculum from a urea-fed donor or from a soybean meal-fed donor was incubated with isonitrogenous Starea or grain plus urea substrates. Starea fermentations produced larger amounts of TCA precipitable protein and smaller ammonia nitrogen concentrations than those produced by grain plus urea. Fermentations using inoculum from the urea-fed donor appeared to give the best utilization of the NPN of either substrate.

Experiment III. Starea was better utilized than grain plus urea under the in vitro conditions of this experiment. Rations fed the donor animals influenced the in vitro results. Inoculum from soybean-fed donors did not utilize NPN as efficiently as when the donors were fed either urea or Starea. Ammonia nitrogen values were lower for fermentations using inoculum from Starea-fed donors, but TCA precipitable protein values were greatest when inoculum was obtained from urea-fed donors.

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EFFECT OF RATIONS FED DONOR COWS ON IN VITRO PROTEIN SYNTHESIS FROM STAREA OR GRAIN PLUS UREA SUBSTRATES.

by

THOMAS BURT AVERY JR

B. S., Kansas State University, 1968
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In vitro techniques are useful for determining how non-protein nitrogen (NPN) is utilized by ruminants. Previously uninvestigated NPN compounds, different energy sources and treatments, or quality control of commercial products can be evaluated without the expense and time required for *in vivo* trials.

Previous work has shown that more protein is synthesized *in vitro* from extrusion processed mixtures of grain and urea (Starea) than from unprocessed mixtures of grain and urea. Slowing urea hydrolysis in the rumen by adsorbing urea on diatomaceous earth and coating the mixture with molasses may enhance protein synthesis. Experiment I compared *in vitro* protein synthesis from a mixture of urea, diatomaceous earth and molasses (UDM) with Starea or a mixture of unprocessed grain plus urea. Experiments II and III defined some of the effects which the ration being fed to the inoculum donor had on *in vitro* protein synthesis.

Experiment I. Substrates were compared at either 16% crude protein (CP) or 44% CP levels. The nitrogen of Starea was better utilized than that of grain plus urea or UDM. Substrates containing 16% CP appeared to be better utilized than those containing 44% CP; however, differences between substrates were magnified at the 44% CP level.

Experiment II. A pair of identical twin cattle was used as inoculum donors to determine if rumen fluid inoculum from animals adapted to urea influence urea utilization *in vitro*. One animal was supplemented with urea and the other with soybean meal. Starea and unprocessed grain plus urea were used as substrates. Microbial protein synthesis was greater using inoculum from the urea-fed donor than from the one fed soybean

meal. The NPN of Starea was better utilized than that of unprocessed grain plus urea.

Experiment III. Starea and unprocessed grain plus urea were used as substrates. Inoculum donor rations were initially supplemented with soybean meal and then changed to either urea or Starea. Both substrates were better utilized during the period the donors were fed urea or Starea than when fed soybean meal. Ammonia nitrogen values were lower for fermentations using inoculum from Starea-fed donors, but trichloracetic acid precipitable protein values were greatest when inoculum was obtained from urea-fed donors.