EFFECTS OF DEFAUNATION AND FAUNATION ON NITROGEN METABOLISM OF RUMINANTS

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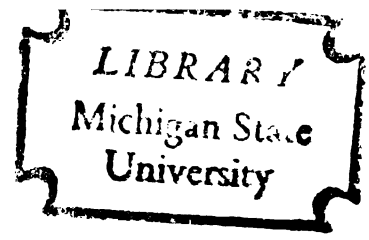
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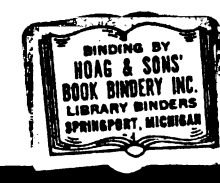
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ABSTRACT

EFFECTS OF DEFAUNATION AND FAUNATION ON NITROGEN METABOLISM OF RUMINANTS

By

JAMES ROBERT MALES

Twelve, two year old Cheviot, wethers were incorporated into four treatment combinations, which consisted of: urea infusion plus protozoa, water infusion plus protozoa, urea infusion with no protozoa and water infusion with no protozoa. Defaunation was accomplished using dioctyl sodium sulfosuccinate. An attempt was made to raise rumen ammonia-N levels of defaunated sheep 4 mg. percent above that of the protozoa water infused control animals by infusion of urea at a rate of .123% of the ration fed per day. Metabolism trials of three weeks duration were initiated to study the effects of the different treatment combinations.

Defaunation resulted in higher rumen dry matter percentages when compared to the faunated sheep. This difference was significant ($P<.01$) at T0 sampling time. Faunated animals had rumen pH values of 6.19 and 5.66 compared to 5.80 and 5.33 for defaunated sheep at the T0 and T2 sampling times, respectively ($P<.10$). The rumen pH values for mean urea infusion were significantly ($P<.10$) higher than the values observed when water was infused. There were no differences observed in any of the nitrogen metabolism parameters for

defaunated sheep receiving either the urea infusion or the water infusion. Urea infusion greatly reduced nitrogen utilization (33.86% vs. 53.28%) and nitrogen balance (3.064 vs. 5.200) in faunated animals and raised fecal nitrogen as a percent of nitrogen intake (31.31% vs. 27.61%). It was concluded that with a high protein ration, the excess nitrogen supplied in the urea infusion could not be adequately used by the microbial population present in faunated sheep.

Sheep with protozoa present in their rumen ecology had a higher level of rumen ammonia-N (11.64 mg./100 ml) than was observed in sheep without protozoa (7.07 mg./100 ml). Urea infusion raised rumen ammonia-N levels above the level that was observed for the water control animals; however, this difference between water and urea infusion was more pronounced for faunated sheep (14.58 mg.% vs. 8.71 mg.%) than for defaunated animals (7.64 mg.% vs. 6.44 mg.%). An *in vitro* fermentation was designed to further study this effect. Ammonia levels *in vitro* were similar for all treatments including urea or for all treatments including only water. It is hypothesised that the greater bacterial concentrations in defaunated ruminants have a more rapid utilization of ammonia-N and therefore levels are observed to be lower in the protozoa free animals.

Pooled data for ammonia production and acetate:propionate ratios, acetate:butyrate ratios, and propionate:butyrate ratios were fitted to linear regression equations. From these regressions it was concluded that at low levels of rumen ammonia-N and molar percent propionate was high

and as rumen ammonia-N levels increased there was a shift from propionate to acetate and butyrate. This trend was observed for three different rations of varying protein levels and also for urea and water infusion for both faunated and defaunated ruminants.

EFFECTS OF DEFAUNATION AND FAUNATION ON NITROGEN METABOLISM OF RUMINANTS

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I. Introduction

A trend toward higher nitrogen retention and digestibility by lambs with protozoa (faunated) compared to lambs that do not have protozoa (defaunated) has been observed, expecially when low protein rations are fed. Previous work has also indicated that the presence of protozoa in the rumen ecology results in significantly higher rumen ammonia-N levels. This suggests two possibilities for the more favorable nitrogen balance observed in faunated animals. The first possibility is that the presence of protozoa in the rumen could directly enhance the nitrogen retention. In other words that the protozoa are of greater nutritional value than are the bacteria. Another possibility may be that the higher ammonia-N levels in the rumen enhance greater bacterial activity and this in turn raises nitrogen retention.

The higher rumen ammonia-N level associated with protozoa raises more interesting possibilities. It suggests that most of the proteolytic activity in the rumen is either due directly to ciliate protozoa or to bacteria that are closely associated and dependent on protozoa. The greater bacteria concentrations in protozoa free animals, on the other hand, could have a much quicker utilization of rumen ammonia-N.

This study was designed to study the effect of an elevated rumen ammonia-N level in protozoa free ruminants. Urea was infused directly into the rumen in an attempt to raise

ammonia-N levels of defaunated lambs to a level similar to that of faunated control animals. Fermentations *in vitro* were also initiated to study the effect of higher bacterial concentrations on digestibility and volatile fatty acid production. The true effect of protozoa on the nutrition of the ruminant has long been questioned and it is hoped that this study will further extend the nutritionist understanding of the effect of protozoa in ruminant nutrition.

II. Literature Review

Due to the problems encountered in culturing protozoa *in vitro*, especially in the absence of bacteria, defaunation of ruminants has become a popular method of studying the effects of protozoa on rumen fermentation.

HCL administration, starvation, milk feeding, cooper sulfate administration, overfeeding, and various combinations of these methods have been used to remove protozoa from ruminants (Hungate, 1966). Another method that has been used, which less drastically affects the animals health, is isolation (Akkada and el-Shazly, 1964; Bryant and Small, 1960; Eadie and Hobson, 1962). This method has a larger animal space requirement and there is some question of whether the rumen bacteria population is typical (Church, 1969).

Two methods of defaunation used on adult animals, which less drastically affects the animal's health, have been used.. Eadie and Oxford (1957) removed the rumen contnets and heated them at 50°C, while washing the rumen with a saline solution. Some problems have been encountered with this method in removing all protozoa. Dioctyl sodium sulfosuccinate, administered on two successive days, eliminated all types of ciliate protozoa without changing the parameters of normal rumen processes which were measured (Akkada *et al*. 1968).

Many parameters have been used to measure the affect of protozoa on ruminal processes. There is some discrepancy in

the results reported in the literature; however, these differences may be due to the method of defaunation used and to the ration being fed. In the following review, the work that has been done with protozoa free animals is summarized. An attempt will be made to associate results with the type of ration fed, level of protein in the ration, and the method of obtaining protozoa free animals that was used.

Growth Data: Nearly all the growth data available in the literature comes from experiments using inoculated and isolated animals; therefore, different bacterial populations in isolated animals compared to inoculated controls may give a difference in observed results. When alfalfa hay was fed free choice with a grain ration, inoculated calves had slightly better gains, while the isolated calves had poorer hair coats and were slightly pot-bellied (Pounden and Hibbs, 1950). In a later trial at the same station (Hibbs and Conrad, 1958), when high roughage pellets were fed, slightly improved gains were obtained from isolated calves. More recently, significantly higher gains for inoculated lambs than for isolated lambs, which were pot-bellied and had a rough hair coat, were reported by Akkada and el-Shazly (1964). Certain large oval organisms and Oscillispira were observed in rumen contents from isolated lambs used in this study, these organisms were not present in inoculated animals. No differences in growth data from isolated and inoculated animals have been observed in two recent studies (Bryant and

Small, 1960; Chalmers et al. 1968); however, Chalmers et al. (1968) did observe a significantly greater body girth measurement from ciliate free lambs. Using copper sulfate as the defaunation agent, Christiansen et al. (1965) observed higher gains and better feed efficiency with faunated lambs.

Bacteria Concentration: The removal of ciliate protozoa from the rumen alters the bacterial concentration in the rumen. Higher bacteria counts were obtained from isolated calves (Bryant and Small, 1960), except when a cellulose medium was used to cultivate bacteria and the highest counts were obtained from inoculated calves. Bacteria concentrations in protozoa free lambs have been found to be nearly two times greater than bacteria concentrations from faunated animals (Eadie and Hobson, 1962; Klopfenstein et al., 1966). Hungate (1966) suggests that this difference in bacterial numbers can be attributed to either competition for food or to consumption of bacteria by protozoa. Very little work has been done to identify bacterial species in defaunated ruminants; therefore, it is not known how the bacteria population in the presence of protozoa compares to the bacteria population when protozoa are not in the rumen ecology. This lack of information as to the bacterial species present in defaunated animals could be an important key to better understanding the effect of protozoa on ruminant nutrition.

Dry Matter Digestion: Dry matter digestion is usually higher when protozoa are present; most deviations from this

trend can be attributed to either ration composition or to the amount of ration fed. In two trials using inoculated and isolated dairy calves, the inoculated calves had dry matter digestibilities 3% higher than those obtained with isolated calves (Conrad and Hibbs, 1953; Hibbs and Conrad, 1958). Dry matter digestibilities of 67.1% for faunated and 65.7% for defaunated lambs on a soybean meal treatment and 66.0% for faunated and 64.1% for defaunated lambs on a urea treatment were obtained by Luther and Perkins (1968). When 1200 g. per day of a semipurified diet were fed, Yoder et al. (1964) obtained dry matter digestibilities of 64.0% for faunated lambs and 58.3% for defaunated lambs; however, these differences were not observed when only 720 g. of the same ration were fed. When a ration consisting of 500 g. of cottonseed cake and rice bran, 3,500 g. of berseem and 250 g. of wheat straw were fed, dry matter digestibilities of 69% for inoculated lambs and 66.5% for uninoculated lambs were observed (Akkada and el-Shazly, 1965). There is an indication that the differences in dry matter digestibility between faunated and protozoa free animals is dependent on the amount of energy present and independent of the amount of protein in the diet. A high protein, low energy diet gave no differences in dry matter digestion, but a low protein, high energy diet gave dry matter digestibilities of 73.7% for faunated lambs compared to 64.2% for defaunated lambs, while a high protein, high energy diet gave dry matter digestibilities of 78.0% for lambs with protozoa and 73.7% for

ciliate free lambs (Klopfenstein et al. 1966).

Cellulose Digestion: Cellulose digestion has been higher with protozoa present in the rumen ecology. Cellulose digestion, of 64.4% and 50.5% for inoculated calves compared to 61.3% and 49.3% for isolated calves, was observed by Conrad and Hibbs in two trials (Conrad and Hibbs, 1953; Hibbs and Conrad, 1958). Higher cellulose digestion, when protozoa were present, 53.5% for inoculated vs. 49.3% for isolated lambs, was observed by Akkada and el-Shazly (1965). A considerably greater cellulose digestion from lambs with protozoa compared to lambs without protozoa, 43.3% and 30.1% respectively, were observed by Yoder et al. (1964). As was the case for dry matter digestion, these differences were only measured at higher feed intakes, suggesting that cellulose digestion may also be dependent on energy levels of the diet. The increased cellulose digestion observed for faunated animals may not be due directly to the protozoa, but may be as a result of bacteria closely associated and dependent on protozoa. As was previously reported when a cellulose medium was used to cultivate bacteria, the highest bacteria counts were obtained from animals with protozoa (Bryant and Small, 1960).

Volatile Fatty Acids: The effects of protozoa on volatile fatty acid production has produced considerable discrepancy in the literature; however, there seems to be a trend towards lower propionate and higher butyrate when protozoa

are part of the rumen ecology. Conrad et al. (1958) obtained a higher propionate and lower butyrate level for isolated calves, while inoculated animals had lower propionate and higher butyrate levels. Using an in vitro fermentation system, Luther et al. (1964) obtained significantly ($P<.01$) lower acetate and propionate when protozoa were added to the fermentation system and significantly ($P<.01$) increased levels of butyrate, valerate and branched chained fatty acids. No differences in total volatile fatty acid production, but an increased proportion of butyrate and a higher acetate to propionate ratio when protozoa were present was observed by Klopfenstein et al. (1966). Higher levels of propionate have been obtained from lambs with protozoa (Akkada and el-Shazly, 1964; Christiansen et al.,1965; Luther and Perkins, 1968), and these lambs have had a higher total volatile fatty acid production (Christiansen et al., 1965; Luther and Perkins, 1968).

Blood Constituents: Blood urea levels have been higher when protozoa are present. There are some exceptions to this trend due to the type of protein supplement used or to the level of protein in the diet. Slightly higher blood urea levels from faunated lambs when a urea supplemented diet was used, were obtained by Luther and Perkins (1968); however, when they fed a soybean meal diet there were no differences in blood urea levels observed. Higher plasma urea levels from faunated lambs on a high protein ration and higher

plasma urea levels from ciliate free lambs on a low protein ration have been reported (Klopfenstein et al., 1966). Isolated lambs had higher blood urea nitrogen levels when they were on a low protein ration (Akkada and el-Shazly, 1965).

Very little work has been done concerning the effect of defaunation on any other blood constituents. Faunation has been shown to increase oleic acid and decrease linoleic and stearic acid (Klopfenstein et al., 1966), while also significantly changing the stearic:oleic and palmitic: stearic acid ratios from those of protozoa free lambs. These results were corroborated by Lough (1968). Protozoa free lambs were suggested to have lysine as a limiting amino acid, while faunated lambs were suggested to have no amino acid consistently limiting (Klopfenstein et al., 1966).

Nitrogen Metabolism

Nitrogen and Crude Protein Digestion: There are considerable differences in nitrogen digestibilities as reported in the literature. Nitrogen digestibility is quite dependent on the level of protein in the ration, the amount of energy in the ration and on the amount of ration that is fed. When feeding a low protein diet, inoculated lambs and calves had slightly higher crude protein digestibilities (Akkada and el-Shazly, 1965; Conrad and Hibbs, 1953); however, this same trend was observed by Hibbs and Conrad (1958) when they fed high roughage pellets and obtained crude protein digestibilities of 65.2% for inoculated calves,

while isolated calves digested 63.1% of the crude protein in their ration. Feeding 1200 g. of a semipurified diet gave nitrogen digestibilities of 62.8% for faunated lambs and 55.6% for protozoa free lambs; however, these differences were not obtained when only 720 g. of the same ration were fed (Yoder et al., 1964). No differences in nitrogen digestibility were observed when a high protein low energy diet was fed (Klopfenstein et al., 1966), but a low protein, high energy feed and a high protein, high energy ration gave digestibilities of 64.3% and 73.0% for faunated lambs and 61.7% and 63.0% for protozoa free lambs, respectively. No differences in nitrogen digestibilities between faunated and ciliate free lambs were observed by Chalmers et al. (1968).

Nitrogen Retention: Higher nitrogen retentions from faunated lambs have been obtained on low protein rations, while high protein rations have tended to give higher nitrogen retention in protozoa free lambs. On a low protein ration, Akkada and el-Shazly (1965) obtained significantly higher nitrogen retention from inoculated lambs and Klopfenstein et al. (1966) observed a significantly ($P<.05$) higher nitrogen retention from lambs with protozoa. On high protein rations ciliate free lambs had higher nitrogen retention (Klopfenstein et al.,1966); however, these differences were not significant.

Ammonia: Ammonia constitutes the main source of non-protein nitrogen in the rumen (McDonald, 1952) and comes from the breakdown of dietary protein to ammonia, carbon

dioxide, and volatile fatty acids (Bryant and Robinson, 1963). A high correlation between rumen ammonia and the concentration of the combined branched chain fatty acids was observed by Jamieson (1959). Seven species of bacteria have been found to be proteolytic (Akkada and Blackburn, 1963) and at least one species of protozoa, Entodinium caudatum, has been shown to hydrolyse amide groups of casein to ammonia (Akkada and Howard, 1962). Warner (1956) suggests that half the proteolytic activity in the rumen is due to bacteria and further postulates that much of the continuing ammonia production in the rumen, in the absence of readily attacked protein, might be due to the endogenous metabolism of rumen protozoa. Ammonia production is of a greater magnitude in the presence of bacteria with protozoa absent. The greatest production of ammonia-N from glutamine was obtained using a bacteria rich in vitro inoculum (Hoshino et al., 1966). Warner (1956) also observed an increased ammonia concentration from bacteria. Ammonia in the presence of carbohydrate can be utilized for production of bacterial protein or it can be absorbed through the rumen wall and then excreted as urea (Hobson, 1959). McDonald (1948) suggests that the absorption of ammonia into the blood stream may be of the magnitude of 4-5 g. per day; however, this absorption is probably dependent on the level of ammonia in the rumen. The highest blood urea levels were obtained with rations that caused a high rumen ammonia-N level (Klopfenstein et al., 1966) and a low plasma urea level was

observed when a low protein ration was fed and rumen ammonia-N level in turn was low. Many species of rumen bacteria require ammonia and are inefficient in utilizing amino acid carbon (Bryant and Robinson, 1963). When given the choice between ammonia and preformed amino acids, rumen bacteria preferentially utilize ammonia (Akkada and Blackburn, 1963). Removal of protozoa from the rumen ecology lowers the ammonia-N levels (Akkada and el-Shazly, 1964; Chalmers et al., 1968; Christiansen et al., 1965; Klopfenstein et al., 1966; Luther and Perkins, 1968). This lower ammonia level from ciliate free animals has been shown regardless of the type ration fed, the amount of ration, or the method of defaunation used; however, the magnitude of the difference between faunated and defaunated animals varies with the amount of protein.

Urea: Feeding urea has long been of interest, due to its availability and economy as a source of nitrogen. An excellent review on non-protein nitrogen as the primary source of nitrogen has been recently published (Oltjen, 1969), and urea as a protein supplement is reviewed by Briggs (1967). This short review is designed to only touch on effects of urea supplementation associated with this work and is far from all inclusive. Feeding urea has been shown to give higher rumen ammonia levels (Hoshino et al., 1966; Hemsley and Moir, 1963; Johnson and McClure, 1964), and a higher concentration of total volatile fatty acids (Hemsley and Moir, 1963). As early as 1939, urea was shown to give

nearly as good gains as did casein and that the most efficient utilization of urea was obtained when some soluble sugars were included in the diet (Hart et al., 1939). Adding urea to the diet has increased dry matter digestion, cellulose digestion, and nitrogen digestion (Johnson and McClure, 1964). Adding urea to a basal ration significantly increased dry matter intake, increased nitrogen digestion and retention, but lowered dry matter digestion (Hemsley and Moir, 1963).

III Materials and Methods

A. Experiment In Vivo

Experimental Design

The original experimental design of this project was a double 4 x 4 latin square with the four variables consisting of urea supplementation vs. a water control and the presence or absence of protozoa. Due to problems encountered with the original eight sheep, only three of these lambs completed the entire experiment. Consequently, a total of twelve sheep were used; six with each of the water infusion treatments and seven with each of the urea treatments, and a lease squares analysis of variance was used to determine statistical significance. The treatments used consisted of urea infusion plus protozoa, water infusion plus protozoa, urea infusion with no protozoa and water infusion with no protozoa. During each feeding period the sheep were allowed a thirteen day adjustment period. After one day in metabolism stalls, feed intake, urine production and fecal output were measured and sampled for analysis. This sampling period lasted for six days; two days later rumen samples and jugular blood samples were obtained at feeding and at two and four hours post-feeding. In later references these times will be referred to as T0, T2 and T4, respectively.

Defaunation

Removal of protozoa was accomplished, using a slightly modified method of Akkada et al., (1968). Dioctyl sodium sulfosuccinate, under the trade name Complemix and furnished

by American Cyanamid Company, was used as the defaunation agent. Twelve c.c. of this compound were infused directly into the rumen on three successive days. Feed was withheld at each administration of Complemix. On the second day of treatment, the rubber cannula was removed, completely cleaned and then disinfected and replaced. At this same time all wool was clipped from around the cannula and this area was disinfected. After two to four days the sheep were consuming their entire ration and were free of ciliate protozoa. These animals were then kept isolated from all other ruminants and the rumen contents were regularly checked for the absence of protozoa.

Urea

An objective of this project was to raise the rumen ammonia-N level of protozoa free wethers 4 mg. percent above that of the control animals. The estimated average rumen size of the sheep used was four liters. In a rumen this size, the desired increase in ammonia-N would require an increase of .16 grams, which could be accomplished with .32 grams of urea. Urea nitrogen was assumed to have a half life of two hours and it was assumed that urea would be recycled at a level of 30 to 50 percent. From this it was estimated that an average of 1.00 gram of urea per day should be adequate to raise rumen ammonia-N levels the desired 4 mg. percent. This was calculated to represent .123 percent of the ration fed per day to a particular sheep and therefore when urea was infused into the animals it was always at this proportion of the ration fed. Sodium sulfide was added to the

urea solution at a ratio of fifteen parts urea nitrogen to one part of sulfur. This mixture of urea and sodium sulfide was infused in 480 ml. of water each day. The control animals received an infusion of 480 ml. of water. A model 954, four channel, infusion/withdrawal pump (Harvard Apparatus Company) was used to make the urea infusion.

Sheep

The ruminants used in this experiment were two-year-old Cheviot wethers that averaged 32 kg. prior to the first two treatment periods and 28 kg. prior to the final two perids. All animals used in this project were fitted with rumen cannulas (Jarrett, 1948) prior to the first experimental period.

Feeding Regime

The sheep were fed twice daily, at 8 a.m. and 5 p.m., receiving six percent of their metabolic body weight ($B.W.^{-.75}$) per day. The ration composition (Table 1) was similar to that fed by Klopfenstein et al. (1966).

Table 1. Ration

Ingredient	Kg. per 100 kg. of Mix
Alfalfa Meal	38.00
Corn Cobs	7.80
Ground Shelled Corn	47.00
Cerelose	4.55
Minerals	2.40
Vitamins	0.25

The minerals and vitamins were supplied in a mix (Table 2).

Table 2. Mineral and Vitamin Mix

Ingredient	Kg. per 100 kg. of Mix
Dicalcium Phosphate (26.5% Ca-20.5% P)	47.38
Trace Mineralized Salt (High Zinc)	47.42
Sodium Sulfate (25.5% Sulfur)	4.78
Vitamin A (10,000 IU/g)	.32
Vitamin D (9,000 IU/g)	.10

Only sheep that consumed their entire ration were used in the statistical analysis. Daily portions were weighed at one week intervals and a sample was taken each week for dry matter determinations. This sample was oven dried at 105°C for 24 hours and saved for later analysis.

Sample Collections

Total fecal collections were made using a canvas bag collection harness. The daily feces were weighed and a sample was taken for dry matter and nitrogen determinations.

Urine was collected in two liter glass bottles. Each bottle contained 25 ml. of 20 percent sulfuric acid. The total volume was measured, then the collection was diluted up to two liters and one sixth of this dilution was retained for further analysis.

Rumen contents (Purser and Moir, 1959) and jugular blood were sampled at T0, T2 and T4 as previously described. Rumen samples were measured for pH using a Corning model 12 pH meter; a portion of the rumen sample was then oven dried at 105°C for 24 hours to determine rumen dry matter. Eleven g. of the T0 rumen sample were diluted in 99 ml. of an anaerobic dilution solution, under anaerobic conditions. Further dilutions to 10^{-8} were made and bacterial counts were measured using the method of Hungate (1966) and the media of Bryant and Robinson (1961). Twenty ml. of whole rumen contents were mixed with twenty ml. of a 50 percent formaldehyde solution and retained for protozoa counts (Purser and Moir, 1959). The T0, T2 and T4 samples of whole rumen contents were strained through two layers of cheesecloth and 19 ml. of the resulting fluid was mixed with 1 ml. of saturated mercuric chloride. This mixture was retained for volatile fatty acid and rumen ammonia-N analyses. The whole blood was centrifuged at 12,100 x g for fifteen minutes and the plasma was retained for plasma urea determinations.

Nitrogen Determinations

The dried feces retained from each day's sample was thoroughly mixed and then ground through a 20 mesh screen in a Wiley mill. Total nitrogen was determined on this ground sample by the micro-Kjeldahl method. Oven dried feed samples were analyzed for total nitrogen by the same method. The urine was thoroughly mixed and a subsample was used to

determine total urine nitrogen using the micro-Kjeldahl procedure.

Rumen and Plasma Analyses

Rumen volatile fatty acid concentrations were determinded on a Packard Gas Chromatograph. Five ml. of rumen fluid were mixed with one ml. of 25 percent metaphosphoric acid, centrifuged at 12,100 x g for 10 minutes and the supernatant retained. The peak heights were converted to micromoles of acid with the aid of standards. Rumen ammonia-N concentrations were determined using the micro-diffusion method of Conway (1950). Plasma urea concentrations were also determined using the micro-diffusion method. Jackbean urease was used to release the urea and samples were corrected for ammonia levels in the plasma.

B. Experiments In Vitro

The fermentation system used was an adaption of the Ohio system (Karn, et al. 1967). Two grams of the ration, used in vivo and ground through a 40 mesh screen in a Wiley mill, was used as the substrate. The media, a mixture of biotin, para-amino-benzoic acid, valeric acid, urea, and mineral mix was similar to that used by Dehority (1961). Rumen fluid inoculum, obtained from the same lambs used in vivo, was strained through two layers of cheesecloth. The strained fluid was then either used as whole contents or centrifuged at 250 x g for 3 minutes and the supernatant used as the inoculum. In all further discussion the inoculum will be

referred to as whole contents or supernatant. The amount of inoculum used varied depending on the experiment. The total volume of each fermentation bottle was 100 ml., with the difference between inoculum levels made up with media. Carbon dioxide was continually bubbled through the system. Every three hours for the first twelve hours and each twelve hours thereafter, pH was adjusted to 6.7.

Dry matter digestion was determined by centrifuging subsamples from each fermentation system in 40 ml. pyrex tubes at 5,000 x g for fifteen minutes. In the last two experiments the supernatant was saved for volatile fatty acid determinations. The sediment remaining in the centrifuge tube was washed twice with distilled water and then oven dried at 105°C for 24 hours. The dried material was then weighed to determine the percent dry matter in the fermentation system. The dried sample was then subjected to cellulose analyses by the method of Crampton and Maynard (1938).

Fermentation A

This experiment was designed to determine the extent of ammonia-N production from urea with defaunated and faunated lambs. Urea was omitted from the media in half the fermentation bottles; these bottles were used as a control. Treatment combinations consisted of: urea plus whole content inoculum, no urea plus whole content inoculum, urea plus supernatant inoculum and no urea plus supernatant inoculum. Inoculum was obtained from both faunated and defaunated lambs. A total of 32 fermentation bottles were used. Subsamples were

taken at zero time, and at three hours, six hours, nine hours and twelve hours after inoculum was added. At T6, pH was adjusted to 6.7; in all other experiments pH was adjusted every three hours for the first twelve hours and thereafter every twelve hours until the end of the experiment. Each subsample was analyzed for ammonia-N using the micro-diffusion method of Conway (1950). Dry matter digestion was determined for the twelve hour period.

Fermentation B

The second fermentation in vitro was designed to determine the effect of varying inoculum levels on dry matter and cellulose digestion. Urea was used in all media for this experiment. The treatments consisted of whole contents and supernatant of rumen fluid from faunated and ciliate free sheep and inoculations of 12.50 ml. and 25.00 ml. were used. Eight sheep were used as inoculum donors and the fermentation system consisted of 32 bottles. Dry matter and cellulose digestion were determined at 24 and 48 hours after the fermentation began.

Fermentation C

This experiment was a repeat of the previous experiment. Only faunated lambs were used as inoculum source. Total volatile fatty acid production was determined in this experiment.

Fermentation D

This fermentation *in vitro* was a continuation of experiment B and C. Inoculum levels were halved to 6.25 ml. and 12.50 ml. Subsamples were taken at 12, 24 and 48 hours. Inoculum was obtained from only two faunated and protozoa free lambs. Thirty-two fermentation bottles were used so there was a replicate of each variable. Volatile fatty acids, dry matter digestion and cellulose digestion were analyzed.

C. Statistical Analyses

The data from *in vivo* experiments were analyzed on the IBM 3600 computer at the Michigan State University Computer Laboratory. Least squares analyses of variance was employed to define the significant relationships in this study. Data from experiments *in vitro* were not analyzed due to small numbers and differences between experiments.

IV Results and Discussion

A. In Vivo

Rumen Dry Matter: Rumen dry matter was higher in protozoa free sheep at all sampling times when compared to sheep with protozoa (Table 3). Faunated sheep had rumen dry matters of 12.22% compared to 16.52% for defaunated sheep, at the T0 sampling time ($P<.01$). Rumen dry matter values were also observed to be greater for defaunated sheep at T2 and T4; however, these differences were not significant. There was no significant interaction between the presence or absence of protozoa and urea or water infusion; however, the mean water values for rumen dry matter were slightly higher than those observed for the urea treated animals. At the T0 sampling time this difference approached significance ($P<.10$).

Rumen pH: Rumen pH was slightly higher for faunated sheep and for the animals receiving the urea infusion (Table 4). The rumen pH for faunated sheep was 6.19 and 5.66 compared to 5.80 and 5.33 for protozoa free sheep at the T0 and T2 sampling times respectively. These differences only approached significance ($P<.10$) and the T4 values were not significantly different between faunated and defaunated animals. At T2 and T4 there were differences approaching significance ($P<.10$) between the mean values for animals receiving the water infusion and those sheep receiving the urea infusion. There were no significant interactions between the presence or absence of protozoa and the urea or water infusions.

Table 3. Rumen Dry Matters[1] (%)

Sampling Time	Treatment						Mean		
	Faunated			Defaunated					
	H2O	Urea	Mean	H2O	Urea	Mean	H2O	Urea	S.E.[2]
TO	12.75	11.70	12.22[A]	17.37	15.67	16.52[B]	15.06[a]	13.68[b]	.55
T2	17.70	15.18	16.44	18.75	17.50	18.13	18.23	16.34	.79
T4	16.10	16.96	16.53	18.54	17.55	18.05	17.32	17.26	1.30

[1]Mean of 26 observations

[2]Standard error of the means

[A,B]Values having different superscripts are significantly different ($P<.01$).

[a,b]Values having different superscripts are significantly different ($P<.10$).

Table 4. Rumen pH Values[1]

Sampling Time	Treatment						Mean		
	Faunated			Defaunated					
	H_2O	Urea	Mean	H_2O	Urea	Mean	H_2O	Urea	S.E.[2]
TO	6.07	6.30	6.19[a]	5.78	5.83	5.80[b]	5.83	5.97	.12
T2	5.46	5.86	5.66[a]	5.29	5.37	5.33[b]	5.37[b]	5.61[a]	.08
T4	5.51	5.92	5.72	5.43	5.48	5.46	5.47[a]	5.70[b]	.08

[1]Mean of 26 observations

[2]Standard error of the means

[a,b]Values having different superscripts are significantly different ($P<.10$).

Volatile Fatty Acids: The effect of the absence of protozoa from the rumen ecology, on volatile fatty acid (V.F.A.) concentrations is outlined in Tables 5, 6 and 7. There was only a slight difference in total V.F.A. concentration observed at any of the sampling times; however, at T0 the total V.F.A. concentration was 66.64 micromoles per milliliter for defaunated sheep and 57.15 micromoles per milliliter for faunated sheep (Table 5). This difference approached significance ($P \leq .10$). At T2 the difference was reversed and faunated wethers showed a higher V.F.A. concentration than did defaunated sheep (Table 6). This slight difference in total V.F.A. concentration is in agreement with most of the work that has been done comparing ruminants with protozoa and those without protozoa. Acetate concentration was also only slightly different, although at T2 (Table 6) the difference between faunated and defaunated sheep approached significance ($P < .10$). This difference in the level of acetate at T2 is also shown in the molar percent acetate (Table 9). The higher molar percent acetate for faunated wethers is significantly different ($P \leq .05$) from the molar percent acetate for defaunated wethers. The greatest and most consistent difference observed in V.F.A. concentration, between faunated and defaunated sheep, was in the level of propionate. Propionic acid was consistently lower in sheep with protozoa, this difference was highly significant ($P < .01$) at T0 and

Table 5. Volatile Fatty Acid Concentration[1,2] TO

	Treatment								
	Faunated			Defaunated			Mean		
V.F.A.	H_2O	Urea	Mean	H_2O	Urea	Mean	H_2O	Urea	S.E.[3]
Total	53.83	60.47	57.15^{al}	67.31	65.97	66.64^{bl}	60.57	63.22	2.48
Acetate	32.86	37.71	35.20	38.06	37.63	37.84	35.37	37.67	1.71
Propionate	11.72	10.05	10.89^{A}	21.21	19.68	20.44^{B}	16.46	14.86	1.58
Butyrate	6.48	8.75	7.61^{a}	4.18	5.03	4.61^{b}	5.33	6.89	.64
Valerate	.64	.76	.70	1.03	.98	1.01	.84	.87	.14

[1]Mean of 26 values

[2]Concentration in micromoles/milliliter

[3]Standard error of means

[A,B]Values having different superscripts are significantly different ($P<.01$).

[a,b]Values having different superscripts are significantly different ($P<.05$).

[al, bl,]Values having different superscripts are significantly different ($P<.10$).

Table 6. Volatile Fatty Acid Concentration[1,2] TO

V.F.A.	Treatment: Faunated H_2O	Faunated: Urea	Faunated: Mean	Defaunated H_2O	Defaunated: Urea	Defaunated: Mean	Mean H_2O	Mean: Urea	S.E.[3]
Total	90.54	91.60	91.07	88.39	82.44	85.41	89.46	87.02	3.51
Acetate	55.73	57.73	56.73^{a1}	50.04	46.76	48.40^{b1}	52.88	52.25	2.19
Propionate	21.77	20.90	21.34	29.06	24.66	26.86	25.42	22.78	2.56
Butyrate	8.68	9.75	9.22	6.04	7.51	6.78	7.26	8.63	.82
Valerate	.87	.78	.82^{a}	1.27	1.64	1.46^{b}	1.07	1.21	.14

[1] Mean of 26 values

[2] Concentration in micromoles/milliliter

[3] Standard error of means

^{a,b} Values having different superscripts are significantly different ($P<.05$).

$^{a1, b1}$ Values having different superscripts are significantly different ($P<.10$).

Table 7. Volatile Fatty Acid Concentration[1,2] T4

V.F.A.	Treatment: Faunated H_2O	Faunated: Urea	Faunated: Mean	Defaunated H_2O	Defaunated: Urea	Defaunated: Mean	Mean H_2O	Mean: Urea	S.E.[3]
Total	74.33	74.47	74.40	81.61	81.99	81.80	77.97	78.23	4.07
Acetate	44.59	46.73	45.66	47.10	46.84	46.97	45.84	46.78	2.34
Propionate	18.34	15.75	17.09[a]	25.45	24.60	25.02[b]	21.94	20.17	1.71
Butyrate	8.41	9.90	9.15	5.12	7.06	6.09	6.76	8.48	1.05
Valerate	.85	.69	.76	1.20	1.46	1.33	1.02	1.07	.21

[1] Mean of 26 values

[2] Concentration in micromoles/milliliter

[3] Standard error of means

[a,b] Values having different superscripts are significantly different ($P < .05$).

Table 8. Volatile Fatty Acid As Molar Percent Of Total[1] TO

V.F.A.	Treatment: Faunated H_2O	Faunated: Urea	Faunated: Mean	Defaunated H_2O	Defaunated: Urea	Defaunated: Mean	Mean H_2O	Mean: Urea	S.E.[2]
	%	%	%	%	%	%	%	%	
Acetate	60.71	62.61	62.66	56.79	57.73	57.26	58.75	59.45	.01
Propionate	22.51	16.21	19.18[a]	31.19	28.69	29.94[a]	26.67	22.45	.02
Butyrate	11.70	14.78	13.24[a]	6.20	7.83	7.02[b]	8.95	11.31	.01
Valerate	1.22	1.16	1.19	1.48	1.42	1.45	1.35	1.29	.00
Branch Chain	4.21	5.23	4.72	4.33	4.31	4.32	4.27	4.77	.00

[1]Mean of 26 values

[2]Standard error of means

[a,b]Values having different superscripts are significantly different ($P<.05$).

Table 9. Volatile Fatty Acid As Molar Percent Of Total[1] T2

V.F.A.	Treatment						Mean		S.E.[2]
	Faunated			Defaunated					
	H_2O	Urea	Mean	H_2O	Urea	Mean	H_2O	Urea	
	%	%	%	%	%	%	%	%	
Acetate	61.96	63.06	62.51[a]	56.84	57.02	56.93[b]	58.40	61.04	.01
Propionate	23.54	22.54	23.04[al]	32.52	29.36	30.94[bl]	28.03	25.95	.06
Butyrate	9.79	10.95	10.37	6.87	9.31	8.09	8.33	10.13	.00
Valerate	.92	.82	.87[a]	1.46	1.96	1.71[b]	1.19	1.39	.00
Branch Chain	2.29	2.53	2.41	2.71	2.35	2.53	2.50	2.44	.00

[1]Mean of 26 values

[2]Standard error of means

[a,b]Values having different superscripts are significantly different ($P < .05$).

[al, bl]Values having different superscripts are significantly different ($P < .10$).

Table 10. Volatile Fatty Acid As Molar Percent Of Total[1] T4

V.F.A.	Treatment						Mean		S.E.[2]
	Faunated			Defaunated					
	H_2O	Urea	Mean	H_2O	Urea	Mean	H_2O	Urea	
	%	%	%	%	%	%	%	%	
Acetate	60.01	62.83	61.42	57.53	57.47	57.50	58.77	60.15	.01
Propionate	24.86	21.74	23.30^{al}	31.16	29.44	30.30^{bl}	28.01	25.59	.02
Butyrate	11.19	13.07	12.13^{a}	6.27	8.79	7.53^{b}	8.73	10.93	.01
Valerate	1.20	.96	1.08	1.48	1.64	1.56	1.34	1.30	.00
Branch Chain	2.72	2.66	2.69	3.08	2.54	2.81	2.90	2.60	.00

[1]Mean of 26 values

[2]Standard error of means

[a,b]Values having different superscripts are significantly different ($P < .05$).

[al, bl,]Values having different superscripts are significantly different ($P < .10$).

significant ($P<.05$) at T4. This difference is also shown in the tables on molar percent (Tables 8, 9, 10), as the molar percent propionate is significantly higher ($P<.05$) for defaunated sheep at T0 and approaching a significant difference ($P<.10$) at T2 and T4. Butyrate was consistently lower when protozoa were not in the rumen ecology; however, this difference was only significant ($P<.05$) at T0 (Table5). The molar percent butyrate was also significantly ($P<.05$) lower for defaunated wethers at this sample time (Table 8).

The concentration of valeric acid was higher for defaunated sheep (Table 6) at the T2 sampling time ($P<.05$). The molar percent valerate was also significantly ($P<.05$) higher at T2 (Table 9). Branched chain volatile fatty acids as a molar percent of total showed no difference between treatments. There were also no observed differences in any volatile fatty acid parameters between mean water and mean urea treatments.

The acetate:propionate ratio was lower for defaunated sheep (Table 11). The difference at T0 was highly significant ($P<.01$) and at T2 and T4 the lower acetate:propionate ratio for wethers without protozoa was significant ($P<.05$). This is in agreement with the findings of Klopfenstein et al. (1966). The acetate:butyrate ratio is higher for sheep without protozoa; however, these differences are not significant. The acetate:butyrate ratio for the defaunated water infused animals was higher than any of the other acetate:butyrate ratios for other treatments.

Table 11. Volatile Fatty Acid Ratios[1]

Ratio	Sampling Time	Treatment						Mean		S.E.[2]
		Faunated			Defaunated					
		H_2O	Urea	Mean	H_2O	Urea	Mean	H_2O	Urea	
AC/PR	T0	3.411	4.073	3.742[A]	1.886	2.214	2.050[B]	2.648	3.144	.251
AC/BU	T0	6.486	4.608	5.547	8.902	7.391	8.146	7.694	6.000	.801
PR/BU	T0	3.391	1.386	2.388	4.754	3.561	4.157	4.072	2.473	.876
AC/PR	T2	3.050	2.962	3.006[a]	1.920	2.174	2.047[b]	2.485	2.568	.185
AC/BU	T2	7.311	6.162	6.736	8.871	6.312	7.592	8.091	6.237	.883
PR/BU	T2	3.383	2.345	2.864	5.375	3.416	4.396	4.379	2.881	.796
AC/PR	T4	2.967	3.153	3.060[a]	2.060	2.235	2.147[b]	2.513	2.694	.594
AC/BU	T4	6.379	5.077	5.728	9.306	6.657	7.981	7.843	5.867	.941
PR/BU	T4	3.510	2.046	2.778	5.145	3.465	4.305	4.327	2.755	.818

[1]Mean of 26 values

[2]Standard error of means

[A,B]Values having different superscripts are significantly different ($P<.01$).

[a,b]Values having different superscripts are significantly different ($P<.05$).

The urea infusion into defaunated animals raised the butyrate concentration to a magnitude similar to that observed for faunated animals; therefore, the acetate:butyrate ratio from defaunated sheep receiving the urea infusion was lower and nearly equal to the ratio for faunated animals.

The propionate:butyrate ratio was higher for sheep without protozoa; however, as was the case for the acetate:butyrate ratios these differences were not significant. The same trend for urea infused defaunated wethers to have a lower ratio of a magnitude similar to that of the faunated animals was observed and was caused by higher butyrate and lower propionate from sheep that had no protozoa and which received the urea infusion. There were no significant differences observed for the mean water values compared to the mean urea values for the three ratios that were analyzed.

Plasma Urea Nitrogen: Plasma urea nitrogen was higher for sheep with protozoa (Table 12), which is in agreement with the previous work. The biggest difference in plasma urea nitrogen was at T4 when faunated wethers had 9.17 mg. per 100 ml. plasma and defaunated wethers had 5.95 mg. per 100 ml. of plasma. This difference was highly significant ($P < .01$); however, at the other sampling times no significant differences were observed. Urea infusion increased the plasma urea nitrogen levels when compared to the water infusion controls. The T0 values were not significantly different; however, at T4 the urea infusion increased plasma urea nitrogen significantly ($P < .05$) over the water control and at T2

Table 12. Plasma Urea Nitrogen[1,2]

Sampling Time	Treatment						Mean		
	Faunated			Defaunated					
	H_2O	Urea	Mean	H_2O	Urea	Mean	H_2O	Urea	S.E.[3]
T0	9.09	10.34	9.71	6.71	10.00	8.35	7.90	10.17	.49
T2	7.95	11.02	9.48	5.72	7.60	6.66	6.83^{al}	9.31^{bl}	.95
T4	7.93	10.40	9.17^{A}	5.50	6.40	5.95^{B}	6.71^{a}	8.40^{b}	1.09

[1]Mean of 26 observations

[2]Mg-N/100 ml. of plasma

[3]Standard error of means

[A,B]Values having different superscripts are significantly different ($P < .01$).

[a,b]Values having different superscripts are significantly different ($P < .05$).

[al, bl,]Values having different superscripts are significantly different ($P < .10$).

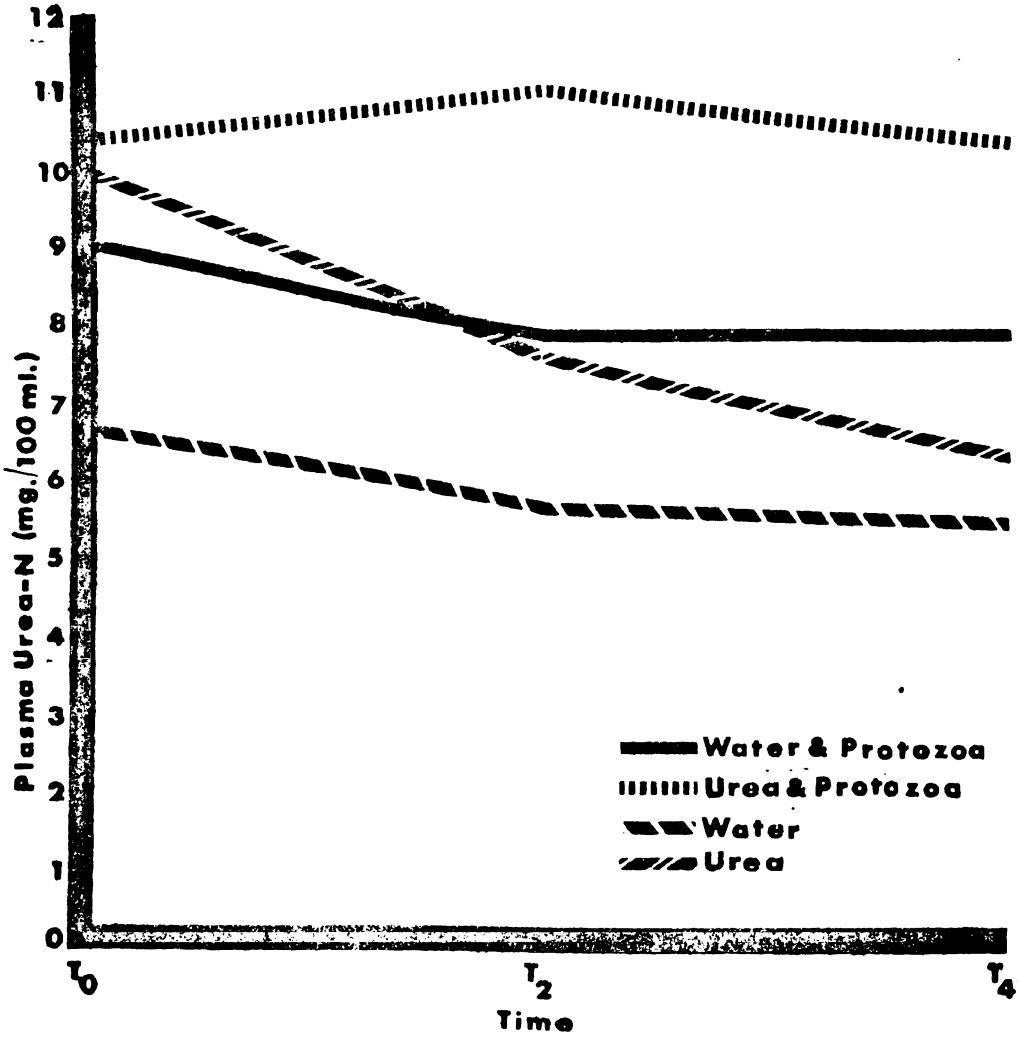

Figure 1. In vivo plasma urea-N levels.

this difference approached significance ($P<.10$). There were no significant interactions between urea infusion and the presence or absence of protozoa. The plasma urea nitrogen results for the four treatment combinations are displayed graphically in Figure 1.

Dry Matter and Nitrogen Digestion: Dry matter digestion was not significantly different when the treatment effects were analyzed (Table 13). Urea infusion slightly decreased dry matter digestion for both faunated and defaunated sheep when compared to the water controls; however, this difference was not significant. Nitrogen digestion was similar for all defaunated animals. The mean nitrogen digestion value for faunated sheep was not signifcantly different from the mean value for defaunated sheep; however, urea infusion significantly ($P<.05$) lowered nitrogen digestion of the faunated wethers when compared to the water infusion controls.

Nitrogen Utilization: The nitrogen utilization figures that were observed for this experiment were extremely high for sheep that were on a maintenance ration; however, there were significant differences observed. Nitrogen utilization for defaunated sheep was significantly ($P<.01$) higher than utilization from sheep with protozoa. In defaunated animals the infusion of urea only slightly increased nitrogen utilization over that of the water infused sheep; however, in faunated animals the urea infusion decreased utilization significantly ($P<.10$). It is postulated that the greater bacterial concentration in defaunated sheep was better able to utilize the excess nitrogen supplied by the urea.

Table 13. Dry Matter Digestion and Nitrogen Metabolism[1]

Treatment	Dry Matter Digestion	Nitrogen Digestion	Nitrogen Utilization	Fecal Nitrogen / Nitrogen Intake	Nitrogen Balance
	%	%	%	%	
Faunated					
H_2O	78.58	71.75^{a}	53.28^{al}	27.61^{a}	5.200^{al}
Urea	76.79	68.04^{b}	33.86^{bl}	31.31^{b}	3.064^{bl}
Mean	77.68	69.90	43.57^{A}	29.46	4.142^{a}
Defaunated					
H_2O	77.61	70.63	71.69	30.19	6.396(a)
Urea	76.64	70.63	75.12	30.20	6.789(a)
Mean	77.12	70.63	73.54^{B}	30.19	6.593^{b}
H_2O Mean	78.09	71.19	62.61	28.90	5.798
Urea Mean	76.71	69.34	54.49	30.75	4.937
S.E.2	.60	.87	4.68	.87	.449

[1]Mean of 26 values

[2]Standard error of means

^{A,B}Values with different superscripts are significantly different ($P<.01$).

^{a,b}Values with different superscripts are significantly different ($P<.05$).

$^{al, bl,}$Values with different superscripts are significantly different ($P<.10$).

Fecal Nitrogen As Percent Nitrogen Intake: There were no significant differences observed for the mean values (defaunated vs. faunated) of fecal nitrogen as a percent of nitrogen intake (F.N./N.I.); however, an interesting trend was observed (Table 13). Regardless of whether urea or water was infused, the F.N./N.I. for defaunated sheep was 30.19%. The faunated wethers that received the urea infusion had a mean F.N./N.I. value of 31.31% compared to 27.61% from faunated sheep receiving the water infusion ($P<.05$). This figure further magnifies the nitrogen inbalance observed with faunated wethers receiving the urea infusion.

Nitrogen Balance: The highest nitrogen balance values were observed in defaunated sheep ($P<.05$); however, these values were also very high for adult sheep on a maintenance ration (Table 13). As was the case for other nitrogen metabolism data, the urea infusion into defaunated wethers did not greatly effect the nitrogen balance results. The lowest nitrogen balance was observed when faunated sheep were infused with urea, a difference that approached significance ($P<.10$). We believe this also shows that lambs with protozoa and therefore lower bacterial concentrations were unable to utilize the excess nitrogen as well as the defaunated sheep.

Rumen Ammonia Nitrogen: Rumen ammonia-N levels showed the same trend in this experiment that has been reported in other literature with defaunated and faunated ruminants. High rumen ammonia-N levels were found when protozoa were present

in the rumen ecology (Table 14). At the T2 sampling time this difference was highly significant ($P<.01$) and at T4 there was a significant difference observed ($P<.05$). The urea infusion raised ammonia-N levels; however, this difference was greater in faunated whethers when compared to defaunated animals. Urea infusion did not raise the rumen ammonia-N levels in defaunated sheep the desired 4 mg. percent, although the ammonia-N levels were raised. This finding is in agreement with Luther and Perkins (1968), who were also unable to raise rumen ammonia-N levels significantly with urea. The ammonia-N levels for the different treatment combinations are displayed graphically in Figure 2. A fermentation *in vitro* was initiated to study the reason for these low ammonia-N levels when urea was infused into sheep without protozoa and the results of this experiment are displayed graphically in Figure 3 and in tabular form (Table 15). Ammonia levels *in vitro* were similar for all treatments including urea or for all treatments including only water. Such a result suggests that differences *in vivo* were the result of differences in ammonia uptake. This suggests that the higher bacterial concentration in defaunated animals utilized the ammonia-N to a greater extent than did faunated animals.

Purser and Dehority in unpublished data, obtained the greatest urease activity with defaunated animals which corraborates the *in vitro* experiment in this project.

Table 14. Rumen Ammonia-N Levels[1] (mg./100 ml.)

Sampling Time	Treatment						Mean		
	Faunated			Defaunated					
	H_2O	Urea	Mean	H_2O	Urea	Mean	H_2O	Urea	S.E.[2]
T0	8.712	14.578	11.645	6.436	7.638	7.037	7.574	11.108	1.58
T2	9.308	12.962	11.135^{A}	2.221	4.563	3.392^{B}	5.765^{al}	8.763^{bl}	1.17
T4	5.812	6.629	6.220^{a}	.136	2.223	1.798^{b}	3.283	4.735	1.02

[1]Mean of 26 values

[2]Standard error of means

^{A,B}Values with different superscripts are significantly different ($P<.01$).

^{a,b}Values with different superscripts are significantly different ($P<.05$).

$^{al, bl,}$Values with different superscripts are significantly different ($P<.10$).

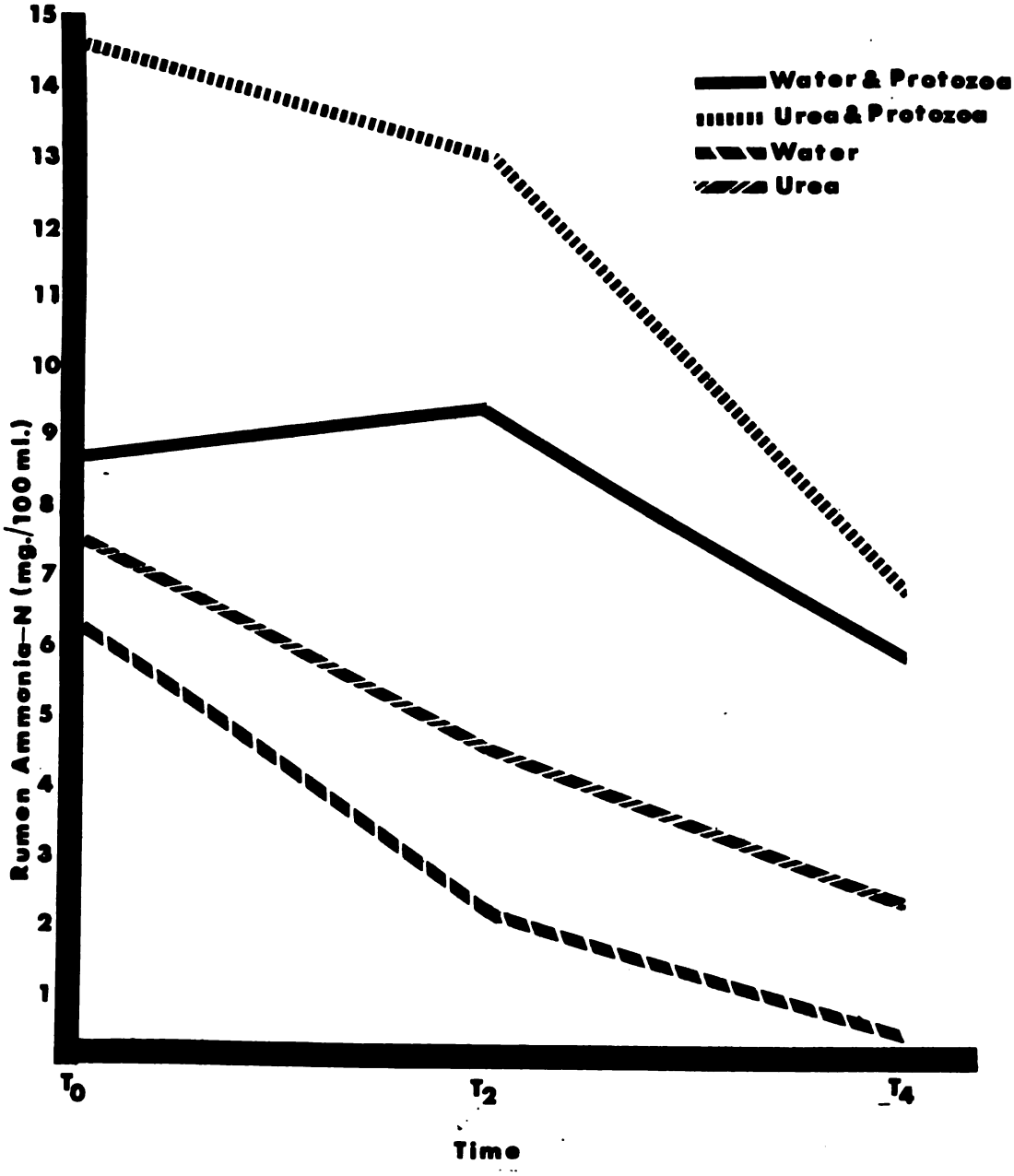

Figure 2. In vivo rumen ammonia-N levels.

Table 15. In Vitro Ammonia-N Levels[1]

Treatment	Time T0	T3	T6	T9	T12
Faunated	Mg%	Mg%	Mg%	Mg%	Mg%
H_2O Whole Contents	2.90	2.44	2.02	4.73	5.77
H_2O Supernatant	1.23	.55	.53	1.41	.95
Urea Whole Contents	49.22	40.80	40.94	42.92	45.11
Urea Supernatant	47.63	38.42	36.47	34.03	34.48
Defaunated					
H_2O Whole Contents	3.17	1.84	1.22	3.91	3.87
H_2O Supernatant	4.31	1.64	1.18	3.59	3.86
Urea Whole Contents	48.19	37.79	35.77	41.90	42.76
Urea Supernatant	53.56	40.38	36.71	38.81	40.64

[1]Mean of 32 values

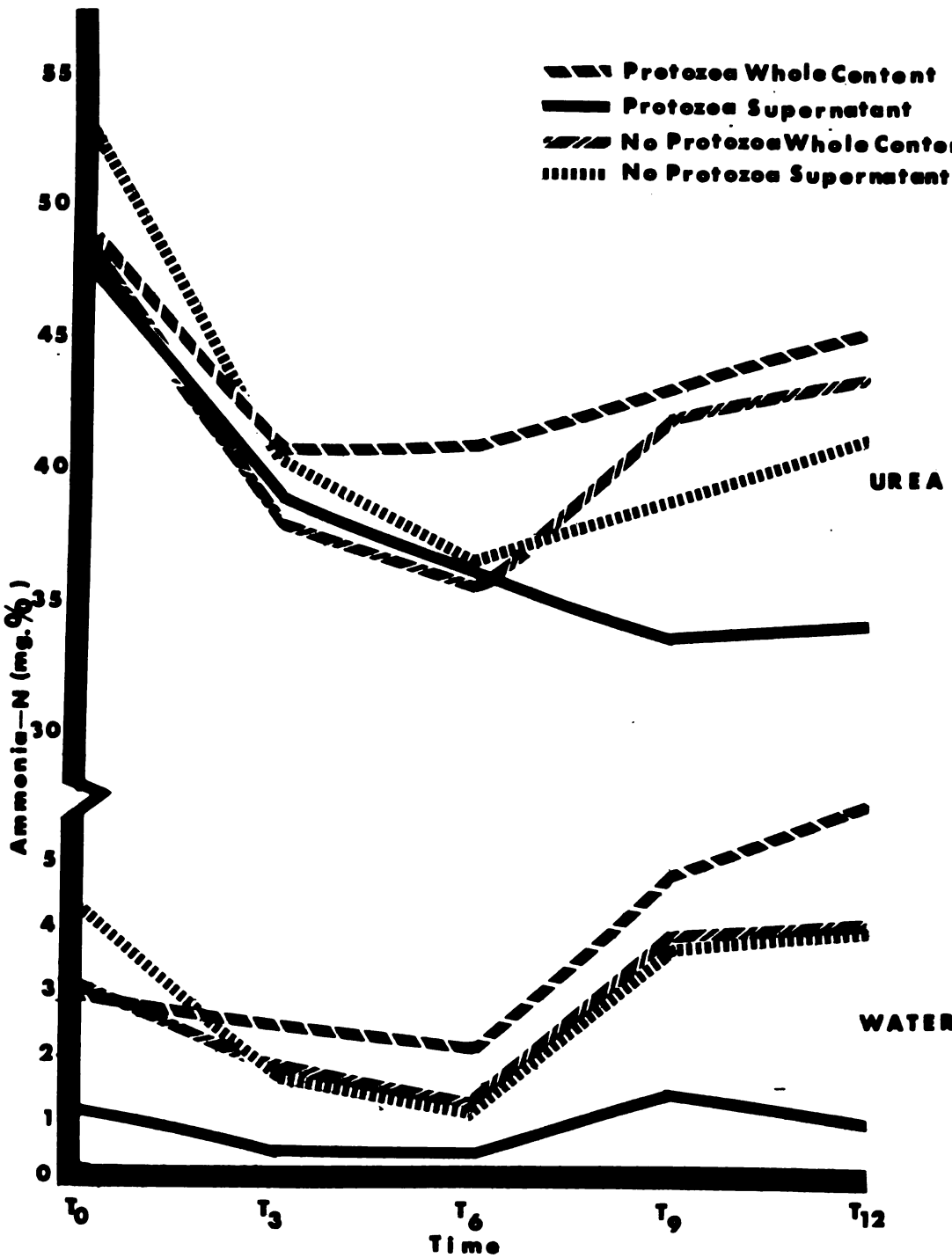

Figure 3. Ammonia-N levels for in vitro fermentation with urea added to media and water added to media.

Data for rumen ammonia-N levels and for the acetate:propionate, acetate:butyrate and propionate:butyrate ratios at T0 and T4 from this experiment were combined with similar data from Klopfenstein et al. (1966) (Appendix Table 1). Regressions for each ratio and rumen ammonia-N levels were calculated. The acetate:propionate ratio at T0 and T4 is plotted in Figure 4 and Figure 5. The regression at T0 is significant ($P < .05$) and the correlation is 0.72. The regression at T4 was significant ($P < .01$) with a correlation of 0.91. This shows that as rumen ammonia-N levels increase, acetate increased and propionate decreased. The acetate:butyrate ratio regression was significant ($P < .01$) at both T0 and T4 (Figures 6 and 7) suggesting that as rumen ammonia increased butyrate increases. There was 0.89 correlation between acetate:butyrate ratio and rumen ammonia at T4. The propionate:butyrate ratio at T0 was not a linear regression; (Figures 8 and 9) however, at T4 it was a curvilinear regression with a correlation of 0.81 These data show a definite trend for an increased molar percent acetate and butyrate with a lower molar percent propionate associated with increased ammonia levels. In neither experiment were any significant differences in total V.F.A. production observed, suggesting the changes that take place in the V.F.A. production are a shift from propionate, at low ammonia levels, to acetate and butyrate at higher ammonia levels. These data cover only a low level of rumen ammonia-N and to verify this finding it would be enlightening to collect similar data at high rumen ammonia-N levels.

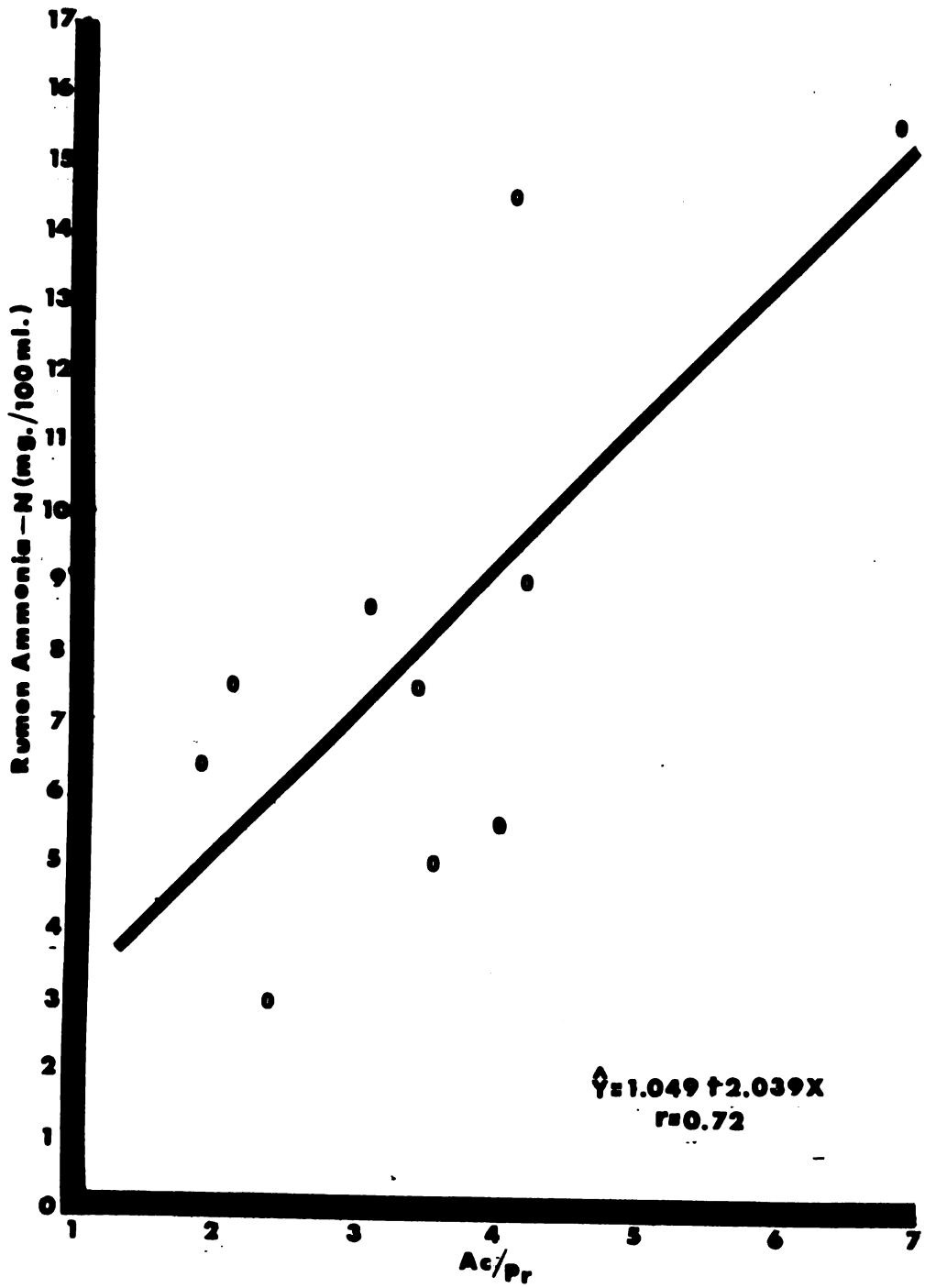

Figure 4. Regression of rumen ammonia-N with acetate:propionate ratio TO.

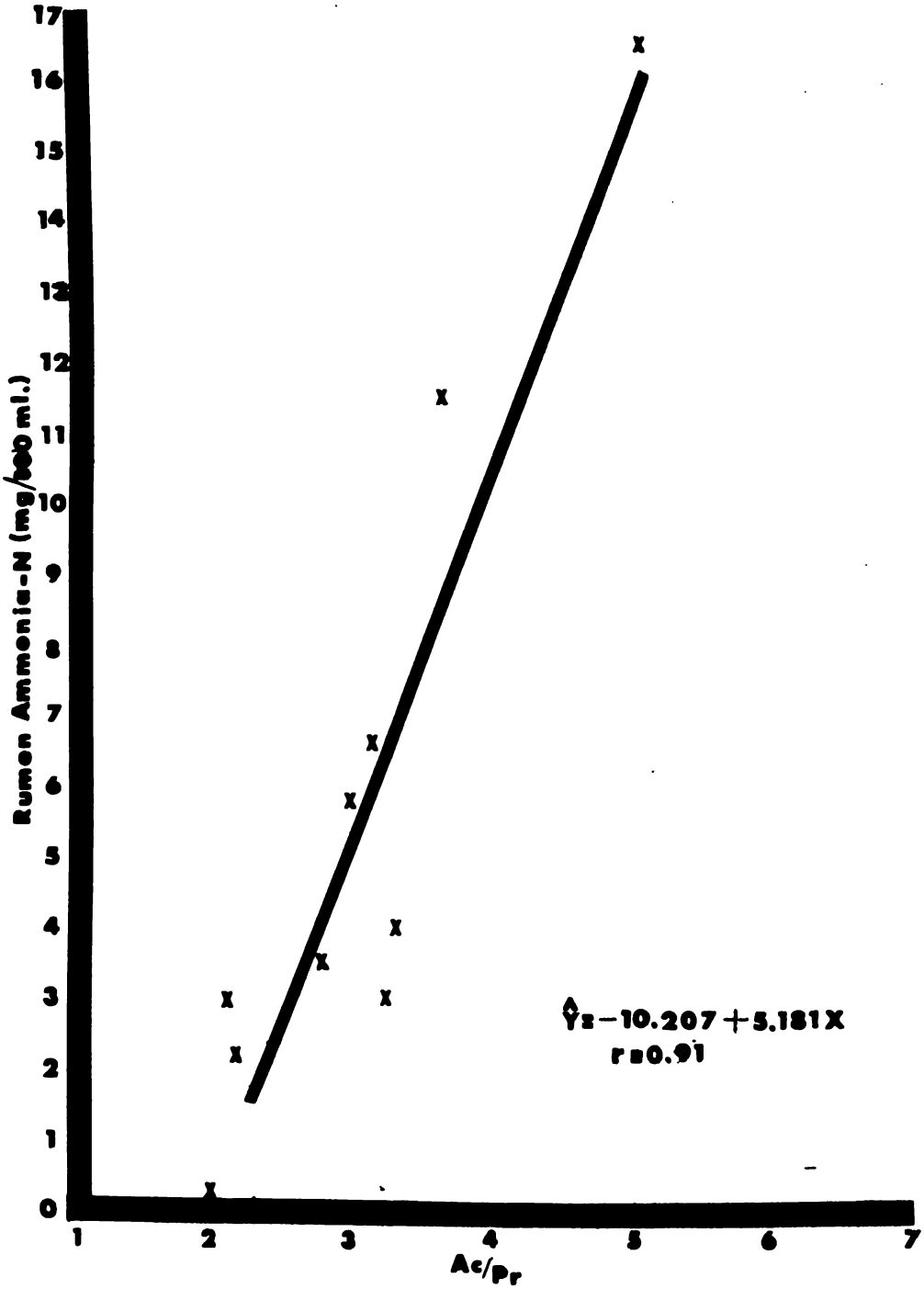

Figure 5. Regression of rumen ammonia-N with acetate:propionate ratio T4.

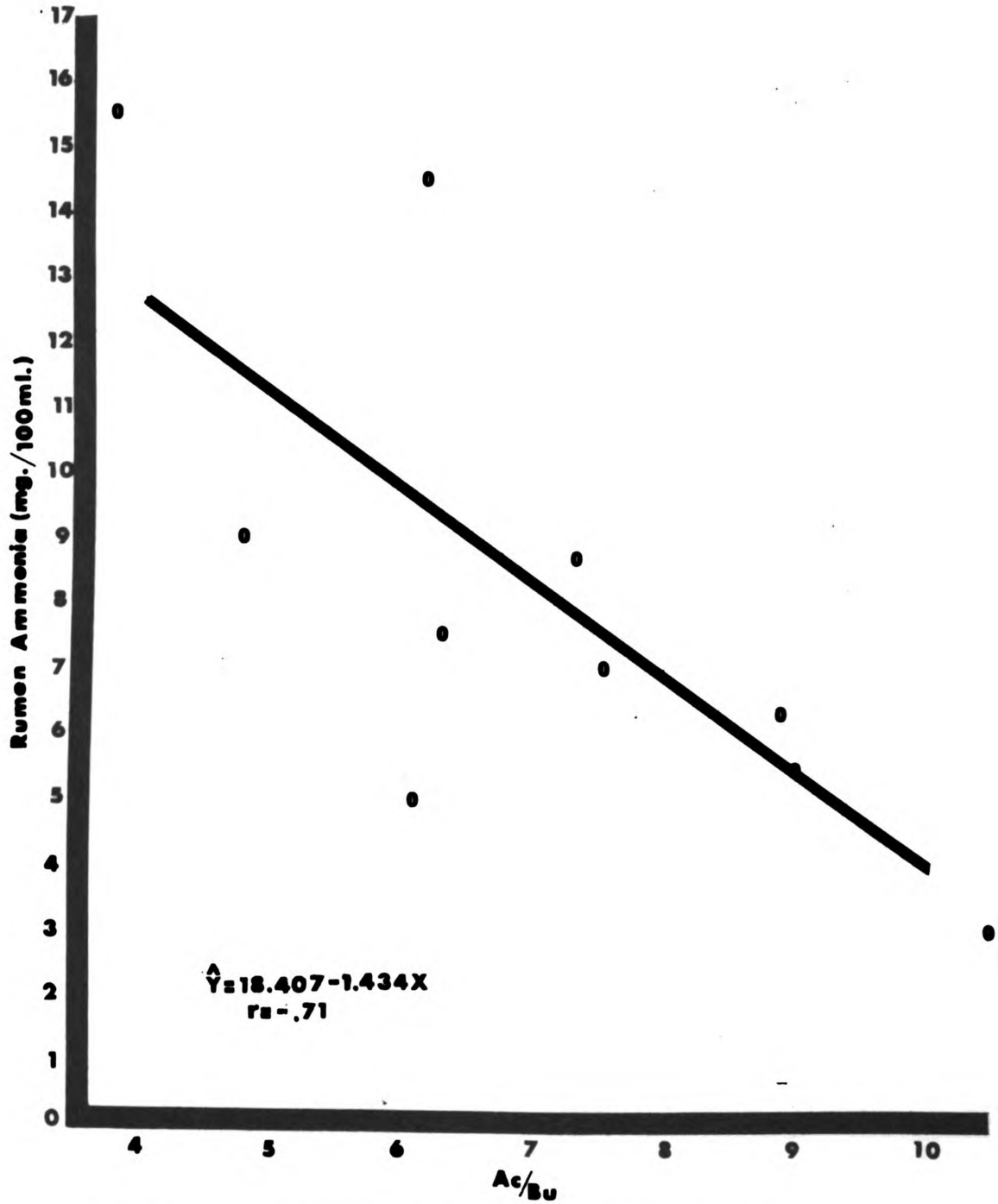

Figure 6. Regression of rumen ammonia-N with acetate:butyrate ratio T0.

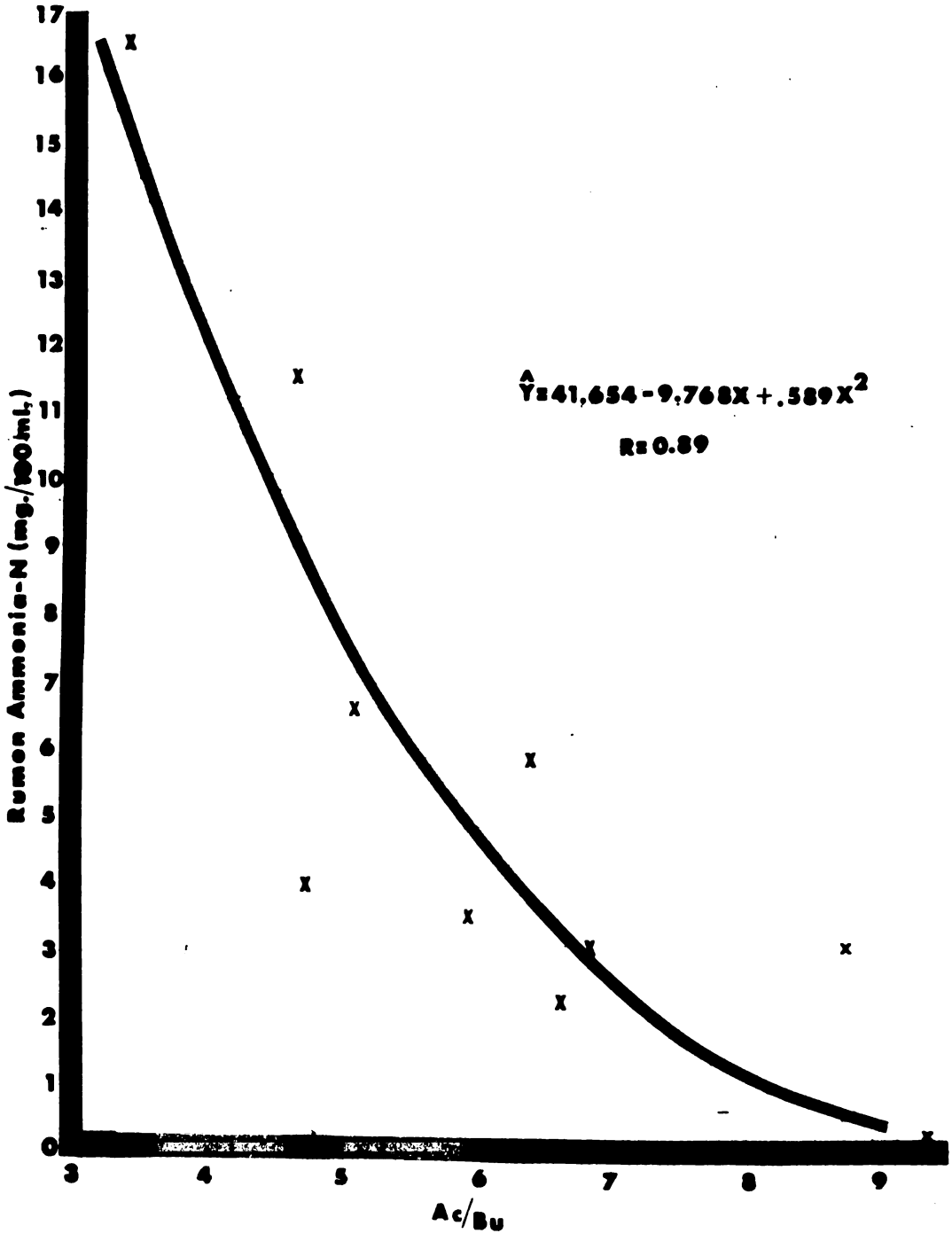

Figure 7. Regression of rumen ammonia-N with the acetate:butyrate ratio T4.

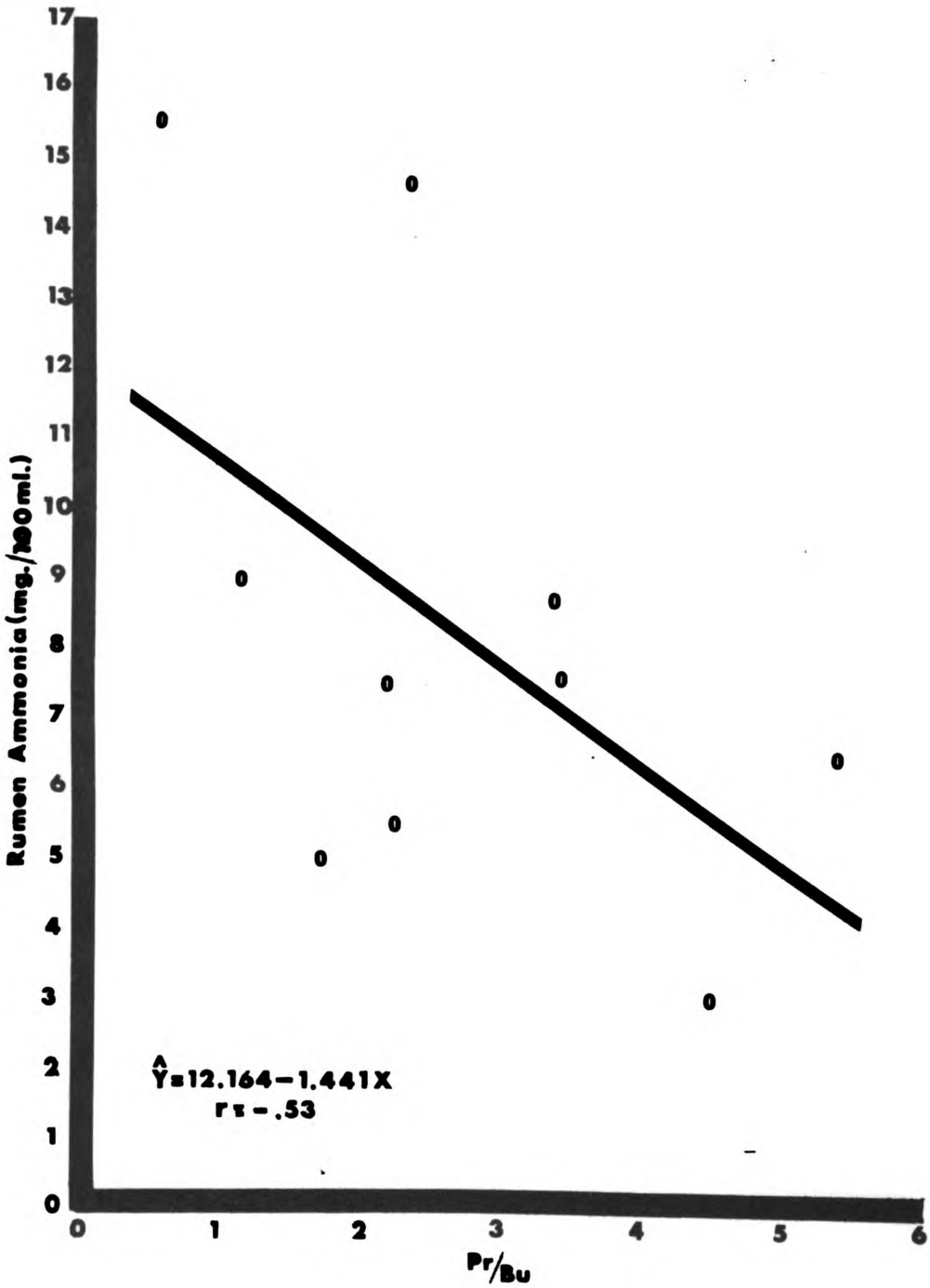

Figure 8. Regression of rumen ammonia-N with propionate:butyrate ratio TO.

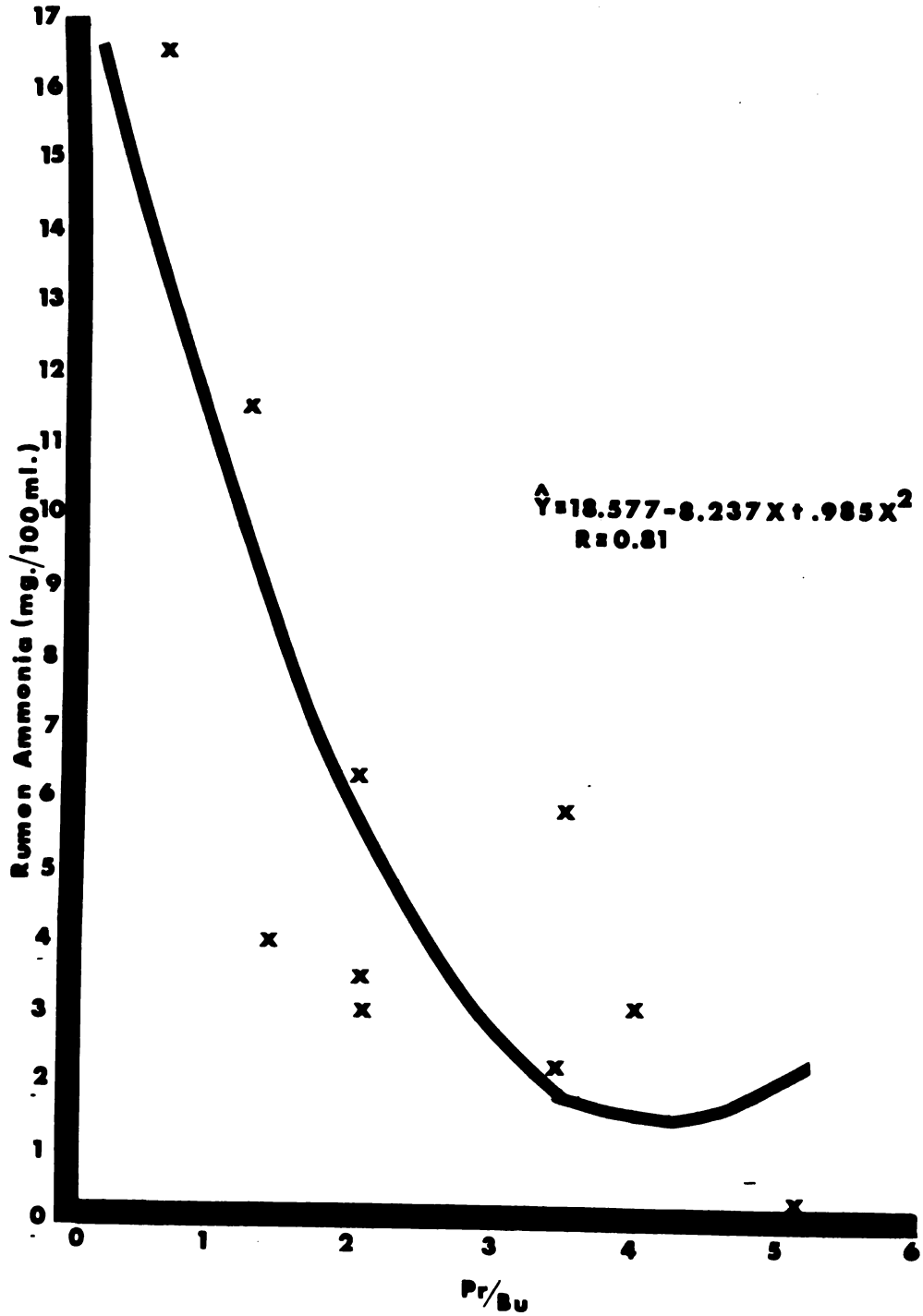

Figure 9. Regression of rumen ammonia-N with the propionate:butyrate ration T4.

B. In Vitro

The dry matter digestibilities, cellulose digestibilities and V.F.A. production from fermentation B, C and D appear in appendix table 2. These data are incomplete due to lack of time on the part of the author. From the results of these three experiments it appears that it would be desirable to either further decrease inoculum size or to increase substrate; however, this will require more fermentations *in vitro* to find the true effect of different bacterial concentrations.

SUMMARY

Sheep with protozoa present in their rumen ecology had a higher level of rumen ammonia-N than was observed in sheep without protozoa. This result is in agreement with previous work that has been done using defaunated and faunated ruminants. Urea infusion raised rumen ammonia-N levels above the level that was observed for the water control animals; however, this difference was more pronounced for faunated sheep than for defaunated animals. This effect was also observed by Luther and Perkins (1968) and was further studied in this experiment using an *in vitro* fermentation system. Ammonia levels *in vitro* were similar for all treatments including urea or for all treatments including only water. Purser and Dehority (unpublished) studied the urease activity in faunated and defaunated animals and found the greatest urease activity in defaunated animals. From these results it is hypothesised that the greater bacterial concentrations in defaunated ruminants make more rapid utilization of ammonia-N, and therefore levels are observed to be lower in protozoa free animals. *In vitro* studies were designed to study the effect of varying concentrations of bacteria on dry matter and cellulose digestion and volatile fatty acid production; however, these studies are incomplete and further work is needed in this area.

Urea infusion and therefore slightly higher rumen ammonia-N in defaunated sheep had no effect on nitrogen digestion,

nitrogen utilization, fecal nitrogen as a percent of nitrogen intake or on nitrogen balance. From this it is concluded that the bacterial population could adequately use the excess nitrogen supplied by the urea infusion. Urea greatly reduced nitrogen utilization and nitrogen balance in faunated animals and slightly raised fecal nitrogen as a percent of nitrogen intake; therefore, the conclusion drawn from this is that with a high protein ration, the excess nitrogen supplied in the urea infusion could not be adequately used by the microbial population present in faunated sheep. Although the protozoal protein is of higher quality than bacterial protein, it is postulated that the larger quantity of bacterial protein in a defaunated animal is of greater use to the host animal, which would explain the nitrogen metabolism data obtained in this study.

Urea infusion into protozoa free sheep tended to raise butyrate levels and lower propionate levels. This led to the possibility that the molar porportions of volatile fatty acids could very linearly with ammonia levels. Pooled data of Klopfenstein et al. (1966) and from this study for ammonia production and acetate:propionate ratios, acetate:butyrate ratios, and propionate:butyrate ratios when fitted to linear regression equations exhibited this trend. From the regressions it was concluded that at low levels of rumen ammonia-N the molar percent propionate was high and as rumen ammonia-N

levels increased there was a shift from propionate to acetate and butyrate. This trend was observed for three different rations of varying protein levels and energy levels and also for urea and water infusion for both faunated and defaunated ruminants.

The ration used in this study was high in protein and therefore the infused urea was probably in excess of the daily crude protein requirement. However, from the results obtained in this study it is obvious that further investigation of bacterial concentration effects on rumen metabolism and classification of bacterial species present in faunated and defaunated ruminants and at varying ammonia levels would be of great interest to the ruminant nutritionist. Such studies could aid in more clearly defining the role of ciliate protozoa in the metabolism of the rumen.

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APPENDIX TABLES

Appendix Table 1. Pooled data of ammonia-N levels and V.F.A. ratios.

Treatment	Ammonia		Ac/Pr		Ac/Br		Pr/Bu	
	T0	T4	T0	T4	T0	T4	T0	T4
Klopfenstein et al. (1966)								
Ration 1								
Defaunated	5.50	3.00	4.00	3.24	9.00	6.80	2.25	2.10
Faunated	9.00	11.50	4.19	3.61	4.79	4.64	1.14	1.29
Ration 2								
Defaunated	3.00	3.00	2.37	2.18	10.67	8.71	4.50	4.00
Faunated	5.00	3.50	3.53	2.83	6.09	5.91	1.73	2.09
Ration 3								
Defaunated	7.50	4.00	3.40	3.30	7.56	4.71	2.22	1.43
Faunated	15.50	16.50	6.80	5.00	3.78	3.42	0.56	0.68
Males (1969)								
Urea								
Defaunated	7.64	2.22	2.17	2.23	6.31	6.66	3.42	3.46
Faunated	14.58	6.63	4.07	3.15	6.16	5.08	2.34	2.05
H_2O								
Defaunated	6.44	0.14	1.92	2.06	8.87	9.31	5.37	5.14
Faunated	8.71	5.81	3.05	2.97	7.31	6.38	3.38	3.51

Appendix Table 2. Fermentation In Vitro

	D.M. Digestion[1]			Cellulose Dig.[1]			V.F.A. Prod.[2]		
	T12	T24	T48	T12	T24	T48	T12	T24	T48
Fermentation B									
Faunated									
12.50 W.C.[3]		38.2	51.0		24.2	44.5			
25.00 W.C.		43.7	52.0		30.7	40.3			
12.50 Super.[4]		44.1	52.8		32.4	49.9			
25.00 Super.		46.2	51.4		37.0	52.5			
Defaunated									
12.50 W.C.		55.7	65.4		47.9	54.2			
25.00 W.C.		53.0	61.7		43.4	49.8			
12.50 Super.		59.3	67.6		47.3	53.1			
25.00 Super.		56.5	65.6		44.6	52.1			
Fermentation C									
Faunated									
12.50 W.C.		67.1	66.6		60.5	65.7		150	104
25.00 W.C.		61.2	51.6		57.2	53.9		140	130
12.50 Super.		57.4	59.8		48.1	57.8		90	98
25.00 Super.		52.9	56.5		48.2	54.3		107	89
Fermentation D									
Faunated									
6.25 W.C.	45.1	53.8	61.2	33.3	56.8	55.3	70	77	109
12.50 W.C.	38.7	51.0	58.8	41.1	54.4	54.6	70	89	132
6.25 Super.	31.7	50.5	55.2	25.0	41.0	48.1	56	71	107
12.50 Super.	32.4	48.4	52.5	26.9	42.1	48.4	63	78	103
Defaunated									
6.25 W.C.	36.5	54.7	66.0	23.7	44.9	56.2	56	83	99
12.50 W.C.	36.0	49.3	61.1	25.0	37.4	49.8	71	84	113
6.25 Super.	41.4	58.7	65.4	13.9	38.6	52.9	53	75	88
12.50 Super.	40.6	54.7	63.2	20.2	36.4	56.0	60	77	89

1 Digestion as % digestibility
2 V.F.A. production in micromoles/milliliter
3 W.C. is Whole Content inoculum
4 Super. is Supernatant inoculum

Appendix Table 3. Rumen dry matter, rumen pH and feed digestibilities for faunated lambs.

Sheep	Dry Matter			pH			Digestibility
	T0	T2	T4	T0	T2	T4	
	%	%	%				%
				Urea Infusion			
13	14.3	17.3	12.9	5.85	5.35	5.75	75.99
14	13.9	16.9	15.9	6.60	6.50	6.50	72.85
17	10.3	14.7	29.6	6.55	6.50	6.50	76.38
21	13.6	13.1	13.2	5.70	5.60	5.40	77.60
36	11.0	15.4	15.9	6.35	5.60	5.65	77.76
38	8.7	9.8	11.0	6.60	5.70	6.00	75.92
57	10.9	17.5	16.8	6.30	5.40	5.40	77.96
				Water Infusion			
14	16.5	16.8	17.7	5.80	5.50	5.75	75.83
17	12.7	22.8	15.6	6.50	5.50	5.70	81.87
21	11.5	14.4	16.1	6.10	5.50	5.40	79.31
36	13.5	18.1	16.0	5.70	5.40	5.40	75.67
38	11.3	14.5	15.0	5.90	5.35	5.15	77.92
57	9.2	15.9	16.8	6.75	5.65	5.55	73.71

Appendix Table 4. Rumen dry matter, rumen pH and feed digestibilities for defaunated lambs.

Sheep	Dry Matter			pH			Digestibility
	T0	T2	T4	T0	T2	T4	
	%	%	%				%
				Urea Infusion			
10	17.5	18.2	19.5	5.65	5.55	5.60	79.31
12	15.9	20.3	19.9	6.00	5.45	5.50	75.94
17	14.0	17.5	16.9	6.10	5.30	5.45	75.29
25	14.1	17.0	16.7	6.00	5.55	5.60	77.21
36	12.7	17.5	17.8	6.00	5.30	5.40	75.67
37	17.8	17.8	21.8	5.65	5.40	5.55	77.17
52	15.4	16.7	16.6	5.70	5.50	5.60	79.41
				Water Infusion			
10	16.1	23.5	18.2	6.30	5.20	5.40	78.20
17	16.0	17.6	17.8	5.90	5.30	5.35	79.27
25	15.6	14.1	15.7	5.60	5.25	5.40	79.04
36	18.6	18.3	20.4	5.55	5.45	5.60	79.24
38	17.7	19.6	22.7	5.85	5.40	5.60	77.23
52	14.2	16.2	15.2	5.85	5.45	5.50	80.11

Appendix Table 5. Nitrogen metabolism data for faunated lambs.

Sheep	Fecal N.	Urine N.	Total N. excreted	N. Intake
	g./day	g./day	g./day	g./day
		Urea Infusion		
13	4.724	3.228	7.952	13.284
14	6.074	8.163	14.237	15.423
17	5.287	5.084	10.371	14.236
21	4.354	7.854	12.208	15.630
36	3.652	5.498	9.150	13.433
38	3.723	5.226	8.949	13.207
57	3.698	5.962	9.660	11.671
		Water Infusion		
14	5.534	4.012	9.546	15.775
17	4.068	3.084	7.152	14.560
21	3.842	3.726	7.568	14.910
36	3.725	6.462	10.187	13.260
38	3.303	6.032	9.335	13.035
57	3.010	4.176	7.186	11.152

Appendix Table 6. Nitrogen metabolism data for defaunated lambs.

Sheep	Fecal N.	Urine N.	Total N. excreted	N. Intake
	g./day	g./day	g./day	g./day
		Urea Infusion		
10	3.553	1.870	5.423	11.802
12	4.534	4.568	9.102	16.490
17	4.061	2.498	6.559	14.648
25	2.826	2.304	5.130	9.821
36	3.795	2.156	5.951	11.633
37	3.988	1.514	5.502	13.063
52	2.937	3.106	6.043	11.717
		Water Infusion		
10	3.406	3.555	6.961	10.584
17	3.966	4.534	8.500	14.557
25	2.550	2.320	4.870	9.760
36	3.814	1.514	5.328	12.640
38	3.905	1.228	5.133	12.336
52	2.858	2.212	5.070	11.094

Appendix Table 7. Rumen Ammonia-N and Plasma Urea (Mg.%)

Sheep	Ammonia-N T0	Ammonia-N T2	Ammonia-N T4	Urea-N T0	Urea-N T2	Urea-N T4	Sheep	Ammonia-N T0	Ammonia-N T2	Ammonia-N T4	Urea-N T0	Urea-N T2	Urea-N T4
					Faunated								
	Urea Infused							Water Infused					
13	5.96	1.18	3.75	7.06	6.59	11.37	14	6.66	2.02	1.23	5.54	6.52	4.82
14	19.91	6.72	.48	10.47	11.14	8.20	17	.45	.25	.50	5.94	3.09	5.32
17	18.93	13.27	7.59	13.72	15.40	12.60	21	9.24	10.67	6.69	18.48	9.63	8.96
21	13.02	11.54	7.53	14.78	12.49	11.70	36	12.94	11.34	9.46	8.18	8.96	7.90
36	7.48	15.99	7.81	10.53	9.46	7.84	38	12.10	13.55	10.47	10.14	10.42	10.53
38	12.04	10.08	4.73	14.62	13.46	11.36	57	14.11	15.48	4.17	19.26	15.01	8.18
57	19.32	17.64	9.30	10.92	10.08	8.85							
					Defaunated								
	Urea Infused							Water Infused					
10	5.24	7.08	8.71	3.58	5.04	4.93	10	11.82	4.14	3.42	6.18	7.53	4.45
12	15.15	11.23	2.41	9.30	9.18	8.62	17	9.77	7.17	5.38	9.18	7.28	6.89
17	6.08	1.15	.11	14.73	8.62	7.17	25	7.03	4.15	4.09	7.28	6.94	6.61
25	6.05	10.02	2.77	12.82	7.00	7.17	36	3.14	1.04	.90	6.50	5.51	5.54
36	10.92	3.28	3.70	9.18	9.47	3.86	38	.45	.84	.42	4.37	4.23	5.26
37	4.20	1.60	.45	4.03	4.03	4.37	52	4.76	5.10	.00	6.10	5.26	5.21
52	9.67	10.75	7.90	8.06	9.18	8.74							

Appendix Table 8. Total V.F.A. Concentration[1] for Faunated Lambs Receiving Urea Infusion.

Sheep	Total	Acetate	Propionate	Butyrate	IsoButyrate	IsoValerate	2Methyl Butyrate	Valerate
				T0				
13	71.74	34.26	30.15	3.96	.83	.35	.28	1.91
14	93.19	56.02	18.84	10.84	2.10	1.22	1.95	2.22
17	59.82	36.22	8.36	9.86	2.52	.87	.98	1.01
21	66.46	39.03	14.63	10.20	.50	.59	.99	.52
36	55.85	35.59	7.75	9.93	.50	.82	.96	.30
38	52.05	33.41	9.90	6.60	.56	.53	.62	.43
57	37.66	23.23	5.74	6.73	.62	.46	.46	.42
				T2				
13	96.04	45.46	42.19	4.87	.24	.19	.00	2.28
14	96.53	58.17	23.73	10.68	.87	.49	1.08	1.51
17	111.72	72.69	26.67	7.94	2.13	.49	.59	1.21
21	59.04	35.38	14.66	7.14	.24	.31	.61	.70
36	91.30	57.05	19.20	13.29	.39	.27	.41	.69
38	68.85	41.54	16.73	9.06	.37	.26	.32	.57
57	95.91	58.99	26.22	9.21	.30	.23	.15	.81
				T4				
13	82.24	37.38	37.92	3.95	.33	.41	.27	1.98
14	92.61	58.46	20.64	9.68	1.20	.39	.88	1.36
17	93.15	55.29	19.35	15.18	1.67	.29	.36	1.01
21	57.47	35.06	13.64	6.86	.28	.42	.69	.52
36	87.40	54.76	17.39	13.27	.39	.40	.46	.73
38	56.26	35.17	13.03	7.58	.17	.00	.00	.31
57	72.83	42.96	19.23	8.98	.34	.28	.21	.83

[1]Concentration in micromoles/milliliter

Appendix Table 9. Total V.F.A. Concentration[1] for Faunated Lambs Receiving H_2O Infusion.

Sheep	Total	Acetate	Propionate	Butyrate	IsoButyrate	IsoValerate	2Methyl Butyrate	Valerate
				T0				
14	63.42	34.54	14.27	11.12	1.07	.57	.57	1.28
17	47.94	22.73	21.66	1.79	.49	.21	.00	1.06
21	63.06	39.74	13.70	6.22	.56	.67	1.74	.43
36	58.19	38.51	8.51	7.84	.56	.66	1.59	.52
38	59.25	35.77	11.64	9.21	.56	.66	.79	.62
57	33.34	22.04	5.45	4.37	.48	.29	.43	.28
				T2				
14	80.20	54.22	17.60	6.27	.21	.38	.38	1.14
17	101.41	53.45	41.81	4.25	.00	.00	.00	1.90
21	70.00	37.16	20.00	10.26	.22	.27	1.20	.79
36	83.11	55.79	15.01	9.43	.49	.31	1.38	.70
38	98.58	57.73	17.83	12.00	.49	.46	.77	.93
57	83.72	53.45	20.19	8.68	.32	.19	.32	.57
				T4				
14	70.38	44.93	14.08	9.82	.21	.26	.32	.76
17	76.49	39.15	32.14	3.04	.24	.24	.19	1.49
21	86.66	48.83	22.50	11.37	.45	.53	1.52	1.46
36	63.74	41.73	11.23	7.85	.39	.42	1.39	.73
38	87.04	55.63	17.64	11.48	.45	.35	.55	.94
57	70.56	38.59	21.75	9.08	.39	.13	.20	.42

[1] Concentration in micromoles/milliliter

Appendix Table 10. Total V.F.A. Concentration[1] for Defaunated Lambs Receiving Urea Infusion

Sheep	Total	Acetate	Propionate	Butyrate	IsoButyrate	IsoValerate	2Methyl Butyrate	Valerate
					T0			
10	73.96	41.60	25.33	5.17	.10	.42	.92	.42
12	50.77	34.88	5.80	4.31	.50	.20	4.77	.31
17	70.93	36.61	26.22	4.98	.46	.25	.38	2.03
25	54.43	32.97	10.00	7.71	.72	1.03	1.85	.69
36	63.37	39.74	16.13	3.61	1.44	.26	1.23	.66
37	70.00	40.33	19.19	7.41	1.02	.42	.78	.85
52	64.00	42.37	11.33	5.16	.62	.53	3.57	.42
					T2			
10	96.12	56.38	31.14	6.53	.32	.32	.96	.47
12	83.17	55.79	10.20	10.07	.37	.23	5.23	1.28
17	89.71	48.19	29.49	7.73	.37	.00	.32	3.61
25	83.34	49.50	20.70	9.67	.44	.55	1.09	1.39
36	69.93	39.01	20.34	7.55	.76	.00	1.02	1.25
37	94.73	58.24	25.09	9.25	.27	.38	.64	.86
52	85.18	56.30	16.13	7.43	.55	.46	3.38	.93
					T4			
10	76.02	43.62	25.09	5.27	.32	.48	.96	.28
12	72.68	49.71	8.27	8.18	.45	.28	4.65	1.14
17	97.56	51.82	32.79	8.87	.21	.00	.26	3.61
25	78.47	47.78	16.74	10.13	.61	.75	1.37	1.09
36	83.97	46.08	26.45	8.72	.22	.27	1.13	1.10
37	79.67	47.10	22.14	7.43	.44	.68	.89	.99
52	77.68	52.84	14.13	6.34	.45	.42	2.98	.52

[1] Concentration in micromoles/milliliter

Appendix Table 11. Total V.F.A. Concentration[1] for Defaunated Lambs Receiving Water Infusion

Sheep	Total	Acetate	Propionate	Butyrate	IsoButyrate	IsoValerate	2Methyl Butyrate	Valerate
				T0				
10	63.93	38.96	17.86	3.83	1.44	.35	.96	.53
17	63.64	37.48	16.65	5.95	.56	.53	2.05	.42
25	72.09	40.30	22.71	4.44	.45	.26	2.58	1.35
36	72.15	40.82	25.07	3.58	.88	.64	.42	.74
38	58.06	29.18	21.41	3.71	1.22	.42	1.06	1.06
52	66.74	43.39	14.15	4.85	.72	.38	1.97	1.28
				T2				
10	90.69	50.08	30.27	7.20	1.24	.00	1.12	.78
17	90.43	54.03	23.17	8.71	.43	.38	2.08	1.63
25	80.44	48.15	22.81	4.86	.30	.31	2.31	1.63
36	81.68	45.17	31.30	3.36	.16	.32	.51	.86
38	105.91	57.39	40.38	5.70	.21	.32	.96	.95
52	94.22	58.70	22.01	8.56	.75	.96	2.19	1.04
				T4				
10	83.28	43.51	30.05	6.95	.50	.27	1.00	1.00
17	82.75	51.11	20.00	7.66	.51	.49	1.94	1.04
25	76.45	46.42	21.09	4.49	.39	.35	2.15	1.56
36	74.67	42.81	26.53	3.23	.32	.48	.52	.78
38	85.44	50.12	28.25	4.45	.37	.44	.99	.82
52	88.93	57.42	18.77	7.96	.61	.75	2.33	1.09

[1]Concentration in micromoles/milliliter