UTILIZATION OF ABOMASALLY INFUSED RIBONUCLEIC ACID AND DEOXYRIBONUCLEIC ACID IN SHEEP

by

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INTRODUCTION

The ruminant animal, because of its unique digestive system, enjoys a symbiotic relationship with the microorganisms in the rumen. Ruminants rely heavily on the rumen microbiota for digestion of the fibrous components of the diets, as well as for the synthesis of microbial protein from dietary protein and nitrogen. With the synthesis of microbial protein, there is a concurrent production of microbial nucleic acids. Between 15 and 20% of the total microbial nitrogen is in the form of nucleic acids. Due to the large populations in the rumen, microorganisms constitute a significant proportion of the digesta entering the small intestine. Upon the digestion of these microorganisms, large quantities of nucleic acids are released into the small intestine. Early work led researchers to suggest that microbial nucleic acids were of no value to the host animal and, therefore, a source of nitrogen wastage to the animal. Later studies indicated some utilization of the nucleic acids. A controversy exists as to the exact role of microbial nucleic acids in the nutrition of the ruminant.

REVIEW OF LITERATURE

Nucleic Acids in the Rumen

Production in the Rumen. Blaxter (1961) reported that ruminants excrete three to six times more purine nitrogen per day than monogastric animals of comparable body weight. Lusk (1931) observed that purine excretion levels remained unchanged in fed and fasted monogastrics. Blaxter and Wood (1951) found that purine excretion levels in the suckling calf were not affected by fasting. Blaxter (1961), however, observed that starvation caused a large decrease in the excretion of allantoin in the ruminant. Knowing that allantoin is the major end product of nucleic acid metabolism in most mammals, Blaxter (1961) suggested that appreciable amounts of nucleic acids are formed in the rumen.

Blaxter and Martin (1961) infused casein (free of RNA and DNA) into either the rumen or abomasum of sheep via cannulae. Allantoin N for the animals receiving the ruminal infusion was elevated over those receiving the abomasal infusion, indicating bacterial production of nucleic acids in the rumen.

Ellis and Pfander (1965) were the first researchers to quantify the importance of nucleic acids in ruminant nutrition. They sampled rumen contents of sheep fed diets essentially devoid of RNA and DNA. The rumen samples were incubated at 39 C in anaerobic incubation tubes and 10 ml sub-samples were removed at intervals up to 12 hours.

Total microbial N, RNA-N, and DNA-N values were determined on each of the samples and each value was found to increase with incubation time.

McAllan and Smith (1968) analyzed rumen digesta samples from fistulated calves for RNA and DNA concentrations using the Schmidt-Thannhauser separation procedure. Concentrations of nucleic acids were more than five times higher than could be explained by the levels of nucleic acids in the diets of the calves.

Effects of Ration on Production. The levels of nucleic acids in the rumen are dependent upon the density of the microbial population which is controlled primarily by the total N concentration in the rumen, and, therefore, by the N intake. Moir and Harris (1962) and Hume et al. (1970) observed a significant positive relationship between rumen bacterial counts and nitrogen intake. Ellis and Pfander (1965) reported microbial nitrogen values of 41.1 to 51.6% of the total ingesta nitrogen in experiments using zein as the nitrogen source. Weller et al. (1958) reported 63 to 82% of the total N in the rumen of sheep fed wheaten hay to be in the form of microbial N. Smith et al. (1968) observed a constant conversion ratio of N in the rumen to microbial N of 65 percent.

Elliott and Topps (1963) concluded that ruminal production of nucleic acids increased as the protein content of the diets fed to African cattle increased. Topps and Elliott (1965) observed increased nucleic acid concentrations in the rumen samples of sheep fed high concentrate, compared to those fed a high roughage ration. Condon and Hatfield (1970b) observed increased ruminal production of purines

with increased intakes of nitrogen and energy. Data collected by Ellis and Pfander (1965) suggested that 5 to 13.3% of the dietary nitrogen of ruminants is converted to rumen microbial nucleic acid N. Higher percentages of dietary nitrogen were converted to nucleic acids when sheep were fed urea as compared to zein. Smith and McAllan (1971) observed an apparent stimulatory effect of ammonia on the production of microbial nucleic acids. They observed that as ruminal ammonia concentration increased, there was an associated increase in nucleic acid concentration. Smith et al. (1968) fed calves a variety of diets with a wide range of nitrogen content. A significant direct correlation was observed between the concentrations of nucleic acid N and total N in the rumen fluid of the calves. Nucleic acid nitrogen ranged from 9.5 to 15.5% of the total nitrogen present in the rumen samples. Analysis of the microorganisms collected from the rumen fluid by centrifugation showed 19% of their total N to be in the form of nucleic acids. This value was relatively constant, regardless of the N content of the ration. This was in agreement with work by Ellis and Pfander (1965) in which nucleic acids accounted for 15% of the microbial nitrogenous compounds formed.

Palmer and Smith (1971) analyzed two different single-cell bacterial preparations and found the nucleic acid N (% of total N) to be 18.4 and 7.5%. McAllan and Smith (1972) analyzed mixed bacteria obtained by centrifugation of rumen contents of calves, sheep and cattle. The ratios of RNA-N : total N and DNA-N : total N were .114 $\pm$ .003 and .066 $\pm$ .004, respectively, for the calves, .076 $\pm$ .003 and .051 $\pm$ .004, respectively, for the sheep and .070 $\pm$ .004 and

.051 ± .003, respectively, for the cattle. Thirteen different strains of rumen bacteria grown in pure culture were also analyzed. RNA-N : total N and DNA-N : total N values were .112 ± .007 and .074 ± .006, respectively. Ratios were found to be similar for each group whether pasture or all-roughage stall diets were fed. Values appeared to be depressed by urea supplementation and elevated by N-free diets by about 15-25 percent. Diurnal variations were apparent in stall-fed animals. Values were 20 to 35% lower when samples were taken before a morning feed. It was observed that standard deviations for the RNA concentrations were about one half of those for DNA.

Bacteria, because of their rapid growth rate have the highest ratio of RNA-N to total N of all cells (Davidson, 1972). McAllan and Smith (1974) found rumen bacteria to contain between 9 to 12% of the total non ammonia nitrogen in the form of RNA-N.

Smith and McAllan (1974) observed that RNA-N : total N ratios were significantly lower and DNA-N : total N ratios slightly elevated for mixed bacteria sampled just before feeding as compared to 4 to 6 hr after feeding.

Topps and Elliott (1965) and Razzague et al. (1973) reported total nucleic acid concentrations in rumen fluid of sheep to be around 50 mg/dl and 80 mg/dl, respectively. Smith et al. (1968) found nucleic acid concentrations in the rumens of calves to be as high as 250 mg/dl of rumen fluid.

Weller (1957) and Purser and Buechler (1966) reported 75 to 85% of the total N in rumen bacteria is in the form of proteins, peptides, or free amino acids. Keyser (1976) reported 76% of the N in bacteria

harvested from steers was associated with amino acid N, and suggested that most of the other nitrogen is in the form of nucleic acids. These values reported by various researchers for nucleic acid nitrogen and amino acid N appear to account for most of the bacterial nitrogen. Smith (1969) proposed that for every 4 parts of dietary nitrogen converted to bacterial protein or amino acids, one part is converted to nucleic acids.

Less is known about the nucleic acid concentrations of rumen protozoa; however, it is suspected that they contain less than half the amounts found in bacteria per unit of total nitrogen. This was supported by the fact that the mixed bacterial and protozoal samples analyzed by Ellis and Pfander (1965) contained significantly less total nucleic acid per unit of total nitrogen than did pure bacterial samples analyzed by Smith et al. (1968).

Effects of Dietary Nucleic Acids. McAllan and Smith (1968) reported pure samples of RNA and DNA were rapidly degraded in the rumen with less than 10% of either being detected after 45 minutes. It was concluded that nucleic acids in rumen fluid are almost entirely microbial in origin.

To further study the effects of dietary intake of nucleic acids on rumen concentrations of nucleic acids, Smith and McAllan (1970) analyzed a variety of feedstuffs for nucleic acid content. The procedure used in the analysis was that developed by McAllan and Smith (1969). Percentages or RNA-N and DNA-N were .107 and .041, respectively, for hay, .086 and .009, respectively, for straw, .106 and .056, respectively, for flaked maize, and .102 and .006 respec-

tively, for decorticated extracted ground nut meal. Six diets formulated from these feedstuffs to provide a wide range of dietary ratios of RNA : DNA, were fed to rumen cannulated steers. Each diet provided the same daily net energy intake. Rumen samples were taken 3 hr after feeding and analyzed for total-N, RNA-N, and DNA-N. RNA : DNA ratios in the rumen samples were unaffected by the different dietary ratios, suggesting that the dietary nucleic acids were degraded in the rumen. Nucleic acid concentrations were found to be closely related to the nitrogen contents of the diets.

Razzague and Topps (1972) studied the effect on digestibility when either RNA or DNA was added to the basal diet of lambs. Five to 6-month-old lambs, equipped with abomasal cannulas, were placed on one of three treatments: basal diet alone, basal diet plus 5 g RNA or basal diet plus 5 g DNA. The digestibility of organic matter, nitrogen, crude fiber and cellulose was significantly elevated for lambs receiving either RNA or DNA compared to lambs receiving the basal diet alone. The authors noted that dietary nucleic acids are highly digestible and rapidly degraded in the rumen.

Razzague and Topps (1972) also reported in vitro cellulose digestibility of hay to be significantly elevated when either RNA or DNA was added to the system. It was concluded that cellulolytic microorganisms of the rumen are stimulated by the addition of either RNA or DNA.

To further study the possible degradation of dietary nucleic acids in the rumen, Smith and McAllan (1970) incubated pure samples of nucleic acids in vitro with rumen fluid at 37 C in an atmosphere

of 95% N_2 and CO_2. Within 1 hr, the RNA had been broken down to ultrafilterable poly- and oligo-nucleotides. Some mononucleotides, nucleosides and purine and pyrimidine bases were also present. After 4 hr, purine and pyrimidine bases accounted for most of the added RNA. When DNA was added, the same products were seen; however, ultrafilterable polynucleotides made up the majority of the products formed and were present after a period of 4 hours. The bases which were formed from the nucleic acids were thymine (from DNA only), uracil, xanthine, and hypoxanthine. Cytosine, quanine, and adenine were apparently deaminated to yield the oxy-compounds.

Smith and McAllan (1970) also studied the degradation products formed when pure samples of RNA and DNA were added to the rumen of the calf. The same products were formed as in the in vitro studies. However, the products did not accumulate as in the in vitro studies, but disappeared, and at a rate greater than that for polyethylene glycol which was added to the rumen as a marker. Two possible reasons for the disappearance of the degradation products were proposed: 1) absorption across the rumen wall, or 2) a more efficient microbial breakdown under in vivo conditions. The authors concluded from this work that the nucleic acids found in rumen fluid are almost solely microbial in origin.

McAllan and Smith (1973b) further quantified and qualitated the degradation products of nucleic acids in rumen fluid in vivo and in vitro. In vitro analyses were conducted with calf, sheep or cow rumen contents with no differences observed between types of rumen contents used. RNA was rapidly degraded with only the bases,

xanthine, hypoxanthine and uracil remaining after 4 hours. DNA was degraded much more slowly, yielding a large proportion of ultrafilterable oligo- and mono-nucleotide material which remained after 4 hours. The bases, thymine, hypoxanthine, uracil and xanthine were also measured.

Degradation of RNA and DNA in vivo yielded the same products as in vitro; however, these products disappeared at a rate much greater than could be explained by digesta flow. Levels of nucleic acid degradation products in the proximal duodenum could account for only a small percentage of those lost in the rumen. It appears these bases are either metabolized more efficiently by the bacteria in vivo than in vitro or they are absorbed across the rumen wall.

Incubation of ground hay with whole rumen contents resulted in the formation of the same nucleic acid degradation products as were seen with the pure nucleic acids.

Further studies by McAllan and Smith (1973a) seem to suggest that the nucleic acids are degraded by the rumen bacteria and the degradation products are absorbed across and metabolized by the rumen wall.

Ruminal Degradation of Bacterial Nucleic Acids. Smith and Smith (1976, 1977) studied the rate of digestion in the rumen of bacteria and the subsequent degradation of their nucleic acids. Mixed rumen bacteria, separated by centrifugation of the rumen contents of a young steer fed flaked maize and hay, were incubated in a growth medium containing [U^{14}C] adenine. The [U^{14}C] adenine was incorporated into the nucleic acid fraction of the bacteria. The bacteria were incubated in rumen fluid and the release of radioactivity was assumed to represent

the rate of bacterial nucleic acid breakdown in the rumen. These authors calculated a turnover rate of bacterial nucleic acid-N in the rumen of 30 percent. Using a steer weighing 115 kg with a rumen volume of 17.5 l, these authors estimated a total nucleic acid-N production of 6.2 g/day, of which only 4.4 g/day would be passed into the duodenum.

Concentrations Reaching Small Intestine. Using polyethylene glycol as a marker, Smith et al. (1968), analyzed ingesta samples and found no major destruction of RNA or DNA in the abomasum. The ratio of nucleic acid N to total N in digesta entering the duodenum of calves was similar to that for rumen fluid with the exception of being consistently lower. A partial explanation for the difference was accredited to the addition of endogenous N in the abomasal secretions. Nucleic acids composed 8 to 12.5% of the total N in the digesta entering the small intestine of ruminating calves aged 4 to 10 months and receiving a variety of diets.

Sutton et al. (1975) measured RNA-N entering the duodenum and observed the amount to be .77 g/day. RNA-N accounted for 7.65% to 11.64% of the total microbial N entering the duodenum of sheep fed hay and concentrates.

McAllan and Smith (1974) found RNA-N to make up 5.5 to 8% of the total non-ammonia nitrogen in the duodenum of a ruminating calf. Microbial N to total N ratios in the duodenum were found to be .60 $\pm$ .08 for flaked maize, .58 $\pm$ .01 for crushed oats and .79 $\pm$ .08 for rolled barley. Smith et al. (1977) reported comparable ratios of .71 and .46 for flaked maize and hay, and dried grass, respectively.

Smith and Smith (1977) estimated the passage of 4.4 g/day of microbial nucleic nitrogen into the duodenum of a steer fed flaked maize and hay.

From the above literature, the following generalizations appear appropriate. The rumen contains higher levels of nucleic acids than can be accounted for by ration intake, in addition, dietary nucleic acids are rapidly degraded in the rumen. Nucleic acids in the rumen are produced through microbial synthesis. The level of nucleic acids in the rumen is therefore dependent upon the density of the microbial population which is related to the nitrogen and energy intake or total nitrogen concentration in the rumen. Between 15 and 20% of the total microbial nitrogen is in the form of nucleic acids. With the exception of those cells which are degraded within the rumen, the microbial cells are passed on to the duodenum with the nucleic acids intact. Once in the duodenum, the cells are hydrolyzed and the nucleic acids are released for degradation. Between 7 and 14% of the total nitrogen in the duodenal digesta may be nucleic acid nitrogen.

Digestion and Absorption of Exogenous Nucleic Acids

Biochemical Pathways of Digestion. Degradation or digestion of nucleic acids in the intestine involves the following primary reactions: 1) nucleic acids are hydrolyzed by nucleases to polynucleotide fragments. These fragments are reduced by phosphodiesterases to single nucleotides. 2) Nucleotidases hydrolyze the nucleotides to nucleosides. 3) The nucleosides are then further reduced by nucleosidases to yield free bases and pentose phosphate.

Prior to 1910, no classification of the enzymes responsible for

the hydrolysis of nucleic acids in the gastrointestinal tract existed. Lavene and Medigreceanu (1911a) used dogs to observe the actions of various enzymes of the gastro-intestinal tract on nucleic acids. They proposed the first enzyme classification which recognized the differences between nucleases, nucleotidases and nucleosidases.

The hydrolysis of nucleic acids by nuclease action, with the resulting formation of polynucleotide fragments, was first demonstrated by Jones (1920) on yeast nucleic acid. The nuclease used was extracted from ground pig pancreas with chloroform.

Phosphodiesterases produced in the pancreas are responsible for the reduction of polynucleotides to individual nucleotides (Khorana, 1961 and Lehninger, 1975).

London and Schittenhelm (1910) studied the digestion of nucleic acids in dogs with fistulas in different locations along the gastrointestinal tract. Rapid dephosphorylation of nucleotides to nucleosides and purine bases was reported in the small intestine. Hydrolysis appeared more active in the jejunum than the ileum; the stomach was found to have no activity.

Levene and Medigreceanu (1911b) demonstrated the hydrolysis of the nucleotide guanylic acid to guanosine by the action of a nucleotidase obtained from intestinal secretions.

Kalckar (1946) reported the conversion of nucleosides to free bases and pentose-1-phosphate by a phosphorylysis reaction. During <u>in vitro</u> studies by Wilson and Wilson (1958) in which the nucleoside uridine was converted to free bases, it was observed that the ribose

moiety disappeared. Since tissue is unable to utilize free ribose, it is reasonable to assume that the ribose exists as ribose-1-phosphate within the cell (Wilson, 1962).

Monogastric Digestion and Absorption. Wilson and Wilson (1958) used everted sacs of rat and hamster small intestine to study the digestion and absorption of pyrimidine nucleotides. Incubation of the pyrimidine nucleotides on the mucosal side of the intestine resulted in rapid hydrolysis to nucleosides and inorganic phosphate. A much slower conversion of the nucleosides to free bases was seen. Smaller amounts of free bases were formed from the incubation of TMP than from CMP or UMP. The formation of the free base uracil from UMP seemed more rapid than the corresponding reactions with TMP and CMP. The main degradation product of CMP was cytidine, however, some of the latter was deaminated to yield uridine, with the subsequent production of uracil.

A mixture of nucleosides and free bases was found to pass through the intestinal wall to the serosal side when either of the three pyrimidines were incubated on the mucosal side. Larger amounts of free base appeared on the serosal side when CMP and UMP were incubated as compared to TMP, however, nucleosides were predominant in all three cases.

In the case of UMP, the authors noted a five to 10-fold greater concentration of uridine on the mucosal side than on the serosal side, however, the concentrations of uracil on the two sides were similar. In a few instances, the concentration of uracil was higher on the serosal side than on the mucosal. This indicated the possibility of

an active transport system for the uracil. Further experiments disproved this possibility, and lead the authors to believe the nucleosides and free bases were absorbed by simple diffusion.

Schanker and Tocco (1960) studied the absorption of the pyrimidines uracil and thymine in the rat. In vivo studies were conducted in which the small intestine of anesthetized rats was infused with solutions containing varying concentrations of uracil and thymine. It was first determined that uracil and thymine are not degraded in the intestine so that any disappearance from the infused solutions could be attributed to absorption. As the concentration of each pyrimidine in the circulating solution increased, the proportion absorbed decreased until, at the higher concentration, it leveled off and remained constant. Thymine absorption leveled off and maintained a constant 19-20% while uracil absorption remained a constant 15-16% at the higher concentrations. The results indicated absorption of uracil or thymine by a combination of two processes: 1) a transport system which operates when concentrations are low but which becomes saturated at higher concentrations at which time the 2) passive process of diffusion takes over.

Uracil was found to be a competitive inhibitor of thymine transport, indicating the same affinity for the transport mechanism. Thymine transport was depressed when hypoxanthine was added to the solution, suggesting that the transport mechanism may be involved in the absorption of purines and purine derivatives (Schanker and Tocco, 1960).

In vitro experiments with everted sacs of rat intestines showed

thymine and uracil to be actively transported across the intestinal wall against a concentration gradient (Schanker and Tocco, 1960).

Schanker and Tocco (1960) circulated for 1 hr, solutions of thymine-2-^{14}C in anesthetized rats cannulated at the end of the duodenum and ileum. Between 20-40% of the circulated thymine was absorbed during the 1-hr period. The small intestine was removed and analyzed for radioactivity. The remaining carcass was homogenized in a blender and also analyzed. The percentage of the absorbed radioactivity recovered in the body ranged from 18 to 78% while the intestine accounted for 8 to 15%. Expired CO_2 accounted for the remainder of the radioactivity. The data suggested that the absorbed thymine readily entered the circulation and was not stored in the intestinal wall to a great extent.

Wilson and Wilson (1962) studied the digestion and absorption of the purine ribonucleotides AMP and GMP by everted sacs of rat or hamster intestine. When GMP was incubated on the mucosal side of the sac, rapid hydrolysis to guanosine occurred. This was followed by a much slower conversion to xanthine and then, uric acid. A small amount of guanine was detected as the intermediate between guanosine and xanthine; active guanase prevented the accumulation of guanine. Guanosine, xanthine and uric acid were the major products which appeared on the serosal side; small amounts of guanine were present in some experiments.

Incubation of AMP resulted in rapid hydrolysis to adenosine which did not accumulate, but was quickly deaminated to inosine. Complete breakdown of AMP and adenosine usually occurred within 30

minutes. Subsequent enzyme reactions converting inosine to hypoxanthine, hypoxanthine to xanthine and xanthine to uric acid occurred at a much slower rate. Inosine, hypoxanthine, xanthine, and uric acid were absorbed across the wall to the serosal side of the intestine.

When either GMP or AMP was incubated, no nucleotides were absorbed across the intestinal wall. Absorption of the nucleoside and bases did not occur until after 30 min of incubation. The concentration of an absorbed compound was never higher on the serosal side than on the mucosal side, indicating absorption was occurring by passive diffusion.

Ruminant Digestion and Absorption. The ruminant animal, because of its unique digestive system, enjoys a symbiotic relationship with the microorganisms in the rumen. Ruminants rely heavily on the rumen microbiota for digestion of the fibrous components of the diet. Due to the large populations in the rumen, microorganisms constitute a significant proportion of the digesta entering the small intestine. Upon the digestion of these microorganisms, large quantities of nucleic acids are released into the small intestine.

Barnard (1969) reported that ruminants are also unique with respect to the levels of ribonuclease (RNase) produced in the pancreas. Production of RNase is very low in many vertebrates and evidence indicates it is nonessential in most. However, the ruminants produce extremely high levels of RNase; twice and three times the levels of the next highest groups, the marsupials and rats, respectively, which are many times higher than the majority of vertebrates. Barnard proposes these levels of RNase are used for the degradation of the

microbial RNA released into the intestine.

Smith et al. (1969) compared the nucleic acid levels entering the duodenum with those leaving the ileum to measure net absorption of nucleic acids in the small intestine of ruminating calves. They reported that 75 ± 2% of the DNA and 85 ± 1% of the RNA was degraded and lost in the small intestine. The net absorption for total N was 67 ± 2%.

Using sheep, Ellis and Bleichner (1969b) studied the growth and digestion of microorganisms in the reticulorumen, abomasum, upper two-thirds and lower one-third of the small intestine and the caecum by measuring the ratio of nucleic acid adenine to chromic oxide in the digesta. Ion exchange chromatography was used to determine the ratio in the digesta. Digestibility of nucleic acids appeared to be around 76%. Microbial nucleic acid synthesis in the caecum was found to be as much as one-third that in the reticulorumen; however, the digestibility of these nucleic acids appeared to be relatively low.

Condon and Hatfield (1970b) reported digestibility values of abomasally infused RNA-N in sheep to average 82%. Condon (1971) reported 88% of abomasally infused RNA to be digested and absorbed.

To summarize the literature on digestion and absorption, the following statements are offered. Nucleic acids are degraded in the small intestine to nucleosides and free bases by a series of enzyme reactions involving nucleases, nucleotidases and nucleosidases. Nucleosides and free bases appear to be the primary products which are absorbed. The primary mode of absorption has been found to be passive diffusion, however, active transport of pyrimidine nucleosides

and bases at low concentrations has been demonstrated.

Large quantities of nucleic acids are released into the intestines upon the digestion of rumen microorganisms in the ruminant animal. Compared to other animals, the ruminant produces extremely large amounts of RNase in the pancreas. Digestion and absorption values reported for RNA and DNA in the ruminant range from 82 to 88% and 73 to 76%, respectively.

Utilization of Exogenous Nucleic Acids

Biochemical Pathways of Utilization. According to Hartman (1970), purine nucleotides may be formed directly from purine bases by a phosphoribosyltransferase reaction involving phosphoribosyl-pyrophasphate (PRPP).

$$\text{Base} + \text{PRPP} \longrightarrow \text{nucleotide} + \text{pyrophosphate}$$

Two different purine phosphoribosyltransferases are involved; one for adenine and one for guanine and hypoxanthine. Flaks et al. (1957) studied the enzymes and reported high concentrations in beef liver.

The nucleotide adenylic acid may be formed by the reaction of adenosine kinase with the nucleoside adenosine. Inosine and guanosine are not substrates of this reaction in animal tissues. This suggests that the conversion of many purine nucleosides to nucleotides involves degradation to free bases followed by a phosphor-ibosyltransferase reaction (Hartman, 1970).

Although purine nucleosides may be formed from free bases by the reversible reaction involving phosphorylase, few nucleosides are formed by this route. This reaction is primarily a degradative one.

$$\text{Nucleoside} + \text{phosphate} \longleftrightarrow \text{base} + \text{ribose-1-phosphate}$$

Adenosine may undergo interconversion to inosine through the action of adenosine deaminase (Hartman, 1970). Brady (1942) reported the intestines of the cow, bullock, heifer and calf to have high adenosine deaminase activity.

In contrast to the purines which are salvaged primarily by the phosphoribosyltransferase reaction, the pyrimidines are salvaged primarily through nucleosides converted to nucleotides by nucleoside kinase reactions. Free pyrimidines are rapidly degraded to CO_2, NH_3, and either β-alanine or β-aminoisobutyrate. This degradation is rapid enough to prevent efficient use of exogenous pyrimidine bases for nucleotide and nucleic acid synthesis.

Monogastric Utilization. Study of the utilization of exogenous nucleic acids in monogastrics was begun as early as 1903 when Mendel et al. (1903) conducted feeding experiments as well as intravenous and intraperitoneal injections of nucleic acids using man and dogs as the experimental subjects. Increased uric acid excretion was observed in man and increased allantoin excretion in the dog.

Studies by Roll et al. (1949) revealed incorporation of ingested ^{15}N - labeled nucleic acids into the purines and pyrimidines of the body nucleic acids of rats. Hammarsten and Reichard (1949) injected rats, subcutaneously, with the ^{15}N - labeled pyrimidine nucleosides, cytidine and uridine. Although both nucleosides were found to be incorporated into the body nucleic acids, cytidine was utilized to a much greater extent than uridine.

Plentl and Schoenheimer (1943) fed ^{15}N - labeled thymine, uracil and guanine to rats. No incorporation of the ^{15}N was found in the

tissue nucleic acids. The isotope was recovered in the urinary allantoin after guanine was fed, and in urinary urea and ammonia after the pyrimidines were fed.

Bendich et al. (1949) found ^{15}N - labeled cytosine not to be incorporated into the body nucleic acids when fed to rats. Gether et al. (1949) studied the utilization of isotopically labeled hypoxanthine and xanthine in feeding experiments with rats. Neither of the compounds was incorporated into the body nucleic acids. Extensive conversion of the compound to allantoin was noted.

Abrams (1951) noted de novo synthesis of purines to be inhibited by intraperitoneal injection of the ^{15}N - labeled purines, adenine and guanine in rats. Adenine was readily incorporated, whereas, guanine incorporation was much slower and in smaller amounts. Adenine was converted and used as a source for guanine. Goldthwait and Bendich (1952) also observed a sparing of de novo synthesis in rats following the interperitoneal injection of ^{15}N - labeled adenine.

Abrams and Goldinger (1951) incubated bone marrow slices from rabbits with adenine-8-^{14}C and guanine-8-^{14}C. Both purines were observed to be rapidly utilized for nucleic acid synthesis. Both were utilized with almost complete exclusion of de novo synthesis.

Lojtha and Vane (1958) suggested that the primary source of purines for bone marrow cells, as well as the cells of other peripheral tissues, is the liver.

Studies by Smellie et al. (1956, 1958) showed the liver to be the primary site of purine synthesis and salvage.

Ruminant Utilization. In early studies, the utilization of the

exogenous microbial nucleic acids by ruminants was evaluated by the excretion of purine derivatives in the urine, primarily allantoin. Blaxter and Martin (1961) associated increased allantoin excretion in sheep with increased levels of nucleic acids in the rumen. Topps and Elliott (1965) observed a highly significant positive correlation between the level of nucleic acids in the rumen and the level of allantoin and uric acid excreted in the urine of sheep.

Ellis and Pfander (1965), noting the high levels of purine excretion in ruminants, suggested that the nucleic acids are of no nutritional value to the ruminant's tissues, thereby creating a source of nitrogen wastage in the ruminant animal. These authors pointed out this source of nitrogen wastage as a necessary cost to the ruminant for the beneficial aspects of the rumen microbes.

Ellis and Bleichner (1969a) examined the absorption and utilization of purines in sheep fed semi-purified, low purine diets containing chromic oxide. Apparent absorption of purine was determined by analysis of digesta samples from various segments of the gastrointestinal tract by ion exchange chromatography for nucleic acid, adenine, quanine and chromic oxide. Urine samples were also analyzed by ion exchange chromatography for the purine excretory products xanthine, hypoxanthine, allantoin, uric acid and creatinine. The purine excretion products accounted for an average of 27% of the purine apparently absorbed from the gastrointestinal tract. After consideration of endogenous purine excretion, it appeared that 70% of the absorbed purines were either utilized or excreted in forms other than allantoin, uric acid, xanthine, and hypoxanthine.

Condon and Hatfield (1970b) abomasally infused lambs with 20 g per day of RNA containing 3.05 g nitrogen. Lambs received one of two basal diets supplying either 8 g or 19 g of dietary nitrogen per day. Nitrogen balance trials were conducted between control and RNA infusions. Nitrogen retention was significantly increased when RNA was infused as compared to the control for those lambs fed the low protein diet. However, no significant differences were observed for those animals on the high protein diet. Urinary excretion of total nitrogen, allantoin, uric acid, xanthine, and hypoxanthine was significantly elevated for the RNA infusions over the controls.

Recovery in the urine of the purine N from the RNA infusions was estimated at 92% for the animal on the low protein and 110% for those on the high protein. It was concluded that the N from the RNA was not well utilized.

Condon (1971) in further discussions of the above work, suggested the following breakdown of nucleic acid fate:

1. Lost to the host animal - 63%
    a. 20% undigested and excreted in the feces
    b. 43% excreted as purine derivatives in the urine
2. Possible value to the host - 37%
    a. 11% into the ammonia pool from degradation of purine bases
    b. 10% into the ammonia pool from degradation of pyrimidine bases
    c. 16% from degradation products of pyrimidine bases, i.e., β-aminoisobutyrate, β-alanine.

Condon and Hatfield (1970a) and Condon (1971) reported on the abomasal infusion of lambs with the following: adenine-8-^{14}C, uracil-2-^{14}C, yeast RNA-UL-^{14}C or glycine UL-^{14}C. An ion chamber was used to continuously measure expired ^{14}C. Twelve hours after infusion, blood samples were taken and lambs were sacrificed. Tissue samples of liver, intestinal mucosa, muscle and adipose tissue were immediately analyzed for ^{14}C activity in perchloric acid soluble, RNA, and DNA fractions. Total radioactivity was determined on the contents from the abomasum, small intestine, caecum and large intestine. Total radioactivity excreted in the urine was also measured. The incorporation of ^{14}C-RNA into tissue nucleic acids was estimated to be 9-10% during the 12 hr period. Since it has been reported in time interval studies in rats that maximum incorporation of adenine into tissue nucleic acids occurs 24 to 48 hr after injection, these authors speculated that 10 to 20% of the ruminally produced nucleic acids may be utilized by tissues of the host. This level of utilization could meet the needs of the animal, thereby diminishing the importance of de novo synthesis. This was supported by the fact that glycine was not incorporated into RNA or DNA fractions, indicating little de novo purine synthesis occurring in the lambs. Adenine and uracil were also incorporated into the host animal tissue nucleic acids. A high percentage of radioactivity from adenine was recovered in the urine indicating excretion of excess purines in the urine. Around 66% of the radioactivity from uracil was recovered in CO_2, indicating the degradation of excess pyrimidines to CO_2, NH_3 and β-alanine.

In a subsequent study, Condon (1971) examined the rates and

modes of excretion of abomasally infused ^{14}C labeled adenine, uracil, RNA and glycine. Three different experiments were conducted; a portion of the work was reported by Condon and Hatfield (1970c). Wethers were infused with single doses of one of the labeled compounds, and total radioactivity in the urine and feces and expired gases was measured. A total of 88% of the infused RNA was digested and absorbed with 12% being excreted in the feces and 22% excreted in the urine. The majority of the RNA infused was metabolized to CO_2. The rate of metabolism was slower than that for any of the other infused compounds.

Uracil was found to be rapidly and extensively metabolized, with 50 to 70% of the radioactivity from the infused uracil appearing in the expired gases. This verified that pyrimidines are degraded to β-alanine, NH_3 and CO_2. Only 5% of the infused uracil was accounted for in the urine.

The majority of the radioactivity from the infused adenine, between 65 and 78%, was found to be excreted in the urine. Not more than 5% could be accounted for in the feces and expired CO_2. During the first 12 hr, the percentage of the total radioactivity in the urine as xanthine, hypoxanthine, allantoin and uric acid was 16, 18.2, 30.4, and 13.1, respectively. The ratio of these components in the urine remained relatively constant with time with the exception of hypoxanthine which decreased with time.

Smith et al. (1974) studied the utilization of radioactive bacterial nucleic acids in sheep. Escherichia coli K12 and rumen bacteria were cultured on a medium containing 8-^{14}C adenine. Analysis of the bacteria showed that 95% of the radioactivity taken up by the

bacteria from the 8-^{14}C adenine was incorporated into bacteria adenine and guanine nucleotides. Two to three times more radioactivity was present as adenosine monophosphate than as guanosine monophosphate. The labeled bacteria were injected into the rumen of sheep and urine and feces were collected until the animals were sacrificed 24 hr after injection. Tissue samples of the liver, kidney, spleen, blood, lung, heart, brain, rumen wall, large and small intestine, tongue, and muscle of the thorax and hind-limbs were taken for analysis. One *E. coli* injected animal, which was not slaughtered, excreted 15% of the radioactivity in the urine during the first 10 days; 50% of this radioactivity was excreted in the first two days. Three to five percent of the injected radioactivity was accounted for in the feces. Analysis of tissues showed 5% of the radioactivity from the bacterial nucleic acids to be contained in the liver, spleen and kidney alone. Using the values obtained from the muscle samples assayed, it was estimated that at least 20% of the radioactivity was incorporated into the muscle tissues of the body. When samples of RNA from the liver were analyzed, only adenine and guanine were found to contain radioactivity. Absence of labeling of pyrimidines indicated that the purines from the bacterial nucleic acids were incorporated into the nucleic acids of the host animal *via* a salvage pathway described by Murray (1971). Labeling of the pyrimidines would have shown the nucleic acids to be degraded to CO_2 and acetate with subsequent resynthesis of the bases.

These authors suggest that ruminants can utilize bacterial nucleic acids for the synthesis of tissue nucleic acids, and, therefore,

have little need for a de novo pathway.

While many aspects of nucleic acid production, and especially utilization, are not well understood, the following statements generally summarize the information available. Between 7 and 14% of the total nitrogen in the duodenal digesta of the ruminant is microbial nucleic acid nitrogen. Upon the hydrolysis of the microbial cells in the small intestine, the nucleic acids are released and degraded to nucleosides and free bases which are absorbed across the intestinal wall. Digestion and absorption values of nucleic acids in the ruminant range from 73 to 88%. Early studies of the utilization of exogenous nucleic acids in monogastrics and ruminants led researchers to believe the absorbed nucleic acids to be of little value to the animal. Increased levels of allantoin, xanthine, hypoxanthine, urea, and uric acid in the urine appeared to account for all or most of the absorbed nucleic acids. Later studies with isotopically labeled purine and pyrimidine nucleosides and bases have shown that animals may utilize exogenous nucleic acids by salvage pathways with resulting inhibition of de novo synthesis.

The primary salvage pathway for the purines involves the direct formation of nucleotides from bases. However, the pyrimidines are salvaged primarily by the conversion of nucleosides to nucleotides. Utilization studies in monogastrics have shown purine bases to be utilized to a greater extent than purine nucleosides and pyrimidine nucleosides to be utilized to a greater extent than pyrimidine bases.

Ruminants have been found to incorporate 15 to 25% of the absorbed nucleic acids into their own tissue nucleic acids.

OBJECTIVE

This study was conducted to compare the utilization of nitrogen from RNA, DNA and a combination of RNA and DNA with that of soy protein in sheep.

EXPERIMENTAL PROCEDURE

Two metabolism trials were conducted with 15 abomasally cannulated wether lambs. Cannulas, made from .5 cm silastic[1] tubing and dacron mesh[2], were sutured into the abomasum and extended to the outside through a hole in the abdominal wall. A period of 2 weeks was allowed for recovery and recuperation from surgery. During this time, the animals were treated for internal parasites in preparation for use in the two metabolism trials. The lambs were blocked by weight and breeding and randomly assigned within block to five treatments for the first trial. For the second trial, the lambs were rerandomized within blocks to treatments with the restriction that no animal receive the same treatment in both trials. False-bottom wooden metabolism stalls similar to those described by Briggs and Gallup (1949) were used for separate collection of urine and feces.

A 5-day period for adaptation to the stalls was allowed during which the lambs were fed twice daily (7:00 A.M. and 7:00 P.M.) 350 g of a ration consisting of 61.4% mixed grass hay, 28.8% corn, 9.6% soybean meal, and 2000 I.U. Vitamin A per .45 kilogram. Iodized salt was fed at the rate of 5 g per feeding. Water was provided ad libitum except during the 1 hr feeding periods.

Following the adaptation period was a 5-day transition period

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[1]Dow Corning Corporation, Medical Products Division, Midland, MI.
[2]E.I. Dupont de Nemours and Company, Incorporated, Wilmington, DE.

during which the animals were introduced to the experimental basal ration and experimental treatments at a rate of 10% per feeding period.

All lambs received the basal ration, fed at a level of 350 g twice per day (total of 700 g/day), which supplied 7.2 g dietary nitrogen per day (table 1). The components for the basal ration were weighed and mixed prior to each feeding period. The components were ground orchard grass hay, cob fractions, and a concentrate mix containing all the other ingredients listed in table 1.

The experimental treatments were abomasal infusions of either soy protein, RNA, DNA, a one-to-one combination of RNA and DNA, or a sham infusion twice daily. Yeast RNA[3] and salmon sperm DNA[3] were used to make the nucleic acid infusions. Tris buffer, adjusted to pH 7 with citrate was found to work well as a solvent for both the RNA and DNA.

The soy and nucleic acid infusions were calculated to be isonitrogenous, supplying 2.5 g of nitrogen per day. A 50-ml infusion of the respective nucleic acid treatments contained 8.33 g for RNA infusion, 9.98 g for DNA infusion, and 4.16 g RNA and 4.99 g DNA for the combination infusion. When soy protein[4] was infused, 9.34 g were infused at each feeding. Since tris buffer was used as the solvent for the nucleic acids, 50 ml of the buffer were used as a carrier for the soy protein, and as the negative control or sham infusion. The RNA, DNA, combination of RNA and DNA, and the sham infusion were in

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[3]INC Pharmaceuticals, Inc., Cleveland, Ohio 44128.

[4]Assay protein C-1, Skidmore Enterprises, Cincinnati, Ohio.

TABLE 1. COMPOSITION OF SHEEP BASAL DIET.

Item	Amount in Diet
Ingredient composition, %	
Corn, dent yellow, grain (4), IRN 4-02-935	48.73
Cob Fractions[a]	39.11
Sugar cane, molasses, mn 48 invert sugar, mn 79.5 degress brix, (4), IRN 4-04-696	5.00
Orchard grass, hay, S-C, cut 1 (1), IRN 1-03-433	5.00
Phosphate, defluorinated grnd., mn 1 pt F per 100 pt P, (6), IRN 6-01-780	1.16
Iodized salt (6)	1.00
Vitamin A palmitate, commercial (7), IRN 7-05-143[b]	+
Chemical Composition	
Dry matter, %	91.86
Composition of dry matter, %	
Crude protein	7.00
Nitrogen	1.12
Crude fiber	19.35
Ether extract	2.21
Ash	4.73
NFE	66.72

[a]Number 4 cob fraction, The Andersons, Maumee, Ohio.

[b]1,100 I.U. per kilogram of ration.

solution. The soy protein, however, was infused as a slurry. Syringes (50 ml) used to make the infusions were loaded just prior to the infusion period. Each animal was infused just prior to being fed and in the same order at each 7:00 A.M. and 7:00 P.M. feeding. After each infusion, the syringe was rinsed by filling with water and this was also infused into the abomasum.

A 10-day preliminary period was followed by a 10-day collection period during which all urine and feces were collected. Samples of the components of the basal ration were taken 2 days prior to the beginning of the collection period and continued until 2 days prior to the end of the collection period.

Feces were collected each day and dried in a forced-air oven for 24 hr at a maximum of 60 centigrade. Upon removal from the oven, the feces were stored in metal containers and allowed to equilibrate with ambient moisture. At the end of each trial, fecal composites were weighed and subsampled.

Urine was collected in plastic jugs containing 15 ml of a 1:1 (w/w) mixture of concentrated sulfuric acid and water, diluted in approximately 500 ml of water. Once each day the urine was diluted to a constant weight with water and a 2% sample by volume was taken. The 2% samples were composited and stored under refrigeration. At the end of the trial, the urine was mixed and subsampled. These samples were frozen until needed for analysis.

Analytical Procedures. Samples of ration ingredients, refusals, and feces were ground in a Wiley mill through a 1 mm screen. Each sample was thoroughly mixed and subsampled for dry matter, ether

extract, ash and Kjeldahl nitrogen determinations by A.O.A.C. (1975) procedures. Crude fiber was determined by the method of Whitehouse et al. (1945) using the ether-extracted samples. Kjeldahl nitrogen was also determined on all urine and infusion samples.

On the last day of each trial, blood samples were obtained from each lamb via jugular puncture at 6 and 12 hr postfeeding. Hematocrits were determined using micro-capillary tubes. Protein-free filtrates were prepared from heparinized whole blood and frozen for later analysis of urea using the method of Coulombe and Favreau (1963).

Protein-free filtrates of plasma were prepared by adding 1 ml of 20% sulfosalicylic acid solution to 4 ml of plasma, shaking vigorously, cooling, centrifuging, and filtering through glass wool. The filtrates were charged with N_2 and frozen for later analysis of plasma free amino acids on the Technicon Model TSM amino acid analyzer.

Serum was prepared and stored frozen for later analysis of serum total protein and uric acid. Serum total protein was determined by a modification of the procedure described by Oser (1965). Uric acid analyses were performed by an enzymatic procedure[5] utilizing uricase.

Urinary urea nitrogen was determined using the procedure of Coulombe and Favreau (1963). Urinary ammonia nitrogen was measured by the Conway (1958) procedure. Creatinine in the urine was

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[5]Sigma Chemical Company, St. Louis, Missouri.

determined by a procedure described by Oser (1965) using a 1% picric acid solution. Uric acid levels in the urine were measured by the same procedure used on the serum. Allantoin was determined in the urine by a modification of the procedure described by Young and Conway (1941).

Statistical Analysis. The procedures of Barr and Goodnight (1971) were used to statistically analyze the data by least squares analysis of variance. The multiple range test of Duncan (1955) was used to test for differences among treatment means.

RESULTS AND DISCUSSION

Initial weights, final weights and weight gains are shown in table 2. The animals averaged 27.9 kg at the beginning of the experiment and 28.6 kg at the finish, with an average gain of .7 kilograms. Differences in weight gain between treatments were not significant.

Apparent digestibilities of proximate components are presented in table 3. No significant differences were observed in the digestibilities of dry matter, crude fiber, ether extract, nitrogen free extract or organic matter. There was a tendency for dry matter, crude fiber and organic matter digestibilities to be lower for the sham treatment as compared to the other infusions. Razzague and Topps (1972) observed increased digestibilities of organic matter, crude fiber and cellulose when either RNA or DNA were added to the diets of lambs. They attributed dramatic increases in crude fiber and cellulose digestibility to the stimulation of rumen cellulolytic microorganisms by the RNA and DNA. The infusion of the nucleic acids into the abomasum in this study could have had the same effect in the caecum and colon. Since the infusion of soy also tended to increase the digestibility of these components, it appears that the additional nitrogen in the infusions was causing the effect rather than simply RNA or DNA.

Ether extract digestibility tended to be elevated for the sham

TABLE 2. INITIAL WEIGHTS, FINAL WEIGHTS AND WEIGHT GAINS.

Treatment	Weights		
	Initial	Final	Gain
	----------kg----------		
Sham	27.55	27.86	.32
Soy	28.32	29.36	1.04
RNA	27.73	29.02	1.29
DNA	27.95	28.49	.54
Combination	27.80	28.14	.38
S.E.[a]	.564	.597	.388

[a]Standard error of means.

TABLE 3. APPARENT DIGESTIBILITIES OF PROXIMATE COMPONENTS.

Component, %	Abomasal infusion					
	Sham	Soy	RNA	DNA	RNA-DNA	S.E.[a]
Dry matter	67.53	72.32	70.93	68.31	68.51	.626
Crude fiber	55.42	63.61	65.09	61.38	61.95	1.518
Crude protein	45.82[b]	61.74[c]	58.34[c,d]	54.92[d]	53.40[d]	.739
Ether extract	81.44	78.76	78.81	76.04	77.31	.501
N.F.E.	74.47	76.93	75.99	74.88	74.93	.569
O.M.	68.58	69.69	72.10	70.21	70.32	.810

[a]Standard error of means.

[b,c,d]Means in the same row with different superscripts are different ($P < .05$).

treatment as compared to the nitrogen infusions. No obvious explanation for the trend can be offered. Keyser (1976) observed a similar response with a sham infusion.

Crude protein digestibility was significantly elevated for the nitrogen infusions over the sham treatment. The soy infusion had a higher ($P < .05$) value than did either DNA or the combination of RNA and DNA. Infusing RNA resulted in an intermediate value.

Nitrogen intake, excretion, absorption and retention data are presented in table 4. Nitrogen intake for the animals receiving the negative control or sham treatment was 7.74 g per day. The nitrogen infusions supplied an additional 2.7 g of nitrogen giving the soy and nucleic acid treatments an average nitrogen intake of 10.4 g per day. The contribution of nitrogen from DNA was higher than desired, the difference was due to calculated nitrogen content vs. analyzed nitrogen content of the DNA.

Fecal nitrogen excretion, expressed as grams per day, was higher ($P < .05$) for the DNA and combination of RNA and DNA infusions than for the other treatments. Urinary nitrogen excretion values in grams per day were elevated ($P < .05$) for the nucleic acid and soy treatments as compared to the sham. Urinary nitrogen excretion for the DNA treatment was higher ($P < .05$) than the soy and combination infusion, but not different from the RNA. Topps and Elliott (1965) found increased levels of nitrogen in the urine to be highly positively correlated with increased levels of nucleic acids in the rumen of sheep. Condon and Hatfield (1970b) observed increased urinary excretion of nitrogen with abomasal infusion of nucleic acid.

TABLE 4. NITROGEN INTAKE, EXCRETION, ABSORPTION AND RETENTION

Item	Abomasal infusion					S.E.[a]
	Sham	Soy	RNA	DNA	RNA-DNA	
Total nitrogen intake, g/day	7.74	10.20	10.21	10.79	10.55	.037
Nitrogen excretion, g/day						
Urinary	2.39[b]	3.98[c]	4.33[c,d]	4.56[d]	3.92[c]	.069
Fecal	4.19[b]	3.99[b]	4.27[b]	4.88[c]	4.92[c]	.077
Total	6.58[b]	7.96[c]	8.60[c,d]	9.45[e]	8.84[d,e]	.183
Nitrogen absorption						
Grams/day	3.54[b]	6.21[c]	5.94[c]	5.90[c]	5.64[c]	.080
Percent of intake	45.82[b]	60.90[d]	58.17[c,d]	54.76[c]	53.40[c]	.728
Nitrogen retention						
Grams/day	1.16[f]	2.23[g]	1.61[f]	1.34[f]	1.71[f,g]	.101
Percent of intake	14.92	21.81	15.76	12.40	16.18	.959
Percent of absorbed	32.62	35.75	27.17	22.86	30.12	1.56

[a]Standard error of means.

[b,c,d,e]Means in the same row with different superscripts are different ($P<.05$).

[f,g]Means in the same row with different superscripts are different ($P<.01$).

Total nitrogen excretion was higher ($P < .05$) for the nitrogen infusions than for the sham. Among the nitrogen infusions, soy had the least total nitrogen excretion and DNA the largest, while RNA and the combination of RNA and DNA had intermediate values ($P < .05$).

Nitrogen absorption was higher ($P < .05$) for the nitrogen treatments as compared to the sham. When expressed as grams per day, differences among nitrogen sources were not significant. However, when expressed as percent of intake, soy had the highest nitrogen absorption, DNA and the combination of RNA and DNA the lowest, and RNA was intermediate ($P < .05$). By comparing fecal excretion for the nitrogen infusions with that for the sham infusion, the apparent absorption of infused N for each treatment was estimated. Absorption of the infused soy was around 100 percent. It appears the infusion of soy increased the absorption of dietary nitrogen as the soy treatment had less fecal nitrogen excretion than the sham treatment. Absorptions of the infused RNA, DNA and combination of RNA and DNA were 97, 77.4, and 72%, respectively. Smith et al. (1969) reported digestion and absorption values of 85 $\pm$ 1% for RNA and 75 $\pm$ 2% for DNA. Condon (1971) found 88% of the abomasally infused RNA to be digested and absorbed.

Nitrogen retention when expressed as grams per day tended to be elevated for the nucleic acid infusions as compared to the sham treatment, however, differences were not significant. The soy infusion had a higher ($P < .01$) retention value than either the RNA or DNA treatment, but was not different from the combination of RNA and DNA. When nitrogen retention was expressed as percent of intake or

percent of absorbed, no significant differences were observed among treatments. However, in each case, there was a tendency for the combination of RNA and DNA value to be higher than for either RNA or DNA alone. By comparing urinary excretion for the nitrogen treatments with that for the sham, the percent of the infused nitrogen excreted in the urine was estimated. From this value, the percent of the infused nitrogen which was retained was estimated to be 35, 18, 7.4 and 18% for the soy, RNA, DNA, and the combination of RNA and DNA, respectively. These values for the nucleic acids agree with those reported by Condon (1971) who speculated 10 to 20% of the ruminally produced nucleic acids are utilized by the tissues of the host animal.

Urinary nitrogen components are presented in table 5. Urinary uric acid levels were below the detection limits of the procedure employed, therefore, it is assumed that the contribution of uric acid to nitrogen excretion products was negligible. Urinary urea nitrogen values for the nitrogen treatments were higher ($P < .05$) than for the sham. The value for the combination of RNA and DNA was lower ($P < .05$) than the other nitrogen treatments. Urea nitrogen accounted for the largest proportion of the nitrogen excreted in the urine. As much as 53 and 56% for the DNA and soy treatments, respectively. It appears that the major form of excretion of the nitrogen from the infused nucleic acids was urea.

Allantoin nitrogen excretion was elevated ($P < .05$) for each of the nucleic acid treatments as compared to the soy and sham. Values associated with the infusion of nucleic acids were approximately three-fold higher than those obtained for the soy and sham treatments.

TABLE 5. UREA, ALLANTOIN, CREATININE, AMMONIA, AND OTHER NITROGEN EXCRETION IN THE URINE

Nitrogen component	Abomasal infusion					S.E.[a]
	Sham	Soy	RNA	DNA	RNA-DNA	
	g/day					
Urea	.48[b]	2.27[c]	2.15[c]	2.51[c]	1.69[d]	.063
Allantoin	.19[b]	.21[b]	.54[c]	.62[c]	.54[c]	.015
Creatinine	.26	.32	.33	.37	.32	.011
Ammonia	.018[b]	.031[b]	.082[c]	.093[c]	.101[c]	.006
Other	1.45	1.15	1.23	.97	1.28	.063

[a]Standard error of means.

[b,c,d]Means in the same row with different superscripts are different ($P < .05$).

Allantoin excretion accounted for the second largest percentage of the total nitrogen excreted in the urine. Values averaged 13.7% for the nucleic acid treatments compared to 8.4 and 5.1% for the sham and soy treatments, respectively. The increased allantoin is indicative of an increased purine catabolism associated with the infusion of nucleic acids. The allantoin values for the soy and sham treatments agree well with those reported for the control treatments of Condon (1971). However, the allantoin values determined in that study associated with nucleic acid infusions were about twice those observed in this experiment.

Creatinine nitrogen values were not affected by treatment. Values averaged 0.32 g per day, accounting for an average of 8.7 percent of the urinary nitrogen excreted.

Ammonia nitrogen excretion was higher ($P < .05$) for the nucleic acid treatments than for the sham and soy. Values were approximately three-fold higher for the nucleic acid treatments than for the soy infusion. Among the nitrogen components analyzed in the urine, ammonia accounted for the least amount of the total nitrogen in the urine. Increased ammonia levels for the nucleic acid treatments may be associated with the degradation of pyrimidines.

"Other" nitrogen in the urine, nitrogen which was not identified, was not significantly different across all treatments when expressed as grams per day. However, when expressed as percent of total nitrogen excreted in the urine, "other" nitrogen was higher ($P < .01$) for the sham treatment as compared to an average of 25% for the nitrogen treatments.

Table 6 shows hematocrit values for blood sampled at 6 and 12 hr postfeeding on the last day of each trial. Values were lower ($P < .05$) for the DNA and combination of RNA and DNA than for the sham. Hematocrit values were intermediate for the soy and RNA treatments. No significant time effects were observed.

Blood urea nitrogen (BUN) concentrations were not affected by time after feeding (table 7). The low nitrogen intake of the sham was reflected in the associated low BUN value. The level of BUN was lower ($P < .05$) for the sham than for other treatments, except the combination. The high BUN values for the RNA and DNA infusions indicate substantial absorption and subsequent catobolism of these substances to yield ammonia. The lower level of BUN associated with the combination of RNA and DNA possibly indicates a greater utilization of these substances, at least concerning nitrogen. This is supported by the data on nitrogen absorption and retention (table 4).

Serum protein concentrations (table 8) were not significantly affected by treatment or time. Values averaged 7.4 g/deciliter.

In addition to the above blood constituents, uric acid measurements were performed on serum. As with urinary uric acid, levels were below the detection limits of the procedure employed. From these results, it appears that uric acid is not an important end product of nucleic acid catabolism in sheep.

Mean essential plasma free amino acid concentrations are presented in table 9; values for the samples at 6 and 12 hr post-feeding are presented in appendix table 1. Tryptophan levels were not determined because levels were below the detection limit of the analytical

TABLE 6. HEMATOCRIT VALUES AT 6 AND 12 HOURS POSTFEEDING.

Abomasal infusion	Sampling time		
	6 hours	12 hours	Mean
	----------%----------		
Sham	32.45	31.28	31.87[b]
Soy	29.72	29.67	29.69[b,c]
RNA	30.55	30.50	30.53[b,c]
DNA	29.18	28.12	28.65[c]
RNA-DNA	30.05	27.08	28.65[c]
S.E.[a]	.690	.580	.346

[a]Standard error of means.

[b,c]Means in the same column with different superscripts are different ($P < .05$).

TABLE 7. BLOOD UREA NITROGEN CONCENTRATIONS AT 6 AND 12 HOURS POST FEEDING.

Abomasal infusion	Sampling time		
	6 hours	12 hours	Mean
	----------mg/dl----------		
Sham	6.06	6.00	6.03[b]
Soy	8.12	7.38	7.75[c,d]
RNA	7.91	8.11	8.01[d]
DNA	8.21	7.67	7.94[d]
RNA-DNA	7.04	6.80	6.93[b,c]
S.E.[a]	.298	.249	.1443

[a]Standard error of means.

[b,c,d]Means in the same column with different superscripts are different ($P<.05$).

TABLE 8. SERUM PROTEIN CONCENTRATIONS AT 6 AND 12 HOURS POST FEEDING.

Abomasal infusion	Sampling time		
	6 hours	12 hours	Mean
	mg/dl		
Sham	7.66	7.49	7.57
Soy	7.15	7.43	7.29
RNA	7.53	7.23	7.38
DNA	7.72	7.42	7.57
RNA-DNA	7.19	7.43	7.31
S.E.[a]	.124	.094	.060

[a]Standard error of means.

TABLE 9. ESSENTIAL PLASMA FREE AMINO ACID CONCENTRATIONS

Amino acid	Abomasal infusion					S.E.[a]
	Sham	Soy	RNA	DNA	RNA-DNA	
	uM/dl					
Threonine	7.82[b]	11.00[c]	13.34[d]	9.22[e]	11.84[c]	.130
Valine	16.77[b]	20.97[c]	19.32[b,c]	19.22[b,c]	17.62[b]	.360
Methionine	3.86	3.68	3.86	3.92	4.19	.066
Isoleucine	8.35	9.95	9.32	8.61	9.13	.210
Leucine	13.24	14.86	14.25	13.72	13.84	.244
Phenylalanine	6.44	6.11	6.02	6.62	6.41	.098
Lysine	5.44	5.41	5.92	6.00	5.90	.154
Histidine	9.50[f]	8.27[g,h]	8.73[f,g]	8.77[f,g]	7.26[h]	.189
Arginine	7.80[b]	10.17[c]	9.26[d]	9.12[d]	9.65[c,d]	.096
Total Essential	80.04[b]	90.87[c]	89.87[c,d]	86.03[e]	87.61[d,e]	.418

[a]Standard error of means.

[b,c,d,e]Average values within a row not having common superscripts are different ($P < .05$).

[f,g,h]Average values within a row not having common superscripts are different ($P < .10$).

system. Low tryptophan levels could be attributed to either naturally low levels in the plasma or destruction of the tryptophan during preparation of the plasma filtrate with sulfosalicylic acid and subsequent storage.

Threonine concentrations were higher ($P < .05$) for the nitrogen treatments than for the sham treatment. Among the nitrogen treatments, RNA had the highest value and DNA the lowest, with soy and the combination of RNA and DNA being intermediate ($P < .05$). Although no differences were observed between the valine concentrations for the nucleic acids and the sham, the nucleic acid values tended to be higher. The levels resulting from RNA and DNA were not different from the soy which was higher ($P < .05$) than the sham. Valine concentrations were lower ($P < .05$) at 12 than at 6 hr postfeeding (appendix table 1).

The concentrations of methionine, isoleucine, leucine and phenylalanine were not significantly affected by treatment or time, there was a tendency for the nucleic acid treatments to be elevated over the sham for each of these amino acids with the exception of phenylalanine. Lysine was also not significantly affected by treatment, however, the nucleic acid treatments tended to be higher than either the sham or the soy. Values for lysine were lower ($P < .01$) at the 12- than at the 6-hr sampling period (appendix table 1).

Histidine did not follow the general pattern established by the other essential plasma free amino acids. Values for the soy and the combination of RNA and DNA were lower ($P < .01$) than for the sham, while RNA and DNA were not significantly different from either the

soy or the sham. Histidine values experienced a time effect in which the concentrations were lower ($P < .01$) at 12 hr postfeeding than at 6 (appendix table 1).

Arginine concentrations were significantly higher for the soy and nucleic acid treatments than for the sham. RNA and DNA values were lower ($P < .05$) than the soy, with no difference between the combination of RNA and DNA and the soy. Arginine concentrations also decreased ($P < .01$) with time. Since arginine is one of the primary components of the urea cycle, the increased arginine concentrations for the nucleic acid treatments may indicate stimulation of the urea cycle by the nucleic acid infusions as a result of the degradation of these to ammonia, especially the infusion of the combination of RNA and DNA.

Concentrations of total essential amino acids were higher ($P < .05$) for each of the nitrogen infusions than for the sham. Among the nitrogen treatments, soy was the highest and DNA the lowest, with RNA and the combination of RNA and DNA being intermediate. Total essential values decreased ($P < .01$) with time for all treatments except the combination of RNA and DNA which actually increased slightly from the 6 hr to the 12 hr sampling period (appendix table 1). This difference in the combination of RNA and DNA is probably responsible for the significant treatment x time interaction for the total essential concentrations (appendix table 1). Another possible cause for the interaction may be that the soy treatment experienced a greater decrease in concentration with time than observed for the RNA, DNA or the sham.

Non-essential plasma free amino acid concentrations are presented in table 10. Individual values for 6 and 12 hr postfeeding are presented in appendix table 2. Although aspartic acid concentration for the combination of RNA and DNA was higher ($P < .05$) than the other nitrogen treatments, no difference was seen between the nucleic acid treatments and the sham. Soy was significantly lower than the sham. Aspartate plays a role in the urea cycle. Possible stimulation of the urea cycle by the nucleic acid and soy infusions could have increased the utilization of aspartate, thereby decreasing plasma levels. Also, these infusions probably resulted in increased protein synthesis which would require aspartic acid. Keyser (1976) noted decreased aspartate concentrations when soy was infused as compared to a sham infusion. Aspartate is also one of the precursors for purine and pyrimidine ring synthesis. With the aspartic acid concentration, the combination of RNA and DNA treatment might have caused a sparing effect on aspartic acid utilization for ring synthesis.

Serine concentrations were lowest for the soy, though not significantly lower than the sham or DNA treatments. RNA and the combination of RNA and DNA were higher ($P < .05$) than the soy. Oltjen and Putnam (1966) reported increased plasma free serine levels when low quality or protein deficient diets were fed. Keyser (1976) found a significant increase in serine concentrations for a sham treatment over a soy treatment. No significant difference was seen between the sham and soy in this study. Serine concentrations increased ($P < .05$) with time for every treatment, with the exception of the soy which

TABLE 10. NON-ESSENTIAL PLASMA FREE AMINO ACID CONCENTRATIONS

Amino acid	Abomasal infusion					S.E.[a]
	Sham	Soy	RNA	DNA	RNA-DNA	
	uM/dl					
Aspartic acid	5.12[b,c]	4.34[d]	4.68[c,d]	4.64[c,d]	5.23[b]	.064
Serine	12.45[b,c]	12.16[b]	13.57[c,d]	13.19[b,c,d]	14.21[d]	.157
Asparagine	3.90[b]	4.74[c,d]	5.18[c]	4.40[b,d]	5.05[c]	.075
Glutamic acid	39.34[b]	38.25[b]	40.55[b,c]	39.15[b]	42.13[c]	.329
Glutamine	4.56	5.27	5.04	4.88	4.78	.166
Proline	7.85	7.98	8.76	8.79	9.01	.428
Glycine	78.21[b]	79.67[b,c]	87.27[d]	76.51[b]	84.47[c,d]	.720
Alanine	35.76[b]	27.64[c]	30.21[c,d]	33.73[b,d]	30.32[c,d]	.611
Citrulline	7.67[b]	10.68[c]	9.12[b,c]	9.22[b,c]	9.16[b,c]	.202
Cystine	2.26	2.33	2.55	2.27	2.92	.078
Tyrosine	5.74[b]	5.84[b]	6.39[c]	6.34[c]	6.62[c]	.052
Ornithine	5.11[e]	5.91[f,g]	5.54[e,f,g]	5.26[e,f]	6.19[g]	.110
Total non-essential	209.15[b]	204.83[b]	219.87[c]	205.71[b]	220.75[c]	1.183
Total overall	288.65[b]	295.62[b]	309.03[c]	291.50[b]	308.39[c]	1.427

[a]Standard error of means.

[b,c,d]Average values within a row not having common superscripts are different ($P < .05$).

[e,f,g]Average values within a row not having common superscripts are different ($P < .10$).

decreased slightly (appendix table 2).

Concentrations of asparagine for the soy, RNA, and the combination of RNA and DNA were elevated ($P < .05$) over the sham. DNA tended to be higher than the sham, but was not significantly different from the sham or the soy.

Glutamic acid concentrations were not different among the sham, soy, RNA, and DNA treatments. The combination of RNA and DNA was highest ($P < .05$), but not significantly different from RNA. Glutamic acid is involved in the urea cycle. The increased glutamic acid values for RNA and the combination of RNA and DNA may indicate increased ammonia production due to these two treatments. This ammonia may be combining with alpha-ketoglutarate to yield glutamic acid. The increased BUN values and urea excretion associated with these treatments support this idea. Although glutamine and cystine concentrations were not significantly affected by either treatment or time, there was a tendency for the nitrogen treatments to be higher than the sham. Glutamine is a precursor of the purine and pyrimidine rings. The tendency for increased glutamine concentrations for the nucleic acids may represent a sparing of glutamine from ring synthesis when the nucleic acids were infused. Proline concentrations also tended to be elevated for the nitrogen treatments, especially the nucleic acid treatments, as compared to the sham. Proline concentrations experienced a dramatic increase ($P < .01$) from the 6-hr to the 12-hr postfeeding sampling period (appendix table 2).

Glycine concentrations were highest for the RNA and the combination of RNA and DNA, lowest for DNA and the sham, with the soy

treatment having an intermediate value ($P < .05$). Glycine concentrations were affected by time ($P < .05$) and values increased with time postfeeding for all treatments except the sham, which decreased (appendix table 2). Since glycine is a precursor for the synthesis of the purine ring, the increased level of glycine for the RNA and combination of RNA and DNA indicates a possible decrease in de novo purine synthesis with the infusion of these nucleic acids. Condon and Hatfield (1970a) attributed the decrease in glycine incorporation when RNA was infused into sheep to decreased purine synthesis. Keyser (1976) attributed higher glycine levels for sham infusion than for soy to the lower protein quality of the sham treatment. This difference was not seen in this study.

Sham had the highest concentration of alanine, soy the lowest, while the nucleic acids were intermediate ($P < .05$). Citrulline concentrations were highest for the soy and lowest for the sham with the nucleic acids again being intermediate ($P < .05$). Concentrations of citrulline were lower ($P < .01$) at 12 than at 6 hr postfeeding. The elevated citrulline values for the nucleic acids over the sham probably indicate increased activity of the urea cycle with the infusion of the nucleic acids as a result of ammonia production.

Infusion of each of the nucleic acids resulted in increased ($P < .05$) levels of tyrosine as compared to the infusion of soy or sham. Tyrosine levels experienced a time effect ($P < .05$). Concentrations decreased with time for each treatment, with the exception of the combination of RNA and DNA, which increased with time.

Ornithine concentrations were significantly elevated for the combination of RNA and DNA over the values for the sham and DNA treatments. However, neither the combination of RNA and DNA nor the DNA treatment was different from the soy and RNA. The higher levels of ornithine for the RNA and combination of RNA and DNA could, as discussed with other amino acids involved in the urea cycle, indicate increased activity of the cycle. The infusion of the nucleic acid treatments, especially the RNA and combination of RNA and DNA, caused increased concentrations of both essential and non-essential plasma free amino acids. One possible explanation appears to be an increased urea production as the result of the infusion of the nucleic acids. This is supported by the increased levels of urea excretion in the urine. Blood urea nitrogen levels were also higher for the RNA and DNA treatments.

Total non-essential plasma free amino acid concentrations were not different for the sham, soy and DNA. The infusion of RNA and the combination of RNA and DNA increased the levels of total non-essential plasma free amino acids significantly. Total non-essential concentrations increased ($P < .01$) for all treatments from the 6-hr to the 12-hr postfeeding samples. The RNA and the combination of RNA and DNA treatments had higher ($P < .05$) total overall concentrations of amino acids than the other treatments. Ammonia is a possible product of degradation of nucleic acids. Increased levels of ammonia were seen in the urine of the nucleic acid treatments. The ammonia produced could be converted to urea via the urea cycle. The elevated levels of ornithine, citrulline, and arginine for the nucleic acid infusions

suggest an increased use of the urea cycle for these treatments as compared to the sham. Once produced, the urea could be recycled to the rumen with the result of increased microbial protein production. Likely, the basal ration was more limiting in nitrogen than energy to support microbial activity. This could explain the increased plasma free amino acid levels for the nucleic acid treatments.

Table 11 presents the essential plasma free amino acid molar proportions. Molar proportion values at 6 and 12 hr postfeeding are shown in appendix table 3. Molar proportions for methionine and phenylalanine were unaffected by either treatment or time.

Threonine molar proportions were higher ($P < .05$) for the nitrogen treatments than the sham. Among the nitrogen treatments, RNA had the highest ($P < .05$) value, followed by DNA ($P < .05$), with soy and the combination of RNA and DNA being similar.

Valine values were significantly elevated for the soy, DNA, and the combination of RNA and DNA over the sham. Soy was higher ($P < .05$) than the DNA which was higher ($P < .05$) than the combination of RNA and DNA. The RNA treatment was not different from the sham or the combination of RNA and DNA. Molar proportions of valine were lower ($P < .01$) at 12 than at 6 hr postfeeding (appendix table 3). A treatment x time interaction ($P < .05$) was also observed. This is probably due to the proportionately larger decrease with time for the soy and the smaller decrease for the combination of RNA and DNA than was seen for the other treatments.

Although the isoleucine values for RNA and the combination of RNA and DNA were not different from the sham, there was a tendency for

TABLE 11. ESSENTIAL PLASMA FREE AMINO ACID MOLAR PROPORTIONS

Amino acid	Abomasal infusion					S.E.[a]
	Sham	Soy	RNA	DNA	RNA-DNA	
	moles/100 moles amino acids					
Threonine	2.68[b]	3.76[c]	4.31[d]	3.14[e]	3.66[c]	.024
Valine	5.80[b]	7.23[c]	6.10[b,d]	6.63[e]	6.20[d]	.047
Methionine	1.35	1.25	1.27	1.33	1.33	.025
Isoleucine	2.92[f]	3.32[g]	3.00[f]	3.27[g]	2.96[f]	.045
Leucine	4.23[b]	5.01[c]	4.60[b,d]	4.71[c,d]	4.52[b,d]	.050
Phenylalanine	2.23	2.07	2.11	2.25	2.03	.027
Lysine	1.87	1.83	1.93	2.06	1.92	.050
Histidine	3.30[b]	2.77[c,d]	2.83[b,c,d]	3.00[b,d]	2.36[c]	.064
Arginine	2.71[b]	3.43[c]	3.14[d]	3.12[d]	3.14[d]	.025
Total Essential	27.09[b]	30.69[c]	29.18[d,e]	29.54[d]	28.36[e]	.146

[a]Standard error of the mean.

[b,c,d,e]Average values within a row not having common superscripts are different ($P < .05$).

[f,g]Average values within a row not having common superscripts are different ($P < .10$).

the former to be higher than the sham. Soy and DNA had higher ($P < .01$) proportions than the other treatments. Values at 12 hr postfeeding were lower ($P < .05$) than those at 6 (appendix table 3).

Leucine values were higher ($P < .05$) for the soy and the DNA than for the sham. Values for RNA and the combination of RNA and DNA tended to be higher than the sham and lower than the DNA, however, differences were not significant. Leucine values were significantly affected by time. Values decreased with time for all treatments except the combination of RNA and DNA, which increased with time postfeeding (appendix table 3).

Although lysine molar proportions were not different ($P > .05$) between the nucleic acid treatments and the sham or soy, there was a tendency for the nucleic acids to be higher. Lysine values did decrease ($P < .01$) with time from 6 to 12 hr postfeeding (appendix table 3).

Histidine values were highest for the sham, lowest for the combination of RNA and DNA, and intermediate for DNA, RNA, and soy. Values for all treatments significantly decreased with time after feeding (appendix table 3).

Arginine molar proportions were significantly elevated when the nucleic acids were infused as compared to the sham. The soy treatment had the highest ($P < .05$) value of all treatments. Values were lower ($P < .01$) at 12 hr postfeeding than at 6 (appendix table 3). The increased arginine values for the nucleic acid treatments suggest increased activity of the urea cycle with the infusion of nucleic acids.

Total essential plasma free amino acid molar proportions for the nucleic acid infusions were higher ($P < .05$) than the sham, but lower ($P < .05$) than the soy. Total essential values were subject to a time effect ($P < .05$) (appendix table 3). Values for all treatments decreased with time after feeding to a greater extent and the combination of RNA and DNA to a lesser extent than did the other treatments.

Nonessential plasma free amino acid molar proportions are presented in table 12; corresponding values for 6 and 12 hr postfeeding are presented in appendix table 4.

Aspartic acid molar proportions were lower ($P < .05$) for the soy, RNA, and DNA treatments than for the sham. The combination of RNA and DNA was not significantly lower than the sham or higher than the DNA. Since aspartic acid is one of the components of the urea cycle, the lower value for the nucleic acid infusions as compared to the sham might be the result of increased activity of the urea cycle. Molar proportions of serine were highest for DNA and the combination of RNA and DNA, and lowest for the soy, with RNA and sham having intermediate values ($P < .10$). Values increased ($P < .05$) from the 6-hr postfeeding sample to the 12. Asparagine values were higher ($P < .05$) for the nitrogen treatments than for the sham. No differences were seen among the nitrogen treatments.

Glutamic acid molar proportions were higher ($P < .05$) for the combination of RNA and DNA than for the other nitrogen treatments, however, the former was not different from the sham which had the highest value. Glutamic acid values experienced a treatment x time

TABLE 12. NON-ESSENTIAL PLASMA FREE AMINO ACID MOLAR PROPORTIONS

Amino acid	Abomasal infusion					S.E.[a]
	Sham	Soy	RNA	DNA	RNA-DNA	
	moles/100 moles amino acids					
Aspartic acid	1.79[b]	1.48[c]	1.52[c]	1.59[c,d]	1.68[b,d]	.019
Serine	4.34[f,d]	4.12[f]	4.36[f,g]	4.53[g]	4.58[g]	.047
Asparagine	1.35[b]	1.60[c]	1.67[c]	1.51[c]	1.63[c]	.021
Glutamic acid	13.89[b]	13.05[c]	13.16[c]	13.21[c]	13.70[b]	.050
Glutamine	1.56	1.78	1.60	1.70	1.59	.054
Proline	2.76	2.77	2.83	2.99	2.90	.158
Glycine	27.29	26.98	28.10	26.21	27.49	.202
Alanine	12.38[b]	9.32[c]	9.76[d]	10.61[e]	9.83[d]	.048
Citrulline	2.69[f]	3.60[g]	3.03[f]	3.14[f]	3.14[f]	.075
Cystine	.80	.78	.83	.78	.94	.047
Tyrosine	1.98[b]	1.98[b]	2.07[b,c]	2.17[c]	2.13[c]	.028
Ornithine	1.75	1.99	1.78	1.71	2.00	.040
Total non-essential	73.01[b]	69.30[c]	70.84[d]	70.46[d]	71.62[d]	.159

[a]Standard error of the means.

[b,c,d,e]Average values within a row not having common superscripts are different ($P < .05$).

[f,g]Average values within a row not having common superscripts are different ($P < .10$).

interaction ($P < .05$) (appendix table 4). Values for sham and soy increased with time while the nucleic acid infusions decreased with time.

Glutamine, cystine, and ornithine molar proportions were not affected by either treatment or time. Proline was also not affected significantly by treatment. There was a tendency for the nucleic acids to be higher than the soy or the sham. Proline proportions increased ($P < .01$) with time after feeding for all treatments (appendix table 4). Glycine values were not affected by treatment. A time effect ($P < .05$) was seen for glycine; values increased with time for all treatments except the combination of RNA and DNA, which decreased slightly (appendix table 4).

Molar proportions of alanine were significantly lower for the nitrogen infusions than for the sham. Among the nitrogen infusions, soy had the lowest value, DNA the highest, and RNA and the combination of RNA and DNA were intermediate. It appears the extra nitrogen in the nitrogen infusions inhibited the production of alanine. A treatment x time interaction was seen for alanine. Values for the sham treatment increased with time while the other treatments decreased (appendix table 4).

Citrulline molar proportions tended to be higher for the nucleic acid infusions than for the sham though not significantly. Soy values were higher ($P < .10$) than the other treatments. Values for all treatments were lower ($P < .01$) at the 12- than at the 6-hr post-feeding period (appendix table 4). The tendency for the nucleic acid treatments to have higher values than the sham suggests an

increase in the activity of the urea cycle for these infusions.

Tyrosine values were higher ($P < .05$) for the DNA and combination of RNA and DNA than for soy and sham. RNA values were intermediate. A decrease ($P < .05$) was seen in tyrosine values between the 6-hr and the 12-hr postfeeding sampling period (appendix table 4).

Nonessential plasma free amino acids expressed as molar proportions were significantly lower for the nitrogen infusions than for the sham. Soy had the lowest ($P < .01$) values among the nitrogen infusions, while the nucleic acid treatments were not different from each other. Both a time effect ($P < .01$) and a treatment x time interaction ($P < .05$) were observed for total nonessential values. All values increased with time after feeding; soy increased to a greater extent and the combination of RNA and DNA to a lesser extent than did the other treatments.

SUMMARY AND CONCLUSIONS

Fifteen abomasally cannulated, growing wether lambs were used in two metabolism studies to evaluate the utilization of RNA and DNA. Wethers were blocked by weight and breeding and randomly assigned within block to one of five treatments with the restriction that no animal receive the same treatment in both trials. All animals were fed 350 g, twice daily, of a basal ration supplying 7.1 g nitrogen. In addition, the lambs received twice daily infusions of either soy protein, RNA, DNA, or a 1:1 combination of RNA and DNA or a sham infusion. Tris buffer was used as the solvent for RNA and DNA, the carrier for soy protein and the sham infusion. Infusions, except for the sham, were calculated to be isonitrogenous supplying 2.5 g nitrogen per day. Following a 10-day preliminary period, all urine and feces were collected during a 10-day collection period. Jugular blood was obtained at 6 and 12 hr postfeeding on the last day of each trial.

Crude protein digestibility was significantly elevated for the nucleic acid infusions over that for the sham. The RNA value, though not different from DNA or the combination, was comparable to that for the soy. Calculated by difference, the absorption values of RNA, DNA, and the combination of RNA and DNA were 97, 77, and 72%, respectively. Nitrogen retention, expressed in g/day, tended to be higher for the nucleic acids than for the sham. The combination of RNA and DNA was

not different from the soy treatment which had the highest ($P < .05$) nitrogen retention. Urinary urea, allantoin, and ammonia levels were elevated ($P < .05$) for the nucleic acid treatments over the sham. Urinary creatinine and "other" nitrogen (nitrogen unaccounted for) were not affected by treatment.

Blood urea nitrogen tended to be higher for the combination of RNA and DNA than the sham, while RNA, DNA, and the soy treatments were higher ($P < .05$) than the sham. Serum protein concentrations were unaffected by treatment.

The amino acid concentrations either tended to be higher or were higher for the nucleic acids, especially the RNA and combination of RNA and DNA, than for the sham. Concentrations for the nucleic acid treatments compared well with those for the soy treatment and in some cases were higher than the soy.

From these data, the following conclusions were made:

1) nucleic acids are well digested and absorbed;
2) retention and utilization of nitrogen from nucleic acids appears to be minimal;
3) the majority of the absorbed nucleic acids are excreted as urea and allantoin; and
4) essential and non-essential plasma free amino acids are increased by exogenous nucleic acids. The effect may be influenced by urea recycling to the rumen as the result of nucleic acid degradation. Urea recycling would stimulate microbial growth with consequent increases in microbial amino acid synthesis. Amino acids associated with the

urea cycle were noted to be affected.

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APPENDIX

APPENDIX TABLE 1. ESSENTIAL PLASMA FREE AMINO ACID CONCENTRATIONS AT 6 AND 12 HOURS POSTFEEDING

	Abomasal infusion										
	Sham		Soy		RNA		DNA		RNA-DNA		S.E.[a]
Amino acid	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	
	uM/dl										
Threonine	7.85	7.79	11.52	10.48	13.68	13.00	9.15	9.29	11.46	12.93	.130
Valine[c]	17.61	15.92	22.86	19.08	20.61	18.03	19.48	18.97	18.55	16.69	.360
Methionine	4.10	3.63	3.55	3.81	3.96	3.77	3.71	4.13	3.96	4.41	.066
Isoleucine	8.92	7.78	10.86	9.03	9.86	8.77	7.75	9.47	8.89	9.37	.210
Leucine	12.80	13.69	15.91	13.81	14.70	13.79	13.87	13.58	13.21	14.47	.244
Phenylalanine	6.34	6.54	6.18	6.04	6.55	5.50	6.62	6.63	6.26	6.57	.098
Lysine[b]	6.72	4.16	6.95	3.88	6.80	5.04	6.80	5.20	5.97	5.84	.154
Histidine[b]	13.00	6.01	12.16	4.38	11.96	5.51	11.95	5.60	9.02	5.51	.189
Arginine[b]	8.38	7.23	11.38	8.96	10.62	7.90	10.30	7.94	10.16	9.14	.096
Total essential	85.83	74.25	102.35	79.41	96.87	82.86	91.27	80.80	87.46	87.76	.418

[a]Standard error of means.

[b]Values at 6 and 12 hours are different ($P < .01$)

[c]Values at 6 and 12 hours are different ($P < .05$).

[d]Treatment x time interaction ($P < .01$).

APPENDIX TABLE 2. NON-ESSENTIAL PLASMA FREE AMINO ACID CONCENTRATIONS AT 6 AND 12 HOURS POSTFEEDING

	Abomasal infusion										
	Sham		Soy		RNA		DNA		RNA-DNA		S.E.[a]
Amino acid	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	
	uM/dl										
Aspartic acid	5.16	5.08	4.29	4.39	4.80	4.57	4.61	4.67	4.86	5.61	.064
Serine[c]	11.94	12.97	12.17	12.15	13.45	13.68	12.40	13.97	13.45	14.96	.157
Asparagine	3.84	3.98	5.26	4.23	5.43	4.94	4.53	4.26	4.92	5.18	.075
Glutamic acid	39.45	39.24	35.35	40.95	41.71	39.38	39.11	39.20	41.45	42.82	.329
Glutamine	4.67	4.46	5.76	4.77	5.23	4.84	4.54	5.22	4.61	4.95	.166
Proline[b]	3.37	12.34	3.72	12.23	3.36	14.15	2.99	14.59	4.09	13.93	.428
Glycine[c]	78.81	77.61	77.24	82.08	83.98	90.56	74.40	78.62	81.90	87.05	.720
Alanine	34.52	37.00	28.39	26.90	30.78	29.63	31.52	35.94	29.46	31.17	.611
Citrulline[b]	8.71	6.62	12.72	8.64	9.85	8.38	10.26	8.17	9.56	8.77	.202
Cystine	2.23	2.28	2.26	2.40	2.48	2.62	2.32	2.22	2.49	3.36	.078
Tyrosine[c,d]	5.87	5.60	6.29	5.39	6.74	6.05	6.43	6.26	6.29	6.94	.052
Ornithine	5.13	5.09	6.51	5.30	5.60	5.47	4.74	5.78	5.95	6.43	.110
Total non-essential[b]	204.61	213.68	200.28	209.38	215.49	224.25	198.24	213.18	208.96	232.55	1.183
Total Overall	292.11	285.20	302.51	288.73	310.87	307.19	289.02	293.98	296.42	320.37	1.427

[a]Standard error of means.

[b]Values at 6 and 12 hours are different ($P < .01$).

[c]Values at 6 and 12 hours are different ($P < .05$).

[d]Treatment x time interaction ($P < .05$).

APPENDIX TABLE 3. ESSENTIAL PLASMA FREE AMINO ACID MOLAR PROPORTIONS AT 6 AND 12 HOURS POSTFEEDING

Amino acid	Abomasal infusion										S.E.[a]
	Sham		Soy		RNA		DNA		RNA-DNA		
	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	
	uM/dl										
Threonine	2.69	2.67	3.82	3.70	4.38	4.24	3.15	3.12	3.70	3.62	.024
Valine[b,d]	6.04	5.54	7.84	6.61	6.35	5.86	6.77	6.49	6.26	6.13	.047
Methionine	1.43	1.28	1.18	1.33	1.30	1.24	1.26	1.39	1.33	1.33	.025
Isoleucine[c]	3.10	2.75	3.61	3.30	3.15	2.84	3.27	3.26	3.01	2.92	.045
Leucine[c]	4.41	4.05	5.25	4.77	4.74	4.46	4.81	4.62	4.46	4.57	.050
Phenylalanine	2.18	2.29	2.05	2.10	2.15	2.08	2.25	2.25	2.25	1.92	.027
Lysine[b]	2.30	1.45	2.32	1.34	2.24	1.62	2.35	1.78	2.03	1.82	.050
Histidine[b]	4.47	2.13	4.01	1.52	3.84	1.83	4.11	1.89	3.07	1.66	.064
Arginine[b]	2.88	2.55	3.76	3.11	3.47	2.82	2.52	2.71	3.41	2.87	.025
Total essential[b,d]	29.48	24.71	33.84	27.55	31.57	26.78	31.59	27.48	29.44	27.27	.146

[a]Standard error the the mean.

[b]Values at 6 and 12 hours are different ($P < .01$).

[c]Values at 6 and 12 hours are different ($P < .05$).

[d]Treatment x time interaction ($P < .05$).

APPENDIX TABLE 4. NON-ESSENTIAL PLASMA FREE AMINO ACID MOLAR PROPORTIONS AT 6 AND 12 HOURS POSTFEEDING

Amino acid	Abomasal infusion										S.E.[a]
	Sham		Soy		RNA		DNA		RNA-DNA		
	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	6 hr	12 hr	
	moles/100 moles amino acids										
Aspartic acid	1.79	1.78	1.44	1.52	1.56	1.49	1.59	1.58	1.65	1.73	.019
Serine[c]	4.19	4.51	4.04	4.19	4.28	4.43	4.30	4.76	4.52	4.65	.047
Asparagine	1.33	1.38	1.74	1.47	1.73	1.60	1.57	1.45	1.63	1.64	.021
Glutamic acid[d]	13.81	13.99	11.89	14.21	13.51	12.80	13.46	12.95	13.98	13.42	.050
Glutamine	1.58	1.55	1.90	1.65	1.65	1.55	1.57	1.84	1.58	1.61	.054
Proline[b]	1.10	4.41	1.23	4.32	1.01	4.64	1.06	4.91	1.37	4.43	.158
Glycine[c]	27.08	27.49	25.49	28.46	26.89	29.33	25.65	26.77	27.70	27.28	.202
Alanine[d]	11.88	12.89	9.40	9.24	9.92	9.59	10.89	10.33	9.94	9.72	.408
Citrulline[b]	3.04	2.35	4.21	3.00	3.21	2.84	3.51	2.77	3.32	2.96	.075
Cystine	.80	.80	.74	.82	.81	.86	.80	.75	.84	1.05	.047
Tyrosine[c]	2.01	1.96	2.09	1.88	2.17	1.98	2.23	2.12	2.10	2.18	.028
Ornithine	1.70	1.80	2.14	1.84	1.77	1.80	1.48	1.95	2.02	1.98	.040
Total non-essential[b,d]	70.74	75.28	66.16	72.44	68.78	72.90	68.41	72.52	70.56	72.69	.159
	70.74	75.28	66.16	72.44	68.78	72.90	68.41	72.52	70.56	72.69	.159

[a]Standard error of the means.

[b]Values at 6 and 12 hours are different ($P < .01$).

[c]Values at 6 and 12 hours are different ($P < .05$).

[d]Treatment x time interaction ($P < .05$).

APPENDIX TABLE 5. EXAMPLE OF ANALYSIS OF VARIANCE AND DUNCAN'S MEAN SEPARATION

General Linear Models Procedure

Dependent variable: CP

Source	DF	Sum of squares	Mean square	F value	PR > F	R-square	C.V.
Model	21	1471.07291333	70.05109111	4.28	0.0201	0.918226	7.3788
Error	8	131.00827333	16.37603417		STD DEV		CP mean
Corrected total	29	1602.08118667			4.04673129		54.84266667

Source	DF	Sum of squares	F value	PR > F
Treatment	4	859.53085333	13.12	0.0014
Block	2	156.27588667	4.77	0.0432
Trial	1	1.58700000	0.10	0.7635
Treat*Block	8	165.56964667	1.26	0.3743
Treat*Trial	4	281.91926667	4.30	0.0378
Block*Trial	2	6.19026000	0.19	0.8314

Duncan's Multiple Range Test for CP
Means with the same letter are not significantly different
Alpha level = .05 DF = 8 MS = 16.374

Grouping	Mean	N	TMT
A	61.743500	6	2 (Soy)
B A	58.338333	6	3 (RNA)
B	54.923333	6	4 (DNA)
B	53.398333	6	5 (RNA-DNA)
C	45.818333	6	1 (Sham)

APPENDIX TABLE 6. EXAMPLE OF ANALYSIS OF VARIANCE WITH A TIME FACTOR AND DUNCAN'S MEAN SEPARATION

Analysis of Variance Procedure

Dependent variable: CITC

Source	DF	Sum of squares	Mean squares	F value	PR > F	R-square	C.V.
Model	51	385.35327000	7.55594647	3.10	0.0462	0.951880	17.0221
Error	8	19.48070333	2.43508792		STD DEV		CITC mean
Corrected total	59	404.83397333			1.56047682		9.16733333

Source	DF	Sum of squares	F value	PR > F
Treat	4	54.57085667	5.60	0.0189
Time	1	66.27606000	27.22	0.0008
Block	2	16.86246333	3.46	0.0826
Trial	1	1.40454000	0.58	0.4694
Treat*Time	4	18.23955667	1.87	0.2087
Treat*Block	8	39.36360333	2.02	0.1698
Treat*Trial	4	14.60474333	1.50	0.2896
Time*Block	2	5.16843000	1.06	0.3901
Time*Trial	1	6.14400000	2.52	0.1509
Block*Trial	2	14.81971000	3.04	0.1040
Treat*Time*Block	8	17.39340333	0.89	0.5617
Treat*Time*Trial	4	17.50641667	1.80	0.2226
Treat*Block*Trial	8	81.43855667	4.18	0.0295
Time*Block*Trial	2	31.56093000	6.48	0.0212

Duncan's Multiple Range Test for CITC

Means with the same letter are not significantly different

Alpha level = .05 DF = 8 MS = 2.4351

Grouping	Mean	N	TMT
A	10.678333	12	2 (Soy)
B A	9.215000	12	4 (DNA)
B A	9.163333	12	5 (RNA-DNA)
B A	9.115833	12	3 (RNA)
B	7.664167	12	1 (Sham)

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UTILIZATION OF ABOMASALLY INFUSED RIBONUCLEIC ACID AND DEOXYRIBONUCLEIC ACID IN SHEEP

by

Jerry Lee Hale

(ABSTRACT)

Fifteen abomasally cannulated, growing wether lambs were used in two metabolism studies to evaluate the utilization of RNA and DNA. Wethers were blocked by weight and breeding and randomly assigned within block to one of five treatments with the restriction that no animal receive the same treatment in both trials. All animals were fed 350 g, twice daily, of a basal ration supplying 7.1 g nitrogen. In addition, the lambs received twice daily infusions of either soy protein, RNA, DNA, or a 1:1 combination of RNA and DNA or a sham infusion. Tris buffer was used as the solvent for RNA and DNA, the carrier for soy protein and the sham infusion. Infusions, except for the sham, were calculated to be isonitrogenous supplying 2.5 g nitrogen per day. Following a 10-day preliminary period, all urine and feces were collected during a 10-day collection period. Jugular blood was obtained at 6 and 12 hr postfeeding on the last day of each trial. Crude protein digestibility was significantly elevated for the nucleic acid infusions over that for the sham. The RNA value, though not different from DNA or the combination, was comparable to that for the soy. Calculated by difference, the absorption values

of RNA, DNA, and the combination of RNA and DNA were 97, 77, and 72%, respectively. Nitrogen retention, expressed in g/day, tended to be higher for the nucleic acids than for the sham. The combination of RNA and DNA was not different from the soy treatment which had the highest ($P < .05$) nitrogen retention. Urinary urea, allantoin, and ammonia levels were elevated ($P < .05$) for the nucleic acid treatments over the sham. Urinary creatinine and "other" nitrogen (nitrogen unaccounted for) were not affected by treatment. Blood urea nitrogen tended to be higher for the combination of RNA and DNA than the sham, while RNA, DNA, and the soy treatments were higher ($P < .05$) than the sham. Serum protein concentrations were unaffected by treatment. The amino acid concentrations either tended to be higher or were higher for the nucleic acids, especially the RNA and combination of RNA and DNA, than for the sham. Concentrations for the nucleic acid treatments compared well with those for the soy treatment and in some cases were higher than the soy. From these data, the following conclusions were made: 1) nucleic acids are well digested and absorbed; 2) retention and utilization of nitrogen from nucleic acids appears to be minimal; 3) the majority of the absorbed nucleic acids are excreted as urea and allantoin; and 4) essential and non-essential plasma free amino acids are increased by exogenous nucleic acids. The effect may be influenced by urea recycling to the rumen as the result of nucleic acid degradation. Urea recycling would stimulate microbial growth with consequent increases in microbial amino acid synthesis. Amino acids associated with the urea cycle were noted to be affected.