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EVALUATION OF THE CONTRIBUTION OF RECYCLED UREA TO THE SYNTHESIS OF THE MICROBIAL PROTEIN IN THE RUMEN USING ^{15}N-LABELLED UREA

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by

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ABSTRACT

EVALUATION OF THE CONTRIBUTION OF RECYCLED UREA TO THE SYNTHESIS OF THE MICROBIAL PROTEIN IN THE RUMEN USING ^{15}N-LABELLED UREA

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Urea-^{15}N was continually infused into the jugular vein for 3d in two duodenally cannulated cows fed diets of 1:2 and 2:1 forage:concentrate. Duodenal digesta samples were taken every 3h, and coccygeal blood and milk were sampled twice daily. Urine was collected for 5d starting 1d before infusion and total feces for 3d during infusion. Fecal samples were also taken twice daily during the 5d of collection.

Urinary excretion of ^{15}N accounted for about 90 percent of that which exited from the body; whereas, feces and milk each accounted for about 5 percent. Recovery of ^{15}N during the infusion period ranged from 30 to 50 percent of that infused. Estimates using ^{15}N ratios, as percent of the total N passing into the duodenum, that was bacterial N, were 50 to 90 percent and appeared directly proportional to DM intake of cows.

Recycled-N incorporated into rumen microbes was greater (24 vs. 14% of N in bacteria passing into the duodenum) in cows fed the high concentrate than the high forage diet. Also, incorporation of recycled N into rumen microbes was higher in the lactating than the dry cow (24 vs. 14%) and the flow of nitrogen from the rumen to the small intestine was greater for the concentrate than the forage diet (122.0 vs. 101.0% of nitrogen intake).

In summary, more recycled-N in duodenal digesta and more N flow from the rumen to the small intestine were observed in cows on the concentrate than the forage diet.

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INTRODUCTION

Importance of NPN to Protein Synthesis

The importance of dietary non-protein nitrogen to ruminants stems from the presence of microorganisms in the rumen which synthesize the essential amino acids for the host from non-protein nitrogen sources (Virtanen, 1963). These microorganisms, in addition to fermenting fibrous plant material, synthesize their own protoplasm from the end-products of protein and carbohydrate catabolism together with non-protein nitrogen from both endogenous and dietary sources. Microbial protein is mainly digested in the abomasum and small intestine, and resultant amino acids are absorbed from the small intestine. The amount of microbial protein synthesized depends on several factors, including the amount of readily available carbohydrate present in the rumen (Pearson and Smith, 1943; Annison et al., 1954; Belasco, 1956; Lewis and McDonald, 1958).

Urea is recycled to the rumen from the blood, either across the rumen wall (Houpt, 1959; Decker, et al., 1960; Engelhardt and Nickel, 1965; Houpt and Houpt, 1968) or in saliva (McDonald, 1948; Somers, 1961). Starch, the main component of grain, is regarded as one of the most suitable carbohydrate sources for maximum microbial protein synthesis because of its rate of breakdown in the rumen

(Chalmers and Synge, 1954). Often soluble carbohydrates, such as glucose, sucrose, and maltose, may be broken down too rapidly (Belasco, 1956) and cellulose too slowly (Lewis, 1957) to be as useful as starch in providing energy for microbial protein synthesis. However, a combination of the several sources will generally result in a good fermentation. This thesis deals with quantifying the amount of urea recycled into the rumen which is used for synthesis of microbial protein on a high grain vs. high forage diet fed to dairy cows.

LITERATURE REVIEW

Quantitative Models for N-Kinetics

Nolan (1974) described a quantitative whole animal model which doesn't contain definitive values but serves as a hypothesis for N kinetics (Figure 1).

Recently, a whole-animal model incorporating values for N transfer by pathways that appear biologically important was developed from results of isotope dilution studies with ^{14}C- and ^{15}N-labelled compounds for sheep given different diets (Nolan and Leng, 1972). This model indicated that transfer of urea from blood to the rumen was quantitatively less important as a mechanism for providing N to the rumen than had been previously suggested, and the results indicated that there was an important site of endogenous urea degradation in the lower digestive tract. These earlier studies have been extended by Nolan *et al*. (1976) to include direct measurements of the exchanges of N between pools in the blood, cecum and rumen (Figure 2).

The pathways of ammonia, amino acid and urea metabolism have been incorporated into a model described by Nolan and Leng (1972). For convenience, the digestive tract has been divided into three areas: (1) the anterior area of microbial colonization (i.e., the reticulo-rumen), (2) the abomasum, duodenum, jejunum and anterior ileum, and

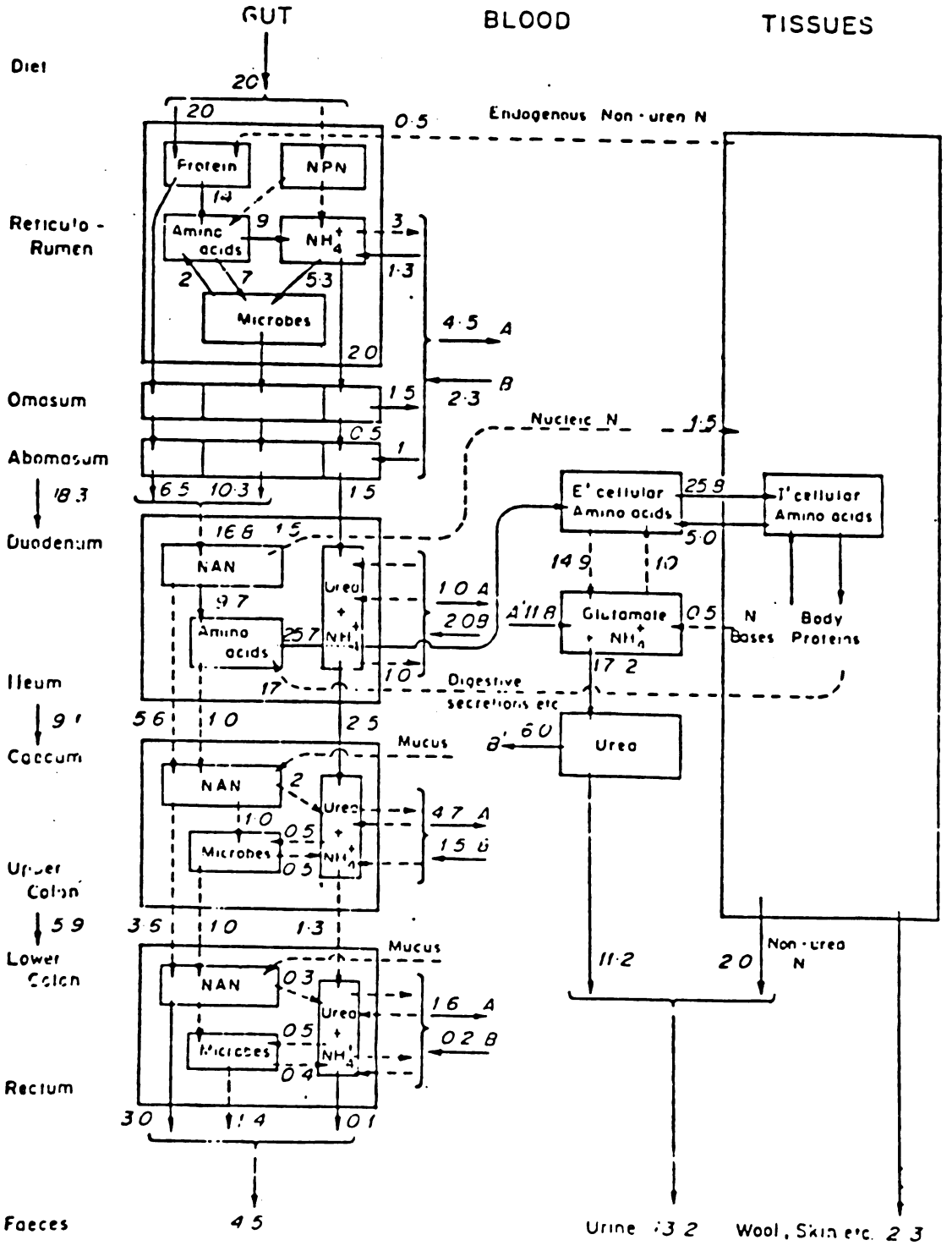

FIGURE 1. A quantitative, whole-animal model of nitrogen transactions in the sheep (from Nolan, 1974)

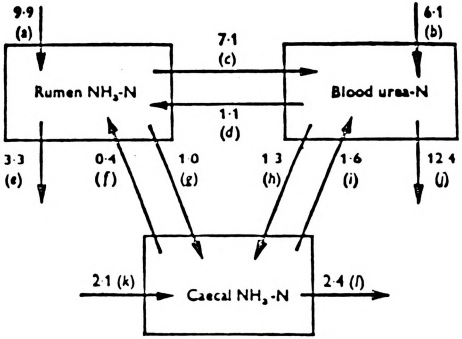

FIGURE 2. A general three-pool, open-compartment model for nitrogen transactions associated with rumen fluid ammonia, plasma urea, and cecal fluid NH_3 in sheep (from Nolan et al., 1976)

(3) the major areas of microbial colonization in the lower digestive tract (i.e., lower ileum, cecum and large bowel). The pools of N in the digestive tract were considered separately from the pools of ammonia, urea and amino acids in the body, which were considered separately from the larger pools with slower turn-over rates such as the muscle proteins and structural protein pathways which were believed to be the major routes for conversion of one nitrogenous compound into another (Figure 3).

The N of compounds digested in the rumen which do not enter the ruminal ammonia pool are changed in the rumen to nitrogenous compounds more complex than ammonia. This could occur by uptake by microbes of peptides and amino acids (Portugal, 1963; Wright and Hungate, 1967; Coleman, 1967) or by absorption of free amino acids across the rumen wall (Demaux et al., 1961; Cook et al., 1965), but the latter is assumed to be quantitatively insignificant.

Ammonia is lost from the rumen (a) by incorporation into microbial cells which pass to the abomasum, (b) by direct absorption through the rumen wall into portal blood, and (c) by loss in fluid entering the abomasum. Ammonia entering the abomasum is probably absorbed when it reaches the small intestine (Smith, 1969).

Nolan (1974) suggested a sub-model of nitrogen metabolism in the reticulo-rumen, omasum, and abomasum (Figure 4). A rumen sub-model which adequately described processes of N metabolism in the reticulo-rumen, omasum,

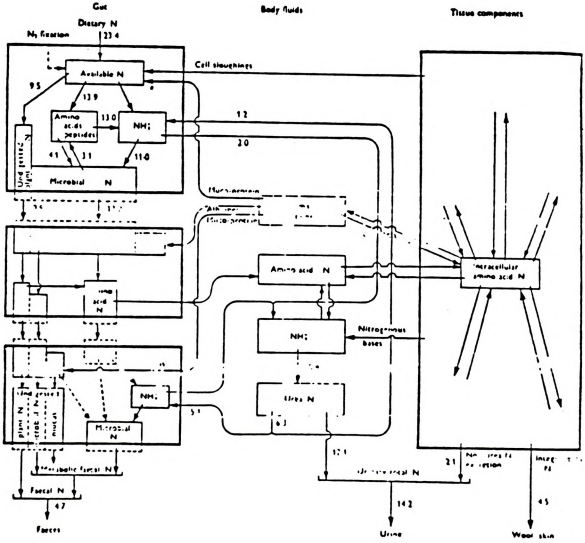

FIGURE 3. A model of nitrogen metabolism in sheep (from Nolan and Leng, 1972)

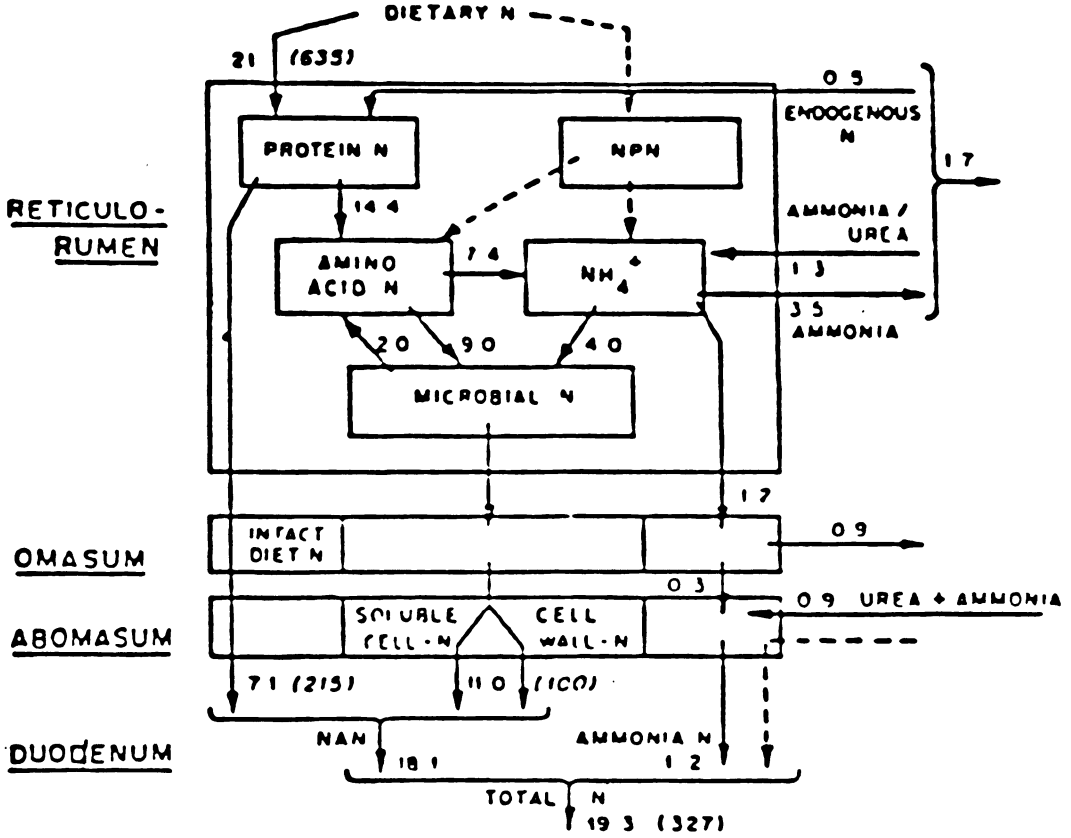

FIGURE 4. A quantitative sub-model of nitrogen transactions in the stomach of a sheep (from Nolan, 1974)

and abomasum might include quantitative assessment of the following:

1. The quantities of soluble and insoluble protein and non-protein nitrogen from the diet. Amount of endogenous urea and other endogenous N compounds, and their contribution to the total pool of N compounds which are available for fermentation in the rumen.

2. The extent to which proteins are degraded to simpler compounds (i.e., petides, amino acids and ammonia) or pass undegraded from the rumen.

3. The extent to which peptides, amino acids, ammonia and nucleic acids are assimilated by bacteria and protozoa, and the net rate of efflux of microbial N to the small intestine.

4. The quantities of soluble N compounds absorbed through the walls of the forestomachs.

5. The quantities of N recycled through pools within the rumen itself (e.g., as a result of ingestion of other microorganisms by protozoa and lysis of bacteria).

In contrast to the rumen, the large intestine has received little attention and there is little quantitative information for which a steady-state sub-model can be determined. Firstly, a conceptual model of N transitions is required. In general, digestive processes appear to be similar to those in the rumen. Cecal contents exhibit cellulase, protease, deaminase and urease activities (Hecker, 1967), and products of fermentation include

volatile fatty acids, ammonia and microbial protein (Williams, 1965).

Proteolytic activity appears to be greater in the contents of the large intestine than in the rumen (Hecker, 1971) and isobutyric and isovaleric acids occur in the cecum in proportions higher than those in the rumen. Orskov et al. (1970) indicated extensive breakdown of protein in the rumen. Although the rumen is the major site of digestion of dietary organic matter (OM), fermentation in the hind gut can play a significant role in overall digestion of structural carbohydrates; for example, in sheep given rations containing high levels of cereals, as much as 30 percent of the cellulose in a ration may be fermented by microorganisms in the hind gut (MacRae and Armstrong, 1969).

Nitrogenous compounds enter the cecum from the upper digestive tract in feed residues, undigested rumen microorganisms and endogenous materials (e.g., mucus) (Hecker, 1973). In addition, substantial quantities of urea-N enter the cecum from the blood. Hence, the total input of N into the cecum from a sheep's blood ranges between 4-15 g/day. There is generally a net absorption of N between the ileum and rectum. It seems probably that hind gut fermentation is energy-limiting, and absorption of N from the hind gut is largely as ammonia (McDonald, 1948). Absorption from the cecum and colon of other nitrogenous compounds, including amino acids (Demaux et al., 1961),

does not appear quantitatively important, but is still an open question.

Detoxication of NH_3 by Liver

It is well established (McIntyre, 1971; Oltjen et al., 1962) that the capacity of the liver to detoxify ammonia increases with increasing N intake. High protein intake resulted in a significantly greater activity of liver ornithine transcarbamylase, arginine synthetase and arginase. Chalupa et al. (1970) found an increase of three urea cycle enzymes of the liver, carbamylphosphate synthetase, ornithine transcarbamylase and arginase, when the animals were given urea. He suggested that urea-fed animals were able to detoxify more ammonia than protein-fed animals since the rate-limiting enzyme of the cycle did not change, and the average urea production rate of the liver in animals fed urea as the sole nitrogen source was significantly higher than in protein-fed animals. It is likely that during short periods after feeding the ammonia load of the liver is higher in urea-fed animals than those which receive an equal amount of protein-N. This, however, is still uncertain. A decrease of total turnover of urea with time was noticed after urea-feeding, and the total urea production was very low from 5-10 hours after feeding.

Experiments were also carried out to study the nature of ammonia toxicosis. The changes of ammonia concentration in the cerebrospinal fluid along with the

rise of the blood ammonia levels were examined. The production of cerebrospinal fluid, and also its pressure, decreased notably in the state of ammonia intoxication. Rise and fall of blood ammonia are followed by similar changes in ammonia content in the cerebrospinal fluid, but with some delay. This accounts for the observation that ammonia level in the nervous symptoms during ammonia toxicosis cannot always be explained by the level of ammonia in venous blood. Clarification of the mechanism of ammonia toxicity might be facilitated by use of ^{15}N (Juhasz, 1972).

N-Excretion

Nitrogen is eliminated from the organs partly through feces and partly through urine. Ninety percent of N-compounds originating in the organs in the course of intermediatary metabolism are excreted in the urine.

Schmidt-Nielsen and Osaki (1958) found that in sheep, urea clearance and excretion of urea suddenly decreased with low protein intake. Therefore, tubular active rediffusion of urea is the mechanism by which the organisms save urea in case of low protein supply.

Urea-N in Saliva

It is known that ruminants produce a large quantity of basic saliva. Ruminant saliva has been known, since 1921, to contain N (Kehar and Mukherjee, 1949). Later, the presence of salivary urea was demonstrated (Krober and Gibbons, 1962), and its concentration increased when higher

rumen ammonia levels occurred (Lewis, 1955). In cattle, urea-N represented an average of 77 percent of the total N in mixed saliva (Lewis, 1957). Higher salivary concentrations of total N resulted in higher percentages of urea-N. The urea content of mixed saliva was about 65 percent of the plasma urea concentration, which ranged from 4-19 mg/dl urea-N. Some data on the sheep partoid-N shows that urea represented 60-70 percent of total N in both mixed or parotid saliva (Lewis, 1962). Sheep differed from cattle in that the percentage of salivary-N as urea N was not affected by concentration of total-N (Lewis et al., 1957). Somers (1961) found that the concentration of urea-N in mixed saliva increased with increasing rate of saliva secretion, and suggested maximum concentrations of approximately 30 mg/dl for total N in saliva. After intake of urea, secretion rate of saliva decreased, blood ammonia concentrations immediately increased, and blood urea level rose after a slight delay (Juhasz, 1972). The rise of urea concentration in the blood was followed by a rise in saliva. The quantity of urea excreted per unit of time, nevertheless, fell considerably during the first hour due to a significant decrease of the salivary secretion rates. These observations show that both the composition and the secretion rate of saliva are influenced by the quantity of ingested nitrogen. If N-uptake is high, less saliva is produced, and the total quantity of urea recycled into the rumen is reduced for some time. Factors responsible for

those phenomena should probably be sought in the rumen (Juhasz, 1972).

Urea flowing from the blood into the forestomach through the wall of the rumen is considered to be an important source of ruminal nitrogen. Urea passes from the blood into the rumen by diffusion across the rumen wall and is split to ammonia by the enzyme urease. The quantity of nitrogen recycled into the rumen by diffusion from blood is about 10 times that secreted through saliva, but this varies with type of diet. At urea concentrations of 20-60 mg/dl of blood, 6-16 g urea will be recycled daily in sheep. The passage of urea across the rumen epithelium from blood to the rumen is not yet fully understood and deserves further study, perhaps by radioisotope techniques (Juhasz, 1972).

In summary, secretion of nitrogen in saliva has an upper limit. Weston and Hogan (1967) showed that following a single intraparotid infusion of urea, the secretion of total nitrogen or urea in parotid saliva reached the limit when blood urea nitrogen was approximately 30 mg/dl and did not increase with further increase in blood urea. Maximum transfer of urea through the rumen wall was found when blood urea nitrogen was about 20 mg/dl (Gartner, 1962; 1963).

Use of Recycled Nitrogen

It is now well established that ruminants use endogenous urea for protein synthesis (Decker et al., 1960;

Houpt, 1959; Simonnet et al., 1957). This special nitrogen conservation cycle involves: 1) transfer of endogenous urea from the blood into the rumen, 2) hydrolysis of this urea to ammonia and carbon dioxide by bacterial urease, 3) use of ammonia nitrogen by rumen microbes for protein synthesis, and 4) digestion and absorption of the microbial proteins from the small intestine. All of the amino acids commonly found in proteins, essential and nonessential, are synthesized by rumen microorganisms. In addition, a portion of the ammonia formed in the rumen is absorbed into the portal blood. This ammonia can be used for hepatic synthesis of nonessential amino acids, similar to what occurs in nonruminants (Rose and Decker, 1956). Through these processes considerable metabolic urea nitrogen may be reclaimed as amino acid nitrogen instead of being excreted in the urine (Houpt and Houpt, 1968).

Lower Threshold Value for Ammonia

Hydrolysis of endogenous urea to carbon dioxide and ammonia occurs in the digestive tract of all animals and depends on urease of bacterial origin (Levenson et al., 1959). In the ruminant, NH_3 is available for microbial cell growth, and microbial protein synthesized from recycled urea-nitrogen provides the animal with an additional source of available protein. Studies by Juhasz (1972) indicated the effect of the ruminal ammonia concentrations on its absorption and that the differences between the ammonia levels of portal and hepatic blood are much greater

in ruminants than nonruminants. The liver is able to extract almost all ammonia from the blood; thus, the maximum ammonia concentrations in rumen liquor at which an increase in the ammonia levels of the peripheral blood occurs is the "liver threshold value." Toxic signs are observed at high ammonia levels in the peripheral blood. Juhasz (1972) indicated that the role of the "liver threshold value" as well as the hepatic utilization or detoxication of ammonia in the synthesis of urea and probably in the synthesis of protein warrant further clarification, perhaps by isotope techniques. Recently Payne and Morris (1969) and Chalupa et al. (1970) have shown that on ingestion of large doses of urea, hepatic enzymes involved in urea synthesis increase. In sheep, as well as in cattle, augmentation of ammonia concentration in rumen liquor resulted in an increase of N in the blood (Juhasz and Kiraly, 1961). Concentration of ammonia in rumen liquor depends on quantity and quality of ingested N (Juhasz, 1972).

Urea Turnover in Blood

Short-term fluctuations in plasma urea concentrations after urea feeding are associated with a simultaneous change in total turnover. Coccimano and Leng (1967) reported a positive linear regression between plasma urea concentration and total urea turnover. Harmeyer et al. (1967) showed that no simple relation exists between plasma urea and endogenous urea turnover rate when considered under various feeding conditions and that variation increases

greatly when plasma urea exceeds 11 mM/ml. Also, they indicated that endogenous urea turnover is influenced by factors other than plasma urea concentrations. These factors are still unknown, and primarily affect long-term changes. Urease activity of the rumen wall (Houpt and Houpt, 1968) and CO_2 concentrations (Thorlacius et al., 1971) have been shown to influence urea permeability of the rumen wall. The mechanisms involved in these changes are yet to be investigated.

Other N Compounds Recycled into the Rumen

Boda and Havassy (1975) think that N, apart from urea-N, can return to the rumen in the following nitrogenous compounds (Figure 5): 1) As glutamic acid, synthesized from ammonia and - ketoglutaric acid in the rumen wall and tissues; 2) As glutamine, synthesized from glutamic acid and ammonia in the rumen wall and tissues; 3) As ammonia, eliminated from glutamine by glutaminase in the rumen wall or from other amides by amidase (glutaminase) activity in the rumen wall. For example, after intravenous administration of acetamide, NH_3 concentration in the rumen was increased (Hoshino et al., 1966; Chomyszynet et al., 1970); 4) As amino acids formed by transamination of the corresponding keto acids with glutamic acid.

Ammonia Use at Various Sites

Boda and Havassy (1975) assumed that blood urea-N utilization takes place during nitrogen recirculation

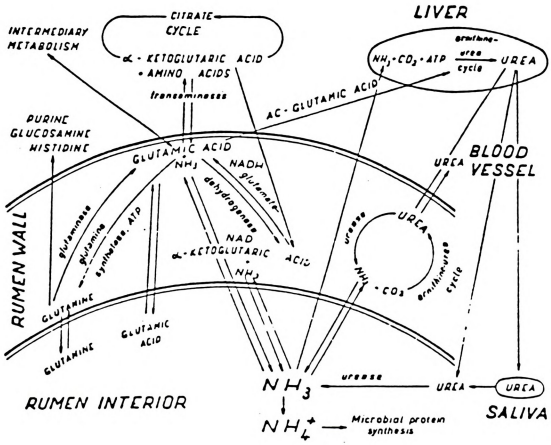

FIGURE 5. Diagram of recirculation and utilization of blood urea and other N compounds in ruminants (from Boda and Havassy, 1975)

between the liver, blood pool and digestive tract (mainly the forestomachs), in such a way that part of the ammonia produced by urea hydrolysis in the digestive tract is not taken up by the rumen microflora and is used in other parts of the digestive tract. This may occur immediately after ammonia resorption by the rumen wall and tissues, with a small portion of ammonia again converted to urea in the rumen wall (Kosarovet et al., 1972), but urea synthesis occurs mostly in the liver. Certain nitrogenous compounds, whose precursors are urea, circulate between the digestive tract and blood pool. Urea-N utilization for protein synthesis by microbes in the rumen may therefore be based on less urea synthesized from the resorbed ammonia during nitrogen recirculation than that hydrolyzed and assimilated in the forestomachs. During 24 hours, less than 50 percent of the administered ^{15}N dose may be excreted and more than 90 percent of that retained is utilized by microbes. The amount of the blood urea-N utilized daily is quantitatively significant, particularly on low protein diets (Boda and Havassy, 1975).

Since a substantial part of blood urea-N may be utilized in the rumen wall and tissues, a clear understanding of the process of this utilization is necessary for clarification of the different aspects of nitrogen metabolism of ruminants. Besides amino-acid synthesis in the rumen epithelium, glutamine synthesis in particular might be quantitatively important because its amidic-N is needed

for synthesis of purine bases (Straub, 1965; Campbell, 1970) and glucosamine (Gottschalk, 1966). These all contribute to the body stores of nucleic acid and glycoproteins.

Urea Diffusion into the Rumen

The mechanism of endogenous urea influx into the rumen has been studied in several investigations. Gartner claimed in 1962 that the flow of urea from the blood through the rumen wall has to be interpreted as an active transport. Data obtained from *in vitro* experiments showed that the amount of urea which appears at the lumen of the mucosa is not a linear function of blood urea concentration. When the urea concentrations exceeded 10 mg/dl, no further increase in ammonia concentrations at the lumen side could be detected. It was concluded that a flux of urea against a concentration gradient was not observed.

These findings of Gartner have not been confirmed by subsequent investigators. Engelhardt and Nickel (1965), as well as Houpt and Houpt (1968), reinvestigated the problem and described the transfer of urea from the blood into the rumen as a diffusion process. *In vitro* experiments with sheep's mucosa by Engelhardt and Nickel (1965) showed a linear relationship between urea flux from the blood to the lumen, and they interpreted that flux as a diffusion process.

Experiments of Engelhardt and Nickel (1965) also indicated that the flux of urea into the rumen of goats was

by diffusion. When blood urea concentration was elevated by an intravenous injection of urea, a corresponding increase of urea influx into the rumen was observed. Houpt and Houpt (1968) worked with sheep and goats using a rumen pouch and studied the changes of urea concentrations in the pouch solution at various initial pouch concentrations. They found that the direction of net flux depended on the concentration gradient, which also suggests a diffusion process. They also found that large differences existed in urea + ammonia fluxes between different animals. Under the experiemtnal conditions the pouch of goats exhibited much greater permeability than that of sheep.

Urea Flux Throughout the Body

Weston and Hogan (1967) conducted similar experiments. They administered increasing amounts of urea into the abomasum of sheep and produced a corresponding increase in blood urea and ruminal ammonia. When blood urea reached about 6.1 uM/ml, further increases failed to increase ruminal ammonia concentrations. This may be interpreted as the "saturation phenomenon" commonly associated with active transport. McIntyre (1971) found that plasma urea concentrations affected urinary urea excretion. He postulated that the kidney increased its rate of urea excretion after plasma concentrations reached about 10.7 uM/ml. Little doubt remains that the transfer of urea nitrogen from the blood into the forestomach of ruminants occurs through a

diffusion process. The data of Engelhardt and Nickel (1965) and Houpt and Houpt (1968) clearly show that the amount of urea nitrogen which enters the rumen is determined mainly by the blood urea concentrations.

Urea influx into the rumen is of quantitative importance, and it may contribute more than 50 percent of the total nitrogen utilized by rumen microbes. Starvation may be regarded as the starting point for varying food and nitrogen intakes (Varady and Harmeyer, 1972). It was shown by Varady et al. (1967 and 1970) that blood urea concentration in ruminants increases during starvation. In goats, but not in sheep, Varady and Harmeyer (1972) also found a slight increase in plasma urea 3-4 hours after feeding. Plasma urea almost doubles in sheep after 30 hours of starvation; but thereafter, concentrations continously decrease. Varady and Harmeyer (1972) suggested that the accumulation of body urea takes place because of a pronounced diminution of endogenous turnover and excretion.

It is somewhat surprising that endogenous urea hydrolysis decreases when blood urea level increases. As mentioned earlier, movement of urea in the alimentary tract is regarded as a diffusion process. Its dependency on blood urea concentration has been shown repeatedly. However, the decrease in endogenous turnover concommitantly with an increase in blood urea level may be due to a change in blood flow through the ruminal mucosa with factors other than blood urea supply being important, e.g., a change in

permeability. This idea is supported by the difference in urea space between fed and starved animals (Varady and Harmeyer, 1972).

McIntyre and Williams (1970) showed that when 12 g of urea was infused intravenously for 8 hours daily for 8 days, there was an improvement in the nitrogen balance of sheep fed low protein rations. This was attributed to increased microbial protein synthesis in the rumen resulting from recycled urea nitrogen.

Concentrations of urea nitrogen in plasma are proportional to the amount of urea nitrogen infused intravenously or intraruminally in ruminants (Weston and Hogan, 1967). Rumen ammonia concentrations in ruminants also were linearly related to the amount of urea infused intraruminally. However, McIntyre and Williams (1970) suggested that a plateau in plasma urea nitrogen concentrations of about 30 mg/dl is reached during intravenous urea infusions.

Carbon Dioxide Effect on Urea Diffusion

Thorlacius et al., (1971) studied the effect of carbon dioxide on urea diffusion through ruminal epithelium and found that the temporary substitution of 100 percent CO_2 for N_2 by gassing the rumen initiated a marked rise in the flux of urea from the blood to the rumen. This response could be divided into three phases. Initially, there was a lag period during which the flux of urea was unchanged, followed by a phase in which the urea flux rose

sharply to a maximum. The third phase was a slow decline toward the prestimulatory level. It was also reported that a graded response could be observed if the CO_2 was diluted with N_2 and the maximum observed was greatly reduced. Moreover, the length of time that flux was enhanced was considerably shorter.

Mechanism of Blood Urea Transfer into the Rumen

Houpt and Houpt (1968) hypothesized that the normal mechanism for blood urea nitrogen transfer across the rumen wall involved a close association of bacterial urease with the rumen epithelium and penetration by urease of the epithelial layers for an unknown distance, with the cornified layers constituting the major barrier to the physical movement of small molecules across the rumen wall. The ammonia molecule is smaller and more lipid soluble than urea and should penetrate cell layers much more rapidly than urea. The essence of this hypothesis is that urea molecules pass by diffusion from the blood vessels to the basal epithelial layers, the site of the bacterial urease action and are hydrolyzed to ammonia and carbon dioxide. The ammonia molecules penetrate the cornified cell barrier by diffusion and enter the rumen interior, thus enhancing urea nitrogen transfer from the blood to the rumen.

Transfer of blood urea nitrogen into the rumen is most important when the animal is on a low-protein ration. Under these conditions, rumen ammonia is extensively utilized for microbial protein synthesis, and rumen ammonia

concentrations are comparatively low. By the proposed hypothesis, the final movement of ammonia from the epithelium into the rumen would depend on a concentration gradient from the site of urea hydrolysis to the rumen interior. A low rumen ammonia concentration would incresae urea nitrogen transfer. Conversely, if concentrations were high, less urea would transfer and more of the ammonia in the epithelium would diffuse back into the blood. This relationship between rumen ammonia concentration and urea nitrogen transfer from the blood into the rumen contributes to the control of rumen ammonia concentrations (Houpt and Houpt, 1968).

Although evidence has been reported (Cocimano and Leng, 1967) that intact urea is transferred from blood to sites of microbial degradation in the digestive tract, there is controversy about the extent of transfer to the rumen and the means by which transfer to the rumen occurs (Allen and Miller, 1976). Weston and Hogan (1967) suggested a maximal transfer of blood urea to the rumen of sheep of approximately 5 g N/d, while Nolan and Leng (1972) concluded that only 1.2 g N/d was transferred.

Amount of Urea Transfer from Blood or Saliva

Secretion of urea in saliva contributes to the transfer of blood urea to the rumen (Somers, 1961), but Houpt (1959) estimated that transport of urea across the rumen epithelium could account for up to 95 percent of the total N transfer. Nolan and Leng (1972) suggested, how-

ever, that virtually all transfer of intact urea occured via the saliva, and dietary factors might explain observed differences in the importance of transfer sites.

Weston and Hogan (1967) showed that sheep on a low nitrogen diet had limited transfer of urea from blood to the rumen at plasma urea concentrations lower than 16-18 mg N/dl, but at higher plasma urea concentrations (caused by infusing urea into the abomasum) rumen ammonia could not be increased above 8-10 mg N/dl.

Recycling of Nitrogen in the Digestive Tract

Nolan and Leng (1972) reported that recycling of ammonia takes place largely within the rumen itself, namely, ammonia -> other nitrogenous compounds -> ammonia. This could result from lysis of bacteria in the rumen due to bacteriophage activity (Hoogenrand et al., 1967), engulfment of bacteria by protozoa which utilize bacterial amino acids (Coleman, 1967) and produce ammonia as an end-product of their intermediary metabolism (McDonald, 1968), or death of bacteria (Hungate, 1967). It was suggested that 30 percent of the ammonia incorporated into rumen microbial protein may have recycled through the amino acid and ammonia pools. One estimate has indicated that 40 percent of ruminal bacteria is engulfed by protozoa (Abe and Kandatsu, 1969). It was assumed that most of the ammonia-N was recycled through the amino acid pool (Nolan and Leng, 1972). If this occurs on all diets, it may be an important process in supplying the requirements for amino acids and

branched-chain fatty acids for some species of bacteria in the rumen. However, if rumen degradation of microorganisms could be prevented, it would increase the quantity of protein leaving the rumen which is available for digestion in the small intestine.

Blood Urea Transfer to the Abomasum and Intestine

Concerning the transfer of blood urea into the abomasum via gastric secretions, Nolan (1974) indicated that in sheep, only 29 percent of the ammonia N in duodenal fluid was derived from ammonia in rumen fluid. It is hypothesized that the other 71 percent is derived from blood urea that passes into the abomasum with gastric secretions (Harrop, 1974).

The transfer of blood urea into the intestines has been studied by many researchers. There is evidence that urea enters the small intestine, and concentrations of urea in intestinal digesta approach those occurring in the blood (Hecker, 1967). Towards the ileal-cecal junction, in an area where bacterial activity occurs (Ben-Gheldalia et al., 1974), urea concentrations in digesta decline (Hecker, 1967), presumably because of microbial urease. The resulting ammonia must either be absorbed from the ileum or pass into the large intestine. Infusion of labelled ammonia into the cecum indicated that urea either passes through the cecal wall or enters the gut just anterior to the cecum. Urea also enters the colon or rectum through the gut wall as indicated by the rapid labelling of ammonia in

feces (Nolan et al., 1976). Other experiments showed that most of the endogenous N passing into the duodenum originates in the forestomachs and not in the saliva. Sources of endogenous N include urea, salivary proteins, mucus from the respiratory tract, and epithelial cells sloughed from the mucosa of the buccal cavity, the esophagus and the stomach itself. Arginine, glutamate and glutamine and other amino acids may also be transferred to the forestomachs (Boda and Havassy, 1975; Harmeyer et al., 1967). Proteins, urea and ammonia are present in gastric secretions and may contribute up to 2.4 g N/d in sheep (Harrop, 1974).

Microbial Protein Synthesis

The urease activity of the rumen is sufficient to convert rapidly to NH_3 all the urea likely to be included in the diet as a partial substitute for protein. The utilization of urea by the ruminant takes place in two stages, first the conversion of urea to NH_3 and then the incorporation of NH_3 into protein.

Pearson and Smith (1943) found that about 100 g of rumen contents converts 100 mg urea to NH_3 per hour. This hydrolysis is affected by many factors, such as temperature, pH, concentration of urea, nature of gases present and presence of starch or certain inhibitory substances. Ammonia, the end product of urea hydrolysis is the preferred source of nitrogen for a large majority of rumen bacteria (Brown et al., 1958, 1960; Browning and Lusk, 1966).

Low contributions of ammonia may limit growth of bacteria. At dietary levels less than 1.2 percent nitrogen, rumen functions were impaired, feed intake was reduced and growth of the host animal stopped or was markedly limited (Bryant and Robinson, 1962; Burroughs et al., 1951a, 1951b; Caffrey et al., 1967a).

Phosphorus is probably the most important mineral for stimulating microbial growth, but others are also effective (Brown et al., 1958; Caffrey et al., 1967b; Colovos et al., 1963, 1967; Conrad and Hibbs, 1961). Sulfur is apparently needed in largest amounts for optimal utilization of urea and other forms of NPN (Colovos et al., 1967) and should be considered when formulating rations relatively high in NPN.

Portugal (1963) used carbon-labelled amino acids and found that only about 10 percent of the carbon in amino acids was incorporated into microbial protein in the rumen; whereas, Weller et al. (1962) concluded that up to 80 percent of the dietary plant N is incorporated into microbial cells. These findings indicate that the carbon and N of dietary protein are separated during metabolism in the rumen before incorporation into microbial cells. Bryant and Robinson (1962) showed both a requirement and preference of certain rumen organisms for ammonia compared to amino acids. Hobson et al. (1968) found that one rumen isolate formed 93 percent of its cellular N from ammonia.

Clearly, rumen ammonia is an important source of N for rumen microbes.

Investigations using heterotrophic bacteria have suggested increased incorporation of amino acids and peptides when these were readily available (Warner, 1956). Since there is usually a positive correlation between non-ammonia and ammonia-N in the rumen (Blackburn and Hobson, 1960), ammonia increases are likely to be accompanied by enhanced amounts of amino acids and peptides available to the rumen microbes.

Of the calculated incorporation of N into microbial cells, it has been generally assumed that 80 percent was derived from ammonia and 20 percent from amino acids. Pilgrim et al. (1970) estimated in sheep given lucerne that 62-64 percent of the N in bacterial protein and 35-41 percent in protozoal protein was derived from ruminal ammonia.

The presence of dietary sources of readily available carbohydrates in the form of starch or sugar enhanced the microbial synthesis of protein, and nitrogen balances in sheep fed such rations were greater than on all-roughage rations (McIntyre and Williams, 1970).

Using ^{15}N-Urea in Tracing N-Metabolism

On the first and third days when ^{15}N-urea was administered intravenously, more than 50 percent was retained and only a small amount (0-6%) appeared in rumen fluid (McIntyre and Williams, 1970). The net passage into the rumen of nitrogenous compounds, whose precursor was blood

urea, was increased when the capacity to retain blood urea was lowered, i.e., when dietary nitrogen intake was high. In other studies by Boda and Havassy (1975) with high and low N diets, blood ^{15}N-urea incorporation into TCA precipitable protein in plasma was slower on low N and blood ^{15}N decreased more rapidly on high N. On the 4th and 5th day after infusion, when net passage of ^{15}N into the rumen was markedly lower for the low-N diet, incorporation of ^{15}N into plasma protein had increased.

When ^{15}N-urea was introduced through a fistula into the anterior part of the jejunum, 63 percent of the dose was retained by the animal. Hence, excretion of ^{15}N in urine and feces totalled 37 percent (Boda et al., 1976). About 81 percent of the intra-jejunally administered ^{15}N participated in metabolic processes (retained N plus urinary excreted N). Apart from the rumino-hepatical circulation, the entero-hapitical circulation of nitrogenous substances, including endogenous urea-nitrogen, plays a quantitative role in recycling of blood urea nitrogen recycling. Boda et al. (1976) also showed that all parts of the digestive tract take part in blood urea-N utilization, and nitrogen compounds synthesized from blood urea-^{15}N were recycled into the alimentary tract. Nitrogen compounds were secreted from blood mainly into the forestomachs, abomasum and duodenum, but were absorbed from the entire intestinal tract.

In other studies, Boda et al. (1976) attempted to confirm the hypothesis that blood urea in ruminants is a qualitatively significant nitrogen source by administering ^{15}N-urea intravenously. It was shown that in the nitrogen pool of sheep, considerable ^{15}N (44-76% from a given dose) was retained in the body and the greater part of the unretained portion was excreted in urine within 24 hours. Excretion in feces amounted to 1.35-2.37 percent of the ^{15}N dose. After low nitrogen intake, more ^{15}N from a given dose was retained in the nitrogen-pool than after high nitrogen intake. The daily ^{15}N excretion in urine and the ^{15}N ammonia level in rumen fluid were parallel.

The ^{15}N from the labelled urea transported in blood passes into the sheep's rumen relatively quickly with maximum enrichment 3 hours after a single infusion. Boda et al. (1976) supposed that considerable amounts of ammonia produced in the rumen wall by means of urea hydrolysis could be used to direct the enzymatic synthesis of amino acids and other nitrogenous substances in the rumen wall, liver or other organs. Most of these presynthesized nitrogenous substances pass into the rumen and probably into the other parts of the alimentary tract, and serve as nitrogen sources in digestive processes. The atom-percent ^{15}N enrichment in blood as well as the percentage of ^{15}N from a given dose decreased in the course of 24 hours and then increased again during the next two days. It may be presumed that for the first 3 hours after a single infusion of

^{15}N-urea into the blood, incorporation of ^{15}N into the blood plasma proteins takes place via synthesis of amino acids from ammonia in the liver after urea hydrolysis in the alimentary tract. This synthesis is realized predominantly by the enzyme glutamate which catalyzes the synthesis of glumatic acid and its subsequent transamination. From the second to the third day, the labelled urea nitrogen was probably incorporated into the blood plasma proteins by two routes: amino acids absorbed from synthesis of microbial proteins and synthesis from non-microbial nitrogen compounds such as amide-N for synthesis of purine bases and histidine (Campbell, 1970), as well as glucosamine (Gottschalk, 1966), which are all necessary for synthesis of nucleic acids and prosthetic groups of glycoproteins.

Excretion of ^{15}N-Urea

Boda et al. (1976) found that of the ^{15}N-urea excreted after a single infusion, most appears on the same day the labelled urea was administered. On the following day, only small amounts of the ^{15}N (1.2-4.2%) were excreted, and on the 3rd-9th day, ^{15}N excretion was imperceptible. The amount of ^{15}N excreted in urine was extremely variable (24-56% of that infused). At the low nitrogen intake, blood urea-N retention was higher (70-76% of infused) than at higher nitrogen intake (44-62% of infused). Lower nitrogen intake was associated with lower ammonia concentrations in the rumen, lower blood urea levels and lower nitrogen excretion in urine.

Excretion of ^{15}N in feces was less than 1 percent of the dose, and was negligible in comparison with that in urine. The ^{15}N urinary excretion of intravenously administered urea practically ceases two days after infusion of a single dose. This indicates that the retained blood urea-N is incorporated into other nitrogen metabolites used for biosynthetic processes.

The percentage of the retained ^{15}N decreased proportionally with increased N excreted in urine in 24 hours, with increased ammonia concentration in the rumen, and with urea in blood plasma before feeding (Boda and Havassy, 1975). However, many metabolic pathways associated with blood-N utilization are still unknown.

Conclusion

Based on the literature mentioned, nitrogen recycling in dairy cows has been demonstrated on both high and low-N diets. Some challenges which remain for further investigation are: 1) To develop more precise methods to quantitatively predict nitrogen recycling; 2) To establish the role of energy level of the diet in affecting the amount of nitrogen recycling that occurs. The following study was designed to use ^{15}N-labelled urea as a precise method to estimate the urea movement from the blood into the rumen microorganisms pool and its contribution to the microbial protein synthesis.

MATERIALS AND METHODS

Animals

Two dairy cows fitted with flexible (T) cannulas in the proximal duodenum were used for two periods (28 days each). During the first period, cows were fed a diet (C) of 1:2 forage:concentrate, while a 2:1 forage:concentrate diet (F) was fed during the second period.

The forage was a mixture of 70 percent of alfalfa cubes and 30 percent of cotton seed hulls. Composition of the concentrate mixture is shown in Table 1.

Table 1. Composition of Concentrate Mixture Fed in Both Diets[a].

Component	Percent
Flaked milo	87.0
Soybean meal	7.0
Molasses	4.0
Dical	1.5
Salt	.5
Vit A	---

a) The concentrate mixture was fed at 1:2 forage:concentrate or 2:1 forage:concentrate.

Diets were calculated to be isonitrogenous, but crude protein was determined to be 14 percent for the C diet and 13 percent for the F diet.

Chromium oxide (Cr_2O_3) was given as a marker in gelatin capsules at feeding (12h intervals). Each capsule contained 12g of Cr_2O_3 powder.

Urea enriched with ^{15}N at 10 atom percent was dissolved in 3,000 ml of sterile saline solution, and continually infused at 2g ^{15}N isotope per day for 3d using infusion tubing placed in the jugular vein which was attached to a bag containing infusate. Bags were placed directly above each cow. Drops of infusate were adjusted regularly so that ^{15}N urea was infused at a constant rate over the entire period.

The infusion apparatus allowed cows full comfort and normal behavior in eating, drinking, and movement. Cows were adjusted to their shaded, cement pens equipped with rubber mats for several weeks before infusion started so as to retain normal habits in eating and drinking.

Sample Collections

Duodenal digesta were collected every 3h starting 1d prior to infusion, and sampling continued for a total of 5d. Samples were collected in clean plastic jars (400 ml digesta each time), pooled for 3, 6, 8, 12, 24 and 30h , and frozen at -20C until analyzed. Coccygeal blood was sampled twice daily using heparinized sterile vacutainer tubes (10ml) and spun immediately in a refrigerated

centrifuge at 4500 rpm. Plasma was then separated and frozen at -20C until analyzed. Milk samples were taken every milking during the 5d collection period in sterile plastic bags and frozen at -20C until analyzed. Fecal samples were taken from the rectum twice daily during collection and frozen in plastic bags at -20C until analyzed during the 3d of infusion. Total feces were collected in clean covered buckets for 3d. The daily fecal production was thoroughly mixed, sampled and frozen at -20C until analysis. Total collection of urine was at 12, 24, and 36h by use of a sterile 24" french urinary catheter equipped with 75 baloon, which was inserted into the cows bladder and then inflated. The catheter was connected to a 2 l collection bag and then to a 20 l plastic bucket. Hydrochloric acid was added daily to the urine collection bucket to prevent escape of ammonia. Samples of urine were collect daily and refrigerated until analysis.

Sample Preparation

Duodenal digesta samples were thawed at room temperature and oven-dried at 50C to avoid heat damage. Fecal samples were prepared similarly. For obtaining bacteria from duodenal and rumen fluid, unfrozen samples of digesta were strained through 3 layers of cheesecloth and wool pyrex, differentially centrifuged at 3,000 and 18,000 g (Smith and McAllan, 1974), and then washed with a buffer solution.

Analytical Procedures

Duodenal digesta, feces, milk, blood, urine and bacteria from the rumen and duodenum were digested with sulfuric acid using a block digestor (Goering and VanSoest, 1970). In preparation for ^{15}N analysis, sample aliquots were steam-distilled (Barker and Volk, 1964), the liberated ammonia was collected into 0.05 MHCL, and the resultant solution was dried on a drying block at 95C for 24 hours. Samples were then analyzed for ^{15}N using mass spectrometry (Consolidated Electrodynamics Corporation model 21-621). The atom percent ^{15}N in the samples was calculated from the N-28 to N-29 mass ratio following the methods of Bremner (1965) and Frota and Tucker (1972, 1978). The main steps of these procedures were summarized as follows by Mohammed and Tucker (1981):

1. About 2 ml of solution were brought to complete dryness.
2. The completely dried sample (now as a salt) was cooled and the air was replaced with argon gas.
3. About .3 ml of argon-saturated deionized water was added to each sample which was held on ice. The water was frozen immediately, forming an ice layer over the sample to keep it free of air. The mixture of ice and sample were then connected to the argon gas system.

4. A similar amount (.3 ml) of argon-saturated sodium hypobromite was then added to each sample and allowed to freeze.

5. The sample tubes were attached to the mass spectrometer. This was followed by a diffusion pump- to insure an air-free condition. Samples were thawed to permit sodium hypobromite to react with ammonia and convert it into N gas. The reaction involved is generally represented as follows:

   $2\ NH_3 + 3\ NaOBr \text{----}> 3\ Na\ Br + 3\ H_2O + N_2$

   (Bremner, 1965)

6. Finally, the N_2 was analyzed for peak heights of N-28 and N-29 mass from which the atom percent of ^{15}N was calculated as follows:

   Total atom percent of $^{15}N = 100/2R + 1$

   Where R = N-28 peak / N-29 peak

All samples were analyzed for nitrogen using the Technicon Auto Analyzer and for dry matter by oven-drying at 100 C according to AOAC (1980).

Sample aliquots were prepared for chromium assay by initial digestion with $H_2S^0_4$ and redigestion using periodic acid and then analyzed by atomic absorption spectrophotometry.

RESULTS

Dry matter intakes were similar for both diets with the lactating cow (no. 6) consuming about 60 percent more than the non-lactating cow (no. 958) (Table 2). Uncorrected dry matter disappearance (across diets) in the rumen averaged (27 and 46%) on the grain and forage diet, respectively. Whereas, 53 and 68 percent of diet dry matter was fermented in the rumen on the grain and forage diet respectively, after correction for de novo synthesis of microbial cellular dry matter (Appendix Table 18).

Dry matter fermented in the rumen of the lactating cow was similar with both diets, while the dry cow showed unexpectedly low values when fed the grain diet, especially for uncorrected DM (Table 2). Total tract digestibility of DM was slightly higher for the grain diet (76 vs. 73%), thus, resulting in more intestinal degradation on this diet (Appendix Table 19).

Bacterial synthesis values (gcp/kgDMc digested in rumen) were (71, 54% on the grain diet and 74, 74% on the forage diet) (Appendix Table 8). Even though slightly more dry matter disappeared on the forage diet which was predominantly fiber, that used on the grain diet was mostly starch which might result in a higher efficiency of microbial synthesis.

Table 2. Intake, Duodenal Flow and Fecal Output of DM of Cows Fed High Grain or High Forage Diets.

	Diet					
	High Grain			High Forage		
COW	958	6	Ave	958	6	Ave
Dry matter (Kg/d)						
Intake	8.1	13.7	10.9	8.5	14.1	11.3
Duodenal Flow						
(Total)	7.5	7.4	7.5	4.6	7.8	6.2
From Feed	4.58	5.14	4.86	2.45	4.84	3.64
From Bacteria	2.87	2.30	2.59	2.13	2.92	2.52
Fecal Output	1.62	3.72	2.67	2.05	4.34	3.20
Digested[a]						
In Rumen	3.52	8.56	6.04	6.05	9.26	7.66
Post Duodenum	5.88	3.68	4.83	2.55	3.46	3.00

a) Corrected for de novo synthesis of microbial cells.

Nitrogen intakes were similar for both diets, with the lactating cow (no. 6) consuming about 62 percent more than the non-lactating (no. 958) (Table 3).

More duodenal-N flow was observed in cows fed the high grain than high forage diet (122 vs. 101% of intake) (Appendix Table 8). Comparing the diets, nitrogen flow was 286 g/d on high grain and 233 g/d on high forage. Total tract N digestibility was similar for the two diets (73.4 vs. 73.2%) (Appendix Table 19).

Table 3. Nitrogen Intake, Duodenal Flow and Fecal Output of Cows Fed High Grain or High Forage Diets.

	Diet					
	High Grain			High Forage		
COW	958	6	Ave	958	6	Ave
Nitrogen (g/d)						
Intake	191.2	295.0	243.1	174.0	294.0	234.0
Duodenal Flow						
(Total)	269.0	303.0	286.0	184.0	282.0	233.0
From Feed	79.2	139.6	109.4	48.4	74.5	61.5
From Bacteria	189.8	163.4	176.6	135.6	207.5	171.5
Post Duodenum	226.6	213.4	220.0	141.1	196.8	168.9
Fecal Output	42.4	89.6	66.0	42.9	85.2	64.1

Nitrogen Enrichment in Biological Samplings

Nitrogen enrichment of all samplings gradually increased with time after initiation of urea ^{15}N infusion. In duodenal digesta, ^{15}N appeared as early as 8h after beginning of infusion, and gradually increased with time and peaked at 60-70h. The average of the highest 3 enrichment values (Table 4) were .071 and 0.74 atom-percent for the two cows on the grain diet (Figures 6 and 10 in Appendix) and .064 and .043 atom-percent for the two cows on the forage diet (Figures 11 and 12 in Appendix).

In duodenal bacteria, ^{15}N also appeared at about 8h after initiation of infusion, and, as expected, the pattern paralleled that of duodenal digesta. The average of the 3 highest values for bacterial enrichment were .092 and .14

atom-percent for the two cows on the grain diet (Figures 6 and 10 in Appendix) and .075 and .077 atom-percent for the two cows on the forage diet (Figures 11 and 12 in appendix).

Table 4. Average of 3 Peak Values for ^{15}N Enrichment of Each Cow in Duodenal Digesta, Duodenal Bacteria, Urine, Feces, Milk, and Blood[a].

		Enrichment (Atom-Percent)					
DIET	COW	Digesta	Bacteria	Urine	Feces	Milk	Blood
Grain	958	.071	.092	.62	.066	----	.015
	6	.074	.14	.44	.072	.062	.017
	Avg	.073	.12	.53	.069	.062	.016
Forage	958	.064	.075	.59	.060	----	.017
	6	.043	.077	.49	.053	.060	.023
	Avg	.054	.076	.54	.057	.060	.020

a) For calculation of ^{15}N enrichment, observed values were subtracted from a standard value of .361 which is the ^{15}N concentration of non-enriched nitrogen that {i.e., for cow 958 on grain diet: .432 (observed value) - .361 = .071 (enrichment value)}.

Nitrogen enrichment in urine appeared as early as 12h after initiation of infusion, rapidly increased with time, and peaked at 60-70h after infusion began. Urinary ^{15}N enrichment was assumed to equal blood urea ^{15}N enrichment. Hence, all calculations for ^{15}N enrichment of recycled N were based on urinary ^{15}N in blood urea from other blood nitrogen components, so ^{15}N was measured in blood plasma. The average of the 3 highest peak values for urinary enrichment were .62 and .44 atom-percent for the two

cows on the grain diet (Figures 7 and 13 in Appendix) and .59 and .49 atom-percent for the two cows on the forage diet (Figures 14 and 15 in Appendix). Most of the ^{15}N excreted from the body exited through the urine with urinary ^{15}N enrichment values 10 times as high as those in bacteria, feces or milk (Table 4).

Nitrogen enrichment in feces and milk appeared as early as 10 to 12h after initial infusion and gradually increased with time to peak at 50-80h (Figures 8, 9, and 16-19 in Appendix). About 15 percent of the fecal and milk-N originated from blood urea (Table 4).

Fate and Recovery of ^{15}N Which was Infused Into Blood

Nitrogen-15 retained in the different tissues and organs of the body was higher in the two cows fed the forage than the grain diets (70.1 vs. 56.9%) (Table 5). These results are in agreement with the work of Smith et al. (1982) who showed that 40-70 percent of ^{15}N dose was retained in the body of cows fed corn silage treated with ammonia-^{15}N.

Table 5. Fate of ^{15}N Infused into Blood[a].

DIET	COW	Urinary Excretion (g/d)	Duodenal Passage (g/d)	Output (g/d) in Milk	in Feces	Retained (%)
Grain	958	.62	.188	---	.05	60.9
	6	.82	.224	.06	.05	52.9
	Avg	.72	.206	.06	.05	56.9
Forage	958	.71	.070	---	.03	74.5
	6	.79	.074	.04	.05	65.7
	Avg	.75	.072	.04	.04	70.1

a) Amount of ^{15}N infused was 2 g/d.

Duodenal and Bacterial Protein N Originating from Blood-Urea (Recycled-N)

As shown in Table 7, more recycled nitrogen appeared in duodenal and bacterial protein of cows fed the grain than the forage diet. It was assumed that essentially all the ^{15}N passing into the duodenum was as bacterial protein. An example of the method used in calculating the amount of duodenal and bacteria N originating from blood urea is given in Table 6. As was mentioned, urinary enrichment which was measured directly, was assumed equal to blood urea enrichment because urinary urea came directly from blood urea with only minimal dilution of other N source.

Percent ^{15}N enrichment in bacteria isolated from the duodenal digesta of cows fed the forage diet was similar to that of bacteria isolated from the rumen.

Also given in Table 7 are measurements of the percent of the total duodenal N as bacterial N, which was calculated as the ratio of ^{15}N enrichment between duodenal and bacterial N.

Table 6. Example of Calculation, Cow No. 6 on Grain Diet

Item	Percent
Urinary enrichment in ^{15}N	.44
Blood urea enrichment in ^{15}N (assumed)	.44
Digesta enrichment in ^{15}N	.07
Percent of N in digesta coming from blood urea (.07/.44)	17
Bacteria enrichment in ^{15}N	.14
Percent of N in bacteria (exiting rumen) coming from blood urea (.14/.44)	32

Table 7. Percent of N Which Originated From Blood Urea (or Recycled N) Recovered in Duodenal or Bacterial N.

DIET	COW	Recycled Duodenal N (total N in digesta) Percent	Recycled Bacterial N (total bacterial N) Percent	Total N as Bacteria Percent
Grain	958	11.9	15.2	78.3
	6	17.3	32.6	53.1
	Avg	14.6	23.9	61.1
Forage	958	11.2	12.6	88.8
	6	8.7	14.9	58.4
	Avg	9.9	13.8	71.7
SEM		2.79	5.34	

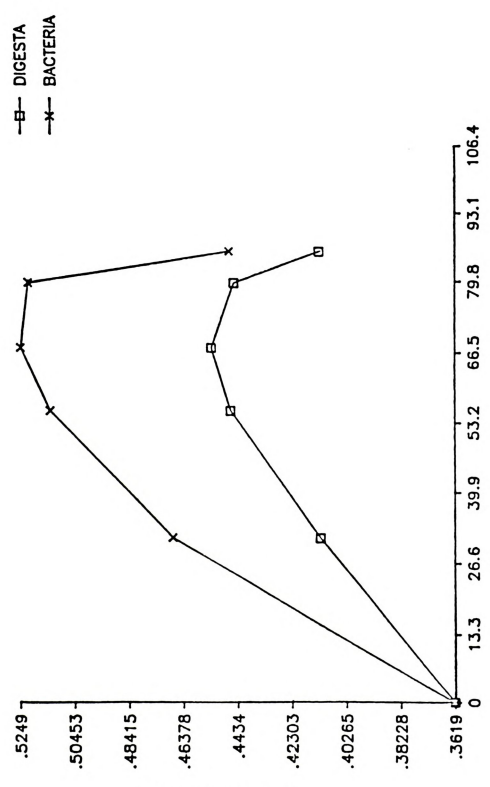

FIGURE 6. ^{15}N-Enrichment in duodenal digesta and duodenal bacteria of cow (6) on grain diet

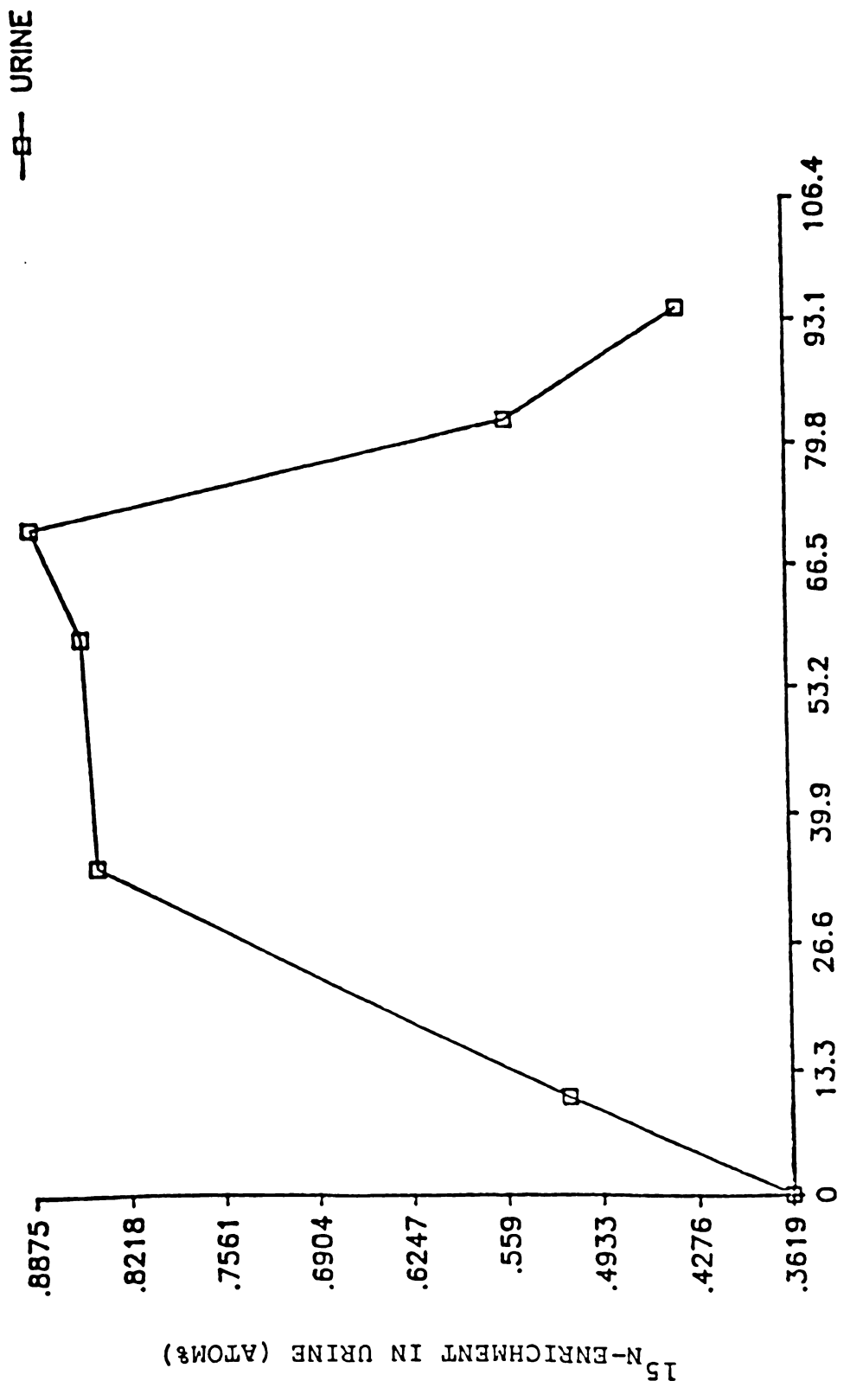

FIGURE 7. ^{15}N-Enrichment in urine of cow (6) on grain diet

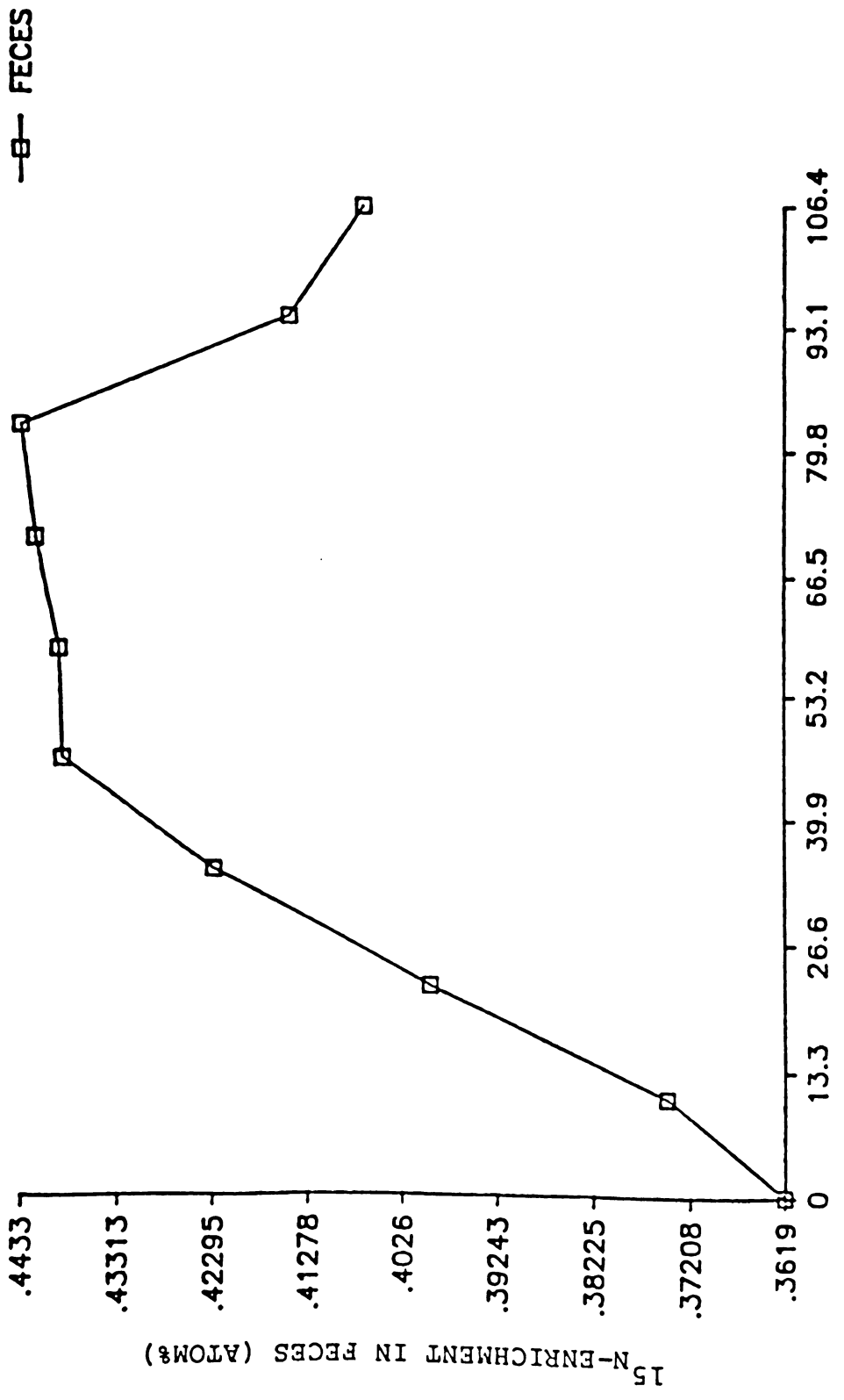

FIGURE 8. ^{15}N-Enrichment in feces of cow (6) on grain diet

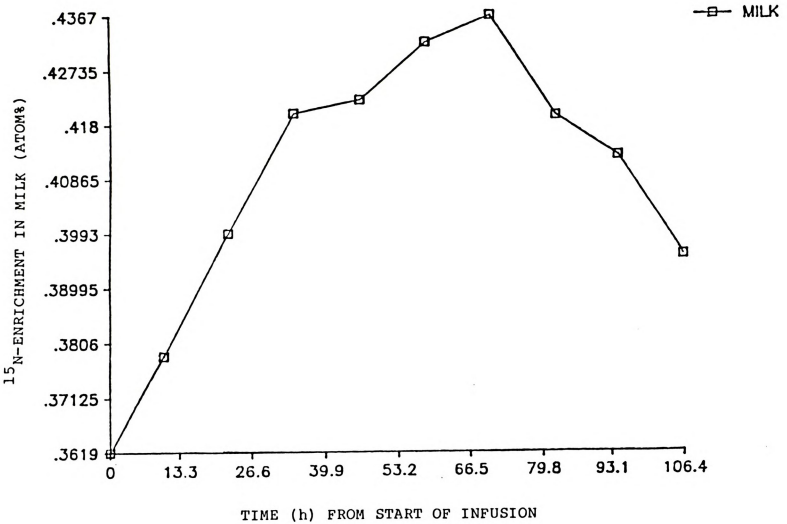

FIGURE 9. ^{15}N-Enrichment in milk of cow (6) on grain diet

DISCUSSION

Duodenal Digesta N-Flow

These data show greater flow of N into the duodenum than there was intake of feed N, and agree with studies by Wanderley and Theurer (1983) in beef steers who showed that the flow of nitrogen reaching the small intestine was greater than that consumed.

Based on Cr_2O_3 flow estimates, Wanderley and Theurer (1983) reported that the average N reaching the small intestine was 140 percent of that consumed in steers fed the grain diet and 101 percent for the forage diet. The flow of all the amino acids, especially DAP, into the small intestine was greater on grain than forage diet. Our data also show greater N flow on the grain diet.

In another study, Wanderley *et al.* (1986) showed that the crude protein flow into the duodenum in beef steers was about 33 percent greater for a grain than forage diet, although protein intake was about 10 percent less on grain. Duodenal bacterial protein, rather than feed protein escaping ruminal degradation, accounted for most of this difference, suggesting that greater amounts of bacterial protein were synthesized in the rumen of the grain-fed than forage fed steers and might be attributed to higher

intake of rumen available energy (Oldham and Tamminga, 1980).

In the studies of Wanderley et al. (1986), microbial-N production exceeded the amount of feed N degraded in the rumen, with the grain diet, presumably by utilizing large amounts of recycled N for ruminal bacterial synthesis. Dietary grain to forage ratios showed a marked effect on ruminal protein digestion and duodenal flow of protein.

Our data support those of Wanderley and Theurer (1983) and Wanderley et al. (1986) in that a mean of 21 percent more N reached the duodenum of cows fed the grain diet than was consumed. Whereas, passage of N into the duodenum on the forage diet equalled N intake. Our data contrast with those of Wanderley et al. (1986) in that we show more passage of feed N on the grain diet but little difference in bacterial flow.

However, due to less feed N degraded in the rumen on the grain diet, there was less NH_3 from the feed N available for microbial synthesis. This difference in NH_3 availability for growth of bacteria was apparently compensated for by greater recycling of blood urea into the rumen. The ^{15}N data suggest that about twice as much recycled nitrogen was captured in bacterial protein when the lactating cow (6) was fed the grain diet as compared to the forage diet, perhaps suggesting a different extent in recycling between diets.

Rate of Nitrogen Enrichment in Duodenal Digesta, Duodenal Bacteria, and Rumen Bacteria.

The N enrichment of duodenal digesta, and duodenal bacteria appeared as early as 8h after infusion. The enrichment in duodenal digesta and bacteria, was greater in cows fed the grain than the forage diet (Figure 6 and 10-12 in Appendix).

The average 3 peak values considered the steady state were reached after 60-70h of infusion. Concentration of ^{15}N then decreased gradually for about 72h when infusion stopped. The enrichment curves of duodenal digesta and bacteria had similar trends in peaking, but the bacterial curves took a longer time to decrease after infusion stopped in comparison with the digesta curve.

Oldham et al. (1980) showed that the enrichment of rumen ammonia N and plasma urea N gradually increased after a continuous infusion of $^{15}NH_4Cl$ into the rumen for 29h and then started decreasing after infusion stopped. The enrichment of rumen ammonia N increased much faster than the plasma urea N, and the ^{15}N enrichment in NH_3 N remained constant for the last 10h of infusion. These data illustrate the difficulties in achieving good mixing of isotope and of taking "representative" samples of rumen fluid from dairy cows. The plateau of their calculated enrichment was the arithmetic mean of the abundances measured in the last nine samples taken before the infusion was stopped. The plateau urea-^{15}N abundance was the arithmetic mean of the

last six samples taken before the infusion stopped. This is likely to be a minimum transfer coefficient, as urea-^{15}N abundance will probably rise slowly for a long time.

Oldham et al. (1980) showed very rapid equilibration of bacterial N with rumen NH_3N. The proportions of bacterial N derived from rumen NH_3N ranged between 0.7 and 0.8.

Nitrogen Enrichment in Urine, Feces, and Milk

Enrichment of urine appeared as early as 12h after infusion and was greater in cows fed the grain than the forage diet. The steady state estimated in our study was the average 3 peak values the animal reached between 60 and 72h after initiation of infusion.

A sharp increase was noticed in the urine enrichment curves compared to enrichment curves for duodenal digesta, bacteria, rumen bacteria, feces, and milk. Also, a sharper decrease was noticed in urinary enrichment after infusion stopped. This was probably due to a much more rapid turnover of urea in blood than NH_3 in the rumen. Most of the ^{15}N dose which was excreted, exited in the urine, which had ^{15}N concentrations about 10 times as high as those in bacteria, feces, or milk (Figure 7 and 13-15 in Appendix).

Feces enrichment appeared as early as 10h after infusion started and was greater in cows fed the grain than the forage diet. The 3 peak values were considered as the steady state and were observed 50-80h after initial

infusion. Milk and feces together accounted for only about 10 percent of the total ^{15}N excreted in this study. Smith et al. (1982) showed that more than one-third of the ^{15}N excreted was voided in feces and a similar amounts in urine and milk of cows fed corn silage treated with ammonia ^{15}N (Figures 8 and 16-18 in Appendix).

The higher fecal and lower urinary excretion in their study was a result of $^{15}NH_3$ being incorporated into the diet and going directly to the rumen; whereas, ^{15}N only entered into the rumen through recycling in our study.

The milk enrichment appeared as early as 12h after infusion and was greater in cows fed the grain than the forage diet (Figures 9 and 19 in Appendix). The steady state for ^{15}N in milk was the avearge of 3 peak values reached 50-70h after initial infusion. Smith et al. (1982) showed that ^{15}N in milk accounted for 25.5 percent of total ^{15}N excretion in cows fed corn silage treated with ammonia, again reflecting direct entry of ^{15}N into the rumen.

Fate of ^{15}N (Excretion vs. Body Retention)

^{15}N-duodenal passage, ^{15}N-output in feces and milk were greater in cows fed the grain than the forage diet. However, none of the cows excreted over 50 percent of the infused ^{15}N during the entire sampling period. In fact, 53-75 percent of the urea-^{15}N dose was retained in the body.

Smith et al. (1982) showed that 40-70 percent of the fed ^{15}N was retained after 3 weeks in the body of cows

fed corn silage treated with ammonia-^{15}N. However, most had been eliminated in one cow sampled at 6 weeks.

Boda et al. (1976) showed that after a single administration, 44-76 percent of ^{15}N was retained in the nitrogen pool when sheep were fed 7.6-24g/d of N using an intravenous injection of ^{15}N-urea.

SUMMARY AND CONCLUSIONS

Two duodenally cannulated cows were used to study the recycled-N from blood urea utilized by rumen bacteria. For 28 days prior to and during infusion, cows were fed diets of 1:2 or 2:1 forage:concentrate. Urea-^{15}N solution was continuously infused into the jugular vein for 3d. Samples of duodenal digesta were taken every 3h for 5d. Blood and milk were sampled twice daily. Urine was collected for 5d starting 1d before infusion and feces were collected for the 3d during infusion. Fecal samples were taken twice daily during the 5d of collection.

Recycled nitrogen incorporated into rumen microbes apeared to be greater in cows fed the grain than the forage diet (23 vs. 14% of microbial-N passing into the duodenum). Also, it was higher in the lactating than the dry cow. The flow of nitrogen from the rumen to the small intestine was greater in cows fed the concentrate than in cows fed the forage diet (122.0 vs. 101.0% of intake).

Within 10-12h after starting ^{15}N infusion, labelled nitrogen appeared in milk and feces with peaks at 50-80h. About 15 percent of the milk-N and fecal-N originated from blood-urea. Urine enrichment of ^{15}N was 10 times that for bacteria, feces or milk. Most of the ^{15}N excretion was in urine.

These results suggest that the N-recycling process in dairy cows can contribute significantly to the animal's nitrogen pool, especially when "normal" diets high in grain or forage are fed. More research is suggested to investigate in great depth the factors which control recycling of N into the rumen.

APPENDIX

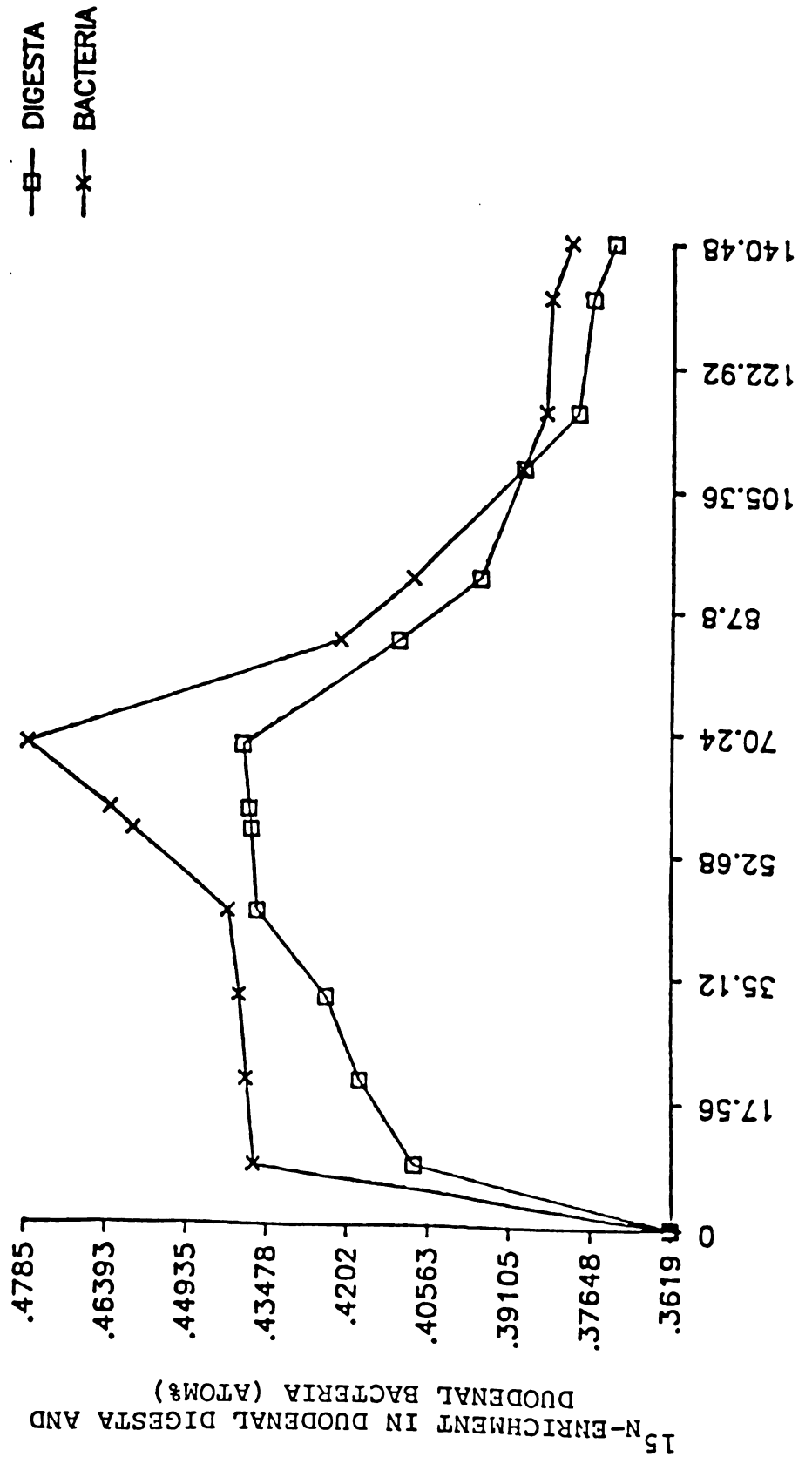

FIGURE 10. ^{15}N-Enrichment in duodenal digesta and duodenal bacteria of cow (958) on grain diet

FIGURE 11. ^{15}N-Enrichment in duodenal digesta and duodenal bacteria of cow (958) on forage diet

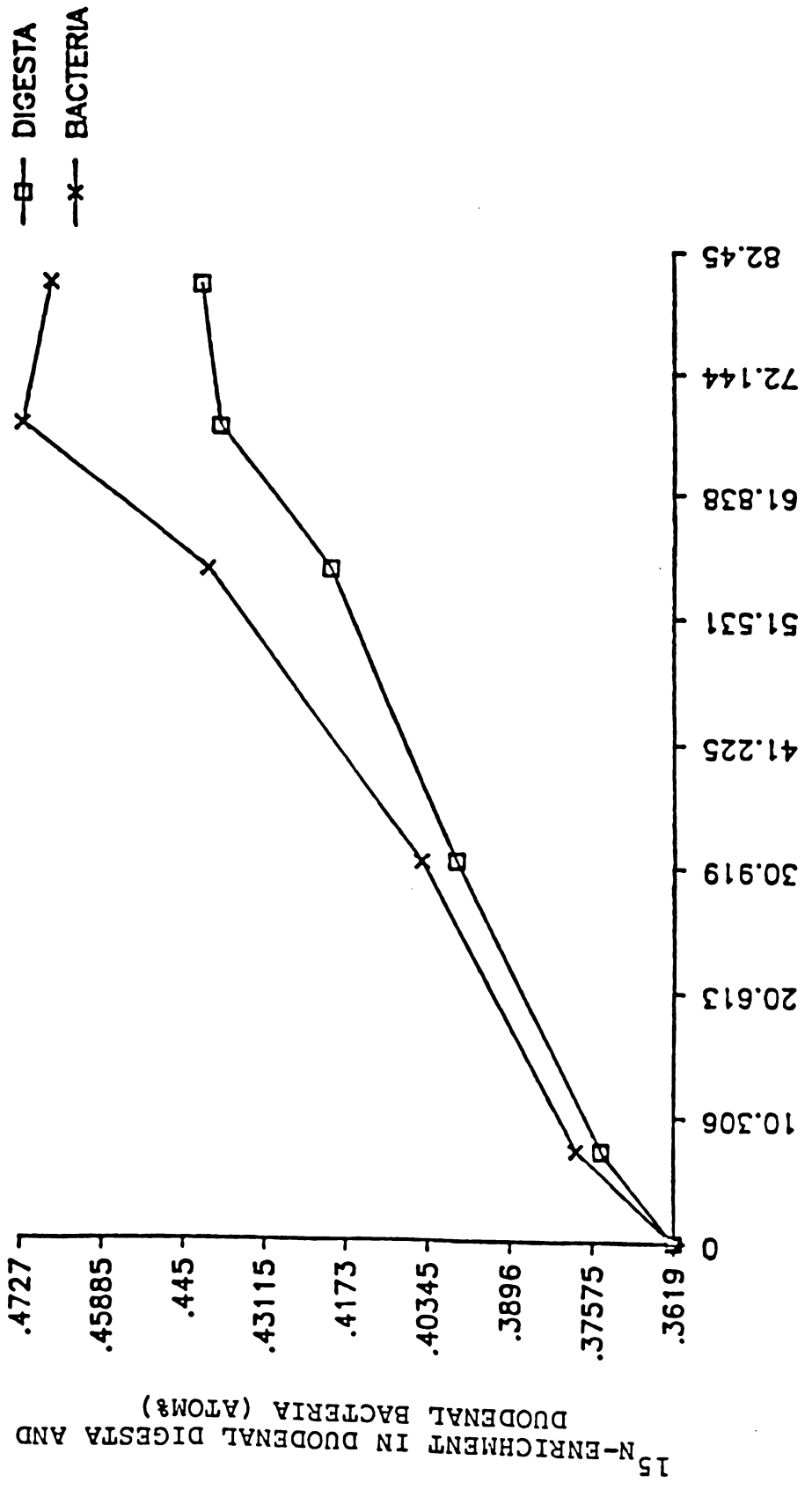

FIGURE 12. ^{15}N-Enrichment in duodenal digesta and duodenal bacteria of cow (6) on forage diet

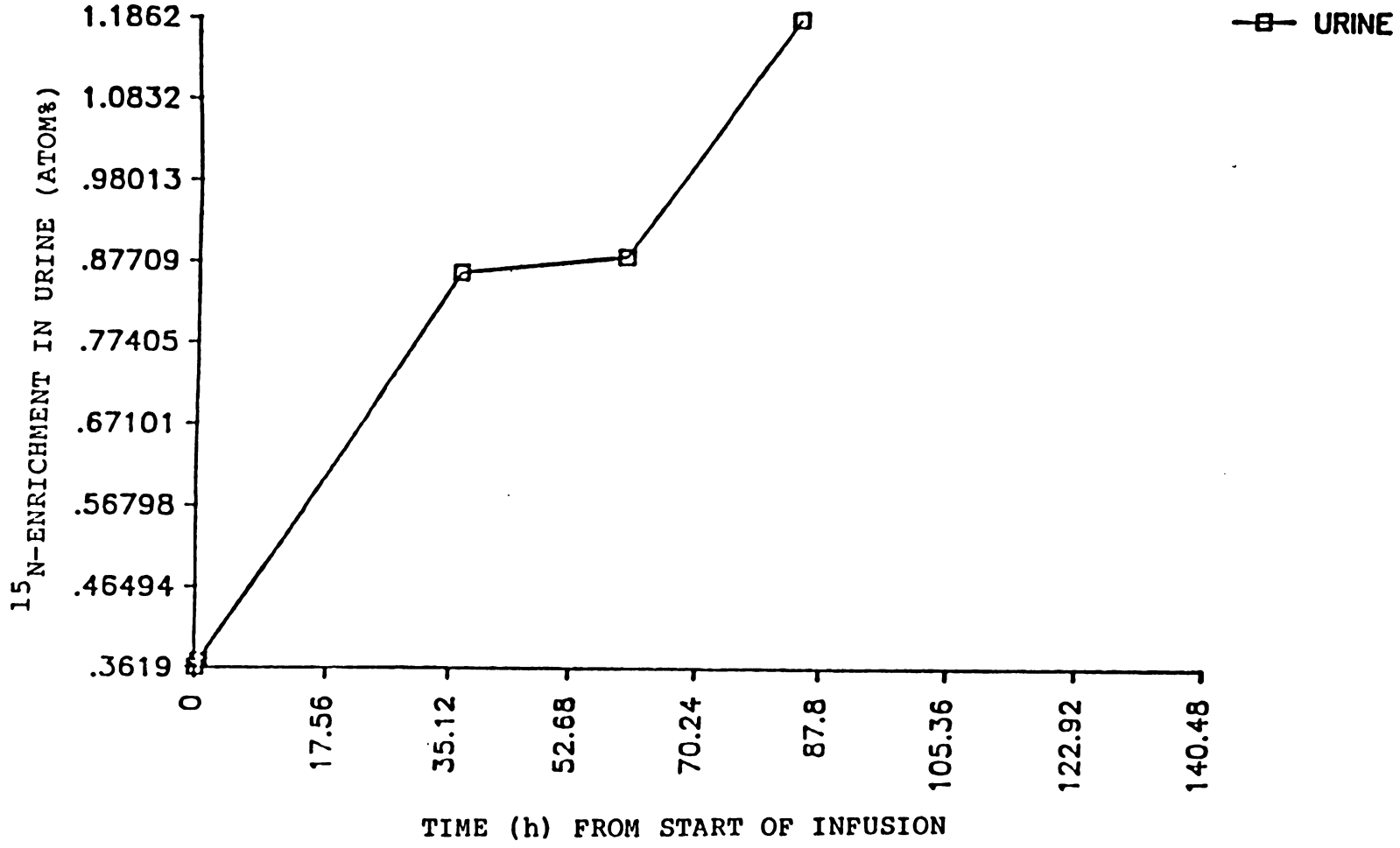

FIGURE 13. ^{15}N-Enrichment in urine of cow (958) on grain diet

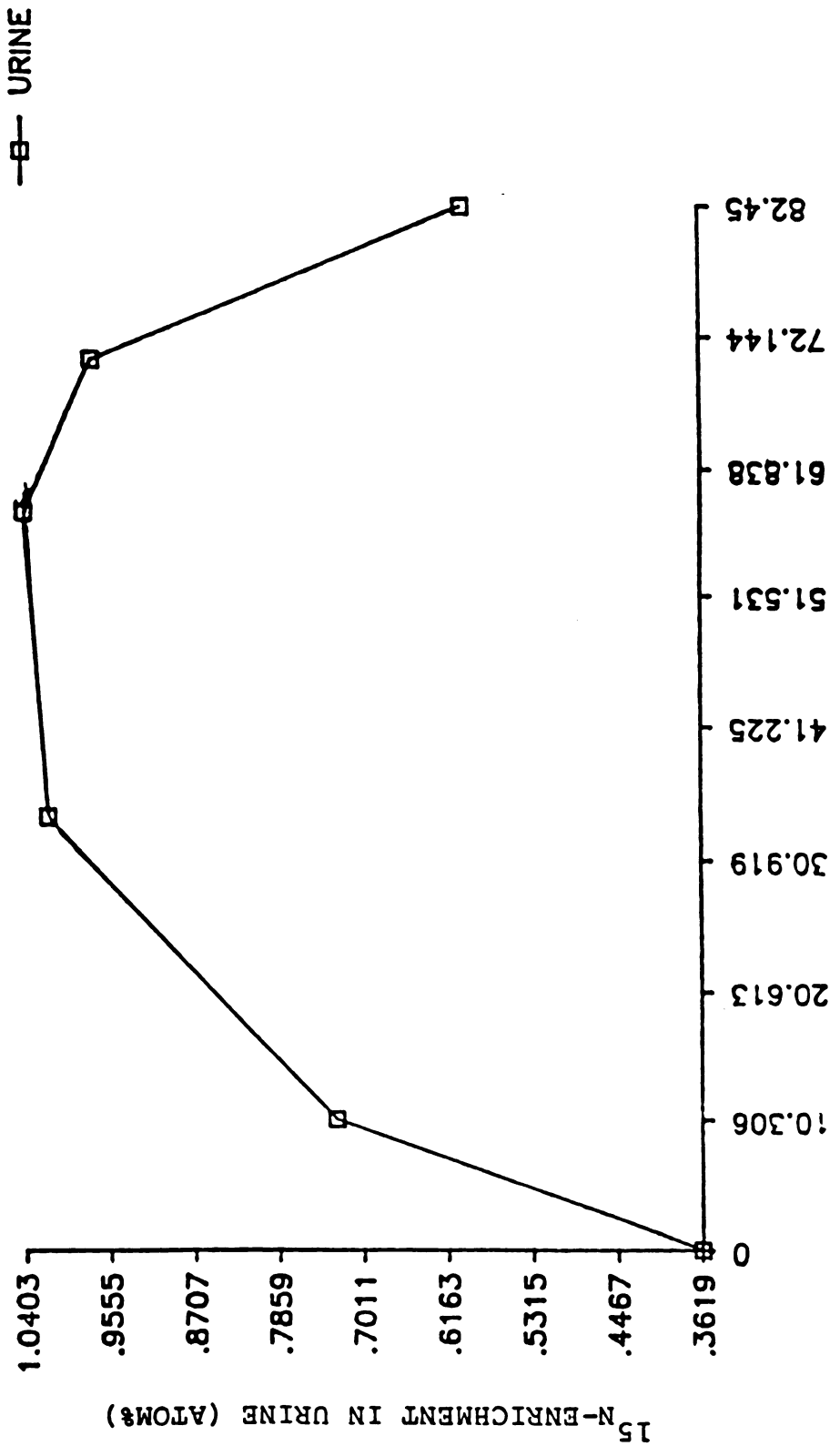

FIGURE 14. ^{15}N-Enrichment in urine of cow (958) on forage diet

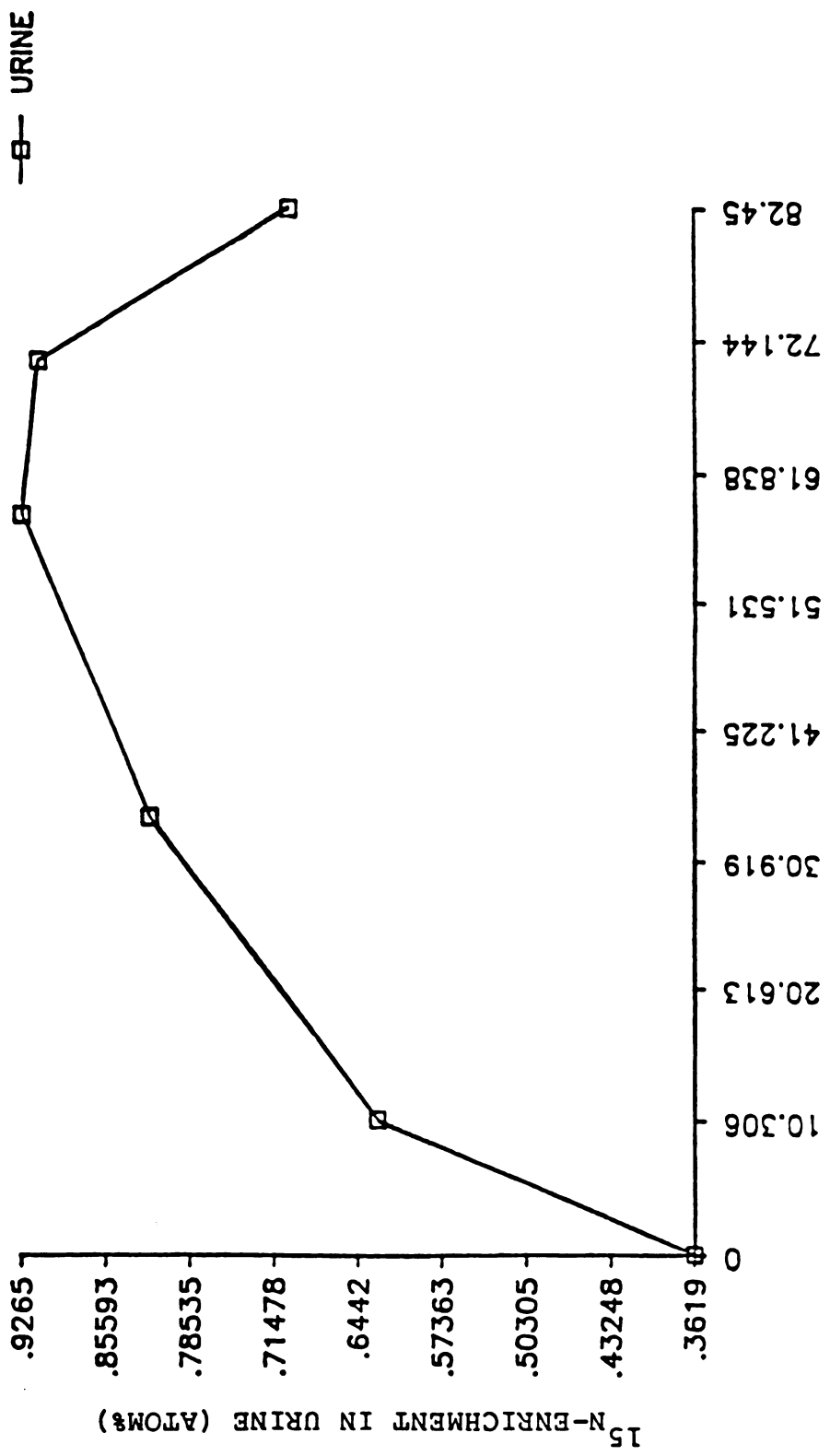

FIGURE 15. ^{15}N-Enrichment in urine of cow (6) on forage diet

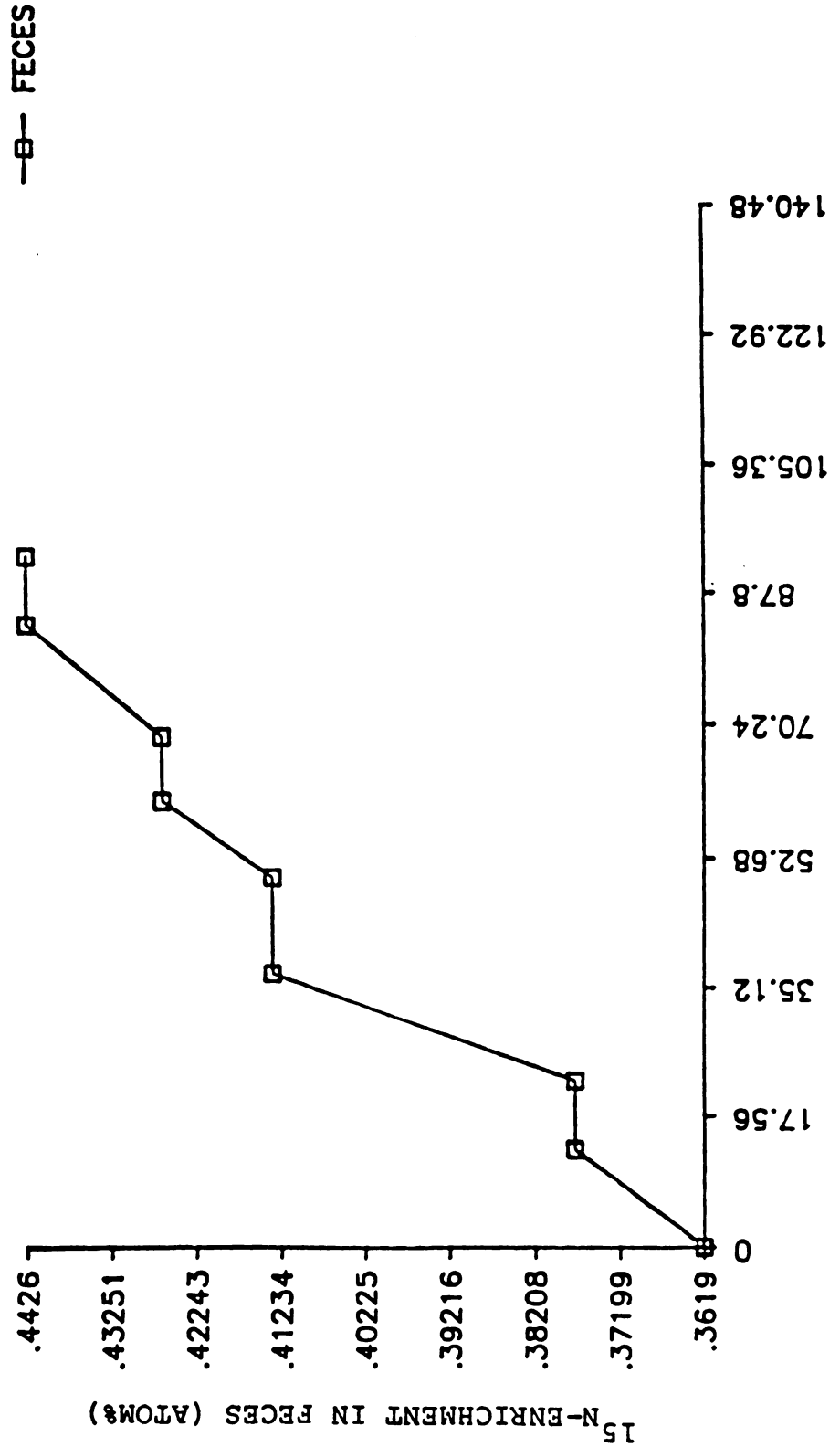

FIGURE 16. ^{15}N-Enrichment in feces of cow (958) on grain diet

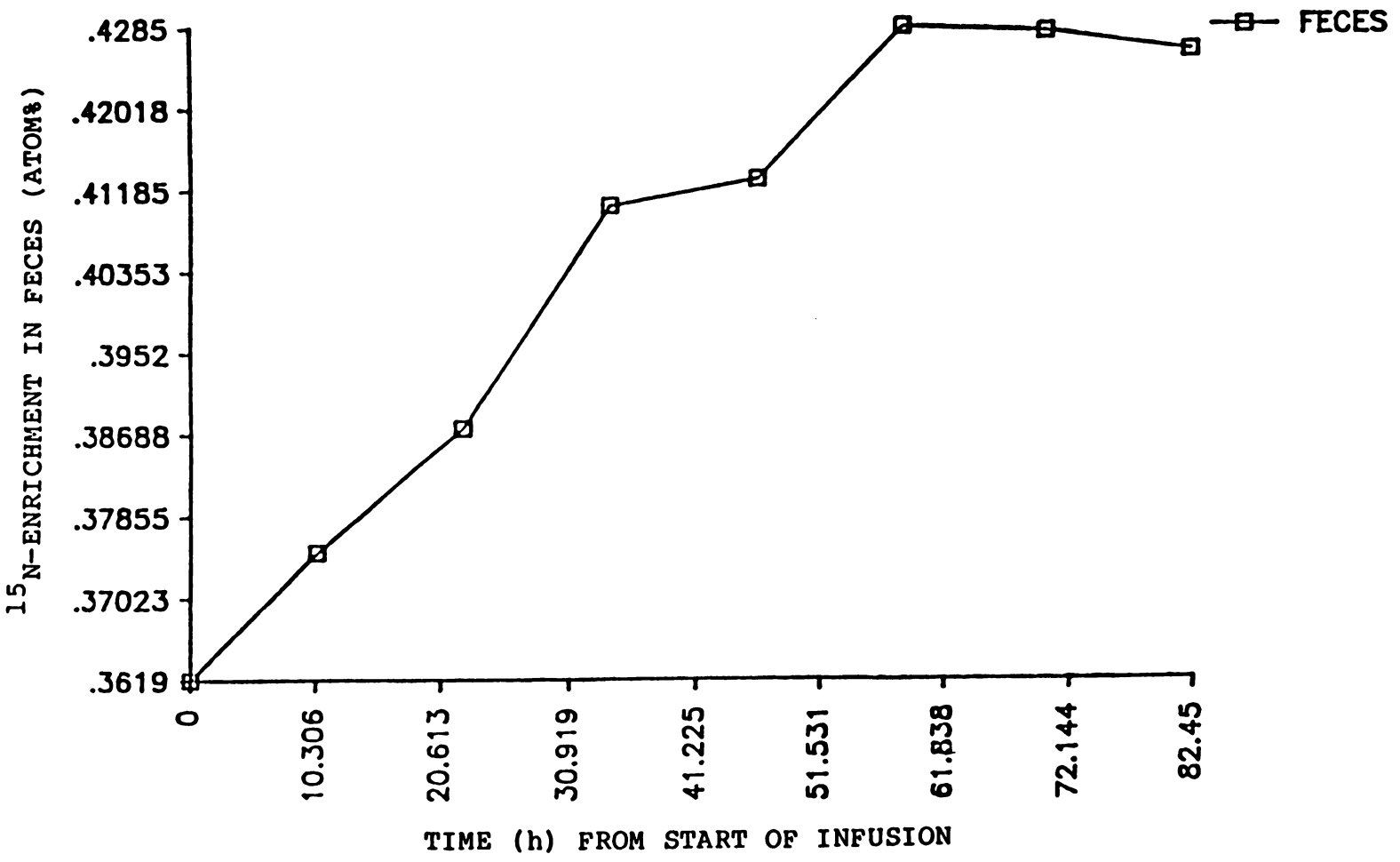

FIGURE 17. ^{15}N-Enrichment in feces of cow (958) on forage diet

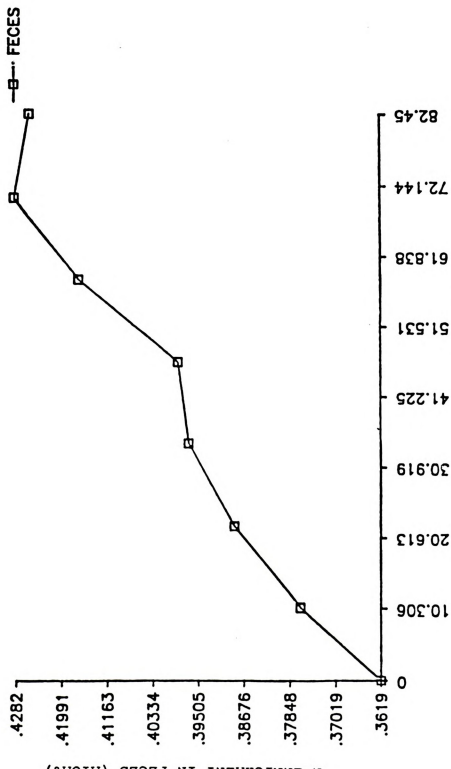

FIGURE 18. ^{15}N-Enrichment in FECES of cow (6) on forage diet

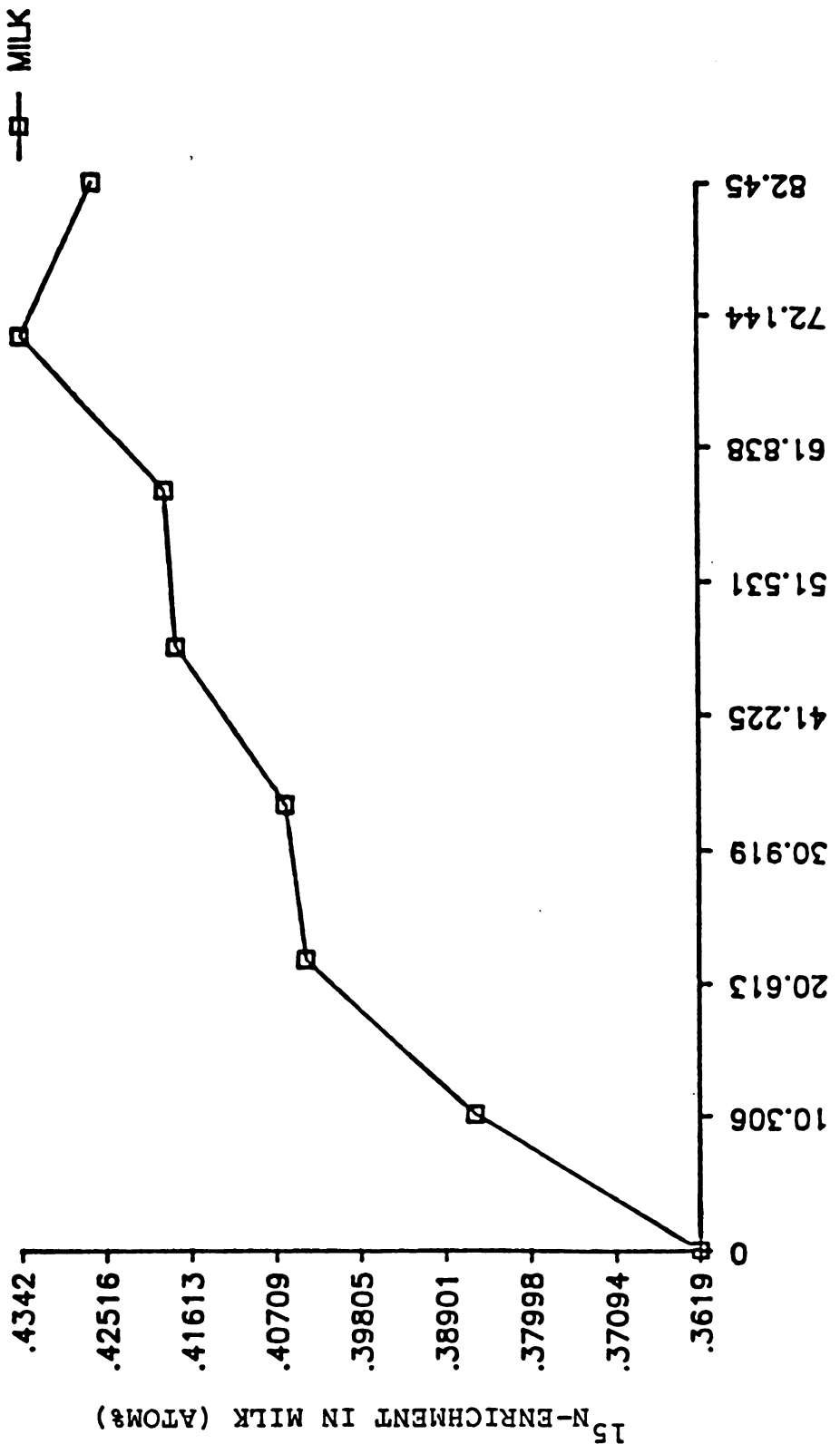

FIGURE 19. ^{15}N-Enrichment in milk of cow (6) on forage diet

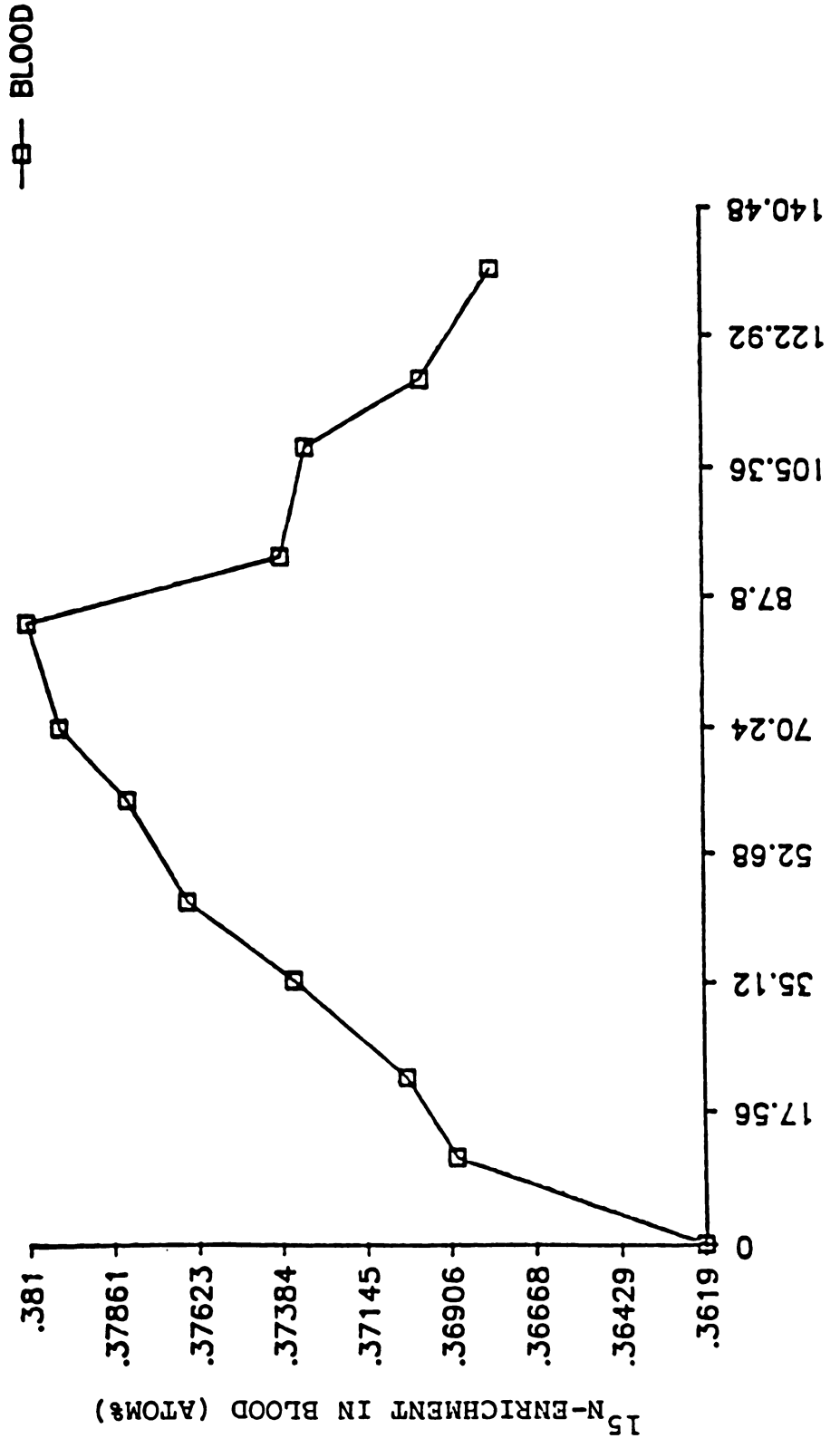

FIGURE 20. ^{15}N-Enrichment in blood of cow (958) on grain diet

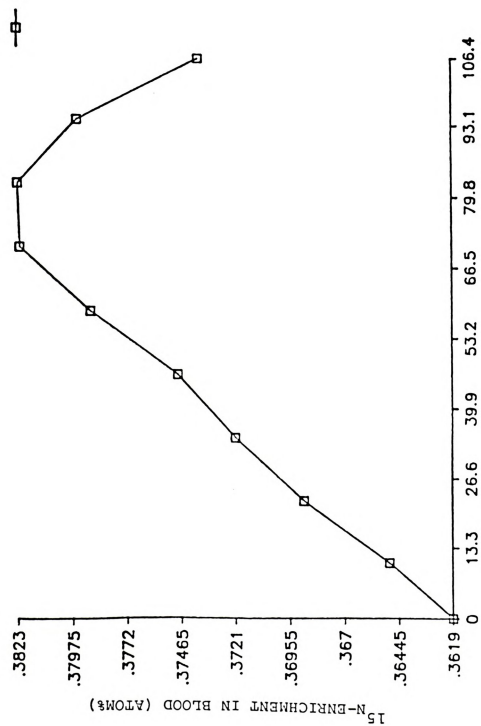

FIGURE 21. ^{15}N-Enrichment in blood of cow (6) on grain diet

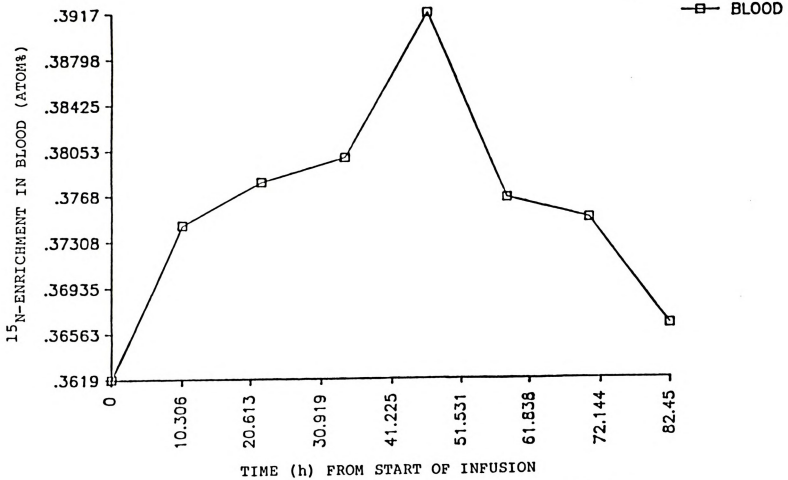

FIGURE 22. ^{15}N-Enrichment in blood of cow (958) on forage diet

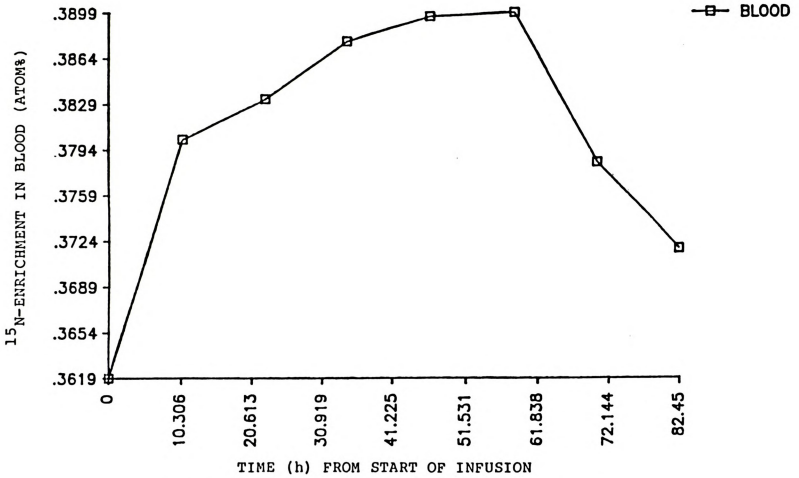

FIGURE 23. ^{15}N-Enrichment in blood of cow (6) on forage diet

FIGURE 24. ^{15}N-Enrichment in rumen bacteria of cow (958) on forage diet

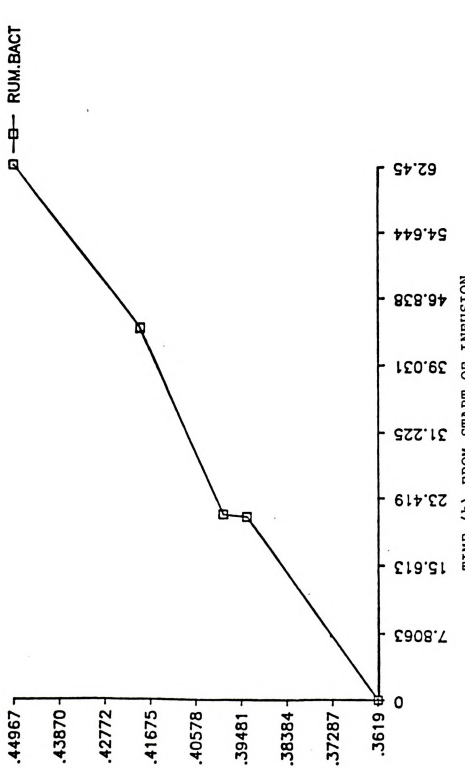

FIGURE 25. ^{15}N-Enrichment in rumen bacteria of cow (6) on forage diet

Table 8. Duodenal N Flow as Percent of Intake, and Fractions of duodenal N from cow infused with ^{15}N Urea.

DIET	COW	Duodenal N Flow (% of Intake)	Duodenal N Fractions From Bacterial N (% of Total)	From Feed N (% of Total)
Grain	958	140.2	70.6	29.4
	6	102.7	54.0	46.0
	Avg	121.5	62.3	37.7
Forage	958	105.6	73.8	26.2
	6	96.0	73.5	26.5
	Avg	100.8	73.7	26.3

Table 9. Average of 3 Peak Values for ^{15}N-enrichment of each cow in rumen bacteria.

DIET	COW	Enrichment in Rumen Bacteria (Percent)
Grain	985	----
	6	----
	Avg	----
Forage	958	.092
	6	.088
	Avg	.090

Table 10. PH, Dry Matter, Nitrogen, and Cr_2O_3 in Duodenal Digesta.

DIET	COW	Duodenal Digesta PH	DM (%)	Duodenal Digesta-DM N (%)	Cr_2O_3 (%)
Grain	958	3.33	6.1	3.6	.34
	6	2.73	5.0	4.1	.34
	Avg	3.03	5.6	3.9	.34
Forage	958	3.22	4.2	4.0	.55
	6	2.56	4.0	3.7	.32
	Avg	2.89	4.1	3.8	.44

Table 11. Fecal-DM Output, Percent of Fecal-DM, Percent of Cr_2O_3 in Fecal-DM.

DIET	COW	Fecal DM (%)	Fecal-DM Cr_2O_3 (%) (spot)	Fecal-DM Cr_2O_3 (%) (total)
Grain	958	20.0	.80	.79
	6	16.8	.70	.73
	Avg	18.4	.75	.76
Forage	958	20.0	.98	.85
	6	17.1	.62	.57
	Avg	18.7	.80	.71

Table 12. Percent of N in Fecal-DM, Fecal-DM Output, and N-Output in Fecal-DM.

DIET	COW	N (%)	Fecal-DM Output (g)	N-Output (Cr_2O_3) (g)
Grain	958	2.7	3017.2	80.1
	6	2.4	3586.1	87.2
	Avg	2.5	3301.6	83.7
Forage	958	2.2	2495.4	53.1
	6	2.1	4052.7	83.8
	Avg	2.1	3274.1	68.4

Table 13. Fecal-Cr_2O_3 Output, and Percent of Fecal-Cr_2O_3 Recovery.

DIET	COW	Fecal-Cr_2O_3 Output (Total) (g)	Recovery (%)
Grain	958	12.8	53.2
	6	27.3	113.8
	Avg	20.0	83.5
Forage	958	17.2	71.8
	6	24.4	101.6
	Avg	20.8	86.7

Table 14. Total Urine Output, Percent of N in Urine, and Total N-Output in Urine.

DIET	COW	Output (l)	Urine N (%)	N-Output (g)
Grain	958	7.8	1.2	101.3
	6	22.9	.9	184.5
	Avg	15.3	1.0	142.9
Forage	958	9.8	1.2	121.0
	6	17.1	1.0	162.1
	Avg	13.5	1.1	141.5

Table 15. Total Milk Output, Percent of N in Milk, and Total N-Output in Milk.

DIET	COW	Output (Kg)	Milk N (%)	N-Output (g)
Grain	958	----	---	----
	6	15.3	.6	87.2
	Avg	15.3	.6	87.2
Forage	958	----	---	----
	6	14.2	.5	70.3
	Avg	14.2	.5	70.3

Table 16. Percent of Nitrogen in Blood.

DIET	COW	Blood-N (%)
Grain	958	2.7
	6	3.0
	Avg	2.8
Forage	958	2.7
	6	2.9
	Avg	2.8

Table 17. Balance of Nitrogen.

DIET	COW	Balance of Nitrogen (%)
Grain	958	8.8
	6	-15.5
	Avg	-6.7
Forage	958	-.64
	6	-8.0
	Avg	-4.3

Table 18. Apparent and True Rumen-DM Disappearance, and Undegraded Rumen N.

DIET	COW	Rumen-DM Disappearance Apparent (%)	Rumen-DM Disappearance True (%)	Rumen Undegraded Nitrogen (%)
Grain	958	8.6	43.8	41.2
	6	45.7	62.5	47.3
	Avg	27.2	53.2	44.3
Forage	958	46.0	71.1	27.6
	6	45.1	65.7	25.5
	Avg	45.5	68.4	26.5

Table 19. Total Tract-DM and N Digestibility.

DIET	COW	Total Tract-DM Digestibility (Total) (%)	Total Tract-DM Digestibility Cr_2O_3 (%)	Total Tract-N Digestibility (Total) (%)	Total Tract-N Digestibility Cr_2O_3 (%)
Grain	958	79.6	61.5	77.3	56.0
	6	72.9	73.9	69.6	70.4
	Avg	76.3	68.0	73.4	63.2
Forage	958	75.8	70.5	75.3	69.5
	6	69.3	71.3	71.0	71.5
	Avg	72.6	71.0	73.2	70.5

Table 20. Intestinal-DM and N-Digestibility

DIET	COW	Intestinal-DM Digestibility (Total) (%)	Intestinal-DM Digestibility Cr_2O_3 (%)	Intestinal-N Digestibility (Total) (%)	Intestinal-N Digestibility Cr_2O_3 (%)
Grain	958	62.0	45.9	80.5	65.6
	6	19.7	28.0	65.2	70.8
	Avg	40.9	36.9	72.8	68.2
Forage	958	24.1	23.4	71.3	69.3
	6	20.6	24.3	66.9	67.5
	Avg	22.4	23.8	69.1	68.4

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