# TRYPTOPHAN METABOLISM IN SHEEP

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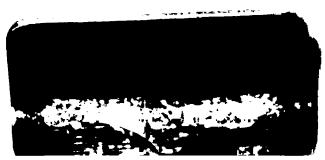

ABSTRACT

TRYPTOPHAN METABOLISM IN SHEEP

By

Constantine Llewellyn Fenderson

Since the essential amino acid, tryptophan, has been ignored in previous amino acid studies in ruminants, two experiments were designed to study the role of tryptophan in protein metabolism in sheep.

Experiment 1 was a 4 x 4 Latin Square in which four rations containing different levels and sources of crude protein were fed to four sheep of approximately equal body weight. Each experimental period consisted of a 21-day ration adjustment which was followed by two sample collection days. On day 22 blood and rumen samples were taken before feeding and at 3, 6, 9, 12, 16, 20 and 24 hours post feeding. Blood samples were analyzed for plasma free tryptophan, total free amino nitrogen and blood urea nitrogen concentrations. Rumen samples were analyzed for rumen ammonia nitrogen and volatile fatty acid concentration. On day 24 of each period, a 2 : 1 starch-glucose mixture in water was instilled into the rumen of each sheep. Blood and rumen samples were secured before and 4 hours after instilling the starch-glucose. Amino acid

levels were determined in the pre-energy instillation ($T_0$) and post energy instillation ($T_4$) blood samples. The resulting $T_4/T_0$ ratio of plasma amino acid levels was used as an indicator of amino acid deficiencies. Plasma tryptophan levels were not affected by dietary treatments in sheep. The range of mean daily tryptophan levels was from 1.86 to 2.11 mg per 100 ml plasma. The finding that rapid availability of energy (VFA) to sheep did not induce a marked depression of plasma free tryptophan suggested that tryptophan was not limiting or deficient in sheep fed the four rations used in this study. The $T_4/T_0$ ratios of tryptophan were 87%, 93%, 98% and 91% for rations 1, 2, 3 and 4 respectively. The total free amino acid nitrogen concentration was not influenced by dietary treatments. The range of mean daily total free amino acid level was from 2.38 to 2.81 micromoles of $\alpha$-$NH_2$-N (as citrulline) per ml plasma. Increased rumen ammonia and blood urea nitrogen concentrations were associated with increased levels of dietary crude protein.

The second experiment was conducted primarily to determine the constancy or variability of rumen microbial tryptophan concentrations. Rumen bacteria and protozoa were isolated from sheep (fed the four rations from experiment 1) before feeding, at 1 1/2 hours and 4 hours post feeding. Samples were analyzed for tryptophan and other amino acids. The results indicated that tryptophan

content of rumen bacterial and protozoal preparations was quite constant and was not affected by dietary treatments or time after feeding. The average tryptophan levels in bacteria and protozoa were 1.38 and 1.00 gm tryptophan per 100 gm protein respectively. The bulk amino acid composition of rumen bacterial and protozoal preparations was constant and was not affected by dietary treatments or time after feeding.

# TRYPTOPHAN METABOLISM IN SHEEP

By

Constantine Llewellyn Fenderson

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# INTRODUCTION

The present unchecked trend in human population growth is toward eventual, if not already existing over-population. It is therefore obvious that adequate food must be provided to cope with the demand of such rapidly increasing population if its growth rate cannot be checked. One way in which this can be achieved is to constantly increase agricultural production by the application of new scientific research knowledge to current systems of agriculture. To reach the present standard of productive efficiency of animals, researchers all over the world have delved deeply into the science of nutrition, breeding and management of farm animals.

Farm animals are important to man in that they provide a source of income for many farmers and high quality animal protein in the human diet. Ruminants form an important class of farm animals which through their rumen microorganisms, possess the unique ability to convert forages and nonprotein nitrogen into animal protein. Proteins form an important class of dietary essential nutrients which are required in every metabolic reaction of the animal's body. Their importance is manifested in indispensable functions

such as cellular construction, catalysis, metabolic regulation and the defense mechanism against pathogens (Mahler and Cordes, 1966). Partial or complete absence of protein from the diet over a period of time leads to severe nutritional disease such as kwashiorkor and marasmus.

Nutritionists have divided the amino acids into essential amino acids and nonessential amino acids based on the ability of the animal's tissues to synthesize these amino acids. Essential amino acids are those amino acids which cannot be synthesized, in sufficient amount by the animal's tissues, to meet the requirement of the animal, whereas nonessential amino acids can be synthesized by the animal's tissues as long as the precursors for their synthesis are supplied in the diet. Thus essential amino acids must be supplied in the diet. The absence of any one essential amino acid from the dietary protein produces no growth. However, this does not hold true for maintenance.

The amino acid supply to ruminants consists of a mixture of undegraded dietary and ruminally synthesized microbial protein. Consequently, the amino acids which are limiting in these proteins will limit the productive performance of the animal. The limiting amino acid may be defined as that essential amino acid that is available in the least amount in relation to the requirement of the animal.

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Tryptophan is an important essential amino acid, but because of difficulties in its determination there are few published data regarding its metabolism in the ruminant. The present study was designed to determine the effect of protein source and level in rations on the plasma tryptophan pool in sheep.

# LITERATURE REVIEW

## The Importance of Micro-organisms in Supplying Protein to the Ruminant

The ruminant animal, because of its importance in supplying food for human needs and because of its symbiotic relationship with its rumen microbiota, has captured the interest of a large number of researchers. The discovery of rumen protozoa by Gruby and Delfond (1843) was the first identification of micro-organism in the ruminant stomach. Pasteur (1863) discovered the role of bacteria in the fermentation of plant materials. Zunt (1879) inferred that rumen micro-organisms ferment fiber anaerobically and thus form acids and gas. One of the unique capabilities of rumen micro-organisms is the conversion of nonprotein nitrogen and β-linked cellulose to microbial protein which is digested and absorbed in the lower gastrointestinal tract of the host. El-Shazly and Hungate (1966) using the diaminopimelic acid concentration of bacteria procedure, found that 69-90 percent of the total nitrogen passing to the small intestine was of microbial origin.

Ammonia produced by rumen micro-organisms from both protein and nonprotein nitrogen is the most important

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nitrogen source for rumen bacteria but is less important for rumen protozoa (Allison, 1969). In vivo isotope studies involving $^{15}N$ labeled ammonia or urea (Allison, 1969) and $^{15}N$ labeled ammonium citrate and urea (Abe, 1968) showed that ammonia nitrogen is first incorporated into bacterial cells and then into protozoal cells following the ingestion of the bacteria by protozoa.

Klopfenstein et al. (1966), Males and Purser (1970) and several other workers reported that rumen ammonia concentration was higher in faunated lambs than defaunated lambs. This can be explained by the fact that there is a greater bacterial concentration in the defaunated animal thus causing greater ammonia utilization than in the faunated animal in which protozoa are extremely poor utilizers of ammonia (Males and Purser, 1970). Barringer (1968) reported that rations, supplemented with soymeal and zein, fed to faunated lambs had higher digestibility coefficient than when fed to defaunated lambs; dry matter intake for all rations used was 9.2 percent higher; higher nitrogen retention and lower urinary nitrogen excretion were noted in faunated lambs on the same rations. On the other hand, there were higher viable bacterial concentrations in the defaunated lambs on semipurified rations, further suggesting that protozoa ingest a portion of the bacterial cells and use it as a nitrogen source and a high degree of competition between protozoa and bacteria for substrate per se. On

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natural type rations microfauna apparently produce a higher percentage of microbial protein than microflora whereas on semipurified rations the converse is true (Bergen et al., 1968).

Bergen et al. (1968b) fed rumen microbial protein to rats and observed a slightly higher biological value of bacterial protein than protozoal protein; a higher true digestibility for protozoal protein and a higher net protein utilization in protozoal protein. He also reported that histidine was the first limiting amino acid in protozoal protein while cystine (sulfur amino acid) was the limiting amino acid in bacterial protein. Lysine content of rumen protozoal preparation is higher than bacterial preparation (Purser and Buechler, 1966; Bergen et al., 1968).

The amount and quality of protein reaching the small intestine of the ruminant depends on the degradation of dietary protein and the amount of microbial protein synthesized in the rumen (Smith, 1969). Bergen et al. (1968a) reported that within a microbial preparation the amino acid composition is not affected significantly by rations, and neither is the bulk amino acid composition and quality of microbial preparation affected by ration change. Purser and Buechler (1966) also reported a constant amino acid composition in rumen bacterial preparation.

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The unique ability of the ruminant to provide its own essential amino acid supply is strictly a function of rumen micro-organisms. Thomas et al. (1949) reported that there is a remarkable synthesis of all amino acids by rumen micro-organisms and that the essential amino acid content of rumen material was similar for urea and casein diets. Downes (1961) working with both growing and mature sheep reported that isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and histidine were metabolically essential to ruminants since they cannot be synthesized by their tissues; but arginine was not an essential amino acid for the sheep. However, whether tryptophan was an essential amino acid was not assessed because of the difficulties in accurate tryptophan determination. Downes (1961) therefore concluded that tissue amino acid metabolism of sheep is similar to that of non-ruminants.

The rate of digestion of various proteins in the rumen has been correlated with the solubility of the protein in salt solutions or rumen fluid (El-Shazly, 1958; Blackburn and Hobson, 1960; Hendricks and Martin, 1963). Ely et al. (1967) after estimating the extent of conversion of dietary zein to microbial protein, concluded that the low apparent digestibility of zein indicated that dietary zein nitrogen was not efficiently utilized. They also reported that all lambs fed zein protein diets showed a negative nitrogen

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balance. Thus because most of the zein protein bypassed the rumen resulting in depressed microbial protein synthesis and because lysine is severely limiting in zein, there were not enough utilizable amino acids to maintain nitrogen equilibrium. Plasma urea levels were higher when urea was used as dietary nitrogen source than when zein was used as a dietary nitrogen source (Ely et al., 1969). The explanation for this lies in the fact that urea is rapidly converted to ammonia by rumen micro-organisms, consequently, a large amount of the ammonia which is not converted to microbial protein by rumen micro-organisms is absorbed through the rumen walls into the ruminal vein and converted to urea by the liver (Lewis, 1961). According to McDonald (1954) only about 40% of the dietary zein is degraded in the rumen and converted to microbial protein. Abou Akkada and Osman (1967) observed that changes in ruminal ammonia and blood urea concentrations were considerably higher three hours after feeding leguminous forages than non-leguminous forages and that most of the nitrogen excretion from legume-fed animals was urinary nitrogen whereas for grasses it was fecal nitrogen. Tagari et al. (1964) reported an increase in rumen ammonia and blood urea concentrations with an increase in protein level in the ration fed to sheep. A significant correlation between protein content of the ration, rumen ammonia and blood urea concentration has been reported by several workers (Preston et al.,

1965; Lewis, 1957; Tagari et al., 1964). Lewis (1957) reported that the major factor controlling blood urea concentration is the concentration of rumen ammonia in that a change in rumen ammonia concentration is usually accompanied by a change in blood urea concentration. Rumen ammonia concentration is dependent on the type of ration, that is, the amount and type of energy source and the quantity and solubility of nitrogenous material in the ration. Consequently, blood urea concentration is indirectly dependent on the type of ration fed to the animal. McDonald (1947) reported that urea occurs in significant quantities in the saliva of sheep. According to Lewis (1957) as rumen ammonia increases there is a greater loss of urea in the urine and a greater return of nitrogen to the rumen via saliva. Lewis and McDonald (1958) reported that rumen ammonia concentration dropped to a low level between eight and sixteen hours after feeding and increased during the last eight hours of the 24 hour period after feeding. They therefore claimed that the increase during the last eight hours may be due to continued uptake of nitrogen through saliva, slowing down of bacterial growth and autolysis of some of the microorganisms. Ørskov and Fraser (1969), studying the effects of protein on nitrogen retention in lambs, observed that liquid protein supplement given directly to the abomasum, using nipple bottle, resulted in decreased urinary nitrogen loss and increased nitrogen retention, whereas the dry

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protein supplement fed in the normal way produced increased urinary nitrogen due to extensive deamination by rumen micro-organism.

Nitrogen for urea synthesis thus arises from ammonia absorption from the rumen but may also arise from deamination of amino acids in body tissues. However, except in those rare cases where a poor quality protein bypasses rumen degradation and a poorly balanced amino acid mixture is absorbed from the small intestine, the ruminal ammonia supplies the major portion of nitrogen for urea synthesis and completely overshadows tissue amino acid deamination. In response to the extra nitrogen load for urea synthesis, sheep appear to have higher activities of hepatic urea cycle enzymes (Payne and Morris, 1969).

## Effects of Dietary Protein on Plasma Amino Acids

The study of plasma amino acid in several species over the past two decades has provided a wide range of valuable information which explains some of the fundamental principles of mammalian protein metabolism. McLaughlan (1963) working with rats, reported that the concentration of plasma amino acids increased after a meal of good quality protein and the duration of this increase was related to the amount and composition of the protein fed. This was somewhat substantiated by the work of Hogan *et al.* (1968) who working with sheep, observed an increase in total plasma

essential amino acid concentration in response to each successive casein infusion in the abomasum, but the increase after the third infusion was less than that of the preceding two. Oltjen and Putman (1966) after feeding urea diets to steers, observed an increase in serine and glycine and a decrease in valine, isoleucine, leucine, and phenylalanine in blood plasma. Such a decrease in the branched chain amino acids may be attributed to less rumen microbial protein synthesis, consequently, a limited amount of protein is available to the animal, thus with adequate available energy such condition resulted in a typical kwashiorkor type of protein calorie malnutrition. According to Klopfenstein et al. (1966) a lower than normal individual plasma amino acid concentration in faunated lambs suggested a greater amino acid utilization rather than deamination. McLaughlan (1963) in his review of protein quality, pointed out that there is usually a good correspondence between the amount of amino acid in plasma and the protein fed, but since other dietary constituents such as glucose or butter may influence plasma amino acid concentration, plasma amino acid per se should not be used to compare protein qualities.

Fasting or imbalanced amino acid diets has a marked effect on plasma amino acid concentration. Ganapathy and Nasset (1962) working with dogs, reported that ingestion of protein may cause an increase or decrease in plasma

amino acid levels. The work of Leibholz and Cook (1967) showed a lower concentration of free α-amino acid nitrogen, a significantly lower urea concentration and a significantly higher concentration of lysine in blood plasma of starved lambs than lambs fed maintenance ration. According to Brown et al. (1961), extended fasting of 88 hours in cattle caused plasma glycine level to increase approximately twofold, a significant increase in the aromatic amino acids, lysine, valine, threonine, leucine, and isoleucine, a decrease in serine and alanine and no significant changes in the plasma concentrations of glutamic acid, cystine, histidine and arginine. Zimmerman and Scott (1967) working with chickens, observed that plasma lysine, methionine, isoleucine, leucine, tyrosine, phenylalanine and histidine concentrations increased with each extension of fasting period whereas plasma cystine decreased and proline, glutamic acid and arginine were unaffected by fasting. These workers also observed that feeding a nonprotein diet to chickens caused a lower plasma amino acid concentration as compared to the plasma amino acid levels of fasted chickens. Feeding nitrogen free diets to rats, Bergen and Purser (1968c) observed a lower total plasma essential amino acid levels than other treatments although the individual histidine levels were higher. Denton and Elvehjem (1954) fed nonprotein diets to dogs and observed

a decrease in all plasma amino acid concentrations of portal blood except tryptophan.

The removal of an essential amino acid from the diet will cause a severe deficiency and consequently, a low plasma concentration of that particular essential amino acid, whereas there will be an increase in plasma levels of all other amino acids. Such increase in plasma level of all the other amino acids can be interpreted as the result of very poor utilization of these amino acids due to the absence of the essential amino acid which impedes protein synthesis. Consequently, high plasma amino acid concentration may indicate proper amino acid absorption but not efficient amino acid utilization. This was substantiated by Purser (1970) who pointed out that the plasma concentration of a specific amino acid does not always reflect nutritional status unless the amino acid is markedly limiting. Zimmerman and Scott (1965), working on the interrelationship of plasma amino levels and weight gain in chicken, reported that the first limiting amino acid in a diet remains at very low and constant level in the blood regardless of the severity of the deficiency and that increments in excess of the amount required to maximize weight gain resulted in a rapid and linear accumulation of that amino acid in the blood. Kumta and Harper (1962) working with rats suggested that if the influx of amino acid to the blood stimulates cellular uptake or protein

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synthesis then the concentration of essential amino acid in short supply should fall much lower than the other essential amino acid. Plasma amino acid may thus serve as a sensitive indication of the limiting or deficient amino acid in diets (McLaughlan, 1964; McLaughlan et al., 1967; and Rao et al., 1968; Purser et al., 1966; Potter et al., 1968).

Harper and Rogers (1965) in their discussion on amino acid imbalance simply defined an amino acid imbalanced diet as one which is a low protein diet and in which there are additional amounts of essential amino acids which cause an intake depression. Two further important conditions distinguish an amino acid imbalance from a simple essential amino acid deficiency. First, both the control diet and the imbalanced diet contain exactly the same amount of the limiting amino acid and second, the concentration of the limiting amino acid (or acids) must be increased in the imbalanced diet to overcome the appetite depression or growth retardation. On the other hand, addition of the limiting amino acid to the control diet produces very little or no additional growth response. An imbalanced diet of casein-gelatin or oxidized casein in which tryptophan was the limiting amino acid produced severe growth depression and marked lowering of plasma tryptophan level which was corrected by addition of tryptophan to the diet (Sauberlich and Salmon, 1955). According to Harper and Rogers (1965), the major effect of amino acid imbalanced diets is a drastic

decrease in intake of the imbalanced diet, so much that rats will eat a nonprotein diet which will not support life instead of eating the imbalanced diet which will support life. After reviewing several of their previous experimental data and data of several other workers, Harper and Rogers (1965) concluded that growth can be improved in rats on amino acid imbalanced diets as long as food intake can be maintained.

## Effects of Energy on Plasma Amino Acids

The utilization of dietary protein by an animal depends on several closely integrated factors and one of these factors is available energy. Using $^{14}C$ labeled glycine, Munro et al. (1962) observed that in diets containing adequate amounts of protein a high caloric intake caused an increase in total amount of glycine taken up by liver protein, but with protein free diets the dietary caloric level had no influence on incorporation. They therefore concluded that the major effect of the caloric content of the diet in influencing protein metabolism is on the utilization of circulating amino acids (plus tissue free amino acid pools) between meals and not during the active phase of amino acid absorption after meals. A similar conclusion was drawn by Knipfel et al. (1969) from amino acids incorporation data when fasted rats were injected with $^{14}C$ lysine and subsequently fed glucose. Rao and McLaughlan (1967) studied the effect of time on the

nitrogen sparing effect of dietary carbohydrates in rats and observed no significant difference in weight gain between rats fed carbohydrates with protein or rats fed casein in the morning and carbohydrates eight hours after. However, rats fed casein alone had an elevated plasma amino acid level until carbohydrates were fed eight hours later. Rats fed carbohydrate with casein had a significantly greater nitrogen retention on the first day but not on the following days, than rats fed protein and carbohydrate separately. They concluded that the effect of time factor on the nitrogen sparing effect of dietary carbohydrates was only transient. Munro (1964) reviewed several reports on the difference in actions of carbohydrate and fat on protein metabolism in several species and then concluded that fat either fails to reduce nitrogen output in fasting animals or is less effective than carbohydrate under comparable circumstances. He hypothesized that the apparent depression of plasma amino acids and their incorporation into muscle protein after a carbohydrate meal is influenced by insulin secretion. Christensen (1964) pointed out that body tissue can absorb amino acids without immediately metabolizing them and that the increase in amino acid transport into tissues caused by insulin secretion in response to glucose ingestion might not necessarily accelerate tissue protein synthesis. Dror et al. (1969) reported that the positive effect of addition of starch on

protein utilization depends on the physiological status of the animal and the composition of the diet, particularly the energy protein ratio. Infusion of energy, in man (Crofford et al., 1964) in sheep (Potter et al., 1968) in the lactating dairy cow, (Halfpenny et al., 1969) and in rats (Knipfel et al., 1969) caused a significant depression of plasma free essential amino acids. The relative depression or depression pattern of essential amino acids depends on the source of energy infused (Potter et al., 1968). Potter et al. (1968) reported more cellular uptake of plasma free amino acid from proprionic acid and glucose than from butyrate and acetate infusion.

## The Role of Tryptophan in Mammalian Metabolism

The role of tryptophan in mammalian metabolism seems to be very important, but unfortunately, enough work has not been done to pinpoint a specific role which is unique to tryptophan, other than for protein synthesis, although several attempts have been made. Munro (1968) summarized all reports on the loss of heavy polysomes and an accumulation of monosomes and oliogosomes which was somewhat unique to the absence of dietary tryptophan alone. He concluded that tryptophan is the essential amino acid which normally determines polysome aggregation in liver cells of intact animals, but not because of some specific or unique role but because tryptophan is least abundant in free amino acid pool and in the protein synthesized from such pool.

Tryptophan is uniquely low in tissue free amino acid pool, body tissue protein and dietary protein (Munro, 1970). Scott et al. (1969) reported that collagen which represents more than 1/2 of the animal's body protein contains absolutely no tryptophan. The absence of dietary tryptophan will more severely limit the charging of tryptophanyl t-RNA than the absence of any other amino acid (Munro, 1970).

The availability of niacin in foods or feed stuffs is often extremely low, consequently, excess dietary tryptophan plays a vital role in satisfying the animal's requirement for niacin if niacin is not supplemented in the diet. The requirement for niacin is dependent on the level of dietary tryptophan (Harmon et al., 1969). Murata and Kimura (1969) feeding threonine free diet, tryptophan free diet or complete amino acid diet to rats concluded that dietary tryptophan which is normally used for protein synthesis is converted to nicotinic acid when protein formulation is limited by omission of threonine.

Tryptophan also seems to be important in the maintenance of pregnancy. Lojkin (1962) feeding different levels of tryptophan in diets to pregnant rats, reported that 100 percent of the rats fed 0.136 percent tryptophan diet had healthy and normal fetuses on the 20th day of gestation but in all rats fed .096 percent diet there was 100 percent resorption of fetus. She therefore, concluded that approximately 0.136 percent tryptophan is required for normal pregnancy to proceed in rats.

# MATERIALS AND METHODS

## A. Experiment 1

### 1. General Design of Experiment

In this study, four mature, rumen fistulated wethers were randomly allotted to a 4 x 4 Latin Square experiment (Table 1), involving four rations (Table 2), which contained different levels of protein (Table 3), and different energy sources. These rations were selected because they were used in previous experiments in this laboratory (Purser et al., 1966; Bergen et al., 1968a,b). However, rations 3 and 4 which were previously isonitrogenous are not isonitrogenous in the present study, because a 50% crude protein soybean meal was used instead of the original 40% crude protein soybean meal. Wethers were housed indoors singly in 4 feet x 6 feet metal stalls and given free access to fresh water. Each period consisted of a 21 day ration-adjustment period and a 3 day sampling period. The sheep were fed 1/2 of the dietary allowance at each feeding, 8 a.m. and 8 p.m., at the rate of 7% of their metabolic body weight (B.W. $^{\cdot 75}$) (Kleiber, 1961).

On each sampling day the animals were fed only in the morning immediately after the first sample was taken. On

TABLE 1

Experimental Design for Experiment 1

| Period | Sheep No. 94 | 990 | 32 | 28 |
|---|---|---|---|---|
| 1 | A | B | C | D |
| 2 | B | C | D | A |
| 3 | D | A | B | C |
| 4 | C | D | A | B |

Treatments: A = Ration 1
B = Ration 2
C = Ration 3
D = Ration 4

TABLE 2

Rations

| Ingredients | 1[a] % | 2[a] % | 3[b] % | 4[b] % |
|---|---|---|---|---|
| Alfalfa meal | _ | 38.0 | 50.0 | - |
| Ground corn cobs | 31.0 | 8.0 | - | - |
| Ground corn | 47.0 | 42.0 | - | 70.0 |
| Cerelose | 3.0 | 5.0 | - | - |
| Starch | 7.0 | - | - | - |
| Solka Floc | 5.0 | - | - | - |
| Urea | 0.5 | - | - | 1.0 |
| Mineral-Vitamin mix[c] | 2.0 | 2.0 | 2.0 | 2.0 |
| Soybean meal | - | - | - | 15.0 |
| Basal Pellets[d] | - | - | 43.0 | 7.0 |
| Molasses | 5.0 | 5.0 | 5.0 | 5.0 |

[a]Purser et al. (1966).

[b]Bergen et al. (1968a).

[c]Mineral-Vitamin mix contained in percent: Dicalcium phosphate, 47.38; high zinc trace mineral salt, 47.38; $Na_2SO_4$, 4.78; Vitamin A (10,000 IU/g), 0.32; Vitamin D (9,000 IU/g), 0.10.

[d]Basal Pellets contained in percent (air dry): Ground hay, 50.0; ground corn cobs, 30.0; Molasses, 5.0; Urea, 1.1; Mineral mix, 2.0; Soybean meal, 11.9.

TABLE 3

Crude Protein and Dry Matter Content of Rations

| | Ration 1 % | Ration 2 % | Ration 3 % | Ration 4 % |
|---|---|---|---|---|
| Crude Protein | 6.9 | 11.1 | 15.8 | 20.2 |
| Dry matter | 93.1 | 93.0 | 94.4 | 92.3 |

each occasion sampling began at 8 a.m. On day 22 of the period blood and rumen samples were taken immediately before feeding ($T_0$) and sequentially at 3, 6, 9, 12, 16, 20 and 24 hours post feeding. On day 24 both blood and rumen samples were similarly taken immediately before feeding, but instead of the a.m. feeding a 2 : 1 starch-glucose mixture was instilled in the rumen at the rate of 1.5% of the metabolic body weight in 500 ml distilled water. Blood and rumen samples were again taken at 4 hours after dosing.

2. Sample Collection and Preparation

a. Blood samples

At each sampling time 10 ml of blood were collected from the right jugular vein of each sheep using a heparinized syringe. Immediately after collection, samples were centrifuged at 4,080 xg for 10 minutes to separate the liquid plasma from the solid portion of red cells. The plasma was then transferred into small test tubes with disposable pasteur pipettes. Two ml of the plasma were stored in the freezer (-70°) for later use in the spectroflurometric determination of tryptophan (see Section 3 below). To the rest of the plasma 1 mM norleucine was added as an internal standard, at the rate of 0.1 ml norleucine per ml of plasma. This was followed by the addition of 50% (w/v) sulfasalycylic acid at the rate of 0.1 ml sulfasalycylic acid per ml of plasma and the mixture

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placed in an ice bath for 30 minutes. The sulfasalycylic acid was added to precipitate plasma protein. After 30 minutes the mixture was centrifuged at 47,000 xg for 15 minutes. The supernatant (protein free filtrate) was removed with a pasteur pipette and stored in the freezer for determination of α-amino nitrogen and complete amino acid analysis (Purser et al., 1966; Makdani et al., 1971; Bergen and Potter, 1971).

b. Rumen samples

A representative sample (50 ml) of rumen content was taken from the rumen through the rumen cannula with a collection tube at each sampling time. Rumen contents were squeezed through two layers of cheese cloth and 19 ml of the liquid portion (rumen liquor) transferred to a test tube containing 1 ml of saturated mercuric chloride. Saturated mercuric chloride prevents further degradation of the included substrates by rumen microbes and stops any further chemical reactions. A 5 ml sample was then taken from the 20 ml rumen liquor-mercuric chloride mixture and 1 ml of 25% metaphosphoric acid added to precipitate soluble protein and the mixture centrifuged at 12,100 xg for 15 minutes. The supernatant was saved for the determination of short chain volatile fatty acids by gas liquid chromatography (see Section 6 below).

3. Tryptophan Determination

Tryptophan was assayed using the "Simplified Spectrofluorometric Micromethod" of Wapnir and Stevenson (1969). Tryptophan standards were made by dissolving 100 mg of L-tryptophan in 100 ml of deionized distilled water to form a stock solution. Aliquots of 1 ml, 2 ml, 3 ml , 4 ml and 5 ml were taken from the stock solution and deionized distilled water added to each to make tryptophan standard solutions of 1, 2, 3, 4, and 5 mg/100 ml respectively. Maximum recovery of tryptophan in plasma was obtained during trial runs, consequently, no conversion factor was necessary.

A 20 µl portion of the sample or standard was placed in the center of a printed circle on filter paper cards (S & S 903-C, Schleicher & Schuell, Inc.) and air dried. A water filled circle was used as blank. Each sample or standard was run in triplicate. The dried circle was cut out, sectioned in four pieces and placed in clean, dry, labeled test tubes. To each test tube 1 ml of 78% (v/v) ethanol was added to elute the free tryptophan from the sectioned circle. Each test tube was stoppered immediately to avoid any evaporation of the ethanolic solution. Occasionally the test tube racks were gently shaken, making sure that each piece of paper was properly covered by the solvent at all times. After not less than 30 minutes 0.5 ml of the ethanolic extract was rapidly transferred to

another small test tube and 3 ml of 0.02 M tris base solution added. The contents were mixed and immediately read on the Aminco-Bowman Spectrophotofluorometer (at an excitation wave length of 287 nm, emission wave length of 357 nm, excitation slit width of 2, emission slit width of 5, photomultiplier slit width of 4, meter-multiplier of 0.01 and a sensitivity of 50, using quartz cuvettes).

To prevent quenching all glassware was acid washed, rinsed once with deionized distilled water, rinsed twice with absolute ethanol and rinsed twice with 78% ethanol.

4. α-Amino Nitrogen Determination (total free amino acid test)

The method of Palmer and Peters (1969) was used. A citrulline standard was made by dissolving 0.3504 g citrulline (M. W. 175.19) in deionized distilled water to form a stock solution of 10 μM/ml. From this stock solution 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 μM/ml citrulline standard solutions were made by diluting the appropriate aliquots to 100 ml with deionized distilled water.

Fresh 0.25% 2, 4, 6, trinitrobenzene sulfonic acid (TNBS) (0.25 g/100 ml $H_2O$) and 0.05 M $Na_2B_4O_7 \cdot 10\ H_2O$ (19.07 g sodium borate per liter of water, buffered at pH 9.2) solutions were made weekly.

To each test tube 0.2 ml of either the citrulline standard or protein free filtrate, 1.6 ml of the 0.05 M sodium borate solution and 0.2 ml of the 0.25% TNBS reagent

were added and mixed. The mixture was incubated at 37°C for 20 minutes. At the expiration of this period, 2 ml of 1 N hydrochloric acid were added to each tube (to stop the reaction), and the content mixed. The optical density (O.D.) was read immediately on the Coleman Spectrophotometer, model 620, at a wave length of 420 nm using a reagent blank.

5. Rumen Ammonia and Blood Urea Nitrogen Determination

a. Rumen $NH_3$-N

Rumen $NH_3$-N was determined by the micro-diffusion method of Conway (1960). All dishes were prepared by placing 1 ml glycerol in the outer well, 1 ml boric acid solution (.04 N) to the inner well, 0.5 ml of the original rumen liquor and 0.5 ml of distilled water to one side of the middle well and 1 ml potassium carbonate ($K_2CO_3$) solution (100% w/v) to the other side of the middle well, making sure that there was no dripping into the other compartments or on the sample. The lid was quickly placed on the plate and gently rotated in the glycerol a number of times to provide a seal, thus preventing the escape of the liberated volatile nitrogenous base. A water blank was prepared for each batch of samples run, in a similar manner, except that instead of the diluted sample or standard 1 ml distilled water was added. Each sample was run in duplicate. The dishes were swirled gently in order

to carefully mix the $K_2CO_3$ solution with the sample, and placed on a rotator for one hour. At the end of this period the content of the inner well (green in color due to the trapped nitrogen) of each dish was titrated with a standard solution of 0.04 N HCl until the color matched that of the water blank (light pinkish red) and burette readings recorded.

b. Blood urea nitrogen

Blood urea nitrogen was determined by the micro-diffusion technique of Conway (1960). The procedure is the same as that used for the determination of rumen $NH_3$-N, except that before the $K_2CO_3$ solution was added, 0.5 ml urease solution (20 mg/ml) was added to the middle well to hydrolyze urea to $NH_3$ and $CO_2$. After urea hydrolysis was completed (45 minutes) 1 ml $K_2CO_3$ solution was then added to the middle well and the procedure continued in the usual $NH_3$-N determination manner.

$NH_3$-N was not determined in the plasma samples since a prior experiment had shown that the amount of $NH_3$-N in normal plasma of peripheral blood was too low to determine with any degree of precision.

## 6. Volatile Fatty Acids Determination

Rumen volatile fatty acid concentrations were determined by Gas Liquid Chromatography using Packard instruments (model 840). The column (6 ft glass) was packed

with chromosorb 101, the flow rate of carrier gas ($N_2$) was 70 ml/min., and the column temperature was maintained at 195°C. Volatile fatty acids in the rumen fluid were identified qualitatively and quantitatively by comparison with a standard solution of volatile fatty acids.

## B. Experiment 2

Four wethers, fitted with rumen cannulae, of approximately equal body weight were fed each at 8 a.m. and 8 p.m. one of the four rations used in experiment 1, for 21 days at the rate of 7% of their metabolic body weight. On day 22 approximately 250 ml rumen content were taken from each sheep immediately before feeding at 8 a.m. and 1-1/2 and 4 hours after feeding and cooled to 3°C in ice.

### 1. Sample Preparation

Rumen protozoa and bacteria were separated from each rumen sample as follows: rumen contents were squeezed through two layers of cheesecloth and the rumen liquor (1) collected. The residue was resuspended in an ample amount of saline solution (0.9% NaCl) and squeezed through two layers of cheesecloth and the liquid portion (rumen liquor (2)) collected and mixed with rumen liquor (1). To the mixture of rumen liquors an equal volume of 37% formaldehyde solution was added to fix and preserve the rumen micro-organisms. The fixed solution was centrifuged at 250 xg for 15 minutes. The supernatant was saved for

subsequent isolation of bacteria and the pellets (protozoa) were washed and then freeze-dried. Supernatant from the first spin was centrifuged at 2,500 xg for 15 minutes after which the residue (feed particles) was discarded and the supernatant centrifuged again at 40,000 xg for 15 minutes. This time the supernatant was discarded and the pellets (bacteria) resuspended in a large amount of saline solution and again centrifuged at 40,000 xg for 15 minutes. The supernatant was discarded and the pellets were freeze-dried. Samples were kept at 3°C throughout the entire sample preparation procedure.

2. Tryptophan Analysis

Tryptophan determination on freeze-dried preparations of protozoa and bacteria were done by a modified $BaOH_2$ protein hydrolysis procedure, followed by a modified colorimetric determination of tryptophan (Miller, 1967; Spies, 1967; Knox et al., 1970). To a screw capped glass test tube (approximately 25 x 150 mm) containing 5 g $Ba(OH)_2 \cdot 8\ H_2O$, 300 mg of the finely ground dry protozoa or bacteria sample were added and the content was properly mixed. The walls of the tube were washed down with 4 ml deionized-distilled water and the tube was saturated with nitrogen gas, tightly capped and then autoclaved at 15 lb/in.$^2$ for 7 hours after which the tube was left to cool overnight. In the following morning the tube was opened,

4 ml 6 N HCl added to the sample and the mixture transferred to a 50 ml volumetric flask. The tube was finally washed twice into the flask with 1 ml portions of 6 N HCl, and 25 ml of 17.5% $Na_2SO_4$ solution were then added to the flask to precipitate the barium. The resulting suspension was mixed and the volume made up to 50 ml with deionized distilled water. Ten ml of the well mixed suspension were centrifuged for 30 minutes at 14,000 xg.

Two ml aliquots of the supernatant were transferred to three stoppered test tubes. To the first two tubes, 5 ml of 0.5% (w/v) p-dimethylaminobenzaldehyde in concentrated HCl were added and mixed. After 20 minutes 0.2 ml aqueous 0.2% (w/v) sodium nitrite solution was added and properly mixed. The resulting solution was filtered through Whatman #2 filter paper after 25 minutes and the O.D. read on a Gilford spectrophotometer at 590 nm within 40 minutes after filtration. To the third tube (blank) 5 ml concentrated HCl were added, followed by the addition of 0.2 ml of 0.2% sodium nitrite, filtration and reading on the Gilford spectrophotometer. A standard tryptophan curve was prepared by determining the O.D. of 2 ml aliquots of L-tryptophan solutions containing 0.5 to 4 mg tryptophan per 100 ml water. A preliminary experiment, using Lysozyme (7.5% tryptophan), revealed a 98.8% tryptophan recovery, consequently, a recovery factor was ignored.

## C. Statistical Analysis

Data were statistically analyzed for Analysis of Variance (Appendix 1) on a CDC, 3600 computer at the Michigan State University Computer Laboratory. Differences between means were determined by the "Duncan's New Multiple Range Test" on the CDC, 3600 computer.

# RESULTS

## Experiment 1

### Plasma Free Tryptophan

Plasma free tryptophan concentrations of sheep fed the four rations are shown in Table 4. The range of mean daily plasma tryptophan levels was 1.86 to 2.11 mg per 100 ml plasma. There were no significant differences in plasma free tryptophan concentrations of sheep fed the four rations before feeding and at 3, 6, 12, 16, 20 and 24 hours post feeding. However, there was a significant difference ($P<.05$) between plasma concentrations of sheep fed ration 3 and sheep fed the other three rations at 9 hours after feeding. The diurnal patterns of plasma free tryptophan concentrations are shown in Figure 1. Tryptophan concentration increased to its maximum level at 9 hours after feeding, then declined to its minimum level at 16 hours after feeding for sheep fed rations 1 and 2 and at 20 hours after feeding for sheep fed rations 3 and 4. Sheep fed ration 3 were the only ones which had a significant difference ($P<.05$) in tryptophan concentration between time after feeding (between $T_9$ and $T_{20}$). Although ration 4 had the highest level of crude protein (Table 3) the plasma

TABLE 4

Plasma Free Tryptophan Concentration[a,b] During 24 Hour Period (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 1.82±.07 | 1.84±.05 | 2.05±.07 | 1.78±.06 |
| $T_3$ | 1.76±.10 | 2.01±.05 | 2.08±.11 | 1.83±.04 |
| $T_6$ | 1.83±.04 | 2.04±.06 | 1.96±.10 | 1.83±.09 |
| $T_9$ | 2.07±.07 | 2.25±.09 | 2.46±.06 | 2.10±.10 |
| $T_{12}$ | 1.89±.04 | 2.05±.10 | 2.41±.08 | 1.98±.14 |
| $T_{16}$ | 1.65±.09 | 1.68±.09 | 1.96±.08 | 1.97±.16 |
| $T_{20}$ | 1.91±.11 | 1.87±.13 | 1.82±.09 | 1.71±.10 |
| $T_{24}$ | 1.90±.15 | 1.87±.03 | 2.11±.05 | 1.86±.11 |

[a]Mg tryptophan per 100 ml plasma.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

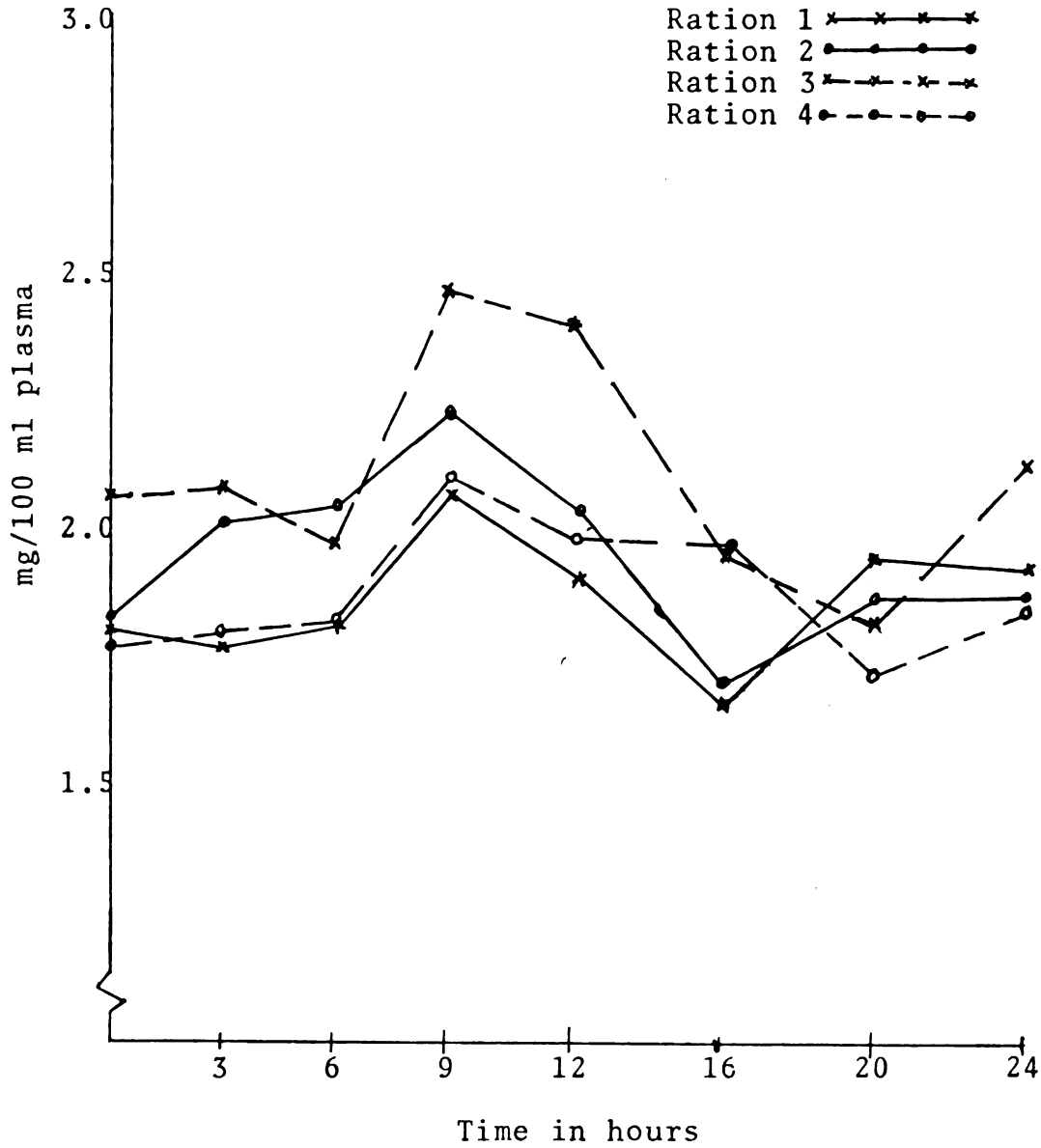


Figure 1. Diurnal pattern of plasma free tryptophan concentrations

free tryptophan levels of sheep fed this ration were lower (although not statistically significant) than the corresponding levels of sheep fed rations 2 and 3 and approximately equal to the corresponding levels of ration 1 which is roughly three times lower in crude protein content.

Plasma free tryptophan concentrations before and after starch-glucose infusion are shown in Table 5. There were no significant differences between tryptophan levels of sheep fed the four rations. The $T_4/T_0$ ratios were 87%, 93%, 98% and 91% for sheep fed rations 1, 2, 3 and 4 respectively.

Plasma α-amino Nitrogen

Plasma α-amino nitrogen concentrations of sheep fed the four rations are presented in Table 6. There were no significant differences in plasma α-amino nitrogen concentrations of sheep fed the four rations before feeding and at 3, 6, 9, 20 and 24 hours post feeding. However, there were significant differences between plasma α-amino nitrogen levels of sheep fed rations at 12 and 16 hours after feeding ($P<.005$). Plasma α-amino nitrogen concentrations of sheep fed the four rations decreased during the first six hours after feeding (Figure 2) and then increased between 9 and 12 hours after feeding; decreased at sixteen hours and then increased between 20 and 24 hours after feeding. Only sheep fed ration 3 had a significant difference ($P<.05$) in α-amino nitrogen concentration at various intervals after

TABLE 5

Plasma Tryptophan Concentration[a,b] Before and After Starch-glucose Infusion (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 1.55±.08 | 1.62±.10 | 1.88±.15 | 1.61±.15 |
| $T_4$ | 1.35±.09 | 1.51±.16 | 1.85±.17 | 1.46±.18 |
| $T_4/T_0$ Ratio | 87% | 93% | 98% | 91% |

[a]Mg tryptophan per 100 ml plasma.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

TABLE 6

Plasma α-amino Nitrogen Concentration[a,b] During 24 Hour Period (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 2.95±.16 | 2.91±.06 | 3.05±.10 | 2.64±.08 |
| $T_3$ | 2.59±.06 | 2.40±.09 | 2.59±.07 | 2.33±.11 |
| $T_6$ | 2.56±.12 | 2.68±.04 | 2.57±.03 | 2.13±.10 |
| $T_9$ | 2.80±.07 | 2.70±.05 | 2.63±.05 | 2.18±.13 |
| $T_{12}$ | 2.81±.10 | 2.98±.05 | 2.86±.07 | 2.44±.16 |
| $T_{16}$ | 2.79±.10 | 2.76±.05 | 2.43±.05 | 2.29±.09 |
| $T_{20}$ | 2.82±.13 | 2.85±.06 | 2.65±.08 | 2.31±.17 |
| $T_{24}$ | 3.16±.10 | 2.80±.12 | 2.86±.07 | 2.73±.12 |

[a]Micromoles of α-$NH_2$-N (as citrulline) per ml plasma.

[b]Mean and standard error of 4 sheep.

[c]Hours after feeding.

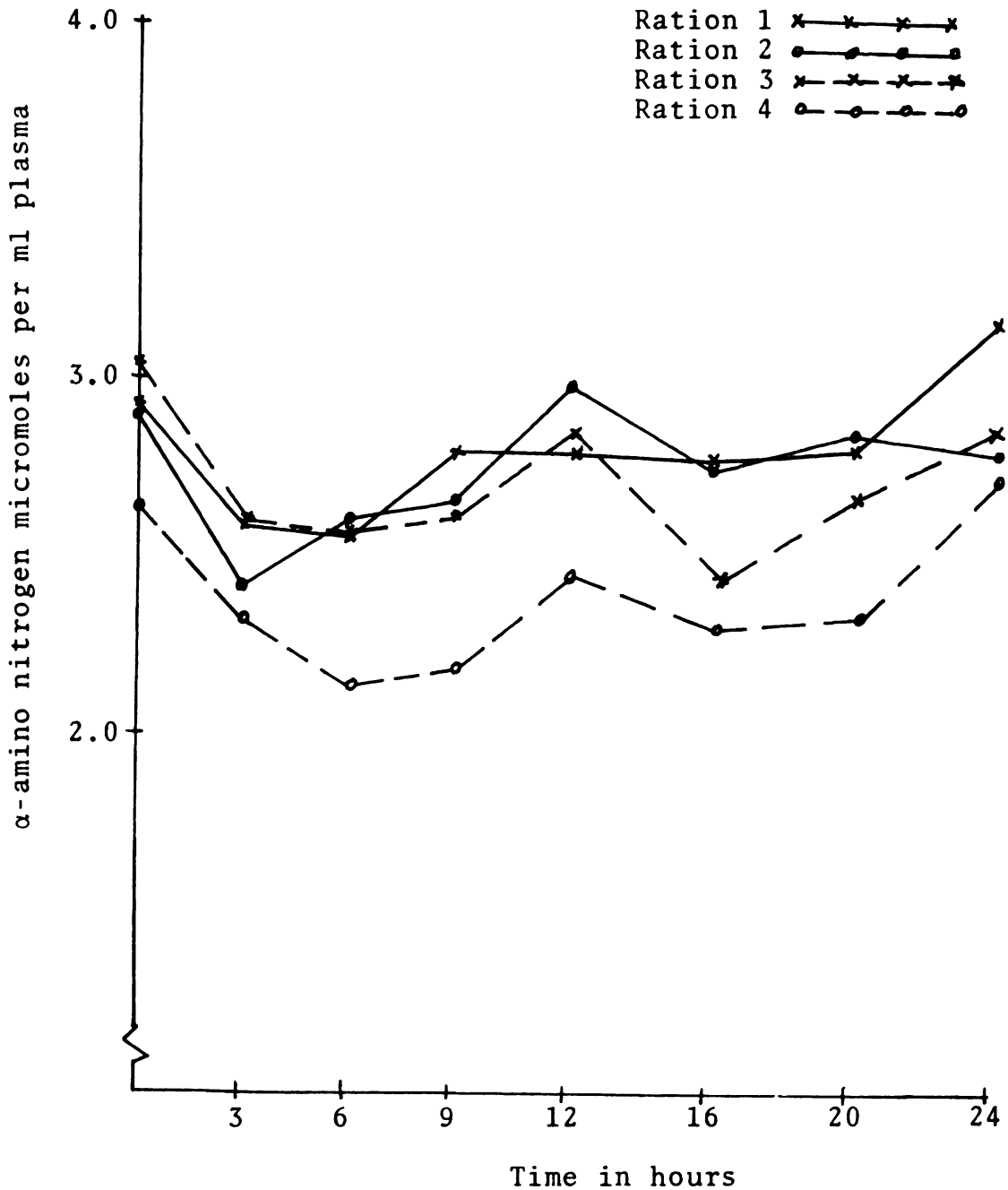


Figure 2. Diurnal pattern of plasma α-amino nitrogen concentrations

feeding (between $T_0$ and $T_{16}$). Plasma α-amino nitrogen concentrations of sheep fed ration 4 were lower than the corresponding values of sheep fed the other three rations throughout the 24 hour period.

Plasma α-amino nitrogen concentrations before and after starch-glucose infusion are shown in Table 7. There was a decline in the α-amino nitrogen levels of sheep fed all rations due to the infusion of energy. The $T_4/T_0$ ratios were 83%, 88%, 84% and 80% for sheep fed rations 1, 2, 3 and 4 respectively.

Rumen Ammonia Nitrogen

Rumen ammonia nitrogen concentrations of sheep fed the four rations throughout the 24 hour period are shown in Table 8. There were significant differences between rumen ammonia concentrations of sheep fed the four rations throughout the period ($T_0$, $P<.001$; $T_3$, $T_6$, $T_9$, $T_{12}$, $T_{16}$, $T_{20}$ and $T_{24}$, $P<.0005$). Higher rumen ammonia nitrogen concentrations were associated with the higher crude protein rations.

The diurnal patterns of rumen ammonia nitrogen concentrations are presented in Figure 3. Rumen ammonia nitrogen concentration increased rapidly to its maximum level at three hours after feeding, then gradually decreased to its minimum level at nine hours, then gradually increased from 16 through 24 hours post feeding.

TABLE 7

Plasma α-amino Nitrogen Concentration[a,b] Before and After Starch-glucose Infusion

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 2.97±.12 | 3.03±.12 | 3.07±.03 | 2.78±.07 |
| $T_4$ | 2.46±.08 | 2.67±.1 | 2.59±.09 | 2.22±.06 |
| $T_4/T_0$ | 83% | 88% | 84% | 80% |

[a]Micromoles of $\alpha$-$NH_2$-N (as citrulline) per ml plasma.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

TABLE 8

Rumen Ammonia Nitrogen Concentration[a,b] During 24 Hour Period (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 5.91±2.2 | 14.76±1.6 | 21.0± 1.0 | 43.87± 3.5 |
| $T_3$ | 7.70±2.1 | 19.04±2.3 | 22.68±1.0 | 66.17± 3.4 |
| $T_6$ | 2.94±1.1 | 11.51±2.5 | 13.34±1.4 | 60.05±14.5 |
| $T_9$ | 3.85±1.1 | 12.15±2.0 | 13.2 ±1.5 | 37.10± 1.1 |
| $T_{12}$ | 3.84±1.2 | 14.06±2.0 | 16.51±0.4 | 39.16± 2.6 |
| $T_{16}$ | 5.85±0.8 | 15.51±2.1 | 16.77±0.5 | 42.65± 3.1 |
| $T_{20}$ | 10.57±1.0 | 18.84±2.2 | 19.02±1.0 | 44.09± 2.1 |
| $T_{24}$ | 13.31±1.0 | 19.15±2.1 | 16.28±0.6 | 47.39± 1.1 |

[a]Mg nitrogen per 100 ml rumen content.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

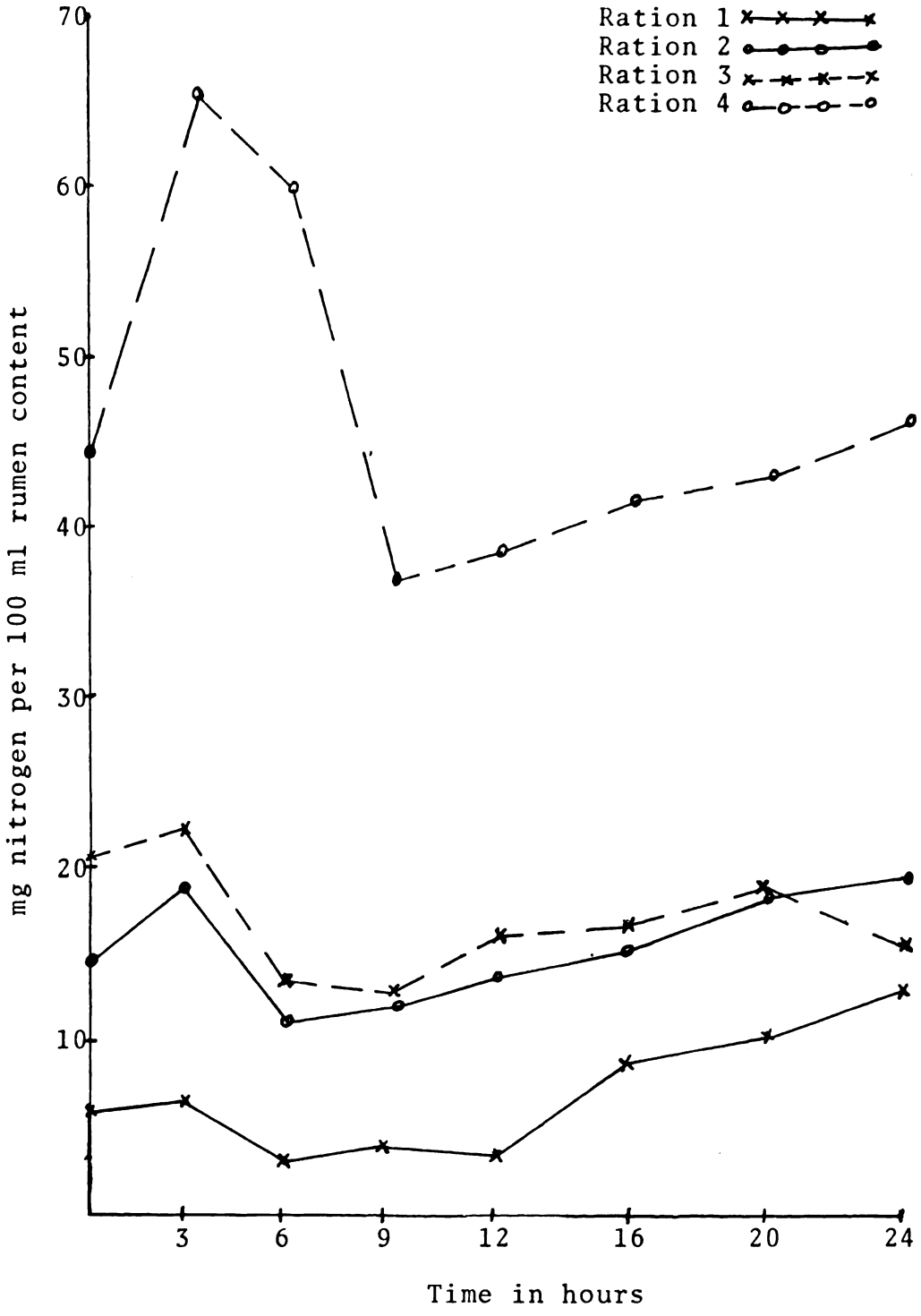


Figure 3. Diurnal pattern of rumen ammonia concentrations

Rumen ammonia nitrogen concentrations before and after starch-glucose infusion are shown in Table 9. There was a significant depression in rumen ammonia concentration four hours after starch-glucose infusion. The $T_4/T_0$ ratios were 25%, 67%, 29% and 44% for sheep fed rations 1, 2, 3 and 4 respectively.

Blood Urea Nitrogen

Blood urea nitrogen concentrations of sheep fed the four rations are shown in Table 10. There were significant differences in blood urea nitrogen levels of sheep fed the four rations throughout the 24 hour period ($T_0$, $P<.04$; $T_3$, $T_6$, $T_9$, $T_{12}$, $T_{16}$, $T_{20}$ and $T_{24}$, $P<.005$). As in the case of rumen ammonia, higher blood urea nitrogen concentrations were associated with the higher crude protein rations.

The diurnal patterns of blood urea nitrogen are shown in Figure 4. Blood urea nitrogen concentrations rapidly increased at three hours after feeding then gradually decreased to their minimum levels at 9 hours in the case of sheep fed rations 1 and 2 and 12 hours after feeding for sheep fed rations 3 and 4, then gradually increased through to 24 hours post feeding.

Blood urea nitrogen concentrations before and after starch-glucose infusion are presented in Table 11. Blood urea nitrogen concentrations were not greatly depressed by energy infusion, when compared with the significant

TABLE 9

Rumen Ammonia Nitrogen Concentration[a,b] Before and After Starch-glucose Infusion (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 9.07±1.9 | 20.18±2.7 | 24.14±1.6 | 45.40±5.8 |
| $T_4$ | 2.30±0.8 | 13.54±2.7 | 7.11±1.3 | 19.99±4.4 |
| $T_4/T_0$ | 25% | 67% | 29% | 44% |

[a]Mg nitrogen per 100 ml rumen content.

[b]Mean and standard error of 4 sheep.

[c]Hours after feeding.

TABLE 10

Blood Urea Nitrogen Concentration[a,b] During 24 Hour Period (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 4.45±1.2 | 9.90±1.1 | 17.56±1.0 | 25.04±2.0 |
| $T_3$ | 5.35±1.5 | 9.67±1.6 | 19.15±2.6 | 29.58±3.1 |
| $T_6$ | 5.17±1.3 | 7.31±0.6 | 19.12±1.4 | 29.33±2.3 |
| $T_9$ | 4.12±0.9 | 8.69±1.0 | 17.60±1.4 | 25.85±1.3 |
| $T_{12}$ | 4.28±0.9 | 9.76±1.0 | 17.36±1.4 | 24.80±1.8 |
| $T_{16}$ | 4.23±0.8 | 11.13±0.7 | 18.80±1.1 | 21.87±0.6 |
| $T_{20}$ | 6.03±0.9 | 14.21±0.7 | 18.85±1.1 | 23.39±1.1 |
| $T_{24}$ | 8.48±1.4 | 17.86±0.6 | 23.31±1.2 | 24.75±1.0 |

[a]Mg nitrogen per 100 ml blood plasma.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

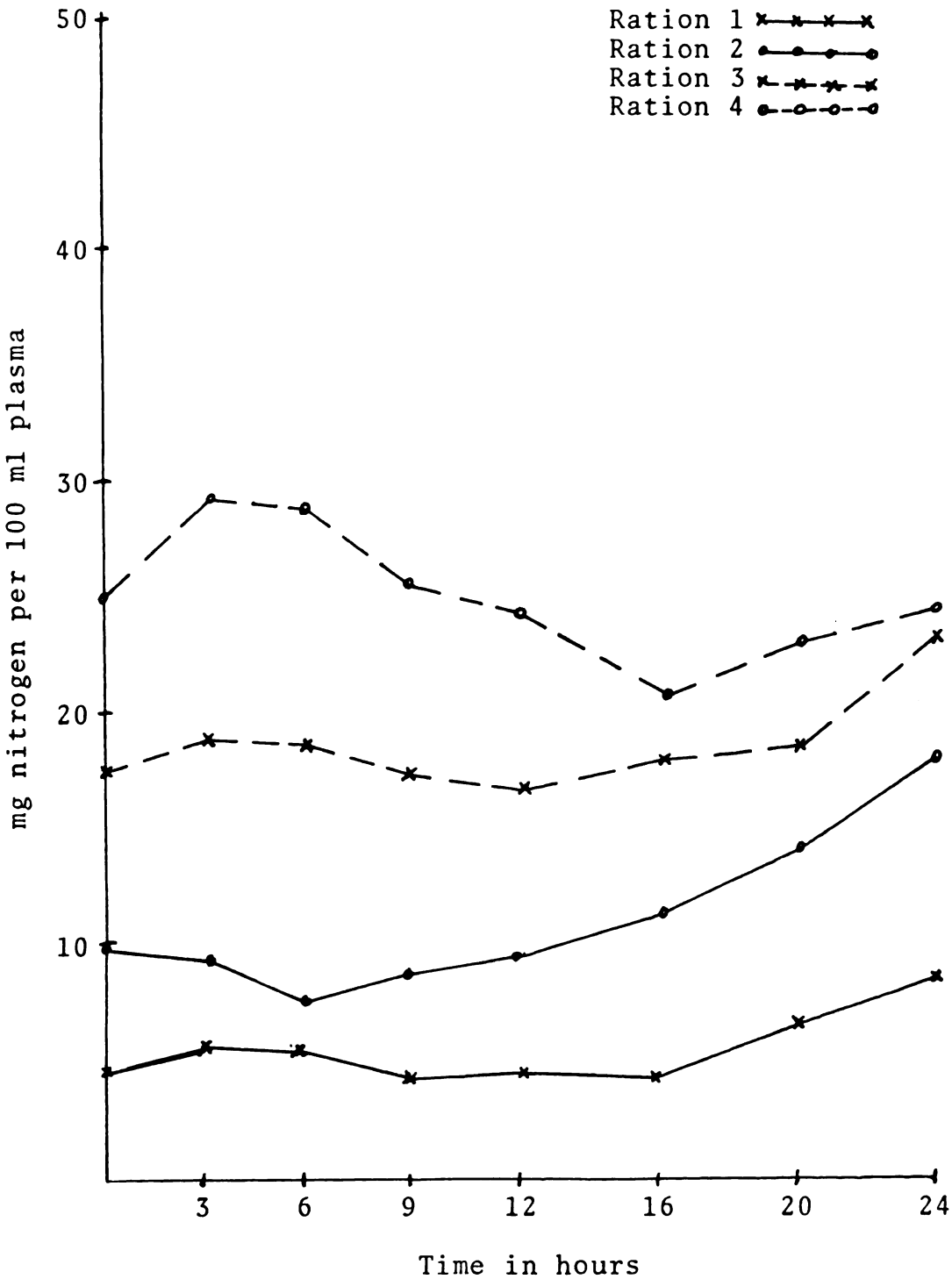


Figure 4. Diurnal pattern of blood urea nitrogen concentrations

TABLE 11

Blood Urea Concentration[a,b] Before and After Starch-glucose Infusion (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 4.85±0.2 | 12.01±1.4 | 16.55±0.9 | 21.38±1.2 |
| $T_4$ | 3.71±0.1 | 9.49±1.1 | 15.07±0.8 | 16.66±1.4 |
| $T_4/T_0$ | 76% | 79% | 91% | 78% |

[a]Mg nitrogen per 100 ml blood plasma.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

depressions noted for rumen ammonia. The $T_4/T_0$ ratios were 76%, 79%, 91% and 78% for sheep fed rations 1, 2, 3 and 4 respectively.

Rumen Volatile Fatty Acids

Rumen acetate concentrations of sheep fed the four rations are shown in Table 12. There were no significant differences in rumen acetate concentrations of sheep fed the four rations throughout the 24 hour period. Rumen acetate concentration increased to its maximum level at three hours after feeding and then gradually decreased throughout the rest of the 24 hour period. The rumen acetate concentrations before and after starch-glucose infusion are presented in Table 13. The data indicate that the starch glucose infusion caused a slight depression in rumen acetate concentration. The $T_4/T_0$ ratios were 75%, 94%, 66% and 80% for sheep fed rations 1, 2, 3 and 4 respectively.

Rumen proprionate concentrations of sheep fed the four rations are shown in Table 14. There were no significant differences between rations in the production of rumen proprionate. Rumen proprionate concentrations reached their maximum levels at three hours then steadily decreased throughout the rest of the 24 hour period after feeding. Rumen proprionate concentration before and after starch-glucose infusion are shown in Table 15.

TABLE 12

Rumen Acetate Concentration[a,b] During 24 Hour Period (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 60.0±5 | 47.0± 5 | 55.0± 7 | 56.0±6 |
| $T_3$ | 67.0±5 | 64.0±12 | 84.0±12 | 75.0±7 |
| $T_6$ | 60.0±7 | 67.0± 8 | 79.0± 9 | 58.0±3 |
| $T_9$ | 59.0±4 | 56.0± 6 | 67.0±11 | 60.0±4 |
| $T_{12}$ | 43.0±4 | 43.0± 8 | 58.0± 4 | 60.0±4 |
| $T_{16}$ | 44.0±2 | 40.0± 9 | 44.0± 1 | 56.0±6 |
| $T_{20}$ | 40.0±3 | 29.0± 3 | 30.0± 2 | 42.0±3 |
| $T_{24}$ | 28.0±3 | 22.0± 2 | 20.0± 1 | 31.0±5 |

[a]Micromoles acetate per ml rumen liquor.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

TABLE 13

Rumen Acetate Concentration[a,b] Before and After Starch-glucose Infusion (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 58.0±4 | 53.0±10 | 58.0±10 | 62.0±10 |
| $T_4$ | 44.0±4 | 50.0± 9 | 38.0± 6 | 50.0± 4 |
| $T_4/T_0$ | 75% | 94% | 66% | 80% |

[a]Micromoles acetate per 100 ml rumen liquor.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

TABLE 14

Rumen Proprionate Concentration[a,b] During 24 Hour Period (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 11.0±2 | 12.0±3 | 12.0±2 | 13.0±2 |
| $T_3$ | 13.0±2 | 21.0±6 | 24.0±2 | 23.0±1 |
| $T_6$ | 10.0±2 | 19.0±4 | 19.0±2 | 17.0±2 |
| $T_9$ | 10.0±2 | 14.0±3 | 14.0±2 | 16.0±2 |
| $T_{12}$ | 10.0±3 | 11.0±3 | 12.0±1 | 13.0±2 |
| $T_{16}$ | 7.0±1 | 10.0±4 | 10.0±1 | 14.0±1 |
| $T_{20}$ | 6.0±0 | 6.0±2 | 7.0±0 | 9.0±1 |
| $T_{24}$ | 5.0±0 | 5.0±1 | 4.0±0 | 7.0±1 |

[a]Micromoles proprionate per 100 ml rumen liquor.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

TABLE 15

Rumen Proprionate Concentration[a,b] Before and After Starch-glucose Infusion (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 10.0±1 | 14.0±4 | 13.0±2 | 16.0±4 |
| $T_4$ | 15.0±3 | 22.0±7 | 12.0±1 | 28.0±5 |
| $T_4/T_0$ | 150% | 157% | 92% | 175% |

[a]Micromoles proprionate per 100 ml rumen liquor.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

The starch-glucose infusion caused a substantial increase in rumen proprionate concentrations of sheep fed rations 1, 2 and 4 and a slight decrease in sheep fed ration 3. The $T_4/T_0$ ratios were 150%, 157%, 92% and 175% for sheep fed rations 1, 2, 3 and 4 respectively.

Rumen butyrate concentrations of sheep fed the four rations are shown in Table 16. There were no significant differences between rations in rumen butyrate concentrations at 6, 9 and 12 hours after feeding. However, rumen butyrate was significantly higher in sheep fed ration 4 than sheep fed the other three rations before feeding and at 3, 16, 20 and 24 hours after feeding ($P<.05$). Rumen butyrate concentrations before and after starch-glucose infusion are presented in Table 17. Starch-glucose infusion caused a slight decrease in rumen butyrate concentrations of sheep fed rations 1 and 4 and a slight increase for sheep fed rations 2 and 3. The $T_4/T_0$ rations were 88%, 118%, 142% and 92% for sheep fed rations 1, 2, 3 and 4 respectively.

Plasma Amino Acid Concentration

Plasma amino acid concentrations before and after starch-glucose infusion are presented in Table 18. There was a substantial depression in the total plasma free essential amino acid concentrations of sheep fed the four rations after the intraruminal infusion of the starch-glucose solution. However, individually, there was an increase in lysine and histidine concentrations in sheep

TABLE 16

Rumen Butyrate Concentration[a,b] During 24 Hour Period (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 8.0±0 | 7.0±1 | 6.0±1 | 12.0±2 |
| $T_3$ | 9.0±2 | 9.0±1 | 9.0±2 | 14.0±3 |
| $T_6$ | 9.0±3 | 10.0±1 | 9.0±2 | 11.0±1 |
| $T_9$ | 9.0±2 | 9.0±1 | 7.0±1 | 12.0±2 |
| $T_{12}$ | 8.0±1 | 8.0±3 | 7.0±1 | 12.0±3 |
| $T_{16}$ | 6.0±1 | 6.0±2 | 5.0±0 | 13.0±3 |
| $T_{20}$ | 5.0±1 | 4.0±0 | 3.0±0 | 9.0±2 |
| $T_{24}$ | 3.0±0 | 3.0±1 | 2.0±0 | 7.0±2 |

[a]Micromoles butyrate per 100 ml rumen liquor.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

TABLE 17

Rumen Butyrate Concentration[a,b] Before and After Starch-glucose Infusion (Experiment 1)

| Time[c] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 8.0±0 | 11.0±3 | 7.0±2 | 12.0±1 |
| $T_4$ | 7.0±0 | 13.0±4 | 10.0±2 | 11.0±1 |
| $T_4/T_0$ | 88% | 118% | 142% | 92% |

[a]Micromoles butyrate per 100 ml rumen liquor.

[b]Mean and standard error for 4 sheep.

[c]Hours after feeding.

TABLE 18

Plasma Amino Acid Concentration[a,b] Before and After Starch-glucose Infusion (Experiment 1)

| Amino Acid | Ration 1 | | | Ration 2 | | |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_4$ | $T_4/T_0$ | $T_0$ | $T_4$ | $T_4/T_0$ |
| **Essential Amino Acids** | | | | | | |
| Lysine | 13.80± 2.7 | 8.64± 2.8 | .63 | 7.59± 2.0 | 4.96± 1.1 | .65 |
| Histidine | 15.88± 2.3 | 15.13± 3.1 | .95 | 11.65± 1.4 | 9.54± 0.3 | .82 |
| Arginine | 12.35± 2.3 | 10.23± 2.4 | .83 | 18.02± 2.2 | 12.71± 0.3 | .71 |
| Threonine | 22.54± 6.8 | 17.53± 6.4 | .78 | 24.22± 6.4 | 16.82± 1.4 | .69 |
| Valine | 29.05± 6.5 | 23.87± 5.0 | .82 | 30.80± 7.2 | 21.04± 5.2 | .68 |
| Methionine | 5.52± 1.5 | 4.93± 1.1 | .89 | 6.27± 1.6 | 4.79± 0.9 | .76 |
| Isoleucine | 13.05± 5.0 | 10.33± 0.5 | .79 | 14.46± 3.0 | 9.87± 2.3 | .68 |
| Leucine | 21.36± 4.5 | 15.12± 2.2 | .71 | 24.79± 6.0 | 15.28± 3.0 | .62 |
| Phenylalanine | 9.92± 2.3 | 7.17± 1.1 | .72 | 11.08± 3.1 | 7.49± 1.1 | .68 |
| Total | 143.48±30.0 | 112.97±20.7 | .79 | 149.00±31.6 | 102.49±13.5 | .70 |
| **Nonessential Amino Acids** | | | | | | |
| N-methyl-lysine | 17.62± 7.8 | 15.11± 4.4 | .86 | 23.86± 6.7 | 22.13± 6.5 | .93 |
| Aspartic acid | 1.78± 0.2 | 2.22± 0.1 | 1.25 | 2.00± 0.3 | 1.80± 0.3 | .90 |
| Serine | 22.31± 2.3 | 18.77± 1.5 | .84 | 22.72± 4.2 | 17.78± 4.0 | .78 |
| Asparigine | 11.85± 2.4 | 6.08± 1.3 | .51 | 10.29± 2.0 | 5.96± 1.8 | .58 |
| Glutamic acid | 28.68± 4.2 | 26.10± 2.8 | .91 | 19.90± 3.6 | 19.67± 6.9 | .99 |
| Glutamine | 32.55± 9.1 | 21.55± 6.1 | .66 | 34.25±14.0 | 24.93± 6.3 | .73 |
| Proline | 25.51± 5.4 | 21.95± 5.0 | .86 | 24.30± 4.3 | 16.04± 2.7 | .66 |
| Glycine | 100.75±23.4 | 110.37±13.6 | 1.10 | 87.54±14.2 | 94.38±19.3 | 1.08 |
| Alanine | 59.39± 6.0 | 55.90± 7.3 | .94 | 59.62±14.0 | 41.30± 8.4 | .69 |
| Cystine | 3.79± 0.5 | 3.53± 0.7 | .93 | 4.38± 1.4 | 3.10± 0.4 | .71 |
| Tyrosine | 19.20± 2.3 | 14.86± 2.3 | .77 | 29.31±14.5 | 12.54± 2.3 | .43 |
| Total | 322.26±42.0 | 296.42±29.9 | .88 | 318.18±63.3 | 259.48±45.3 | .77 |
| N.E./E[c] | 2.25 | 2.62 | | 2.14 | 2.53 | |

TABLE 18 (Continued)

| Amino Acid | Ration 3 | | | Ration 4 | | |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_4$ | $T_4/T_0$ | $T_0$ | $T_4$ | $T_4/T_0$ |
| **Essential Amino Acids** | | | | | | |
| Lysine | 9.01± 2.0 | 9.56± 1.4 | 1.06 | 9.56± 2.3 | 7.65± 1.8 | .80 |
| Histidine | 7.47± 0.9 | 9.58± 1.5 | 1.28 | 11.53± 0.7 | 10.60± 1.7 | .92 |
| Arginine | 13.36± 4.1 | 16.67± 1.5 | .91 | 14.95± 4.1 | 12.94± 1.4 | .87 |
| Threonine | 36.37± 6.2 | 28.04± 4.4 | .77 | 25.70± 5.0 | 16.96± 2.6 | .66 |
| Valine | 41.97± 7.1 | 30.86± 3.2 | .74 | 28.82± 5.2 | 21.91± 4.8 | .76 |
| Methionine | 5.77± 1.5 | 5.18± 1.5 | .90 | 5.72± 0.9 | 5.94± 2.0 | 1.04 |
| Isoleucine | 14.98± 2.4 | 11.59± 0.7 | .77 | 13.55± 2.6 | 9.73± 2.7 | .72 |
| Leucine | 20.91± 3.7 | 16.05± 1.7 | .77 | 26.15± 4.9 | 17.43± 4.3 | .67 |
| Phenylalanine | 10.04± 1.7 | 6.79± 0.4 | .68 | 13.54± 1.9 | 10.71± 2.4 | .79 |
| Total | 164.85±26.5 | 136.31±10.9 | .88 | 149.50±26.3 | 113.88±21.4 | .80 |
| **Nonessential Amino Acids** | | | | | | |
| N-methyl-lysine | 23.39± 8.8 | 23.34± 5.6 | 1.00 | 13.85± 4.2 | 12.67± 2.3 | .91 |
| Aspartic acid | 1.81± 0.5 | 1.72± 0.3 | .95 | 1.62± 0.1 | 1.70± 0.3 | 1.05 |
| Serine | 16.09± 1.8 | 12.60± 0.6 | .78 | 22.02± 1.5 | 19.67± 4.1 | .89 |
| Asparigine | 7.69± 2.1 | 5.62± 0.2 | .73 | 9.85± 1.8 | 8.91± 2.3 | .90 |
| Glutamic acid | 15.17± 6.6 | 11.83± 3.3 | .78 | 21.90± 1.5 | 17.98± 3.6 | .82 |
| Glutamine | 25.08± 5.6 | 15.54± 3.4 | .62 | 37.46± 5.6 | 26.59± 6.7 | .71 |
| Proline | 16.30± 2.6 | 10.32± 3.4 | .63 | 23.84± 1.6 | 19.13± 4.8 | .80 |
| Glycine | 84.28±13.8 | 62.88± 9.1 | .75 | 116.56±14.0 | 101.06±25.1 | .87 |
| Alanine | 56.98±12.9 | 42.92± 4.4 | .75 | 60.91± 2.2 | 49.01± 8.9 | .80 |
| Cystine | 3.91± 1.0 | 2.65± 0.7 | .68 | 4.90± 1.1 | 4.18± 0.6 | .85 |
| Tyrosine | 15.04± 2.9 | 10.49± 1.5 | .70 | 22.33± 3.6 | 17.34± 3.6 | .78 |
| Total | 268.29±37.5 | 199.90±21.5 | .76 | 335.20± 5.5 | 278.19±57.6 | .86 |
| N.E./E[c] | 1.63 | 1.47 | | 2.24 | 2.44 | |

[a]Micromoles per 100 ml plasma.
[b]Mean and standard error for four sheep.
[c]Ratio of nonessential to essential amino acids.

fed ration 3 and methionine in sheep fed ration 4 at $T_4$. The overall total essential amino acid $T_4/T_0$ ratios were 79%, 70%, 88% and 80% for sheep fed rations 1, 2, 3 and 4 respectively. The ratios of nonessential to essential amino acids were: $T_0$, 2.25, 2.14, 1.63 and 2.24; $T_4$, 2.62, 2.53, 1.47 and 2.44 micromoles per 100 ml plasma for sheep fed rations 1, 2, 3 and 4 respectively.

## Experiment 2

### Rumen Microbial Amino Acid Concentration

This experiment was designed primarily to determine the constancy or variability of rumen microbial tryptophan concentrations when the rations described in Table 2 were fed to sheep. Rumen bacterial tryptophan content from sheep fed the four rations are shown in Table 19. The average bacterial tryptophan level was 1.38 gm tryptophan per 100 gm protein. Bacterial tryptophan content was not affected by the type of ration nor the time of sampling.

Rumen protozoal tryptophan content is presented in Table 20. The average tryptophan level in rumen protozoa was 1.00 gm tryptophan per 100 gm protein. Rumen protozoal tryptophan was also not affected by the type of ration nor the time after feeding.

The bulk amino acid composition of bacterial (Table 21) and protozoal (Table 22) preparations were not affected by dietary treatments or time of sampling, however the formaldehyde treatment destroyed some lysine by making

TABLE 19

Tryptophan Levels in Isolated Ruminal Bacteria[a]
(Experiment 2)

| Time[b] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 1.5 | 1.4 | 1.3 | 1.3 |
| $T_{1-1/2}$ | 1.4 | 1.4 | 1.7 | 1.3 |
| $T_4$ | 1.3 | 1.4 | 1.4 | 1.2 |

[a]Gm of tryptophan per 100 gm protein (Nx6.25).

[b]Hours after feeding.

TABLE 20

Tryptophan Levels in Isolated Ruminal Protozoa[a]
(Experiment 2)

| Time[b] | Ration 1 | Ration 2 | Ration 3 | Ration 4 |
|---|---|---|---|---|
| $T_0$ | 0.80 | 1.09 | 1.16 | 0.75 |
| $T_{1-1/2}$ | 0.77 | 1.08 | 1.18 | 0.84 |
| $T_4$ | 0.87 | 1.07 | 1.20 | 1.05 |

[a]Gm of tryptophan per 100 gm protein (Nx6.25).

[b]Hours after feeding.

TABLE 21

Amino Acid Levels[a] in Isolated Ruminal Bacteria (Experiment 2)

| Amino Acid | Ration 1 | | | Ration 4 | | |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_{1-1/2}$ | $T_4$ | $T_0$ | $T_{1-1/2}$ | $T_4$ |
| Lys. | 7.65 | 3.67 | 3.97 | 3.78 | 3.69 | 4.07 |
| His. | 0.56 | 1.02 | 0.55 | 0.27 | 0.51 | 0.85 |
| Arg. | 6.51 | 4.84 | 4.11 | 4.37 | 4.50 | 4.31 |
| Asp. | 10.29 | 11.73 | 9.32 | 12.41 | 7.62 | 13.62 |
| Thre. | 8.10 | 6.35 | 8.93 | 6.72 | 6.82 | 8.49 |
| Ser. | 3.42 | 3.95 | 4.05 | 3.97 | 4.21 | 3.85 |
| Glut. | 15.35 | 16.25 | 15.95 | 13.49 | 16.53 | 15.25 |
| Pro. | 4.95 | 4.95 | 4.55 | 5.54 | 4.43 | 3.93 |
| Gly. | 6.25 | 6.90 | 6.86 | 7.08 | 7.18 | 6.28 |
| Ala. | 7.15 | 7.38 | 7.57 | 7.88 | 7.70 | 6.88 |
| Val. | 7.56 | 7.91 | 8.15 | 8.44 | 8.44 | 7.43 |
| Met. | 3.06 | 3.21 | 3.16 | 2.77 | 3.59 | 2.81 |
| Ileu. | 6.82 | 7.33 | 7.34 | 7.63 | 8.33 | 7.11 |
| Leu. | 7.97 | 8.84 | 8.69 | 9.21 | 9.28 | 8.67 |
| Phe. | 4.35 | 5.59 | 6.81 | 6.37 | 7.24 | 6.45 |

[a]Mg resolved amino acid per 100 mg resolved amino acid in the hydrolysate.

TABLE 22

Amino Acid Levels[a] in Isolated Ruminal Protozoa (Experiment 2)

| Amino Acid | Ration 1 | | | Ration 2 | | | Ration 3 | | | Ration 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $T_0$ | $T_{1-1/2}$ | $T_4$ | $T_0$ | $T_{1-1/2}$ | $T_4$ | $T_0$ | $T_{1-1/2}$ | $T_4$ | $T_0$ | $T_{1-1/2}$ | $T_4$ |
| Lys. | 7.44 | 9.87 | 7.28 | 9.82 | 8.99 | -- | 7.53 | 9.83 | 8.22 | 9.25 | 9.30 | 9.05 |
| His. | 1.42 | 1.50 | 1.50 | 1.04 | 1.07 | -- | 1.38 | 2.38 | 1.76 | 0.88 | 1.09 | 1.23 |
| Arg. | 4.02 | 3.34 | 2.37 | 4.18 | 4.08 | -- | 4.84 | 6.49 | 5.43 | 3.39 | 3.52 | 4.03 |
| Asp. | 10.29 | 10.60 | 11.16 | 11.06 | 10.75 | -- | 10.44 | 9.08 | 10.96 | 9.31 | 10.21 | 10.57 |
| Thre. | 5.35 | 5.05 | 5.45 | 5.06 | 5.16 | -- | 5.52 | 4.98 | 5.63 | 4.56 | 4.53 | 4.76 |
| Ser. | 3.53 | 3.38 | 4.23 | 3.22 | 3.34 | -- | 3.47 | 3.28 | 3.33 | 3.17 | 3.05 | 3.11 |
| Glut. | 21.90 | 21.78 | 18.44 | 20.23 | 20.53 | -- | 19.60 | 18.82 | 15.38 | 22.17 | 21.69 | 18.42 |
| Pro. | 4.97 | 4.94 | 5.08 | 4.81 | 4.23 | -- | 5.24 | 4.89 | 5.16 | 5.94 | 5.22 | 5.51 |
| Gly. | 5.57 | 5.42 | 5.66 | 5.25 | 5.30 | -- | 5.56 | 5.47 | 6.38 | 6.02 | 5.42 | 5.29 |
| Ala. | 5.94 | 6.39 | 8.29 | 5.51 | 5.78 | -- | 5.80 | 5.41 | 6.69 | 6.14 | 6.46 | 7.43 |
| Val. | 5.93 | 5.93 | 7.12 | 5.93 | 6.11 | -- | 6.22 | 6.18 | 6.64 | 5.61 | 6.14 | 6.62 |
| Met. | 0.93 | 1.15 | 1.37 | 1.37 | 1.21 | -- | 1.24 | 1.05 | 1.18 | 0.99 | 0.84 | 1.66 |
| Ileu. | 8.21 | 8.12 | 7.57 | 9.27 | 8.98 | -- | 8.52 | 7.84 | 9.18 | 7.98 | 8.35 | 7.35 |
| Leu. | 8.67 | 8.22 | 10.10 | 8.93 | 8.67 | -- | 9.41 | 9.17 | 8.71 | 10.65 | 9.89 | 11.27 |
| Phe. | 5.83 | 4.31 | 4.37 | 4.33 | 5.82 | -- | 5.23 | 5.12 | 5.33 | 4.59 | 4.29 | 3.71 |

[a]Mg resolved amino acid per 100 mg resolved amino acid in the hydrolysate.

ε-Formyl-lysine and also a formyl derivative of histidine. Tyrosine was completely destroyed. Thus the data for lysine and histidine are of little value for interpretation and comparison to previous results.

# DISCUSSION

Results from this study indicated that levels of plasma free tryptophan in sheep were not affected by the type of ration or the quantity of crude protein fed.

In humans, Young et al. (1971) found little change in fasting or postprandial plasma tryptophan concentrations at tryptophan intakes below 3 mg per kilogram body weight per day or at tryptophan intakes above 5 mg per kilogram body weight per day. However, they reported that the concentration of tryptophan in fasting plasma increased linearly between intakes of 3 and 5 mg tryptophan per kilogram body weight per day. In ruminants the amount and quality of protein reaching the small intestine depends on the extent of dietary protein degradation and the extent of resynthesis of microbial protein (Smith, 1969). When feeding practical type rations (dietary protein which is readily degradable), the quantity of protein reaching the lower gut is not markedly affected by dietary nitrogen intake in ruminants. This levelling effect has been ascribed to the activity of the ruminal microbes (Hungate, 1966). Hogan and Weston (1969) summarized data showing that the amount of protein reaching the lower gut in sheep did not vary between

dietary intakes of 8 to 20% crude protein. According to Purser and Buechler (1966), Bergen et al. (1968) and Purser (1970) the bulk amino acid composition of rumen microbial protein reaching the lower gut is constant and is not affected by change of ration. The results of the second experiment of the present study indicated that the content of tryptophan in rumen bacterial and protozoal protein was fairly constant and was not affected by dietary treatments or time after feeding. Since the quantity of rumen microbial protein and the bulk amino acid and tryptophan content of rumen microbial protein reaching the lower gut was quite constant at the levels of dietary protein intakes used in this study, it can be postulated that the amount of tryptophan reaching the small intestine and available for absorption was pretty much constant among the 4 rations used. The above postulation is only correct if little or no protein bypassed the rumen and if there were no large differences in tryptophan digestibility or availability from the microbial protein (Bergen et al., 1967). A considerable proportion of the dietary proteins in rations 1, 2 and 4 were in the form of zein protein (a protein which is not degraded extensively in the rumen, McDonald, 1954) and thus a large amount of this protein will pass out of the rumen undegraded to the lower gut. Since zein contains low levels of tryptophan, intestinal digestion of this protein would depress the level of

tryptophan being absorbed from the small intestine. The constant level of tryptophan in the plasma circulating pool and other tissue pools may also be attributed to the fact that any excess in the absorbed tryptophan which is not immediately used for protein synthesis or other cellular metabolic processes will be rapidly deaminated and catabolyzed, establishing an equilibrium between the entry and the removal of tryptophan from the plasma pool.

The diurnal pattern of plasma tryptophan levels for sheep was similar to the patterns reported for nonruminants (Wurtman, 1970). When compared to the fasting level, the concentration of tryptophan in the plasma pool increased gradually (although such a change was not statistically significant) to its highest level by 9 hours postprandial, declined to its lowest level between 15 and 20 hours postprandial and then rose back to the fasting level by 24 hours postprandial. Chester (1971) reported that diet induced alterations observed in plasma amino acid concentrations vary with time after feeding and that the plasma amino acid pattern reflects the incoming dietary amino acid more than the animal's amino acid requirements. Feigin et al. (1971) reported a definite and constant pattern of blood amino acid rhythmicity in humans despite variations in the amino acid concentrations. A 12 hour shift in the sleep and wakefulness cycle resulted in a rapid reversal of such diurnal rhythmicity. The diurnal

pattern of plasma tryptophan throughout the 24 hour period of this study indicated that tryptophan concentration reached its maximum level at nine hours after feeding whereas the bulk amino acid concentration, as indicated by the α-amino nitrogen concentration, reached its maximum level at 12 hours after feeding. The differences in the diurnal patterns of tryptophan and $\alpha$-$NH_2$-N may be related to the fact that tryptophan is involved in some form of metabolic process or processes such as polysome aggregation (Munro, 1970) or acts as the precursor for tryptamine, serotonin, melatonin and nicotinamide adenine dinucleotide synthesis (Sourkes, 1971) which precede protein synthesis or that tryptophan is more rapidly absorbed and degraded than the other amino acids.

It has been claimed by Potter et al. (1968) and shown by Purser et al. (1966) and Munro (1964) that administration of a dose of energy to a fasted animal will cause a depression in plasma free essential amino acids, in particular those low in the diet and also the branched chain amino acids. Purser et al. (1966) stated that the limiting amino acid for the ruminant may be indicated by the essential amino acid which showed the largest percent decline after the administration of readily available energy into the rumen. Such a method for determining the limiting amino acid may not be precise, however, if the level of an essential amino acid is not greatly depressed by the sudden

influx of energy. This finding may suggest that such an essential amino acid was not among those acids which were low in supply. Based on this assumption the $T_4/T_0$ ratio of plasma tryptophan concentrations (Table 5) indicated that tryptophan was not limiting in the lower gut for sheep fed the four rations in question. Using Purser's et al. (1966) $T_4/T_0$ ratio method to determine the limiting amino acid (see Table 18) it would appear that lysine was the most limiting amino acid for sheep fed ration 1 and second limiting for sheep fed ration 2. Leucine on the other hand seemed to be the limiting amino acid for sheep fed ration 2 and the second limiting for sheep fed ration 4. Phenylalanine seemed to be the limiting amino acid for sheep fed ration 3 and threonine for sheep fed ration 4. The $T_4/T_0$ ratio of rations 1 and 2 compared well with those reported by Klopfenstein et al. (1966) who used the same two rations in their studies.

The α-amino nitrogen data presented in Table 6 indicated that the total plasma amino acid concentration was not influenced by dietary treatments. Depending on the quantity of dietary protein fed and the quality of protein reaching the abomasum of ruminants there may or may not be differences in the total amino acid concentration due to dietary treatments (Purser, 1970). One could argue that in ruminants, if the dietary protein sources are such that there is little or no rumen bypass, then the plasma amino

acids should not be influenced by dietary treatments. However, if a large proportion of the protein bypasses the rumen then one may expect to find variations in plasma amino acid levels due to quality and quantity of protein fed. Ørskov et al. (1971a), using soybean meal as a protein supplement, observed an increase in total amino acids reaching the small intestine with each increasing level of dietary soy protein up to 19% crude protein, beyond which there was no further increase. In another study using fish meal and urea as dietary crude protein supplements, Ørskov et al. (1971b) reported an increase in total amino acids reaching the small intestine with each increasing level of dietary fish meal but no increase when increments of urea supplement were used. They concluded that the increase in amino acids in the small intestine when fish meal supplement was used was due to the fact that most of this protein bypassed the rumen. Increased levels of urea did not increase microbial protein synthesis hence the total amino acid reaching the small intestines when urea supplements were used was constant (Hogan and Weston, 1969). Hume et al. (1970) reported a linear increase in flow rate of protein (i.e., tungstic acid precipitable nitrogen x 6.25) out of the rumen with increasing levels of nitrogen intake between 2 and 9 grams nitrogen per day, no further increase between 9 and 16 grams nitrogen per day and a net nitrogen loss (as a percent of intake) at intakes greater than 16 grams

nitrogen per day. In the present study the lack of significant differences in plasma total free amino acid nitrogen between sheep fed the four rations can be attributed to the fact that the supplies of amino acids to the lower digestive tract were the same for sheep fed the four rations. Thus the changes observed in the diurnal pattern of total amino acid nitrogen were mainly changes in protein reaching the intestine due to time after feeding and metabolic rhythmicity. Sheep fed ration 4 which contained the highest level of crude protein and digestible energy had the lowest levels of total plasma amino acid nitrogen. This can be interpreted to mean that there was greater protein synthesis or cellular uptake of amino acids in sheep when ration 4 was fed (Halfpenny et al., 1969).

The observed levels of rumen ammonia and blood urea nitrogen which were associated with the higher crude protein rations in this study were in agreement with the report of Lewis (1957); Tagari et al. (1964) and Preston et al. (1965) who reported a significant correlation between the crude protein content of the ration, rumen ammonia and blood urea concentrations. The drop in rumen ammonia and blood urea concentrations to a low level between 9 and 16 hours after feeding and the subsequent increase during the last 8 hours of the 24 hour period was in accordance with the work of Lewis and McDonald (1958) who claimed that the increase in rumen ammonia and blood urea levels during the

last 8 hours may be due to continued ruminal uptake of nitrogen through salivary secretion, slowing down of bacterial growth and autolysis of some micro-organisms.

Purser and Buechler (1966), Bergen et al. (1968) and Purser (1970) reported that the amino acid composition of rumen microbial preparations was constant. The amino acid data of rumen bacterial and protozoal preparations presented in Tables 21 and 22 respectively, indicated not only a constancy of the amino acid composition of rumen microbial protein but that the amino acid composition was not influenced by levels of dietary nitrogen intake or the time after feeding (at least during the first four hours after feeding). These findings further strengthen the suggestion made earlier in this discussion that lack of significant differences in plasma total free amino acid nitrogen among sheep fed the four rations was due to a constant supply of amino acid to the lower gut for digestion and absorption.

# GENERAL CONCLUSIONS

1. Plasma free tryptophan concentration in sheep was not affected by dietary treatments or levels of crude protein fed.
2. Tryptophan did not appear to be deficient or limiting in sheep when fed four rations containing different sources and levels of nitrogen.
3. Tryptophan content of rumen bacterial and protozoal preparations was constant and was not affected by dietary treatments or time after feeding.
4. The diurnal rhythmicity of plasma tryptophan was the same for sheep fed the four rations and was only slightly influenced by time after feeding.
5. The bulk amino acid composition of rumen bacterial and protozoal preparations was constant and was not affected by dietary treatments or time after feeding.
6. Normal rations such as those used in the present study will not markedly influence the total plasma amino acid nitrogen concentration in sheep.
7. Increased rumen ammonia and blood urea nitrogen concentrations were associated with increased levels of dietary crude protein.

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# APPENDIX

## ANALYSIS OF VARIANCE

APPENDIX TABLE 1

Analysis of Variance (Experiment 1)

| Source of Variation | d.f. | TRAITS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | M.S. | F | Sig. | M.S. | F | Sig. | M.S. | F | Sig. |
| | | Tryptophan $T_0$ | | | Tryptophan $T_3$ | | | Tryptophan $T_6$ | | |
| Treatment | 3 | 0.06 | 2.09 | 0.20 | 0.13 | 1.84 | 0.24 | 0.04 | 0.83 | 0.53 |
| Error | 6 | 0.03 | | | 0.07 | | | 0.05 | | |
| | | Tryptophan $T_9$ | | | Tryptophan $T_{12}$ | | | Tryptophan $T_{16}$ | | |
| Treatment | 3 | 0.13 | 5.64 | 0.04 | 0.20 | 3.7 | 0.08 | 0.13 | 0.52 | 0.68 |
| Error | 6 | 0.02 | | | 0.06 | | | 0.24 | | |
| | | Tryptophan $T_{20}$ | | | Tryptophan $T_{24}$ | | | Tryptophan $T_0$ (D24) | | |
| Treatment | 3 | 0.30 | 0.60 | 0.64 | 0.06 | 0.65 | 0.61 | 0.09 | 1.17 | 0.39 |
| Error | 6 | 0.05 | | | 0.09 | | | 0.08 | | |
| | | Tryptophan $T_4$ (D24) | | | α-Amino Nitrogen $T_0$ | | | α-Amino Nitrogen $T_3$ | | |
| Treatment | 3 | 0.19 | 1.96 | 0.22 | 0.11 | 1.54 | 0.30 | 0.07 | 1.56 | 0.29 |
| Error | 6 | 0.10 | | | 0.07 | | | 0.04 | | |
| | | α-Amino Nitrogen $T_6$ | | | α-Amino Nitrogen $T_9$ | | | α-Amino Nitrogen $T_{12}$ | | |
| Treatment | 3 | 0.23 | 2.56 | 0.15 | 0.28 | 3.60 | 0.09 | 0.49 | 22.48 | 0.001 |
| Error | 6 | 0.09 | | | 0.08 | | | 0.02 | | |
| | | α-Amino Nitrogen $T_{16}$ | | | α-Amino Nitrogen $T_{20}$ | | | α-Amino Nitrogen $T_{24}$ | | |
| Treatment | 3 | 0.32 | 14.23 | 0.004 | 0.25 | 1.65 | 0.27 | 0.21 | 2.87 | 0.13 |
| Error | 6 | 0.02 | | | 0.15 | | | 0.07 | | |
| | | α-Amino Nitrogen $T_0$(D24) | | | α-Amino Nitrogen $T_4$(D24) | | | Rumen Ammonia $T_0$ | | |
| Treatment | 3 | 0.07 | 0.57 | 0.65 | 0.18 | 1.78 | 0.25 | 1044.24 | 24.31 | 0.001 |
| Error | 6 | 0.12 | | | 0.10 | | | 42.96 | | |
| | | Rumen Ammonia $T_3$ | | | Rumen Ammonia $T_6$ | | | Rumen Ammonia $T_9$ | | |
| Treatment | 3 | 2626.62 | 42.93 | 0.0005 | 2043.02 | 7.46 | 0.02 | 818.99 | 57.88 | 0.0005 |
| Error | 6 | 61.18 | | | 273.87 | | | 14.15 | | |

APPENDIX TABLE 1 (Continued)

| Source of Variation | d.f. | TRAITS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | M.S. | F | Sig. | M.S. | F | Sig. | M.S. | F | Sig. |
| | | Rumen Ammonia $T_{12}$ | | | Rumen Ammonia $T_{16}$ | | | Rumen Ammonia $T_{20}$ | | |
| Treatment | 3 | 887.33 | 38.49 | 0.0005 | 991.16 | 33.11 | 0.0005 | 840.80 | 43.25 | 0.0005 |
| Error | 6 | 23.05 | | | 29.94 | | | 19.44 | | |
| | | Rumen Ammonia $T_{24}$ | | | Rumen Ammonia $T_0$(D24) | | | Rumen Ammonia $T_4$(D24) | | |
| Treatment | 3 | 977.40 | 60.20 | 0.005 | 924.67 | 6.98 | 0.02 | 237.28 | 5.52 | 0.37 |
| Error | 6 | 16.24 | | | 132.53 | | | 42.95 | | |
| | | Blood Urea $T_0$ | | | Blood Urea $T_3$ | | | Blood Urea $T_6$ | | |
| Treatment | 3 | 320.65 | 77.88 | 0.0005 | 463.87 | 60.55 | 0.0005 | 484.34 | 33.75 | 0.0005 |
| Error | 6 | 4.12 | | | 7.66 | | | 14.35 | | |
| | | Blood Urea $T_9$ | | | Blood Urea $T_{12}$ | | | Blood Urea $T_{16}$ | | |
| Treatment | 3 | 373.95 | 255.71 | 0.0005 | 320.26 | 89.41 | 0.0005 | 251.65 | 77.23 | 0.0005 |
| Error | 6 | 1.46 | | | 3.58 | | | 3.26 | | |
| | | Blood Urea $T_{20}$ | | | Blood Urea $T_{24}$ | | | Blood Urea $T_0(D_{24})$ | | |
| Treatment | 3 | 255.71 | 47.82 | 0.0005 | 217.19 | 29.99 | 0.001 | 197.44 | 26.21 | 0.001 |
| Error | 6 | 4.72 | | | 7.24 | | | 7.53 | | |
| | | Blood Urea $T_4(D_{24})$ | | | Rumen Acetate $T_0$ | | | Rumen Acetate $T_3$ | | |
| Treatment | 3 | 138.19 | 25.28 | 0.001 | 117.22 | 1.11 | 0.413 | 269.39 | 1.41 | 0.328 |
| Error | 6 | 5.47 | | | 104.90 | | | 190.65 | | |
| | | Rumen Acetate $T_6$ | | | Rumen Acetate $T_9$ | | | Rumen Acetate $T_{12}$ | | |
| Treatment | 3 | 325.56 | 1.62 | 0.282 | 89.40 | 0.37 | 0.776 | 328.90 | 2.43 | 0.163 |
| Error | 6 | 201.48 | | | 231.73 | | | 135.22 | | |
| | | Rumen Acetate $T_{16}$ | | | Rumen Acetate $T_{20}$ | | | Rumen Acetate $T_{24}$ | | |
| Treatment | 3 | 180.23 | 1.33 | 0.350 | 189.73 | 3.41 | 0.094 | 103.17 | 2.78 | 0.132 |
| Error | 6 | 135.73 | | | 55.56 | | | 37.08 | | |

APPENDIX TABLE 1 (Continued)

| Source of Variation | d.f. | TRAITS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | M.S. | F | Sig. | M.S. | F | Sig. | M.S. | F | Sig. |
| | | Rumen Acetate $T_0(D_{24})$ | | | Rumen Acetate $T_4(D_{24})$ | | | Rumen Proprionate $T_0$ | | |
| Treatment | 3 | 48.73 | 0.23 | 0.870 | 125.23 | 2.14 | 0.196 | 2.73 | 0.11 | 0.950 |
| Error | 6 | 209.23 | | | 58.48 | | | 24.56 | | |
| | | Rumen Proprionate $T_3$ | | | Rumen Proprionate $T_6$ | | | Rumen Proprionate $T_9$ | | |
| Treatment | 3 | 91.75 | 2.20 | 0.189 | 69.06 | 2.35 | 0.171 | 23.17 | 1.02 | 0.447 |
| Error | 6 | 41.75 | | | 29.31 | | | 22.67 | | |
| | | Rumen Proprionate $T_{12}$ | | | Rumen Proprionate $T_{16}$ | | | Rumen Proprionate $T_{20}$ | | |
| Treatment | 3 | 7.56 | 0.27 | 0.842 | 26.17 | 0.99 | 0.457 | 6.90 | 1.24 | 0.375 |
| Error | 6 | 27.56 | | | 26.33 | | | 5.56 | | |
| | | Rumen Proprionate $T_{24}$ | | | Rumen Proprionate $T_0(D_{24})$ | | | Rumen Proprionate $T_4(D_{24})$ | | |
| Treatment | 3 | 4.73 | 1.73 | 0.295 | 24.75 | 1.01 | 0.451 | 204.5 | 2.61 | 0.146 |
| Error | 6 | 2.73 | | | 24.50 | | | 78.25 | | |
| | | Rumen Butyrate $T_0$ | | | Rumen Butyrate $T_3$ | | | Rumen Butyrate $T_6$ | | |
| Treatment | 3 | 31.73 | 4.83 | 0.05 | 26.91 | 5.30 | 0.040 | 4.06 | 0.79 | 0.542 |
| Error | 6 | 6.56 | | | 5.08 | | | 5.14 | | |
| | | Rumen Butyrate $T_9$ | | | Rumen Butyrate $T_{12}$ | | | Rumen Butyrate $T_{16}$ | | |
| Treatment | 3 | 20.73 | 3.3 | 0.098 | 20.83 | 1.52 | 0.302 | 58.56 | 7.26 | 0.020 |
| Error | 6 | 6.23 | | | 13.67 | | | 8.06 | | |
| | | Rumen Butyrate $T_{20}$ | | | Rumen Butyrate $T_{24}$ | | | Rumen Butyrate $T_0(D_{24})$ | | |
| Treatment | 3 | 27.89 | 11.64 | 0.007 | 20.50 | 10.69 | 0.008 | 21.73 | 3.54 | 0.088 |
| Error | 6 | 2.40 | | | 1.92 | | | 6.15 | | |
| | | Rumen Butyrate $T_4(D_{24})$ | | | | | | | | |
| Treatment | 3 | 20.73 | 1.83 | 0.242 | | | | | | |
| Error | 6 | 11.31 | | | | | | | | |