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"DIGESTION AND UTILIZATION OF PROTEIN  
IN THE WEST AFRICAN DWARF SHEEP"

BY

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A Thesis in the Department of  
ANIMAL SCIENCE

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## A B S T R A C T

West African dwarf sheep maintained on Cynodon nlemfuensis/Centrosema pubescens hay and concentrate supplements were used to study intake and digestibility of dry matter and nitrogen metabolism.

Levels of ruminal metabolites of nitrogenous origin and blood urea were examined on the rations fed to the sheep.

[ $^{15}\text{N}$ ] ammonium chloride and [ $^{15}\text{N}$ ] urea were used to study the production of ammonia and its utilization in the rumen, and the flow of blood urea into the digestive tract.

Shredded paper impregnated with chromic oxide was used to partition digestibility in the stomach and intestines of the sheep.

The results for the West African dwarf sheep were compared with those of other breeds of sheep in intake and digestibility of organic matter and nitrogen metabolism.

The intake of dry matter by the West African dwarf sheep was similar to that of other breeds when expressed per metabolic size.

Nitrogen retention values were high and this shows that absorbed N was being utilized efficiently.

The metabolic faecal N values of 3.0 to 3.7g N/kg dry matter intake and the endogenous urinary N value of 0.0238 g/day/W<sub>kg</sub><sup>0.734</sup> were obtained for this class of livestock.

The biological values of the rations ranged from 85.7 to 100.0%.

The digestible crude protein requirement for maintenance over the experimental period was 0.74g/day/W<sub>kg</sub><sup>0.734</sup> by the N balance method.

and  $0.22\text{g/day/W}^{0.734}$   ~~$0.734$~~  kg by the factorial method.

The levels of nitrogenous metabolites in the rumen varied with levels of dietary crude protein. Ruminal ammonia was highly correlated ( $r = 0.99$ ) with blood urea.

The amino acids present in lowest concentration in bacterial and protozoal protein are methionine and histidine while there are high levels of lysine and leucine.

Isotopic studies with  $[^{15}\text{N}]$  ammonium chloride and urea shows that 4 - 7% of  $[^{15}\text{N}]$  ammonium chloride administered into the rumen was recovered in the faeces, and 3.1% was recovered in milk. Also 30.5% of  $[^{15}\text{N}]$  urea administered into the blood was recovered in the urine and the isotope was not recovered in the faeces.

Ruminal ammonia contributed 26 - 33% of the bacterial N and 15 - 19% of protozoal N ten hours after feeding.

Urea was synthesized in the body at the rate of 9.4 to 10.1g/day, and 4.7 to 7.3g/day were degraded in the digestive tract of the sheep.

The chromic oxide - impregnated paper method showed that 72.5% of digestible dry matter and 72.6% of digestive organic matter of the rations were digested in the stomach. The corresponding values for small intestine were 10.1% and 11.4% for dry

matter and organic matter respectively, while in the caecum and colon, the values were 17.5% and 16.0% for dry matter and organic matter respectively.

Substantial amount of N of endogenous origin were secreted in the proximal small intestine but were efficiently absorbed before the distal portion was reached.

The results show that the West African dwarf sheep utilize the hay and supplement rations efficiently and are adapted for survival in areas where the intake of N might be inadequate.

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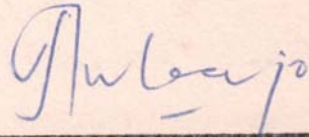
Special appreciation is extended to the author's wife for her encouragement in the course of the research work.

We certify that this work was carried out by  
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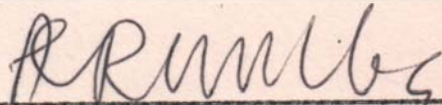
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## CHAPTER ONE

### INTRODUCTION

1.

#### 1.1 General Introduction.

It is generally agreed that the most pressing problem in the developing countries like Nigeria is the shortage of proteins, particularly the animal proteins in the human diets. The developing countries of the world have a population of 2,100 million persons of an estimated world population of 3,000 million people. The FAO (1960) reported that the developing countries and the developed countries had an average daily intake per head of about 58g and 90g respectively of total protein in 1960, and that the daily animal protein intake per head in developing countries was about 9g compared with about 45g in the developed countries. Thus the average person in the developed countries has about five times as much animal protein as the average person in developing countries. The ratio of plant protein to animal protein (P/A) in developing and developed countries are 5.3 and 0.85 respectively. This shows that the developing countries consume approximately 5.3 times as much plant protein as animal protein.

Wright (1961) raised two main objections to the use of plant proteins to help to solve the world food problem:

- (i) the foods containing them are less concentrated sources of protein on dry weight or calorie basis than meat and milk.
- (ii) the quality of animal protein is stated to be superior to that of plants. Cereals have been found to be deficient in essential

amino acids like lysine and tryptophan, and this results in low biological value for these proteins. Mitchell (1927) showed that the biological value of whole egg is 94 and that of whole corn is 60. This shows that proteins from animal products have higher biological value than proteins from plant sources.

In the developing countries there is a protein-deficiency disease called kwashiokor in infants who have been weaned on starchy diet low in protein or of high calorie/protein ratio.

The FAO targets for world food production in 1975 visualize a total annual consumption in human diets of about 125 million tonnes of protein of which about 38 million tonnes will be animal protein giving a P/A ratio of about 2.3. The huge increases over the 1960 figure are to be obtained by an increase of about 13 million tonnes of animal protein. The major problem is the production of enough animal protein to meet the needs of those in developing countries.

The FAO has found that the proportion of livestock to humans is higher in developing countries than in the developed countries. The problem therefore is not that of increasing the number but the productivity of the livestock. Some of the factors militating against livestock production in the tropics are lack of properly managed pastures, disease pests, unfavourable climatic conditions, lack of technical know - how, poor economic condition of the people and their government, and cultural factors. One of the most important of these

factors in Nigeria is lack of properly managed pastures. By far the most important natural grassland of the country is the savannah, which can be divided according to the luxuriance of the growth of the grasses, the rainfall, the length of the dry season and humidity, into the Derived **guinea**, the Guinea, the Sudan and the Sahel savannah. The savannah as a whole is characterized by a continuous sea of grasses with scattered trees.

Oyenuga (1957) noted that while most of Nigeria (83%) is covered by natural grassland no serious attempts have been made to bring these grasses into cultivation. Nigerian pastures are left in their wild natural condition without the application of the well-tested findings on the cultivation of pastures that are in use in other parts of the world. In consequence, the natural grass species rapidly decline in nutritional value, becoming fibrous and coarse since they are neither grazed nor cut for fodder at their optimum stages of growth. Moreover, these tough, dry grasses are subject, during the dry season, to periodical burnings which result in an appreciable destruction of organic matter. Burning also destroys the surface organic matter of the soil, damages the trees scattered all over the savannah land, annihilates low bush, removes the shade which protects the land against the sun to conserve moisture and thus intensifies soil erosion. If pastures are properly managed, then the protein levels in them could be increased.

Most of the cattle are extensively managed and are still in the hands of the pastoral herdsmen whose system of management is largely

traditional. Although most of the sheep and goats in the Northern states of Nigeria belong to settled farmers, these animals have not become integrated into the farm economy. The direction of movement tends southwards as the dry season approaches and northwards during the rainy season. During this period, the animals put on weight, most of which is lost during the dry season when the grasses are dry and the crude protein content is very low. The FAO (1962) studies on livestock production in Rhodesia, showed that considerable economic losses occur because of the seasonal variation in the availability of pastures for cattle. They also showed that rapid gain in summer is lost during winter. If the loss per animal is multiplied by the number of animals which suffer such losses, the overall economic losses would be appreciated. Oyenuga (1957) has shown that the tropical grass species are low in crude protein and high in crude fibre when compared with temperate grasses cut at similar stages of growth.

Protein is an essential body - building component of the animal. Maynard and Loosli (1969) showed that protein contributes 15 - 21% of the gross composition of the body of domestic animals. Protein is required for maintenance, growth and production. The maintenance requirement must replace the endogenous urinary and the metabolic faecal losses. While the urinary losses are considered to be reasonably constant per unit metabolic size ( $W_{kg}^{0.734}$ ), the faecal losses are variable according to the nature of the ration and dry matter consumed.

It is directly proportional to the dry matter consumed by the animal. To prevent undue weight loss in domestic animals during adverse conditions and to enhance maximal growth during the rainy season, protein supplements are often supplied to livestock. Such concentrates as soybean meal, groundnut cake, palm kernel cake and other by-products of industry are often fed to ruminants. It is known that the rumen microorganisms convert these into high quality microbial protein which is digested in the intestines. The amino acids derived from the microbial protein are absorbed. The endowment of the ruminant animals with the capability of converting such plant proteins and even non-protein nitrogenous substances into proteins of high biological value is a great asset for the development of the livestock industries. Conversion of grass and other forages which are normally not required by other farm animals and certainly not acceptable to man will go a long way to increased livestock production and increased output of meat and milk to augment the much needed animal proteins in developing countries like Nigeria.

#### 1.1.1 Breeds of sheep in Nigeria.

The breeds of sheep in Nigeria are classified into two main groups:

- (a) the West African long-legged sheep of which the West African Uda is an example.
- (b) the West African dwarf sheep.

##### The West African long-legged sheep:

There are many types within this group, such as the Arab, the

**Fuareg**, and the Uda or the Fulani. They are all characterized by their long legs, and are kept in the savanna north of latitude  $10^{\circ}\text{N}$  by the nomads. There were some 3.2 million heads of Uda or Fulani in Northern Nigeria in 1960 (Oyenuga, 1967).

The Uda stands between 65 cm and 90 cm in height with body weights varying from 30 kg to 50 kg. They vary in colour between brown and white and white spotted.

#### The West African dwarf sheep:

The West African dwarf sheep is widely scattered around human settlements in the forest and derived Guinea savanna zones of West Africa south of latitude  $14^{\circ}\text{N}$ . The Federal office of statistics estimated the population of the breed in Southern Nigeria to be 1.82 million in 1960.

The West African dwarf sheep is small, varying between 40 cm and 60 cm in height and between 20 kg and 30 kg in weight. The colour may be white, white spotted with black, black or brown. The rams are heavily maned at the neck and chest and have close, spiral horns. The ewe is hornless.

#### 1.1.2 Importance of sheep to the economy of Nigeria.

The Federal Office of Statistics estimated the livestock population of Nigeria in 1960 to be 10 million cattle, 15 million goats and 5 million sheep. It was also estimated that 1.6 million cattle, 6 million goats and 3.2 million sheep are slaughtered annually

in Nigeria. The great importance of sheep to the economy of Nigeria would be appreciated if the cost of the sheep slaughtered is given a modest estimate of ₦32 million.

Shaw and Colville (1950) reported that 1.5 million raw untanned hides of sheep were exported from Nigeria in 1947. Thus, the sheep is not only a supplier of the much - needed animal protein in Nigeria but the hides also earn foreign exchange to strengthen the economy of Nigeria.

### 1.1.3 The Management of the West African dwarf sheep.

The West African dwarf sheep owe their existence to their ability to survive periods of drought and semi - starvation. The sheep in Nigeria have not been subjected to any good management but are left to roam around feeding on poor grasses supplemented with waste products available from kitchens or traditional food - processing industries, such as the bran from the milled maize, Guinea corn or millet or the peel from cassava and yams or other root crops. Considerable energy is expended in seeking food, and this reduces from the limited food intake the amount available for productive purposes. They bed down at night in market sheds or other secluded places, by the trunk of shade trees, in the dry season, outside.

It is evident from this lack of management programme that the economy of their living is not such as results in rapid growth and early maturity.

If Nigeria is to supply her rapidly increasing population with adequate animal protein, the present state of lack of management of sheep

should not continue. There is a need for intensive management programme. This will bring about rapid growth and early maturity, and decreased mortality of lambs due to diseases and poor nutrition.

Efforts aimed at intensive management of sheep have been few and confined to government agricultural stations and the universities. Reports of the performances of the West African dwarf sheep have been few but rate of live weight gain of 1.1 kg per week had been obtained with lambs (Oyenuga, 1967). This shows that with good management, the quality and quantity of sheep and sheep products could be increased in Nigeria.

## 1.2

### LITERATURE REVIEW

#### 1.2.1 MICROBIAL FERMENTATION AND PROTEIN DIGESTION IN THE RUMINANTS.

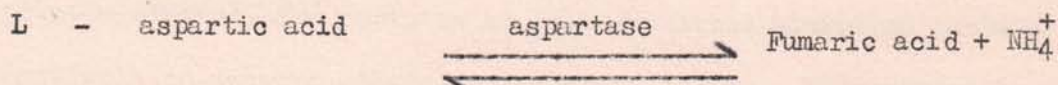
The digestion, absorption, metabolism and utilization of nitrogenous compounds in the ruminant is very much dependent upon the rumen microbial population. Nitrogenous compounds are degraded to varying extents in the rumen and some of the products of microbial degradation are metabolized by the microorganisms, some are absorbed through the rumen wall, while others move down the alimentary tract.

##### 1.2.1.1 Proteolysis and deamination of amino acids.

McDonald (1948) demonstrated that the rapid degradation of casein and the much slower degradation of zein was accompanied by the formation of ammonia and fatty acids in the rumen.  $C^{14}$  - labelled casein used in in vitro bacterial studies resulted in the release of  $C^{14}$  - labelled

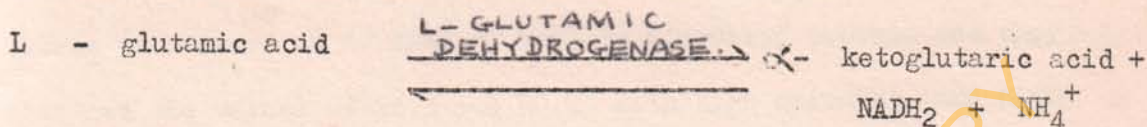


fatty acids as well as ammonia. The proteolytic action of the rumen micro-organisms resulted in the liberation of amino acids from protein when toluene was added to the reaction mixture to inhibit the deaminase which otherwise would have caused the liberation of ammonia. However, amino acids are not all rapidly deaminated in the rumen. Of the amino acids tested individually, aspartic acid was observed by Lewis (1955) to be more rapidly deaminated by washed bacterial cells than other amino acids. El - Shazly (1952) observed that when alanine and proline were incubated together with washed rumen bacterial cells, more ammonia production occurred than when they were incubated separately. This suggests the possibility of a "stickland type" of reaction which involves the oxidative deamination of one member of an amino acid pair and the reductive deamination of the second member. Decarboxylases have been known to effect the decarboxylation of lysine and ornithine. The  $\alpha$ - amino groups of these two amino acids were removed by rumen micro-organisms to yield the corresponding amine derivative of the fatty acids. Tryptophan was found to yield indole. Looper, Stallcup and Reed (1959) showed that in vitro deamination of amino acids occurs rapidly with aspartic acid, followed by glutamic acid and  $\beta$ - alanine. It is suggested that aspartase brings about rapid deamination of aspartic acid as follows:

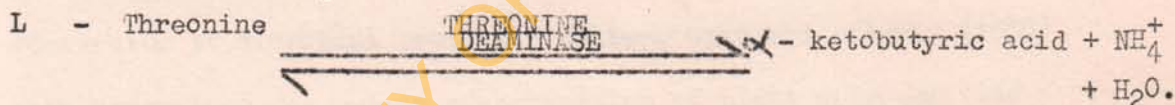


Hoshino, Sarumaru and Morimoto (1966) suggested that deamination of

glutamic acid occurs through the action of NAD - linked enzyme, glutamic dehydrogenase and the action is as follows:



Threonine and serine were also shown to be deaminated by specific deaminases or dehydrases. The enzymes were obtained from rumen micro-organisms and require pyridoxal phosphate as co-enzyme. The deamination of these two amino acids involves the removal of elements of water from the alpha and beta carbon atoms (Chargaff and Sprinson, 1943) and the reaction is as follows:



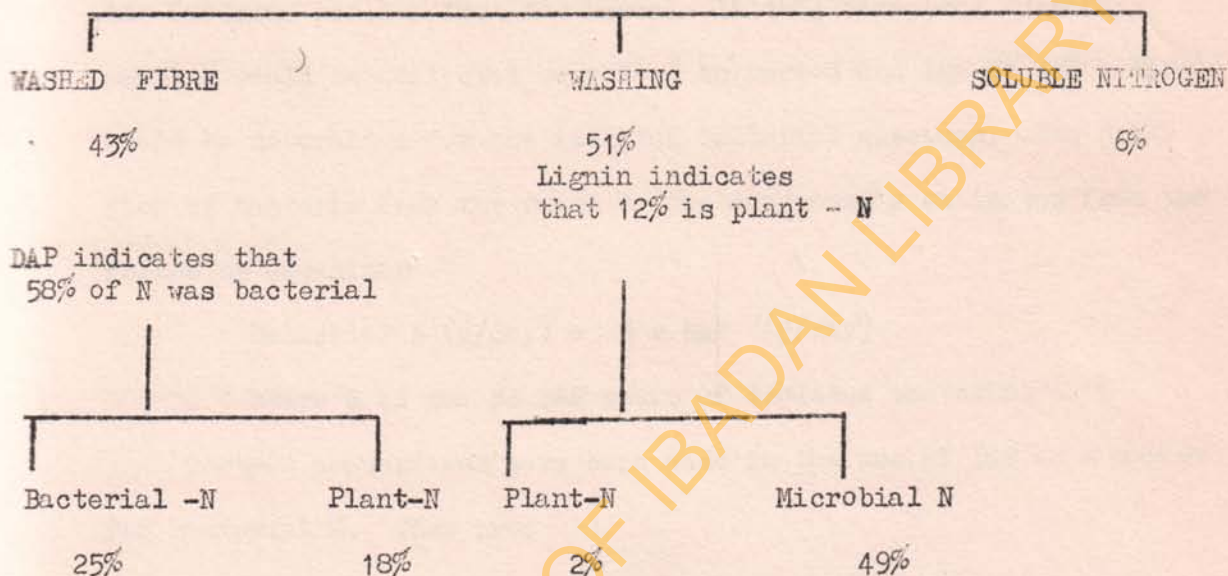
#### 1.2.1.2 Conversion of Feed Protein to Microbial Protein in the Rumen.

The reports of McDonald (1954) and McDonald and Hall (1957) indicate that the extent of conversion of dietary protein to microbial protein can vary considerably among the protein supplements. These investigators used zein to determine the extent of conversion of zein to microbial protein. The use of zein facilitates the separation of feed protein and microbial protein because zein is soluble in aqueous ethanol, and contains no lysine whereas microbial protein is insoluble in aqueous ethanol but contain lysine. The proportion of zein in the abomasal sample is the proportion not degraded in the rumen.

The investigators found that about 40% of zein is converted into microbial protein. Ely, Little, Woolfolk and Mitchell (1967) also estimated the extent of conversion of dietary zein to microbial protein and they obtained the values of 26.3 and 30.5% with high cellulose and high starch rations respectively. The amino acids present in the abomasal fluid result from a mixture of dietary and microbial protein present in the fluid. The observed conversion of zein to microbial protein is probably less than would occur in animals fed ordinary ration. This is because of the low solubility of zein in rumen liquor, its formation of a glutinous, fibrous mass when warmed to body temperature, thus reducing the surface area for enzymic attack, and also its deficiency in lysine and tryptophan. All these render zein unsuitable for conversion to microbial protein. Weller, Gray and Pilgrim (1958) have determined the extent of conversion of plant nitrogen into microbial nitrogen in the rumen of the sheep. They were able to separate plant nitrogen from microbial nitrogen by using  $\alpha$ -E diaminopimelic acid (DAP), as a marker. The marker has been found to be present in bacteria (Work and Dewey, 1953) but not in protozoa or fodder. Lignin was also used as a marker to determine the extent of contamination of bacteria by fine plant particles. This is possible because if the amount of lignin is known, and the ratio of lignin to plant nitrogen is also known, the plant nitrogen present in the bacterial sample can be estimated. The following schematic representation shows

the distribution of nitrogen in the rumen content of a sheep killed 7 hrs after feeding.

### NITROGEN IN RUMEN.



#### DISTRIBUTION:

Plant N - 20%

Microbial - N - 74%

Soluble - 6%

There was a steady increase in microbial N at the expense of plant N as the time after feeding increased. It was assumed that the concentration of DAP - N in bacteria did not vary widely from the value of 0.62% used. Microbial N formed 82% of the total N 16 hrs after feeding and this was the ~~maximum~~<sup>maximum</sup> for the day, the minimum being

61%. It indicates that the extent of conversion of plant N to microbial N is between 61 and 82%. Use can also be made of  $\alpha$ -E diaminopimelic acid to estimate the quantity of bacterial N entering the duodenum per day from the rumen. In this case, the duodenal samples would be collected over 24 - hr period and the N: DAP ratio would be determined for the isolated bacterial specimen. The daily flow of bacteria from the rumen to the duodenum is estimated from the following equation:

$$\text{Bacterial N (g/day)} = R \times \text{DAP (g/day)}$$

where R is the N: DAP ratio of isolated bacteria.

Certain assumptions have been made in the use of DAP as a marker for bacterial N. They are:

- (1) DAP is present in bacteria but absent from other nitrogenous components of the rumen.
- (2) The N: DAP ratio determined for the sample of bacteria is truly representative of the total bacterial population.
- (3) The isolated bacterial preparation is not contaminated with feed residues.

Since the microbial proteins constitute the major portion of the nitrogen - containing compounds that reach the lower gastro - intestinal tract (Weller et al, 1958), factors which affect the microbial population also affect the availability of microbial protein to the ruminant. Blackburn and Hobson (1960) and Warner (1965) showed that

the changes in the rations fed to ruminants can exert a modifying effect on the rumen microbiota. Both semi - purified diets and antibiotics have been shown to influence concentrations of protozoa in the rumen (Bryant and Small, 1960) and to modify the bacterial population or metabolic activity (Purser, Klopfenstein and Cline, 1965). Changes in ration did not however, modify the amino acid compositions of rumen bacteria or rumen protozoa (Weller, 1957; Meyer, Bartley, Deyoe and Colenbrander, 1967). However, the amino acid composition of protozoa has been found to vary (Poley, 1965; Heller and Harmeyer, 1964) while such values for bacteria have not been shown to vary (Purser and Buechler, 1966).

Bergen, Purser and Cline (1968) investigated the effects of different rations on the amino acid composition, pepsin digestibility and protein quality of the rumen bacteria (B), rumen protozoa (P) and the recoverable rumen microbial cell mass (P + B). They found that the total protozoal counts were not significantly affected by ration changes. Within a microbial preparation, the amino acid compositions were not affected significantly by rations. The lysine content was higher in protozoa than in the bacteria. It was also suggested that for conventional type of rations, the microfauna represented a higher percentage of the total microbial protein whereas for a semi - purified diet, the microflora represented most of the total microbial protein. However, this suggestion cannot be accepted unequivocally without data

on total bacterial and protozoal distributions and recovery from rumen contents. It was found that the pepsin digestibilities of protozoa and protozoa plus bacteria preparations from sheep fed semi - purified rations were significantly lower than the pepsin digestibilities of equivalent preparations from sheep fed conventional type of rations. There were no noticeable differences in protein quality of rumen bacterial preparations on different rations. This may be due to a failure to establish different bacterial population or to an incomplete recovery of bacteria of widely divergent protein quality. The cellulolytic bacteria which are generally more difficult to remove from rumen contents may be of relatively low protein quality (Bergen et al., 1967).

Meyer et al. (1967) showed that feed processing does not alter the quality of the protein of bacteria or protozoa but affect the quantity of protein synthesized in the rumen.

Hume, Moir and Somers (1970) investigated the quantity of microbial protein produced by the rumen micro-organisms by measuring the daily flow of protein through the omasum from the rumen. They fed virtually protein - free diets at levels of 2, 4, 9, 16g nitrogen/day in the form of urea in the ration. Casein hydrolysate was infused continuously into the abomasum through a length of surgical quality vinyl tubing. The rate of flow of digesta was estimated by reference to polyethylene glycol (PEG) when steady state conditions have been closely approached in the rumen. The marker was injected into the rumen, and rumen fluid

samples were taken for analysis before injection (To) and 1, 2, 4, 8, 12, 16, 20, 24 hr after injection. From this, the rate of flow of digesta was estimated.

When a protein-free diet is given under equilibrium conditions, the amount of protein flowing from the rumen to the omasum daily may be equated with the daily production of microbial protein in the rumen. The small amount of protein entering the rumen in the saliva (McDonald, 1948) and by desquamation of the rumen epithelium (Phillipson, 1964) will introduce little error into this assumption. The amount of protein flowing from the rumen daily is then the product of the rate of flow of digesta and the concentration of protein in the digesta. Hume (1970) found that there was a linear response in the rate of flow of protein out of the rumen to increasing levels of N intake ranging from 2 to 9g nitrogen/day but there was no further increase between 9 and 16g N/day intake. In the protein - free diet, the protein flowing out of the rumen has low concentration of four and five - carbon branched chain and straight chain volatile fatty acids (VFA), particularly, isobutyric. It was suggested by Allison (1965) that branched - chain carbon skeletons cannot be synthesized by most rumen cellulolytic bacteria. Hence protein synthesis in the rumen may have also been limited by the supply of these nutrients. The ration used by Hume (1970) contained 418g dry organic matter (O.M.) ingested daily. Assuming a protein yield of 15 - 16g/100g O.M. digested in the rumen,



the potential production of protein could be as much as 67g. However, the investigators found that only 48g protein were synthesized. Protein production in the rumen could be limited by factors other than energy and nitrogen. Sulphur and branched - chain volatile fatty acids have been suggested. The amount of protein produced on a protein - free diet is capable of satisfying the animal's requirement for protein if the intake of energy is adequate.

Hume (1970) found that the supplementation of a virtually protein - free diet with a mixture of higher VFA resulted in the increased production of microbial protein from 71g to 81g/day. The flow of total N out of the rumen was also increased. There were no differences in N balance values. A negative correlation was found between acetic acid proportions and protein production ( $r = - 0.62$ ). The addition of VFA did not result in the increase in efficiency of protein production from the energy available. The increased production of microbial protein when protein - free diets are supplemented with VFA has been explained as being due to the fact that the cellulolytic bacteria require branched chain fatty acids which could only arise from amino acids (El-Shazly, 1952). It is suggested that it was the higher VFA that was limiting on protein - free diets.

Hume (1970) showed that dietary protein also affects microbial protein synthesis in the rumen. He found that protein production in the rumen of sheep fed on a virtually protein - free diet supplemented with

urea and higher VFA and yielding 600g organic matter/day amounted to 90g/day. When gelatin was substituted for the higher VFA and 50% of the urea - N, microbial protein production remained at similar level (91g/d), with casein, production increased to 101g/day, and with zein to 104g/d. The protein production on VFA/urea and on gelatin ration may have been limited by the rate of synthesis of one or more amino acids by the rumen bacteria. This has been suggested to be the case with methionine by Loosli and Harris (1945). Zein, being resistant to microbial proteolysis could contribute a great percentage of the protein passing the abomasum. It is however, deficient in lysine and tryptophan. Casein has amino acid composition not very different from that of microbial protein ( B.V 79). However, its high degree of degradation in rumen, and consequent loss through rumen wall makes a portion of it unavailable for microbial protein synthesis.

The investigation by Purser (1970) on the micro-organisms as a source of protein for ruminants has been divided into three general areas.

- (a) a consideration of the amino acid composition and nutritive value of the rumen micro-organisms.
- (b) a discussion of the factors influencing the availability of the amino acids to the host animal's metabolic system.
- (c) a discussion of the importance of supply of energy and protein to the host animal.

### 1.2.1.3 Nutritive values of microbial proteins.

McNaught, Owens, Henry and Kon (1954) obtained the values of 74, 81 and 60 for bacterial true digestibility (TD), biological value (BV) and net protein utilization (NPU) respectively, and 90, 80 and 73 for protozoal TD, BV and NPU respectively. Loosli, Williams, Thomas, Ferris and Maynard (1949), Black, Weiber, Smith and Stewart (1957) and Donnes (1961) showed that the amino acids isoleucine, leucine, lysine, methionine, phenylalanine, tyrosine, threonine, valine and histidine were metabolically essential to the ruminant and could be synthesized from urea by the rumen microbial population. The reported amino acid compositions of rumen bacteria are strikingly similar. Similar agreement between the amino acid composition of microbial preparations from animals receiving different rations has also been reported (Weller, 1957; Purser and Buechler, 1966). A similar type of relationship has been observed with the amino acid composition of rumen protozoa by Weller (1957) and Purser and Buechler (1966). Slight differences are apparent in the amino acid composition of protozoa and bacteria. Lysine, leucine, phenylalanine and tyrosine are slightly higher in protozoa than in bacteria. These differences have been used by a number of investigators to explain the difference between the NPU values of protozoa and bacteria. However, this is an incorrect interpretation as the BV's of the fauna and flora do not differ; their digestibilities only differ, giving rise to higher NPU for protozoa.

#### 1.2.1.4 Factors influencing Microbial Amino Acid Availability

Limiting amino acids of rumen microbial proteins have been determined using the plasma amino acid score (PAAS) technique of McLaughlan (1964) which is reasonably reliable when these proteins were fed to rats (Bergen et al., 1968a). The application of such results to ruminants may not be strictly applicable. Extremely low plasma levels for histidine in the rats fed protozoal protein were recorded. Valine was also low in rats on this dietary treatment. Histidine was in fact indicated as the limiting amino acid in animals fed protozoal protein and cystine gave the lowest value for the animals fed bacterial protein, with arginine, histidine, leucine and lysine also being low. These results implicate histidine, cystine, leucine, arginine, and lysine as potentially limiting amino acids in rumen microbial proteins.

Protein utilization and hence the quality may be influenced by a number of factors.

- (a) In ruminants, energy (VFA) and amino acids are absorbed from different sites in the alimentary tract, that is, from the rumen and from the intestine whereas in a monogastric animal they are absorbed from the same site (intestine).
- (b) rate of release of specific amino acids from microbial protein.
- (c) amino acid composition effect upon absorption, and
- (d) selective absorption of essential amino acids as compared with non-essential amino acids.

Miller and Payne (1964) have discounted the practical importance of the timing of nutrients in large animals, but since amino acids and energy are absorbed from different sites in the ruminant, this aspect of nutrient timing and a possible effect upon nutrient utilization is worthy of investigation.

Differences in rates of release of three amino acids from protozoal, bacterial, and egg protein have been studied by digesting each in pepsin for 180 min and then in pancreatin, (Purser 1970). It was shown that pepsin is particularly ineffective in releasing arginine from protozoal and bacterial proteins but arginine was freely released from egg protein. Glutamic acid was released fairly steadily over the entire digestion period. Alanine was released very easily from bacterial protein, about twice the rate of release from protozoal and egg protein. The composition of the amino acids presented to the intestine can markedly influence both the rate of absorption and the composition of the amino acids absorbed. The amino acid content of both the duodenum and ileum was expressed as a percentage distribution and the ileal value expressed as a percent of that of the duodenum. The duodenal values are thus shown as 100. It was found that the essential amino acids were lower in the ileal content and the non-essential amino acid higher. Consequently in the passage of digesta from duodenum to ileum, a greater relative quantity of essential amino acids (EAA) than non-essential amino acids (NEAA) were absorbed. The EAA comprise about 50% of the total duodenal amino acids and only 44% of the ileal amino acids.

There is some evidence for the existence of an interaction between amino acid utilization and specific metabolic energy as shown by Porter, Purser and Cline, (1968). In their investigation, a specific energy source was infused into the carotid artery of sheep and the plasma amino acid changes then expressed as ratios of original values (Plasma amino acid indices). Glucose was found to be more effective than propionic acid in decreasing the amino acid concentration of blood. Butyric acid was less effective than propionic acid, and acetic acid has no effect on the plasma amino acids. This means that of all the sources of energy mentioned above, glucose is best utilized in the metabolism of amino acids.

#### 1.2.1.5 Relative Supply of Amino Acids and Energy.

It has been found necessary to know the interaction between amino acid utilization and availability of energy source. The following assumptions have been made.

- (1) Microbial cell material synthesized in the rumen contain 65.4% crude protein, (Hungate, 1966).
- (2) Microbial protein passed from the rumen to the alimentary tract has a digestibility of 80%, (McNaught et al, 1954).
- (3) Abomasal secretions amount to 1 to 2g N/day and must be deducted from quantities calculated from duodenal material (Hogan and Philipson, 1969).
- (4) Adjustment has to be made for some protein that escapes rumen microbial degradation and digested in the intestine. In most cases,

maximal conversion of available dietary protein to microbial protein was nearly achieved.

(5) It is assumed that 75% of the energy absorbed by the animal is absorbed from the rumen and 25% from the rest of the tract (Hogan and Weston, 1967a).

(6) That 1g of digestible dry matter contains 4.3 kcal.

Hungate (1966) stressed the fact that the rumen system is an<sup>a</sup>erobic which places a limitation upon the maximum possible conversion of dietary nitrogenous material to microbial cellular material. An<sup>a</sup>erobic fermentation takes place with a maximum cell yield of 15% (Hungate, 1966) which is equivalent to 9.84% (65.4% of 15) yield of protein in material leaving the rumen. Hungate (1966) has calculated the ratio of digestible protein to energy to be 18: 1 when protein is expressed in grams and energy in megacalories. He calculated it as follows using the assumptions previously mentioned that is assuming 15% cell yield per 100g dry matter fermented in the rumen.

(1) Protein yield per 100 g fermented =  $15 \times .656 = 9.84 \text{ g.}$

(2) Digestible protein per 100 g fermented =  $9.84 \times 80 = 7.87 \text{ g.}$

(3) Digestible protein per 430 (1000 x 4.3) digestible kilocalories = 7.87 g.

(4) Therefore, digestible protein per 1000 digestible

$$\text{kilocalories} = \frac{1000}{430} \times 7.87 = 18.3 \text{ g.}$$

Therefore, digestible protein per digestible megacalorie = 18.3g.

Some of the factors influencing the conversion of dietary N to microbial N in the rumen according to Hogan and Weston (1967a) are:

- (a) the time spent by the feed particles in the rumen (the longer the time, the greater the conversion).
- (b) The resistance of dietary N source to deaminative degradation, the more resistant, the less the conversion.
- (c) Availability of N for microbial protein synthesis.
- (d) Energy availability for rumen fermentation.
- (e) Presence of growth factors for instance, minerals such as cobalt, and also vitamins such as B12.
- (f) The population composition of rumen micro-organisms.

Ruminant animals are poorly suited to the use of sources of nitrogen when present in large amounts because of the degradation of these nitrogenous sources and subsequent loss of N through the rumen epithelia in the form of ammonia. However, they efficiently utilize sources of N when these are present only in adequate amounts. When ruminants are fed once daily, there are a number of phases of digestion in the fore-stomach which correspond to the fermentation of various constituents of the ration at different rates (Walker, 1965). Consequently, during the day, there are periods when energy becomes available to the microbial population at differing rates. Using rumen fluid, Walker (1965) has shown that when an excess of readily fermentable carbohydrate is



available as is the case shortly after feeding, only a small proportion of the energy made available by fermentation to VFA is used for growth, the greater part being used for intracellular polysaccharide synthesis. A similar low rate of protein synthesis in relation to energy supply has been demonstrated in whole rumen contents collected shortly after feeding, while later in the day, the protein/energy ratio increased (Walker and Nader, 1968).

Walker (1965) introduced the concept of an interrelationship between rumen microbial cell synthesis and adenosine triphosphate (ATP) made available during the degradation of feed materials. The initial and considerable decline in the protein/ATP ratio in the early part of the feeding period indicated wastage of available energy or diversion to purposes other than microbial growth. Part of the available sugar is utilized by rumen micro-organisms for the synthesis of intracellular polysaccharide. There has been found a dramatic increase in the proportion of polysaccharide per unit of DNA in the rumen liquor organism during the first phase of digestion and this corresponds to the part of the decline in protein/ATP ratio. Unfortunately, because of the lack of methods for distinguishing between microbial and plant polysaccharide, present in whole rumen content, it is not possible to quantitate polysaccharide synthesis and relate the energy used for this purpose to that used for protein synthesis.

The protein/energy ratio increases between 8 and 12 hrs after

feeding. At the same time the polysaccharide content of the cells returns slowly to the pre-feeding level, which would suggest that the environment of the microflora is becoming energy-limiting. Under such conditions, it would be expected that reserve polysaccharide could be used as a readily available energy source. Forest (1969) has shown for a great many organisms in pure culture that under energy-limiting conditions, the growth of an organism results in the production of 10 - 11g dry weight of cell material per mole of ATP available. Since in general, bacteria contain about 60% protein, 6 - 6.6g protein per mole of ATP would be expected. If 81% of the crude protein of bacteria is true protein, then the maximum true protein to ATP ratio is 5.3 g (0.81 x 6.6) protein per mole of ATP.

## 1.2.2 UTILIZATION OF NON - PROTEIN NITROGEN BY THE RUMINANT.

### 1.2.2.1 Non - Protein Nitrogen in Ruminant Rations

591 The concept that micro-organisms play a useful role in protein metabolism was put forward by Zuntz (1991) who expressed the view that rumen bacteria use by preference amides, amino acids and ammonium salts instead of protein, and that the protein supplied by a given ration was augmented as a result of the formation of protein in the bodies of bacteria and protozoa which were later digested. These early observations showed that the protein requirement of animals especially Herbivora could be met in part by such non-protein nitrogenous (NPN) compounds as asparagine, urea and ammonium salts. Loosli et al (1949) obtained specific evidence that microbial action in the rumen can synthesize from urea all of the ten amino acids which are essential for rat growth. In so far as the microbial protein arises from NPN compounds such as urea, a distinct gain in amino acids available to the body results. The microbial protein is of high biological value (BV) as measured by rat growth. McNaught et al. (1954) got the values of 81 and 80% for the biological values of bacteria and protozoa respectively. This means that through the rumen microbial activities, rations of poor quality are enhanced in quality. Amino acids deficient in the ration are supplied by microbial synthesis. This explains why the protein quality of the rations as fed is much less important in the case of the ruminant than in non-ruminant animals. However, the microbial action results

in some losses also. Some of the ammonia produced in the rumen by protein degradation or from NPN compounds such as urea is absorbed into the blood stream and converted to urea in the liver. Most of the urea is lost in the urine and the rest is recycled to the rumen via the saliva and the walls of the rumen.

Virtanen (1967) had shown that milk production could be maintained in cows given purified, protein-free feed using urea and ammonium salts as the sole sources of nitrogen provided energy and minerals are adequate. Deif, El - Shazly and Abou Akkada (1968) fed urea, casein and gluten in the diet of the sheep at levels which supplied 1.33g, 3.33g, 5.33g, 7.33g, 11.33g and 14.33g nitrogen per day to each animal. A nitrogen - balance experiment was carried out for each nitrogen level with each of the three sources of the nitrogen supplements. They found that the faecal nitrogen was lowest when urea or casein was given whereas it was highest with gluten at levels of 11.33 and 14.33g/day. This is to be expected because urea and casein are rapidly degraded in the rumen with the formation of ammonia, some of which is used for the synthesis of microbial protein while the rest is absorbed through the rumen wall into the blood stream and converted to urea in the liver. Thus, a large portion of the urea and casein nitrogen is lost in the rumen, hence, the low faecal nitrogen on these two diets. Gluten is not degraded in the rumen to a great extent and this accounts for greater faecal nitrogen on this diet than on diets of urea and casein.

The fact that casein and urea are highly degraded in the rumen,

and gluten is not, also explains why the urinary nitrogen was higher on casein and urea than on gluten. It also explains why absorbed nitrogen is greater on casein and urea than on gluten. A linear relationship existed between nitrogen intake and nitrogen retention up to nitrogen intakes of 5.33, 11.33 and 7.33g/day for urea, casein and gluten respectively. A linear relationship was also found to occur between absorbed nitrogen and nitrogen retention up to levels of 1.33, 5.33 and 7.33g/day for gluten, urea and casein respectively.

Leibholz and Naylor (1971) using early weaned calves found that the replacement of 20.1 and 39.2% of the meat meal protein nitrogen by urea was associated with a significantly greater weight gain of calves between 5 and 11 weeks of age. The inclusion of urea in the ration to 55.6% of the total nitrogen depressed both weight gain and the intake of the concentrate mixture. The source of carbohydrate was sorghum and at levels of 62.3 to 77.8% of the ration. Also the faecal nitrogen was lowered, and the urinary nitrogen greater than in other urea rations. The concentration of branched chain amino acids in plasma was low on urea rations, so also was the concentration of free essential amino acids. Limiting factors in the experiment might have been carbohydrate to provide energy and carbon skeleton.

#### 1.2.2.2 Metabolism of Ammonia Nitrogen by Rumen Micro-organisms.

The manner in which the liberated ammonia from protein and non-protein is utilized in the synthesis of amino acids is poorly understood but available evidence suggests that ammonia is a starting

material for the synthesis of amino acids which are subsequently incorporated into microbial protein (Loosli et al. 1949).

On the basis of available information on the synthesis of amino acids by animal tissues and bacteria, it seems probable that in the presence of ammonia and a keto acid such as  $\alpha$ -ketoglutaric acid, rumen micro-organisms synthesize glutamic acid through reductive amination. The occurrence of many keto - acids including pyruvic acid and  $\alpha$ -ketoglutaric acid in the rumen liquor may be offered in support of this view. Synthesis of other amino acids would be expected to occur through transamination reactions involving the appropriate keto acids and glutamate. Evidence of transaminase activity has been presented by Otagaki, Black, Goss and Kleiber (1955).

Investigations by Allison and Bryant (1963) have shown that cellulolytic rumen bacteria, Ruminococcus flavefaciens required either isovalerate or isobutyrate for growth but that neither 2 - ketoisovalerate, 2 - ketoisocaproate nor leucine supported the growth of these organisms. The organism failed to incorporate labelled leucine into protein but labelled isovalerate or isobutyrate is required because of inability of the organism to synthesize isopropyl group.

Sulphur - containing amino acids (SAA) are structural units of rumen micro-organisms as well as of ruminant tissue protein. It has been shown that these amino acids can be synthesized by the rumen micro-organism utilizing inorganic sulphur to synthesize cysteine, cystine and methionine.

Lambs fed rations containing urea and inorganic sulphur as the sole source of sulphur were found to produce normal wool growth. Orally administered labelled sulphur has been found to appear in the cystine of wool. Block, Stekol and Loosli (1957) reported that  $S^{35}$  fed as Sodium sulphate to a lactating goat was detected in cystine and methionine of milk protein. These investigators also showed that  $S^{35}$  was incorporated into rumen micro-organisms of the sheep. Emery, Smith and Huffman (1957) found that  $S^{35}$  inorganic sulphate was synthesized more rapidly into cystine than into methionine. Lewis (1954) reported that reduction of sulphate to sulphide was brought about by rumen micro-organism, and the sulphide was believed to be used in sulphur amino acid synthesis.

Although the ability of rumen microbial population to synthesize amino acids from ammonia nitrogen has been shown (Loosli *et al.* 1949), evidence exists to indicate that some supplementary organic nitrogen is required for maximum nitrogen utilization. The nitrogen requirement of most bacteria can be met by ammonia but some bacteria also require amino acids and evidence has been presented to show that growth stimulation of some species may be brought about by peptide (Bryant and Robinson, 1961). Supplementation of high urea ration with organic nitrogen may result in the development of a broader spectrum of rumen bacteria by providing nutrients required by some of the most fastidious species. The possibility also exists that a general improvement in rumen microbial metabolism might occur by virtue of the supplementary organic nitrogen

supplying a rate-limiting nutrient. Ammonia is an essential nutrient for the growth of Bacteroides succinogenes, Ruminococcus flavefaciens, Ruminococcus albus, Bacteroides amylophilus, Methanobacterium ruminantium and Eubacterium ruminantium (Bryant and Robinson, 1963; Hungate, 1966). Addition of nitrogenous sources yielding ammonia stimulated in vitro digestion of cellulose and starch. Both cellulolytic and amylolytic activities in vitro of mixed rumen micro-organisms were increased when urea replaced soybean meal as the sole crude protein supplement, showing that ammonia is important in the nutrition of both cellulolytic and amylolytic rumen bacteria.

Synthesis of amino acids from ammonia by rumen micro-organisms requires the presence of ammonia, carbon skeleton and energy. Utilization of carbon from carbohydrate, (Hoover, Kesler and Flipse, 1963), carbon dioxide (Huhtanen, Carleton and Roberts 1954; Otogaki, et al 1965), Isovaleric acid, acetate and other volatile fatty acids (Hoover et al. 1963) indicates that carbon from a wide variety of sources could be used for synthesis of amino acids. However, synthesis of leucine from isovalerate (Allison, Bucklin and Robinson, 1966), isoleucine from 2 - methylbutyrate (Hungate, 1966), valine from isobutyrate (Allison and Bryant, 1963), phenylalanine from phenylacetate (Allison, 1965) and tryptophan from Indole - 3 - acetate (Allison and Robinson, 1967), indicates a requirement for certain specific carbon skeleton in the synthesis of certain amino acids.



Energy for amino acid synthesis is provided by carbohydrates and other organic compounds in the form of ATP. Hungate (1966) estimated that 1.1g microbial nitrogen is utilized for synthetic purposes for each 100g of carbohydrate fermented.

### 1.2.2.3 Factors Affecting the Utilization of Ammonia in the Rumen.

Recent studies have been concentrated on factors which will promote the maximum bacterial synthesis of protein in the rumen to provide for the more effective use of rations of poor quality protein and particularly non - protein sources of nitrogen such as urea. A readily available source of energy is necessary for the efficient utilization of the end-products of protein fermentation. Pure starch or starch feeds such as cereals, cassava and potatoes are usually most satisfactory. Molasses or sugars are less satisfactory because they pass out of the rumen too rapidly. On the other hand, cellulose is made available too slowly. Rations low in protein and high in readily available carbohydrate are most favourable to protein synthesis in the rumen. In ruminants, it is generally considered that soluble carbohydrates exert a positive influence on protein metabolism. Addition of readily available carbohydrate to protein - rich rations fed to ruminants, was followed by a depression in the concentration of ammonia in the rumen (Chal<sup>m</sup>ers and Synge, 1954). The yield of protein produced by incubating ammonium salts with rumen liquor can be markedly increased by the addition of readily available carbohydrate. Nitrogen retention also increases consistently by supplement of readily available carbohydrate. Lower

concentrations of blood urea are also observed in ruminant receiving a supplement of readily available carbohydrate. The observed inhibition of ammonia accumulation in the rumen has been explained by the fact that unionised ammonia molecules pass through rumen epithelia much quicker than the ionised form (Lewis, Hill and Annison 1957). At high pH, the ammonia molecules are mostly present in the unionised form. The presence of glucose or its derivative, lactic acid, lowers the pH and the ammonia molecules are mostly present in the ionized form and their passage through the rumen epithelia is much delayed, giving time for the rumen micro-organism to incorporate ammonia for microbial protein.

The pH of the rumen liquor also affects utilization of ammonia. The pH affects the production of ammonia and also the absorption of ammonia. Reis and Reid (1959) found that high pH favours ammonia production in the rumen. The optimum pH for ammonia production in the rumen varied between 6.0 and 7.0. The observed effect of pH is on deamination as well as on proteolysis. The enzymes concerned with the deamination of amino acids are affected by the pH of rumen liquor. Warner (1955) has shown that pH affects the rate of proteolysis and that optimum pH range is 6.5 to 7. The pH also affects the rate of growth of bacteria in the rumen.

Annison (1956) showed that the formation of ammonia in the rumen varies with the type of protein - rich supplement. He compared casein,

groundnut meal, herring meal and flaked maize gluten. He found that the groundnut meal can be deaminated to an extent equal to or greater than casein. This is because groundnut meal is also very soluble in rumen liquor. Maize gluten yielded very low level of ammonia. Herring meal is intermediate between groundnut meal and maize gluten. Protein supplements such as casein and groundnut meal are easily degraded giving high levels of ruminal ammonia. Urea is easily hydrolysed by the urease of the rumen giving high levels of ammonia. Therefore, the more soluble the protein supplement in the rumen liquor the more ammonia is produced. The method of processing of the protein supplement also affects the rate of degradation of the protein supplements in the rumen. Formaldehyde - treated casein has been shown to be less soluble than untreated casein and therefore gives lower levels of ammonia in the rumen than untreated casein.

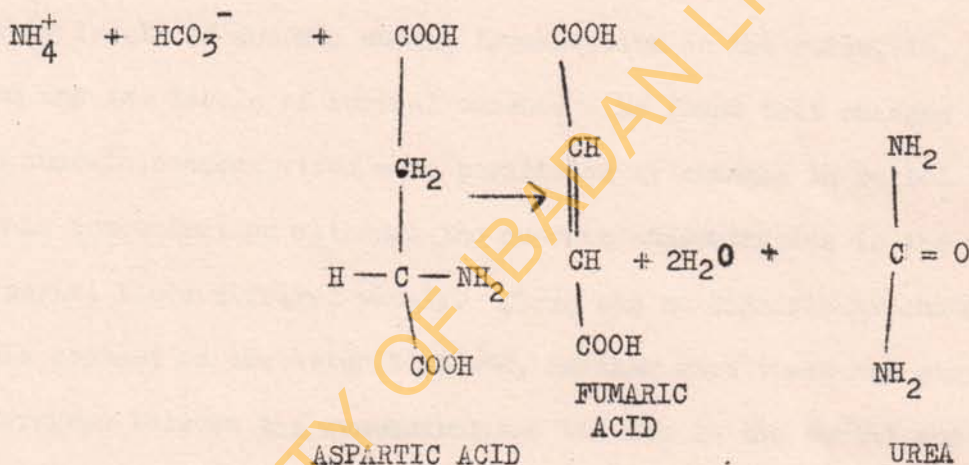
#### 1.2.2.4 Absorption of Ammonia Through the Rumen Wall.

The absorption of ammonia across the rumen wall was reported by McDonald (1948). It is influenced by both the concentration gradient (Lewis, Hill and Annison, 1957) and pH of the rumen liquor. Ammonia is a weak base with a pKa of 8.80 to 9.15. An increase in pH causes the ammonium ion ( $\text{NH}_4^+$ ) to be converted to ammonia ( $\text{NH}_3$ ), and this is rapidly absorbed. Absorbed ammonia is carried via the portal circulation to the liver where it is converted to urea. Hogan (1961) has estimated that

if efferent blood contains 1.5 mg  $\text{NH}_3$  - N per 100 ml and the rate of flow is 200 ml/minute, then ammonia absorption is 4.3 g/day. The rate of saliva urea secretion has also been estimated at 0.5g/day (Hogan, 1961). The deamination of protein and the hydrolysis of (NPN) substance such as urea in the rumen forms large amounts of ammonia which if allowed to accumulate would be highly toxic to the animal. Ruminant blood contains about 1.5 mg  $\text{NH}_3$  - N/100 ml blood in the ruminal vein but only traces about 0.1 m - mole/litre in the peripheral circulation (Chalmers, 1954). It has been shown that a concentration of 0.4 to 0.5 m - mole per litre is toxic to the sheep. It is therefore, important that the animal detoxifies this ammonia in the liver before releasing it into the systemic circulation. This is done by its conversion into urea.

Sp | Krebs and Henseleit (1932), working with liver slices, established the general chemical mechanism by which ammonia is converted to urea. They discovered that the rate of urea production in liver slices incubated with ammonium salts, bicarbonate and lactate, was increased by addition of ornithione or citrulline, and that arginine was an intermediate product of the reaction. It was also observed that the quantity of ammonia disappearing was equivalent to the urea formed. On the basis of these observations, Krebs and Henseleit (1932) proposed a cyclic mechanism for urea synthesis, involving ornithine, citrulline, arginine, ammonia and carbon dioxide. It was found that 2 molecules of ammonia and

1 molecule of carbon dioxide are converted to a molecule of urea for each turn of the cycle and the ornithine is regenerated. Therefore, synthesis of urea involves the primary fixation of carbon dioxide and ammonia. It must be known that of the two nitrogen atoms present in a molecule of urea, an atom comes from ammonia, and the other from aspartic acid and could be shown as follows:



#### 1.2.2.5 Influence of Ruminal Ammonia Level on the Concentration of Ammonia and Urea in the Blood

Comparatively, little attention has been given to the quantitative treatment of the relationship between the ammonia concentration in the rumen and in the portal blood or to the loss of dietary nitrogen in the form of ammonia. Lewis (1955) showed that there was a correlation between rumen ammonia and portal blood ammonia concentration over a wide range of rumen ammonia concentration and that spill - over

of ammonia into the systemic circulation occurred at relatively low portal ammonia concentration. At higher rumen ammonia levels, it was possible to correlate rumen, portal and peripheral blood ammonia and to relate the levels to the onset and development of toxic symptoms. Lewis (1955) determined rumen ammonia, portal blood ammonia, peripheral blood ammonia and blood urea of sheep fed rations giving rise to varying levels of ammonia during fermentation in the rumen, ie, high, medium and low levels of ruminal ammonia. He found that changes in rumen ammonia concentration were paralleled by changes in portal blood ammonia concentration although the ammonia concentration in the rumen and portal blood differed widely. There was no significant change in the ammonia content of the arterial blood, neither were there any significant differences between the concentrations of urea in the portal and peripheral blood. However, blood urea levels were found to increase with increase in ruminal ammonia levels. The investigator could find no regular pattern between the varying concentrations of rumen ammonia and corresponding concentrations of ammonia in the peripheral, venous and arterial blood samples. Whereas the ruminal ammonia rose rapidly and reached a peak in 2 hrs after feeding and later declined slowly over the next 6 hrs, the portal ammonia concentration showed a distinct lag period of 2 hrs before any increase in ammonia concentration occurred

(Lewis 1955). In all these experiments, no significant changes in the ammonia concentration of peripheral blood was reported although relatively high (60 m - moles/litre) rumen ammonia levels were attained on a number of occasions. However, at high ammonia concentrations, some ammonia could pass into the systemic circulation. It has been suggested that a hepatic ammonia threshold exists in the sheep and that if this threshold is exceeded, the liver can no more cope with the high level of ammonia brought to it and therefore the ammonia concentration in the peripheral blood rises sharply. In case where ammonium acetate was used to induce varying levels of ruminal ammonia, no significant changes in arterial ammonia concentration took place until the portal blood contained about 0.8 m - mole  $\text{NH}_3$ /liter of blood. Above this level, the arterial ammonia concentration increases at almost the same rate as the portal blood. When the arterial ammonia concentration reaches 0.4 to 0.5 m - mole/litre, respiratory difficulties arise in the animals, and beyond this death occurs, probably due to a disturbance in acid - base equilibrium caused by excessive  $\text{NH}_4^+$  ions. It is estimated that the amount of ammonia carried to the liver per day is about 14g. Even if a portion of this is returned to the rumen via the saliva and through the rumen epithelia as urea, the nitrogen loss still represents an appreciable proportion of the total nitrogen intake.

Lewis et al., (1957) have shown that the level of blood urea in sheep is relatively constant and is dependent upon the ration. The blood urea tends to reflect the over-all changes in ammonia production in the rumen. They used blood urea determination to assess nitrogen losses following the absorption of ammonia from the rumen and its use has been found to be of practical importance. Blood urea concentration has been found to be uniform over 24 hr period but that greater diurnal variation is found in those instances where the ruminal ammonia is very high, (Lewis et al., 1957). They could find no significant difference between the venous and arterial blood urea concentration but found a close association between ruminal ammonia and blood urea levels. To show that the rise in blood urea level was a direct result of ammonia absorption from the rumen and not to changes in total nitrogen intake, Lewis et al. (1957) used casein and zein at the same level of nitrogen intake but which give different patterns of ammonia production in the rumen. The same general correlation has been found between ruminal ammonia and blood urea levels whatever the ration is. Chalmers and Synge (1954) suggested a partial reduction of the rate of attack of feed protein by rumen micro-organisms by altering the solubility, degree of denaturations and particle size. Houpt (1959) replaced the rumen content of the sheep with saline solution. Arteriovenous urea differences indicate that blood urea moved into the saline solution and was hydrolysed by the traces of bacterial urease present in the



**rumen.** Accumulation of ammonia in the rumen was measured and concurrent absorption of ammonia from the saline was calculated. The total of these two rates equals the urea nitrogen transfer rate into the rumen. He also found that sheep whose rations were supplemented with readily available carbohydrate utilized 53% of urea injected into the blood streams whereas those whose rations were supplemented with poor quality hay utilized only 22%. This shows that readily available carbohydrate is essential for utilization of urea. As a result of urea entering the rumen through the saliva and rumen epithelia, the nitrogen intake necessary to maintain life would be considerably lower for ruminant than non-ruminant animals. The feed - back of blood urea across the rumen epithelia depends very much on the levels of dietary N, the lower the level of dietary N, the more blood urea is being recycled; the higher the level of dietary N, the lower the rate of recycling from the blood. The observation that the decrease of blood urea concentration occurs at constant rates has enabled calculation to be made of the estimates of urea transfer into the rumen. The amount of urea which disappeared from the serum minus the amount excreted in the urine is the amount that moves into the rumen. Houpt (1959) using the above method of calculation obtained a value of 8.3 m - moles as the amount of blood urea recycled to the rumen per hour. The ability of ruminant animals to utilize blood urea would enable their survival time to be prolonged especially those which live in habitats where for most part of the year, the vegetation is mature, dry, tough and contains very little nitrogen.

1.2.3 INTESTINAL DIGESTION OF PROTEIN - N AND  
UTILIZATION IN THE RUMINANT

Interest in the digestive tract of ruminant animals has usually centred on the forestomach, for it is the characteristic digestive organ of the ruminants and about two-thirds of the digestible organic matter is fermented there. The ruminant intestine has been neglected because of the assumption that it resembles that of monogastric animals in its functions. However, the capacity of the rumen and the metabolic activities of its micro-organisms affect the flow and composition of digesta passing to the intestines to an extent that makes intestinal digestion in ruminants a distinctive process. Kay (1969) showed that

- (1) food is retained in the rumen for a long time and only flows to the lower gut slowly;
- (2) microbial activity in the rumen transforms the diversity of protein in the diet to a more uniform product passing to the abomasum; it also removes most of the digestible carbohydrate from the food so that very little sugar is absorbed from the intestine;
- (3) flow of digesta from abomasum is enormous, almost continuous and fairly constant in consistency and composition; pancreatic secretion is equally continuous, abomasal secretion of digestive fluid is continuous and the intestinal content remains acid throughout the upper part of the small intestine.

(4) large amounts of water and salts are secreted into the gut especially by the salivary glands and these must be efficiently re-absorbed mostly in the small and large intestines. The nitrogenous digesta flowing to the duodenum are largely of rumen microbial origin, though variously supplemented with unfermented food residues and digestive secretions. The factors affecting the digestion of food in the intestine, therefore, are the extent of protein degradation in the rumen, the nature and quantity of microbial protein synthesized from dietary and endogenous nitrogen and the amount of endogenous protein flowing to the duodenum.

Hecker (1971) compared the deaminative, ureolytic and proteolytic activities and rates of cellulolysis, carbon dioxide and methane production in the rumen with that of the large intestine. He found that the proteolytic activity of the large intestine is greater than that of the rumen contents. Some proteolytic activity was present in caecal cell-free liquor. Deaminase activity was greater in rumen than in caecal contents. The urease activity of rumen contents was greater than that of caecal contents. The rate of carbon dioxide and methane production was, however, higher in caecal contents than in rumen contents. The rate of cellulose breakdown in vivo were similar for rumen and caecal contents. Thus it is seen that the large intestine, though little studied, is also capable of enormous digestion, the principal difference

being that since hydrolytic digestion of protein, starches, sugars and fats occur before the digesta reaches the caecum, the amounts of these substances reaching the caecum are likely to be small or negligible.

Hogan and Weston (1967) have shown that the amounts of non-ammonia crude protein (NACP) passing the abomasum was similar whether the ration contained 7.8 or 19.8% crude protein (CP). Ørskov, Fraser and McDonald (1971) found that the amount of NACP ( $Y_1$  g/day) disappearing from the small intestine increased with protein intake ( $X$  g/day) according to the equation

$$Y_1 = 2.12 X - 0.0057 X^2 - 83.$$

reaching a maximum when there was 19% CP in the dry matter of the feed.

Andrews and Ørskov (1970a) showed that when the protein concentration of the diet was increased at high constant energy intakes, the growth rate and the retention of nitrogen in the body increased. The level of protein was found to have no significant effect on the disappearance of NACP from the large intestine. The apparent digestibility of crude protein increases with protein concentration. It was not known whether increased absorption came from increase in microbial protein or from dietary nitrogen escaping fermentation. Since they found that the protein used, soya bean has higher digestibility than microbial protein, they concluded that the

increments were due to soy-bean protein escaping fermentation in the rumen.

### 1.2.3.1 The Rate of Flow of Digesta in the Digestive System of Ruminant Animals

A mathematical study of the movement of particles and solutes through the digestive tract of the ruminant has been presented by Warner (1966). From his study, he showed that in a 'steady - state' system,

$$F = \frac{0.693V}{T} \quad (1)$$

where

F = rate of flow from the rumen.

V = volume of liquid in the rumen.

T = Time for the equivalent of half of the liquid in the rumen to be transferred to omasum.

When a water - soluble marker is infused continuously into the rumen, it was shown that

$$F = I/C \quad (2)$$

$$R = V/F = 1.44 T \quad (3)$$

$$P = I \times R \quad (4)$$

$$\text{and } \lambda = I/R \quad (5)$$

where I = rate of infusion of marker into the rumen.

$C$  = concentration of marker in the liquid leaving the rumen.

$R$  = the mean retention time of a population of marker molecules in the rumen.

$P$  = the quantity of marker present in the rumen (the rumen marker pool).

$k$  = is the fraction of the rumen volume transferred to omasum per unit time.

In a "steady-state system", one estimate of  $F$ ,  $V$  and  $T$  may be obtained by administering a single dose of an appropriate marker into the rumen and studying its rate of disappearance. If the marker is infused continuously at a constant rate, a number of estimates of rate of flow from the rumen can be made from the concentration of marker in the liquid leaving the rumen by using equation 2. After the continuous infusion is stopped, an estimate of  $T$  may be obtained by studying the rate of disappearance of marker from the rumen. The value of  $T$  together with the estimates of the rate of flow during the continuous infusion, may be used to calculate the volume ( $V$ ) of water in the rumen as indicated by equation (1). It was assumed that the concentration of marker in the liquid leaving the rumen and abomasum were the same as those obtained in samples of liquor taken from those organs. Rate of flow from the abomasum was calculated from equation (2) by substituting the marker concentration

in abomasal liquor for the marker concentration rumen liquor.

The digestibility coefficients of the dietary nutrient for the entire tract have frequently been obtained by determining the ratio of a given food constituent to some indigestible marker in the food itself, such as lignin and the ratio of the constituent to the marker in the faeces. From them, the percentage of the nutrient digested is given as

$$100 - \left[ 100 \times \frac{\% \text{ Lignin in Faeces}}{\% \text{ Lignin in food}} \times \frac{\% \text{ Nutrient in faeces}}{\% \text{ Nutrient in food}} \right]$$

This method does not require quantitative collection of faeces, provided representative samples can be obtained.

The markers most frequently used in ruminant digestion studies are lignin, polyethylene glycol, and chromic oxide. Chromic oxide is used either in powdered form mixed with the ration (Drennan, Holmes and Garrett, 1970) given in gelatin capsules (Putnam, Loosli and Warner, 1958) or impregnated on to paper (Gowlishaw and Alder, 1963).

Chromic oxide has been shown to be associated with the solid phase of intestinal digesta (Harris and Phillipson, 1962) and can be easily and accurately determined. Polyethylene glycol associates itself with the liquid phase of digesta (Hyden, 1956)

and the method of its determination have not given consistent results. Lignin has the advantage of being a plant constituent but suffers the disadvantage of being an ill-defined entity, the estimation of which is empirical.

Johnson, Dinuson and Bolin (1964) examined the concentration of chromic oxide in all the sections of the gut of sheep after feeding and measured the rate of excretion of a single dose when given in paper form or as powder mixed with a pelleted ration. They found that the powder form moved through the gut significantly faster than the paper form and that this difference was established by different rates of passage from the rumen. From their results, it seemed as if the passage from omasum to abomasum of the paper form was similar to that of lignin. Consequently, chromic oxide concentration in the abomasum might be used to give an accurate estimation of digestion anterior to this point if the paper form were used. Johnson et al. (1964) also found that the powder form yielded an abomasal concentration of chromic oxide only 70% of that found when the paper was used and would lead to a large underestimation of digestion.

Balch (1957) used the lignin-ratio technique to determine the extent of digestion in the reticulo-rumen of the cow. The results showed that about 4.3% of the herbage dry matter was digested in the reticulo-rumen, and that in cows fed entirely on hay, the amount of nitrogen flowing out of the reticulo-rumen was



greater than the nitrogen intake. Rogertson (1958) also used the lignin-ratio technique to determine partial digestion in sections of the alimentary tract of sheep using a slaughter method. He showed that 40%, 50% and 75% of the dry matter of hay, mixed diet and concentrate respectively occurred in the rumen. Bines and Davey (1970) also using the same technique found that 60% of straw diet dry matter was digested in the rumen.

Drennan, Holmes and Garrett (1970), and Holmes, Drennan and Garrett (1970) compared the use of lignin and powdered chromic oxide as markers for estimating the magnitude of digestion in the rumen and intestines using slaughter technique in sheep. They found that the results obtained using lignin as marker was higher and more consistent than those obtained using chromic oxide powder, and suggested that the poor results obtained by using powdered chromic oxide might be due to its very rapid or uneven passage from the rumen. They found that about 70% of the organic matter digested occurred in the rumen.

For studies with ruminants, chromic oxide paper appears to be suitable and promising, no doubt owing to the slow and sustained release of the oxide as the paper undergoes microbial digestion (Corbett, Greenhalgh, McDonald, and Florence, 1960). This has been confirmed by Langlands, Corbett, McDonald and Reid (1963) and Lambourne and Reardon (1963) who showed that chromic

oxide in the form of impregnated paper gave a more even release of marker into the faeces.

Digestibility of nutrients in the sections of the digestive tract is also estimated by the techniques of the re-entrant cannulation. For studies of digestion in the reticulo-rumen, the cannula is placed at the abomasum or duodenum so that all digesta from the reticulo-rumen can be collected. Digestibility of a nutrient is then calculated as the difference between the nutrient in food and the nutrient recovered at duodenal collection point. Similarly digestion in the small intestine is determined by placing cannulae at duodenum and terminal ileum, and the nutrient passing through the duodenal cannula minus the nutrient reaching the terminal ileum is the amount of nutrient apparently digested in the small intestine. Digestion in large intestine is the difference between the total nutrient in terminal ileal point and in the faeces. Digesta may be totally collected at the collection points or samples of digesta may be collected at suitable intervals and pooled to give representative samples of digesta flowing through the portion of digestive tract. Chromic oxide either in powdered form or impregnated on to paper, or lignin is usually given so that digesta could be adjusted to percent recovery of the marker. This technique of the re-entrant

cannulation has been widely used (Topps, Kay and Goodal, 1968; Nicholson and Sutton, 1969; McRae and Armstrong 1970; and McRae, 1970). It is not only useful for determining digestibility in sections of the digestive tract but also in studying biochemical reactions in the sections of the digestive tract, thus Hecker (1971) used sheep with ruminal and caecal cannulae to compare metabolism in the rumen and the caecum.

Using the re-entrant cannulation method for determining digestibility, Hogan and Phillipson (1960) found that of the total dry matter digested in the sheep, 70% disappeared in the stomach, 11% in the small intestine and 19% in the large intestine whereas the corresponding values as obtained by Topps et al. (1968) are 67%, 22% and 11% for hay, and 69%, 17% and 11% for concentrate - fed animals in the stomach, small intestine and large intestine respectively. Several investigators (Nicholson and Sutton, 1969; Topps et al., 1968) have reported that more nitrogen is recovered at abomasum than fed when sheep are given diets low in nitrogen but that substantial loss of nitrogen occurs in the rumen when the diet is rich in nitrogen.

Ben - Ghedalia, Tagari and Bondi (1974), by means of cannulae placed in portions of the small intestine, were able to show that there were substantial increases in water, dry matter and total nitrogen in the section immediately distal to the

pylorus and that these were caused by the inflow of bile, and pancreatic and duodenal juices. The net increase found beyond the entry of the common bile duct was 2.7g protein N and 2.0g non-protein N per day. The region 7 - 15m from the pylorus was found to be the region of most intensive absorption of amino acids, 60.5 of the essential, and 43% of the non-essential amino acids passing through the region being absorbed. They also showed that only small changes occurred in the region after 15m distance from the pylorus.

McRae, Ulyatt, Pearce and Hendtlass (1972), in ten 24 hr. collections of digesta entering the duodenum and eleven 24 hr. collections of digesta reaching the ileum of sheep given dried grass showed that there were highly significant correlations between the 24 hr. flows of chromium marker and the corresponding flows of dry matter, organic matter, nitrogen, gross energy, hemicellulose and cellulose at both sites. This has enabled the investigators to estimate the quantitative intestinal digestion in sheep.

The reactions in the digestive tract are very complex and a knowledge of how they take place, the products formed, the utilization of the products formed, and the factors that enhance the production is essential for adequate feeding of ruminant animals and hence meat production.

#### 1.2.4 ISOTOPIC METHODS OF DETERMINING THE UTILIZATION OF NITROGEN IN THE RUMINANT

The isotopes that have been commonly used in nutrition studies are  $^{15}\text{N}$  either in urea or ammonium salt form,  $^{35}\text{S}$ , usually used as sulphate, and  $^{32}\text{P}$  usually in the form of phosphates. These isotopes have been used to study the synthesis or utilization of protein in the ruminant. In addition  $^{14}\text{C}$  is also used to study the utilization of carbohydrates or other carbon-bearing materials. It is used either as  $^{14}\text{C}$  in urea or in glucose. White, Steel, Leng and Luik (1969) have used  $^{14}\text{C}$  glucose to study the kinetics of glucose metabolism in the sheep. Harrison, Beever and Thomson (1972) and Beever, Harrison and Thomson (1972) have used  $^{35}\text{S}$  as sodium sulphate to estimate the proportion of food and microbial protein in the duodenum of the sheep, while Landis (1968) had also used sulphur - 35 as sodium sulphate to study quantitative aspects of sulphur metabolism in the ruminant. Mathison and Milligan (1971) and Nolan and Leng (1972) have used  $^{15}\text{N}$  as Ammonium chloride or sulphate to study the ruminant digestion, while Land and Virtanen (1959) have used  $^{15}\text{N}$  as Ammonium nitrate to study the synthesis of milk protein from Ammonium salts. Lofgreen and Kleiber (1953) used  $^{32}\text{P}$  or N: P<sup>32</sup> ratio to determine the value of the metabolic faecal nitrogen of young calves.

All the isotopes in common use satisfied the basic requirement that:

- (a) the compound studied for instance urea, ammonia, glucose can be labelled in the required position with a suitable isotope,
- (b) the label is firmly attached to the molecule or or at least to that part of the molecule which is of interest to the investigator,
- (c) the amount of isotopic material introduced into the initial compound is such that it allows for considerable dilution before the concentration of the isotope is too low for accurate determination,
- (d) when radioactive, the rate of decay is sufficiently low to permit all the radioactivity determinations to be made with reasonable accuracy, while on the other hand, the radioactivity does not persist sufficiently long and with sufficient intensity to cause significant radiation damage to the tissues or cells under investigation or to any part of the experimental animal during the course of the experiment, otherwise the experiment is not truly physiological.

The isotopic tracer method is one suited par excellence for the study of biochemical reactions in the living cells. The major advantages of the method are:

- (a) The experiment can often be carried out under strictly physiological conditions on intact normal animals.
- (b) With a few exceptions, the labelled compound has, for all purposes, the same biological properties and the same metabolic fate as the unlabelled compound.
- (c) The amount of isotope required is usually extremely small, particularly with radioactive isotopes.
- (d) Laborious separation of radioactive compounds from tissues and tissue extracts is often unnecessary.
- (e) The precise origin of individual atoms in a compound produced by living tissues can often be determined by isotope studies for instance the N of urea in blood or urine, the S or P atoms in proteins.

There are some limitations, however, even though these are not of such a character as to reduce seriously the value of the isotopic tracer method as a general technique for agricultural research. These disadvantages are:

- (a) There is need for special technique and specialized equipment and these are usually expensive, for example Geiger counter, Mass spectrometer, Emission spectrometer. The compounds themselves for instance  $^{15}\text{NH}_4\text{Cl}$  or  $\text{Na}_2^{32}\text{SO}_4$  are expensive.

(b) The method only involves following the label. The determination gives a measure of the amount of label in the sample analysed. It should not be automatically concluded that the amount of isotope present in a particular tissue or cell gives a true measure of the concentration in that tissue of the substance originally administered to the animal. Also the isotope determinations give no direct information about the fate of any non-labelled parts of labelled molecules which have undergone disruption. These difficulties can often be overcome by the use of two or three different labels attached to different parts of the molecule of the compound studied, for example, Nitrogen and Sulphur in Ammonium  $\left[^{15}\text{NH}_4\right]_2^{32}\text{SO}_4$ .

(c) Radioactive isotopes may cause serious radiation damage to the tissues. This may apply to the tissues of the experimental animal or of the experimenter. In the former case, the experiment may no longer be normal or physiological, and the results obtained may be largely due to a disordered metabolism of the irradiated or some indirectly affected tissues.



(d) Lack of a suitable isotope-

There are few instances where it is impossible to find a suitable isotopic tracer for a biological investigation. Sometimes, however, a radioactive isotope which might otherwise be suitable has rate of decay of activity which is too short or too long for the experiment planned. In the former case, there would be great difficulty in completing all the radioactive measurements before the labelled compound and its metabolites lose their radioactivity and in the latter case there will be a correspondingly greater risk of radiation damage to the tissues.

- (e) Chemical non-identity of isotopes may not be strictly true. The physico-chemical differences between the isotope used as a label and the most abundant stable isotope of the same element may occasionally be sufficiently great to cause significant, quantitative differences between the metabolism of the labelled and unlabelled compound, for example, Heavy water  $D_2O$  penetrates into red blood cells more slowly than does ordinary water.

However, there is no evidence that these 'isotope effects' are normally of great magnitude in the complex biochemical systems of animal and plant organisms.

Isotopic methods have been extensively used in the study of utilization of ruminal ammonia and blood urea by rumen micro-organisms. Mathison and Milligan (1971) used the isotopic tracer technique to determine the proportion of microbial protein derived from ruminal ammonia.  $^{15}\text{NH}_4\text{Cl}$  solution (2L/24 hour) was continuously infused for periods of 120 - 216 hours into the rumen of sheep which were allowed to feed 2 out of every 10 minutes. These treatments achieved 'steady metabolic states' in the rumen in the period of the investigation. They found that 50 - 65% of bacterial N and 31 - 55% of protozoal N were derived from ruminal ammonia; 60 - 92% of the daily N intake was transformed into ammonia, and 17 - 54% of the ammonia formed was absorbed. The generation time of bacterial protein was found to be between 38 and 42 hours. The investigators showed that increase in ruminal ammonia leads to a decrease in the conversion of protein into ammonia in the rumen, and was given by this relationship:

$$Y = 123 - 0.44 x$$

where

Y = Nitrogen converted into ammonia expressed as percentage of N intake.

X = Concentration of ruminal ammonia (mg  $\text{NH}_3$  - N/litre).

These results were similar to those of Pilgrim, Gray and Weller (1970). Nolan and Leng (1972) used isotopic dilution

techniques with [ $^{15}\text{N}$ ] Ammonium sulphate, [ $^{15}\text{N}$ ] urea, and [ $^{14}\text{C}$ ] urea, and obtained similar results. They went further and showed that 59% of the dietary N was digested in the reticulo-rumen; 29% of the digested N was utilized as amino acids and 71% was degraded to ammonia. They also showed that urea was synthesized at the rate of 18.4g N/day from 2.0g N/day of ammonia absorbed through the rumen epithelium and 16.4g N/day apparently arising from deamination of amino acids and ammonia absorbed from the lower digestive tract. They obtained similar results using continuous infusion and single injection techniques and therefore showed that both techniques were valid in isotopic dilution studies. The investigators also used this isotopic technique to determine body urea space.

Landis (1968) using  $^{35}\text{S}$  sulphate given intra-ruminally to lactating dairy goat showed that 32 - 41% of administered sulphur was utilized for synthesis of protein sulphur in the rumen when the ration contained ample amounts of protein but the value was 70% when low protein ration was fed. Analysis of tissues of experimental animals showed that the rumen mucosa tissue protein had the greatest specific activity followed by the proteins of red bone marrow, liver, pancreas and kidney. This shows the sites of greatest utilization of

administered sulphur. Milk was found to be strongly labelled, and the specific activity of individual amino acids of milk was similar to that of the amino acids of tissue proteins. This has brought the suggestion that the amino acid needed for the synthesis of body and milk proteins are drawn from a common amino acid pool. Piva and Silva (1968) used  $^{15}\text{N}$  - Diammonium phosphate to study the utilization of the compound to produce milk and meat proteins. The nitrogen of the essential amino acids of ruminal bacteria and protozoa and milk of the sheep except tryptophan were significantly labelled. Only serine and cystine were found to be significantly labelled of the amino acids of the tissues of the sheep. They also found that about 6% of administered  $^{15}\text{N}$  Diammonium phosphate was metabolized in milk proteins. Land and Virtanen (1959) reported that feeding  $^{15}\text{N}$  Ammonium nitrate to lactating cows resulted in the labelling of milk within one hour, and that of the milk amino acids, histidine and cystine were very weakly labelled. They interpreted the low content of  $^{15}\text{N}$  in histidine to be due to the incapability of the ruminal bacteria to synthesize the imidazole ring. They found that about 17% of administered  $^{15}\text{N}$  was used for milk protein synthesis. Black, Egan, Anand and Chapman (1968) used C - 14 amino acids to show that amino acids play some role

in gluconeogenesis in lactating ruminants, that the process is metabolically important for all animals, and becomes essential for survival when the body's glucose requirements exceed the alimentary supply. This may be so in ruminants where the glucose supply is always tenuous because the rumen micro-organisms rapidly ferment dietary carbohydrate converting it into short - chain fatty acids, leaving the animal very little glucose for absorption. Coccimano and Leng (1967) and Mugerwa and Conrad (1971) using intravenous infusion of C - 14 urea have calculated the urea pool size, rate of entry of urea into body urea pool, rate of degradation of urea in the rumen<sup>and</sup> amount entering the body pool, that is degraded. Their results have shown the complexity of urea kinetics in the ruminant. The findings that have been described, using isotopic dilution techniques have shown how useful the method is and how promising it still is in biochemical and nutritional research. It has made possible the quantitative determination of the utilization or rate of transfer of metabolites in and between body compartments, the estimation of which could not otherwise have been possible without the use of isotopes.

## GENERAL OBJECTIVES

The main objectives of these studies are:

1. To determine the extent to which supplementation of a basal ration of hay (Cynodon nlenfuensis/ Centrosema pubescens) with protein concentrates affects production of ruminal metabolites of nitrogenous origin and blood urea levels, and to assess the efficiency of utilization of hay and concentrate supplements.
2. To determine the utilization of dietary nitrogen for the synthesis of microbial protein, blood urea and milk protein using  $\left[ {}^{15}\text{N} \right]$  ammonium chloride, and the extent of recycling of blood urea into the digestive tract of the sheep using  $\left[ {}^{15}\text{N} \right]$  urea.
3. To estimate the digestibility, nitrogen retention, metabolic faecal nitrogen (MFN), the endogenous urinary nitrogen (EUN), the biological value of the rations and the digestible crude protein requirement for maintenance.
4. To determine the digestibility of the rations in the stomach and intestines of the sheep using chromic oxide - impregnated paper as the marker and to show the reliability of the method for partitioning digestibility in the stomach and intestines of the sheep.

## CHAPTER TWO

## 2. RUMINAL AND BLOOD METABOLITES OF THE WEST AFRICAN DWARF WETHER SHEEP MAINTAINED ON BASAL HAY AND CONCENTRATE SUPPLEMENTS.

2.1 INTRODUCTION

McDonald (1948) had shown that dietary protein and non-protein are degraded by the rumen microbial population and that ammonia is the major end product of the degradation. The nitrogenous substances in the rumen are the feed and microbial protein, ammonia, amines, amides and amino acids. Several investigators (Chalmers and Synge, 1954; Annison, 1956; Lewis, 1957; and Elliott and Topps, 1964) have shown that the levels of these metabolites in the rumen are dependent on the type of ration fed to the animal.

In the present report, ruminal metabolites and blood urea were examined in four fistulated West African dwarf wether sheep maintained on basal hay and concentrate supplements to find the effect of varying levels of dietary protein on the ruminal and blood metabolites.

## 2.2 MATERIALS AND METHODS.

2.2.1 Animals and their management.

Eight West African dwarf wether sheep, 1 - 1.5 years old and with live weights ranging from 13.2 to 26.3 kg were used. Four of the animals were fitted with permanent rumen cannulae. Each sheep

was kept in a metabolism cage as described by Oyemuga (1961).

The animals were usually fed at 8.00 a.m. every day. The residues were collected at 8.00 a.m. every day, weighed, and stored for chemical analysis (in order to estimate nutrient intake). The animals had free access to salt licks and fresh clean water ad lib.

2.2.2 Diets: There were six diets. The basal diet consisted of cynodon nlemfuensis/centrosema pubescens hay. The grass/legume mixture was cut on the field and left to dry for two days, after which the hay was packed and stored in the barn. There were five concentrates ( $C_1 - C_5$ ) composed of cassava flour, groundnut meal, molasses and mineral mixture. The concentrates varied in the crude protein content of dry matter from 1.6 to 17.5%. The chemical composition of the concentrates and hay are shown in Table 2.1. Ration A consisted of 1.0kg of basal hay. Ration B consisted of 0.50kg of the basal hay and 0.50kg of concentrate  $C_1$ . Similarly, rations C, D, E and F consisted of 0.50kg of basal hay and 0.50kg respectively of concentrates  $C_2$ ,  $C_3$ ,  $C_4$  and  $C_5$ .

### 2.2.3 Plan of experiment.

The experiment was divided into two trials. The first trial consisted of two periods and the second trial consisted of four periods in a 4 x 4 Latin square design. (Table 2.2).

In the first trial, the eight animals were divided into two groups of four animal. Each group was made up of two fistulated and



TABLE 2.1

Components and chemical composition of rations fed to the  
West African dwarf sheep.

COMPONENTS	CONCENTRATES				
	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
Groundnut meal	-	4.0	10.0	18.0	28.0
Cassava flour	92.5	88.5	82.5	74.5	64.5
Molasses	5.0	5.0	5.0	5.0	5.0
*Mineral mixture	2.5	2.5	2.5	2.5	2.5
Total	100.0	100.0	100.0	100.0	100.0

%	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	HAY
CP	1.57	5.10	8.35	12.69	17.48	7.70
CF	2.98	4.82	5.08	4.94	4.30	36.30
EE	0.46	0.50	0.60	0.75	0.50	1.40
NFE	89.59	82.71	79.11	75.03	71.95	47.10
ASH	5.40	6.87	6.36	6.59	5.77	7.50
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0

\* 2.5kg of C<sub>23</sub><sup>A</sup> supplies the following:

Vit. A	(1.0)	=	1.25	Fe	(g)	=	15
Vit. D	"	=	0.63	Cu	"	=	10
Mn	(g)	=	40	Co	"	=	0.75
Zn	"	=	30	I	"	=	3.0
				Mg	"	=	5000

TABLE 2.2

Plan of experiment

TRIAL 1

	GROUP 1	GROUP 2
	259, 179	173, 263
PERIOD	186, 268	184, 301
1	A	B
2	B	A

TRIAL 2

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
PERIOD	259, 179	186, 268	173, 263	184, 301
1	C	D	E	F
2	D	E	F	C
3	E	F	C	D
4	F	C	D	E

two intact animals. One group was put on a ration of hay (ration A) and the other group was put on a ration of hay supplemented with cassava flour, molasses, and minerals (ration B).

During the second trial, the eight animals were divided on a live weight basis, into four groups of two animals. Each group consisted of a fistulated and an intact animal, and each pair was allocated at random to each of rations C, D, E and F (Table 2.2). Each trial consisted of a 14-day preliminary period followed by a 6-day collection period. The animals were weighed at the beginning and at the end of each trial period.

#### 2.2.4 Collection of faeces and urine.

A day prior to collection, each animal was fitted with harnesses to which was attached a collection bag for the separation of urine and faeces. The removable trays in the metabolism cage permitted the urine to drain freely into the aluminium tray below it. This was sloped so as to allow easy drainage of urine to its tube at the centre. The tube led to a funnel placed at the mouth of a small plastic bucket below the cage in which urine was collected. The bucket contained 5 ml of 10% Mercuric chloride to prevent microbial breakdown of the nitrogenous components of the urine. Urine volumes were measured immediately after collection and 10% of the daily output was retained. The daily samples of urine for each animal were bulked and stored in a deep freezer at  $-5^{\circ}\text{C}$  until required for analysis.

A polythene bag was placed in the collection bag to allow for easy collection of faeces. The bag was emptied daily before the morning feeding. Faeces were dried to constant weight in the forced-draught oven at 70°C for 48 hours. The daily dried faeces were bulked for each animal, milled with Christy Norris Hamner mill and stored in air-tight glass bottles until required for analysis. However, fresh samples of faeces stored in a deep freezer at -5°C were used for the analysis of N of non-dietary origin.

#### 2.2.5 Sampling of blood and rumen liquor

Samples of rumen liquor and blood were collected during the last three days of the collection period. The animals were allowed to feed from 8.00 to 9.00 a.m., and then the feed was removed. Rumen samples were then taken at 10.00 a.m., 11.00 a.m. and 12.00 noon.

Rumen samples were collected with the method of Alexander (1969) as modified by Mba and Olatunji (1971). The sampling lasted 5 mins during which about 300 ml were obtained. The samples were stored at -5°C until required for analysis.

Blood samples were collected from each fistulated animal at 1.00 p.m., using sterilized needles. The blood samples were obtained from the jugular vein and 5 ml of each blood sample was kept in a sample bottle containing some heparin. The blood was centrifuged at 2000 r.p.m. to obtain the plasma which was stored in the deep freezer at -5°C until required for analysis.

### 2.2.6 Isolation of rumen bacteria and protozoa

Ruminal digesta was strained through six layers of cotton cloth to remove plant debris. Bacteria and protozoa were then separated from the liquid fraction by differential centrifugation method (Blackburn and Hobson, 1960). The microbial samples were freeze-dried at  $-20^{\circ}\text{C}$  for five days.

### 2.2.7 Analytical procedure.

The N contents of feeds, faeces, and urine as well as crude fibre, ether extracts and ash content of the feeds were determined according to AOAC (1970) method, except that Markham's (1942) semi-micro-Kjeldahl apparatus was used for the N determination. Nitrogen of non-dietary origin in faecal samples was determined according to the method of Van Soest and Wine (1967) as modified by Mason (1969). The rumen protein -N after precipitating with 10% TCA and the total ruminal N were determined using Markham's (1942) semi-micro-kjeldahl method. Ammonia -N and blood urea -N were determined by the method of Fawcett and Scott (1960) as modified by Chaney and Marbach (1962).

Amino acid composition of freeze-dried microbial samples was determined by the column chromatographic technique using the automatic Hitachi-Perkin-Elmer amino acid analyser (model KLA - 3B, Hitachi Ltd., Tokyo, Japan), after hydrolysis of 100 mg of each sample with 10 ml of

6N HCl at 110°C for 24 hours. However, small quantities of 2 - Mercaptoethanol (0.5 ml per litre of 6N HCl) were added to the samples to improve the recovery of the amino acids, particularly methionine. The concentration of free  $\alpha$ - amino N in the rumen liquor was determined by the method of Michel (1968).

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## 2.3 RESULTS

The concentrations of the various nitrogenous metabolites in the rumen and blood are given in Table 2.3. Each value with its standard error is a mean for four fistulated animals.

2.3.1 Total ruminal nitrogen (mg/100 ml)

Total ruminal nitrogen, expressed in mg per 100 ml of rumen liquor (mg/100 ml) for ration A was not significantly different from ration B ( $P > 0.05$ ), the mean values being  $40.8 \pm 3.8$  and  $29.9 \pm 2.3$  for rations A and B respectively. The mean differences were highly significant ( $P < 0.01$ ) for the rations in Trial 2. Ration F gave significantly higher levels of total ruminal nitrogen. ( $124.9 \pm 18.5$  mg/100 ml) than rations C, D and E the means of which were  $44.8 \pm 6.4$ ,  $68.4 \pm 5.4$  and  $78.2 \pm 2.2$  respectively.

The mean values of total ruminal nitrogen increased with increasing intake of dietary nitrogen, and also with increasing crude protein content of the ration. Total ruminal nitrogen was correlated with digestible crude protein intake ( $r = 0.56$ ,  $P < 0.05$ ). The highest value of total ruminal nitrogen occurred one hour after feeding and then declined; in some cases, no decline was observed after two hours.

Table 2.5: Ruminal and blood metabolites<sup>†</sup> of the West African dwarf wether sheep maintained on basal hay and concentrate supplements

Metabolites	Trial 1		Trial 2			
	A	B	C	D	E	F
TOTAL RUMINAL N (mg/100ml)	$\bar{x}$ 40.8 ± 3.8	$\bar{x}$ 29.9 ± 2.3	$\bar{a}$ 44.8 ± 6.4	$\bar{a}$ 68.4 ± 5.4	$\bar{a}$ 78.2 ± 2.2	$\bar{b}$ 124.9 ± 18.5
RUMINAL PROTEIN - N (mg/100ml)	$\bar{x}$ 29.4 ± 2.4	$\bar{x}$ 21.7 ± 1.5	$\bar{a}$ 31.5 ± 4.4	$\bar{a}$ 50.6 ± 2.8	$\bar{ab}$ 55.9 ± 1.8	$\bar{b}$ 79.1 ± 9.8
RUMINAL NON-PROTEIN-N (mg/100ml).	$\bar{x}$ 11.4 ± 2.6	$\bar{x}$ 8.2 ± 1.0	$\bar{a}$ 13.3 ± 2.2	$\bar{a}$ 17.8 ± 3.2	$\bar{a}$ 22.3 ± 0.7	$\bar{b}$ 45.8 ± 10.0
* RUMINAL AMMONIA-N(mg/100ml)	$\bar{x}$ 4.7 ± 0.3	$\bar{y}$ 1.2 ± 0.3	$\bar{a}$ 1.8 ± 0.4	$\bar{a}$ 2.4 ± 0.5	$\bar{b}$ 3.8 ± 0.5	$\bar{b}$ 4.5 ± 0.5
RUMINAL RESIDUAL-N (mg/100ml)	$\bar{x}$ 6.8 ± 2.7	$\bar{x}$ 7.1 ± 0.9	$\bar{a}$ 11.6 ± 1.9	$\bar{a}$ 15.2 ± 2.5	$\bar{a}$ 18.7 ± 0.8	$\bar{b}$ 40.2 ± 9.1
RUMINAL $\alpha$ - AMINO-N ( $\mu$ mole/ml)	$\bar{x}$ 3.27 ± 0.21	$\bar{y}$ 1.6 ± 0.16	$\bar{a}$ 2.31 ± 0.44	$\bar{a}$ 3.02 ± 0.31	$\bar{ab}$ 4.79 ± 0.43	$\bar{b}$ 6.29 ± 0.78
PERCENT PROTEIN-N/TOTAL N	$\bar{x}$ 72.7 ± 4.4	$\bar{x}$ 73.1 ± 1.5	$\bar{a}$ 70.3 ± 1.6	$\bar{a}$ 72.5 ± 2.6	$\bar{a}$ 71.4 ± 1.8	$\bar{a}$ 64.6 ± 3.7
PERCENT RESIDUAL-N/NON PROTEIN-N)	$\bar{x}$ 52.0 ± 8.3	$\bar{x}$ 88.5 ± 5.6	$\bar{a}$ 87.1 ± 2.0	$\bar{a}$ 79.2 ± 4.1	$\bar{a}$ 83.9 ± 3.4	$\bar{a}$ 86.8 ± 3.0
AMMONIA-N/TOTAL-N	$\bar{x}$ 11.7 ± 1.5	$\bar{y}$ 3.13 ± 1.50	$\bar{a}$ 3.77 ± 0.92	$\bar{a}$ 3.91 ± 1.80	$\bar{a}$ 4.63 ± 1.93	$\bar{a}$ 4.39 ± 1.59
BLOOD UREA-N (mg/100ml)	$\bar{x}$ 5.3 ± 0.5	$\bar{y}$ 1.4 ± 0.2	$\bar{a}$ 1.9 ± 0.3	$\bar{a}$ 2.6 ± 0.5	$\bar{ab}$ 3.9 ± 1.0	$\bar{b}$ 4.9 ± 0.9

+ EACH VALUE IS A MEAN FOR FOUR ANIMALS

MEANS BEARING THE SAME SUPERSCRIPIT IN THE ROW ARE NOT SIGNIFICANT (P > 0.05)



### 2.3.2 Total Ruminal Protein Nitrogen (mg/100 ml)

The total ruminal protein nitrogen values of  $29.4 \pm 2.4$  and  $21.7 \pm 1.5$  mg/100 ml respectively for rations A and B are shown in Table 2.3. Though the mean value was higher for ration A than for B, the difference was not significant ( $P > 0.05$ ). The mean differences in Trial 2 were highly significant ( $P < 0.01$ ) with the mean values of  $31.5 \pm 4.4$ ,  $50.6 \pm 2.8$ ,  $55.9 \pm 1.8$  and  $79.1 \pm 9.8$  mg/100 ml for rations C, D, E and F respectively. In sheep fed on concentrate-based rations, ruminal protein nitrogen increased with increasing crude protein intake and level of dietary crude protein. The highest concentrations of ruminal protein nitrogen occurred one or two hours after feeding. Individual observations were not significantly variable either within animals ( $P > 0.05$ ) or between periods ( $P > 0.05$ ).

The protein nitrogen expressed as percentage of total ruminal nitrogen were  $72.7 \pm 4\%$  for ration A and  $73.1 \pm 1.5\%$  for B. The mean differences were not significant ( $P > 0.05$ ). The mean value of  $64.6 \pm 3.7\%$  for ration F was lower than the mean values of  $70.3 \pm 1.6$ ,  $72.5 \pm 2.6$  and  $71.4 \pm 1.8$  for C, D and E respectively, although the mean differences were not significant ( $P > 0.05$ ). Ruminal protein nitrogen as percentage of total ruminal nitrogen did not seem to be influenced by intake of nitrogen or by the levels of

dietary crude protein but was remarkably constant for the rations especially from rations A to E. Differences between mean values were not significant either within the experimental animals ( $P > 0.05$ ) or between periods ( $P > 0.05$ ). The lower value for ration F may be due to the fact that for this ration, the products of microbial breakdown of protein was not being as rapidly incorporated into microbial protein in ration F as in the other concentrate rations.

### 2.3.3 Non-Protein Nitrogen (mg/100 ml)

Supplementation of hay with concentrate  $C_1$  depressed the level of non-protein nitrogen (NPN) from  $11.4 \pm 2.6$  to  $8.2 \pm 1.0$  but this depression was not significant ( $P > 0.05$ ). The NPN concentration was higher for ration F ( $45.8 \pm 10.0$ ) than for rations C, D and E which were  $13.3 \pm 2.2$ ,  $17.8 \pm 3.2$  and  $22.3 \pm 0.7$  mg/100 ml respectively, and the mean differences were significant ( $P < 0.05$ ). The NPN increased with increasing dietary nitrogen intake and dietary crude protein percentage of the rations. It is only at the highest nitrogen intake, and when the percentage crude protein in the ration was highest (ration F) that the levels of non-protein nitrogen was high enough to assume significance, due probably to a more rapid breakdown of dietary protein in the rumen than corresponding incorporation of non-protein nitrogenous materials to microbial protein.

### 2.3.4 Ruminal Ammonia Nitrogen (mg/100 ml)

Supplementation of hay with concentrate C<sub>1</sub> (ration B)

mainly cassava flour, very significantly depressed ruminal ammonia nitrogen from  $4.7 \pm 0.3$  mg/100 ml down to  $1.2 \pm 0.3$  mg/100 ml ( $P < 0.01$ ). The mean differences for ruminal ammonia nitrogen were also very highly significant for the rations in Trial 2 ( $P < 0.01$ ) and these were  $1.8 \pm 0.4$ ,  $2.4 \pm 0.5$ ,  $3.8 \pm 0.5$  and  $4.5 \pm 0.5$  mg/100 ml for rations C, D, E and F respectively. Ruminal ammonia nitrogen levels increased with increasing intake of dietary crude protein and also with increasing percentage of crude protein in the rations. Ruminal ammonia nitrogen was correlated with the level of non-protein nitrogen ( $r = 0.91$ ,  $P < 0.05$ ) when concentrate-based rations were fed, and the relationship can also be shown by the following regression equation

$$Y = (0.086 \pm 0.015) X + 0.88$$

$$(r = 0.91) \dots\dots\dots (2.1)$$

where,

Y is the concentration of ruminal ammonia nitrogen (mg/100 ml) and

X is the concentration of ruminal non-protein nitrogen (mg/100 ml).

Even though the mean differences were significant for treatments, low levels of ruminal ammonia were observed even with the ration highest in crude protein content and this shows that ammonia formed

TABLE 2.4

The regression equations showing the relationship between ruminal and blood metabolites and nitrogen utilization in the West African dwarf sheep maintained on hay and concentrate supplements.

	Y	X	REGRESSION EQUATION	r
2.1	RAN	NPN	$Y = (0.086 \pm 0.015) x + 0.88$	0.91*
2.2	$\alpha$ -AA	NPN	$Y = (0.125 \pm 0.020) x + 0.87$	0.95**
2.3	$\alpha$ -AA	RAN	$Y = (1.401 \pm 0.040) x - 0.25$	0.99**
2.4	BUN	NR	$Y = (4.87 \pm 0.14) x - 0.38$	0.99***
2.5	BUN	ND	$Y = (4.44 \pm 0.12) x - 0.32$	0.99**
2.6	BUN	RAN	$Y = (1.10 \pm 0.02) x - 0.05$	0.99**

RAN = Ruminal ammonia nitrogen, mg/100ml

NPN = Non-protein nitrogen, mg/100ml

$\alpha$ -AA =  $\alpha$ -Amino nitrogen,  $\mu$  mole/ml

BUN = Blood Urea nitrogen, mg/100ml

NR = Nitrogen retained, g/day/ $w_{kg}^{0.734}$

ND = Nitrogen digested, g/day/ $w_{kg}^{0.734}$

\* = Significant at 5% level ( $P < 0.05$ )

\*\* = Significant at 1% level ( $P < 0.01$ )

as a result of protein breakdown was efficiently fixed into microbial protein. The high correlation ( $r = 0.91$ ) between ruminal ammonia nitrogen and non-protein nitrogen shows that ammonia formation was very much dependent upon the nitrogen consumed that was converted into non-protein nitrogenous materials.

There were no significant ( $P > 0.05$ ) variations within animals in their ruminal ammonia nitrogen concentration.

The percentage total nitrogen that was ammonia -N was markedly depressed from  $11.7 \pm 1.5\%$  for ration A down to  $3.1 \pm 1.5\%$  for ration B, ( $P < 0.01$ ) by supplementation of hay with concentrate C<sub>1</sub>. In Trial 2, the mean values of the total nitrogen that was ammonia were not different ( $P > 0.05$ ), being  $3.77 \pm 0.92$ ,  $3.91 \pm 1.80$ ,  $4.63 \pm 1.993$  and  $4.39 \pm 1.59\%$  for rations C, D, E and F respectively. Mean values, however, greatly varied between periods and within experimental animals ( $P < 0.01$ ). The mean value was lower in period one than in the other three periods of Trial 2. Mean values were not affected by nitrogen intake or percentage crude protein in the rations. The only ration where ammonia nitrogen formed an appreciable percentage of total ruminal nitrogen was ration A. Mean values for concentrate-based rations were low and seemed to be relatively constant during the first three hours after feeding.

### 2.3.5 Ruminal Residual Nitrogen (mg/100 ml)

The ruminal residual nitrogen or non-ammonia non-protein nitrogen was not affected ( $P > 0.05$ ) by rations in Trial 1, the values being  $6.8 \pm 2.7$  and  $7.1 \pm 0.9$  for rations A and B respectively. In Trial 2, the mean differences were significant ( $P < 0.05$ ). Ration F gave rise to higher concentration of residual nitrogen,  $40.2 \pm 9.1$  mg/100 ml, than rations C, D and E concentrations of which were  $11.6 \pm 1.9$ ,  $15.2 \pm 2.5$  and  $18.7 \pm 0.8$  mg/100 ml respectively. The residual nitrogen concentration increased with increasing levels of dietary crude protein, with total nitrogen intake and also with the concentration of non-protein nitrogen. The concentration of residual nitrogen did not show much variation within animals, between periods or the first three hours after feeding even though the levels were consistently higher one hour after feeding. The residual nitrogen comprises amines, amino acids and some peptides (McDonald, 1948).

Since the residual nitrogen is part of the non-protein nitrogenous materials, it is of interest to note that supplementation of hay with cassava flour increased the percentage of non-protein nitrogen less ammonia from  $52.0 \pm 8.3\%$  to  $88.5 \pm 5.6\%$ . The mean differences were not significantly ( $P > 0.05$ ). For the protein-based rations in Trial 2, there were no significant variation in the percentage of residual nitrogen in the non-protein fraction ( $P > 0.05$ ) and the

mean percentages were  $87.1 \pm 2.0$ ,  $79.2 \pm 4.1$ ,  $83.9 \pm 3.4$  and  $86.8 \pm 3.0\%$  for rations C, D, E and F respectively. The percentage of non-protein nitrogen that was residual -N was not influenced by nitrogen intake or level of dietary crude protein. The results showed that the residual -N formed the major fraction of the non-protein nitrogen of sheep fed hay/concentrate rations especially if the concentrates were rich in readily fermentable carbohydrates. There were no variations in the mean values for experimental animals or periods ( $P > 0.05$ ).

#### 2.3.6 Ruminal $\alpha$ - amino nitrogen ( $\mu$ mole/ml)

The concentrations of ruminal  $\alpha$ - amino nitrogen ( $\mu$ mole/ml) was  $3.27 \pm 0.21$  and  $1.61 \pm 0.16$   $\mu$ mole/ml on rations A and B respectively and the mean differences were highly significant ( $P < 0.01$ ). The mean values were  $2.31 \pm 0.44$ ,  $3.02 \pm 0.31$ ,  $4.79 \pm 0.43$  and  $6.29 \pm 0.78$  for rations C, D, E and F respectively, and the mean differences were significant for the treatments ( $P < 0.05$ ). The levels of  $\alpha$  - amino N increased with percentage crude protein in the ration, dietary nitrogen intake and levels of ruminal non-protein nitrogen. The concentration of  $\alpha$ - amino nitrogen was correlated to that of non-protein nitrogen in the rumen of sheep fed on concentrate - based rations, relationship can be represented by the following regression equation:

$$Y = (0.125 \pm 0.020) X + 0.87 \dots\dots\dots (2.2)$$

where, Y is the concentration of  $\alpha$ - amino nitrogen ( $\mu$ mole/ml) and

X is the concentration of non-protein nitrogen in the rumen (mg/100 ml).

From this equation, it is seen that an increase in the concentration of non-protein nitrogen will lead to a corresponding increase in the concentration of  $\alpha$ - amino acids, showing that  $\alpha$ - amino acids are derived from ruminal non-protein nitrogen. Since a similar relationship was obtained with ammonia and non-protein nitrogen, that increased concentration of non-protein nitrogen caused increase in ammonia nitrogen, it is expected that increase in ruminal ammonia will lead to increase in  $\alpha$ - amino nitrogen. This is in fact so, as shown by the regression equation showing the relationship between  $\alpha$ - amino nitrogen (Y) in  $\mu$ mole/ml and ruminal ammonia nitrogen (X), in mg/100 ml of rumen liquor of sheep maintained on concentrate based rations.

$$Y = (1.401 \pm 0.040) X - 0.25.$$

$$(r = 0.99, P < 0.01) \dots\dots\dots (2.3)$$

It was also observed that supplementation of hay with concentrate ( $C_1$ ) caused a very significant depression of  $\alpha$ - amino nitrogen. Even on the ration richest in digestible crude protein, the  $\alpha$ - amino nitrogen concentration was still low ( $6.29 \pm 0.78$



( $\mu\text{mole/ml}$ ). From this result, it is seen that the levels of  $\alpha$ - amino nitrogen were low on all the rations. There were no variations within experimental animals or between periods ( $P > 0.05$ ).

### 2.3.7 Blood urea nitrogen (mg/100 ml)

The animals on ration B had significantly lower levels of blood urea nitrogen ( $P < 0.01$ ) than those maintained on ration A (Table 2.3). The mean values of blood urea nitrogen (mg/100 ml) were  $5.3 \pm 0.5$ , and  $1.4 \pm 0.2$  mg/100 ml for rations A and B respectively. Thus, supplementation of hay with cassava flour - based concentrate caused a reduction in the concentration of blood urea nitrogen. In trial 2, the mean differences for treatments were significant ( $P < 0.05$ ) and the mean concentrations were  $1.9 \pm 0.3$ ,  $2.6 \pm 0.5$ ,  $3.9 \pm 1.0$  and  $4.9 \pm 0.9$  for C, D, E and F respectively. Blood urea nitrogen increased with increasing dietary nitrogen retained when concentrate-based rations were fed to sheep and the following equation shows the relationship:

$$Y = (4.87 \pm 0.14) X - 0.38 \dots\dots\dots (2.4)$$

$$(r = 0.99 \quad P < 0.01)$$

where, Y is the mean value of blood urea nitrogen (mg/100 ml) and X is the amount of nitrogen retained ( $\text{g/day/w}_{\text{kg}}^{0.734}$ )

Blood urea nitrogen (Y) was also positively correlated with nitrogen digested,  $\text{g/day/w}_{\text{kg}}^{0.734}$  (X) with r value of 0.99 and the

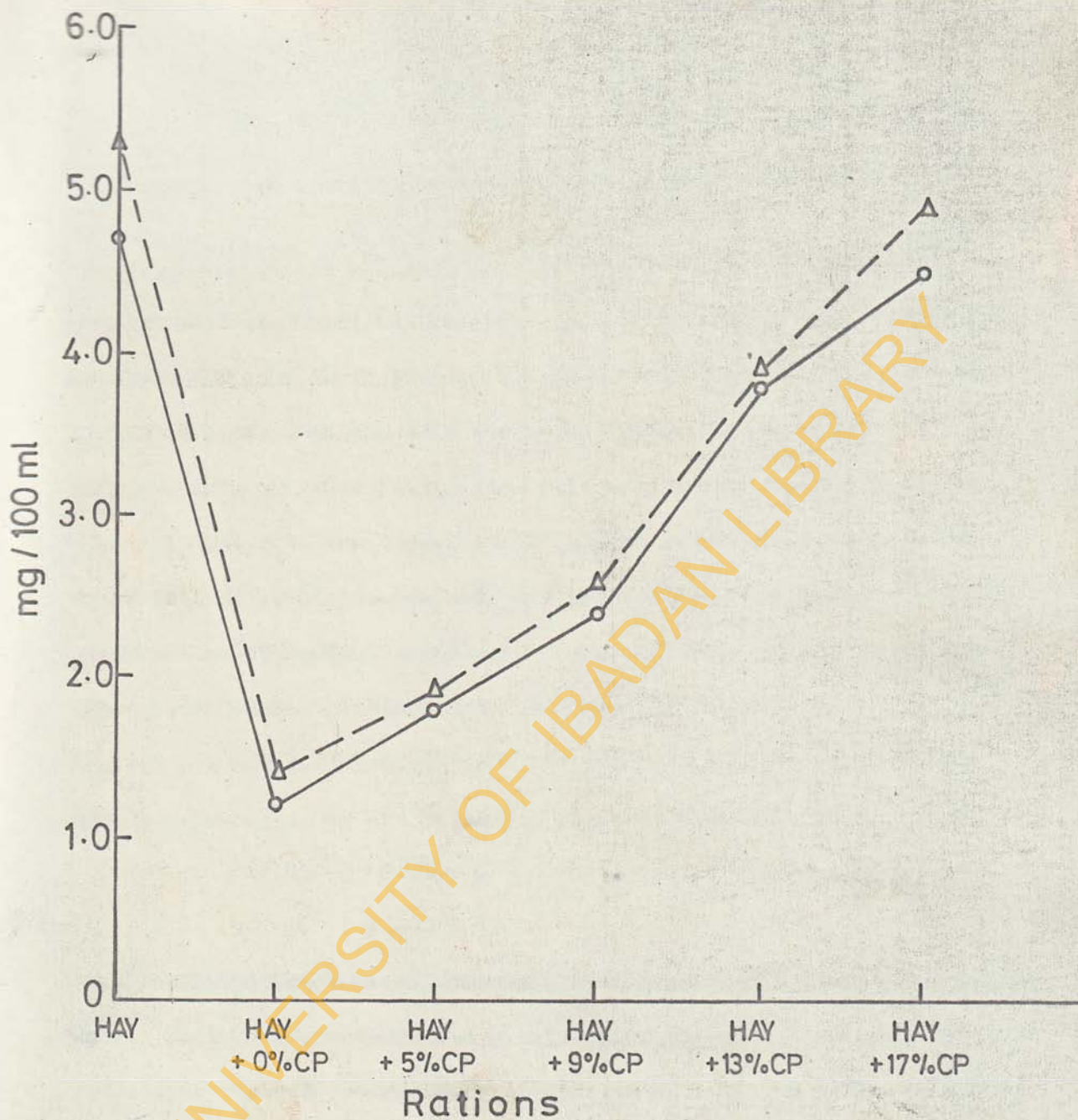


FIG. 2.1 LEVEL OF RUMINAL AMMONIA N (○—○) AND BLOOD UREA N (△—△) IN THE SHEEP MAINTAINED ON HAY AND CONCENTRATE SUPPLEMENTS

relationship can be represented by the regression equation:

$$Y = (4.44 \pm 0.12) X - 0.32 \dots\dots\dots (2.5)$$

when concentrate-based rations were fed to the sheep. The regression equation was similar to the one obtained when blood urea was regressed on nitrogen retained and this shows that blood urea nitrogen was influenced by nitrogen retained as well as nitrogen digested. It was observed that with the experimental rations in trial 2, the highest concentration of blood urea nitrogen was still as low as  $4.9 \pm 0.9\text{mg}/100\text{ ml.}$  Observations of ruminal ammonia nitrogen and blood urea nitrogen showed that concentration of blood urea nitrogen (Y) followed very closely the ruminal ammonia nitrogen concentration (X) and the relationship can be represented by the following regression equation:

$$Y = (1.10 \pm 0.02) X - 0.05 \dots\dots\dots (2.6)$$

$$(r = 0.99, P < 0.01).$$

Thus low levels of ruminal ammonia are associated with low levels of blood urea. There were no variation within experimental animals ( $P > 0.05$ ) but variations between periods were significantly lower in period 1 than in the other periods ( $P < 0.05$ ) of Trial 2.

#### 2.3.8 Amino acid composition of ruminal bacteria and protozoa:

Table 2.5 shows the amino acid composition of ruminal bacteria and protozoa of sheep fed ration F. The results are expressed in g per 16g N and also in g/100g amino acids (g/100g AA). From Table 2.5,

TABLE 2.5

Amino acid composition of ruminal bacteria and protozoa for the West African dwarf wether sheep maintained on basal hay and concentrate supplements compared with reported values

AMINO ACIDS	BACTERIA			PROTOZOA		
	g/16gN	g/100g Amino acid		g/16gN	g/100g Amino acid	
	B	B	B*	P	P	P*
Lysine	8.51	12.60	8.83	8.53	10.40	12.98
Histidine	0.54	0.79	1.95	0.86	1.05	1.60
Arginine	3.14	4.65	5.30	3.73	4.55	3.70
Aspartic acid	8.66	12.81	12.00	8.01	9.77	13.53
Threonine	3.38	5.00	6.00	2.83	3.40	4.75
Serine	2.76	4.08	4.65	3.89	4.77	5.10
Glutamic acid	10.20	15.10	13.95	16.56	20.19	16.28
Proline	2.89	4.27	2.80	5.11	6.23	5.95
Glycine	3.54	5.23	5.88	2.80	3.42	4.10
Alanine	5.62	8.32	7.50	4.96	6.05	4.43
Valine	4.49	6.65	5.20	3.86	4.71	4.03
Methionine	1.52	2.24	1.95	0.71	0.87	1.63
Isoleucine	3.42	5.06	5.23	4.02	4.90	6.10
Leucine	4.64	6.87	7.73	9.96	12.14	7.45
Tyrosine	1.75	2.60	4.33	1.96	2.40	4.23
Phenylalanine	1.90	2.82	4.70	2.97	3.62	5.70

B\*, P\* - Rumen bacterial and Protozoal amino acid composition from Bergen, Purser and Cline (1968), Ration 4.

the amino acid present in the least amount in ruminal bacteria is histidine (0.54g/16gN) and followed by methionine (1.52g/16gN).

Glutamic acid, aspartic acid and lysine are present in the greatest concentration of 10.20, 8.66 and 8.51g/16gN respectively. Of the essential amino acids determined, lysine, leucine, valine and isoleucine were present in greatest concentrations of 8.51, 4.64, 4.49, 3.42g/16gN respectively, while histidine, methionine and phenylalanine were present in lowest concentrations of 0.54; 1.52, and 1.90g/16gN respectively.

The concentration of glutamic acid was very high in ruminal protozoal amino acid (16.56g/16gN). It accounts for about 20% of the protozoal amino acids. Leucine, lysine and aspartic acid followed with concentrations of 9.96, 8.53, 8.01 g/16gN respectively.

Of the essential amino acids, leucine and lysine were present in high concentrations of 9.96 and 8.53 g/16g N respectively, and methionine, and histidine were present in low concentrations of 0.71 and 0.86 g/16g N respectively.

#### 2.4 DISCUSSION:

The concentration of total ruminal nitrogen in the sheep maintained on hay (7.7% crude protein) was  $40.8 \pm 3.8$  mg/100 ml. This value is in very good agreement with the value of 40.6 mg/100 ml obtained by Elliott and Topps (1964) who maintained Persian wethers on

a mixture of hay (8% crude protein). Supplementation of hay with cassava flour-based concentrate significantly reduced ( $P < 0.05$ ) the total ruminal nitrogen to  $29.9 \pm 2.5$  mg/100 ml. This value is ~~is~~ however slightly lower than 34.4 mg/100 ml obtained by Elliot and Topps (1964) for a cassava flour-based ration. They showed that the total ruminal nitrogen was correlated with the crude protein content of the diet. Their values of 49.9, 92.0 and 99.3 mg/100 ml are higher than the present reported values of 44.8, 68.4 and 78.2 mg/100 ml in sheep maintained on rations C, D and E, similar to their low roughage rations. Both this present report and that of Elliott and Topps (1964) showed that maximum level of total ruminal nitrogen occurred about 1 hour after feeding. The unusually high variability associated with the N content of samples of the rumen liquor from sheep given low-roughage diets observed by Elliott and Topps (1964) was not observed in the present work as the mean differences were not significant within animals ( $P > 0.05$ ) or between periods ( $P > 0.05$ ). No sharp increases were observed in the total nitrogen concentration even in the sheep receiving the supplement of the highest crude protein content between 1 and 2 hours after feeding, but there were sharp decreases three hours after feeding.

The concentration of ruminal protein nitrogen,  $29.4 \pm 2.4$  and  $21.7 \pm 1.5$  for rations A and B respectively were higher than 19.8 and

17.1 mg/100 ml obtained for ~~the~~ cassava flour-based rations by Elliott and Topps (1964) but the values of  $31.5 \pm 4.4$ ,  $50.6 \pm 2.8$  and  $55.9 \pm 1.8$  for rations C, D and E respectively are comparable to 29.0, 59.1 and 59.3 obtained by these investigators.

There seemed to be no difference in the percentage of N as protein in the rumen liquor which varied from 64.6% with ration F to 73.1% with ration B. These values are higher than 45 - 65% obtained by Elliott and Topps (1964). They found that percentage of N as protein was negatively correlated with levels of ammonia. No such correlation was observed in the present investigation. Protein-N in the rumen may be derived from feed, bacteria or protozoa. Weller, Gray and Pilgrim (1958) using Di-aminopimelic acid as marker for bacterial protein showed that bacterial protein formed 46%, protozoal 21% and feed 26% of ruminal nitrogen. Freitag et al. (1970) using the same indicator showed that the amount of bacterial protein in the rumen fluid was affected by the dietary nitrogen source. They showed that bacterial protein formed 99% of the rumen fluid protein 7 hours after feeding urea-supplemented ration, the corresponding value for cotton seed meal - supplemented diet was 71%. The rations used in the present investigation contained groundnut meal as source of protein apart from that supplied by the basal hay. The presence of readily fermentable carbohydrates such as cassava flour and molasses enhanced rapid microbial growth. It is therefore reasonable to assume

that a greater percentage of ruminal protein-N would be microbial proteins.

The non-protein fraction (NPN) of rumen liquor is made up of amines,  $\alpha$ - amino acids, ammonia and peptides (McDonald, 1948). The concentration of Non-protein nitrogen was highest 1 or 2 hours after feeding and declined 3 hours after. Annison (1956) showed that casein and groundnut meal were rapidly degraded in the rumen with the formation of non-protein nitrogenous substances. The failure to observe a rapid increase in non-protein nitrogen was due to the presence of readily fermentable cassava flour in the rations, which is in line with the well established observation that the utilization of Non-protein nitrogen is improved when fermentable carbohydrates are also present. Only with ration F containing the highest level of crude protein was an appreciably **high** level of non-protein nitrogen obtained. It may be that in this ration, the rate of proteolysis of dietary protein was greater than the rate of assimilation of the non-protein substances formed.

The supplementation of hay with cassava flour caused a very significant ( $P < 0.01$ ) depression in ruminal ammonia -N. This is in agreement with the findings of Chalmers and Synge (1954), that addition of starch greatly depressed ruminal ammonia production. When rations B to F were fed to the sheep, ruminal ammonia concentration increased with increasing dietary nitrogen intake and percentage of crude protein



in the rations. This is in agreement with the results of Elliott and Topps (1964). Ruminal ammonia concentration was highly correlated ( $r = 0.91$ ) with concentration of non-protein in rumen liquor, which shows that ammonia was obtained by hydrolysis of non-protein substances. Elliott and Topps's (1964) value of 5.9 mg/100 ml. is higher than 4.7 mg/100 ml obtained for ration A but their value of 1.3 mg/100 ml was in very good agreement with present report of 1.2 mg/100 ml for ration A. However, their values of 2.1, 5.2 and 9.7 mg/100 ml are higher than the values of 1.8, 2.4 and 3.8 mg/100 ml obtained in the present investigation. In any case, their rations contained less fermentable carbohydrate than those used in the present experiment.

The highest level of ruminal ammonia nitrogen occurred 1 or 2 hours after feeding. No sharp decline was observed in their levels and this is attributable to efficient fixation of ruminal ammonia by ruminal microbial population (Chalmers and Synge, 1954). The high correlation ( $r = 0.99$ ) between ruminal ammonia nitrogen and ruminal

$\alpha$ -amino nitrogen indicates that both metabolites are dependent and were probably formed from the non-protein nitrogen fraction of the rumen liquor.

The relatively high levels of ammonia -N in the rumen of sheep maintained on only hay may be due to the fact that nitrogen in the

form of urea and mucoprotein was added to the rumen by the saliva and the degradation of these produced high levels of ammonia. This would tend to remain high as microbial protein synthesis would be restricted by a deficiency of available carbohydrate in the ration.

The percentage of total nitrogen in the rumen liquor present in the ammoniacal form was very high in animals given hay ( $11.7 \pm 1.5$ ) and this was depressed to  $3.1 \pm 1.5\%$  when cassava flour was given as supplement. For the concentrate-based rations, there were no differences between the rations even though the tendency was for the percentage to increase with increasing crude protein intake or as total nitrogen in rumen liquor increased. These observations agree with the results of Elliott and Topps (1964). Low levels of ammonia-N ( $4.39 \pm 1.59$ ) as percentage total nitrogen even on ration F showed that very little ammonia-N accumulated in the rumen which could subsequently be lost from the rumen; it indicates efficient utilization of the protein contents of the rations.

Ruminal residual nitrogen, also known as the Non-protein non-ammonia nitrogen, comprises mainly peptides and low levels of  $\alpha$ -amino acids, and amines. Residual nitrogen was low on all rations except on ration F. The mean values for rations A and B, 6.8 and 7.1 mg/100 ml, were lower than those obtained by Elliott and Topps (1964), which were 14.9 and 12.8 mg/100 ml respectively. Similarly,

the values obtained for rations C, D and E which are 11.6, 15.2 and 18.7 were lower than 18.8, 27.7 and 30.3 mg/100 ml obtained by the same investigators. Only on ration F ( $40.2 \pm 9.1$ ) were high levels of residual nitrogen observed.

The residual nitrogen as a percentage of non-protein nitrogen was lower ( $52.0 \pm 8.3$ ) with ration A than with other rations (79 - 88%) although there were no significant differences ( $P > 0.05$ ). This is due to the relatively high levels of ammonia in the rumen of hay-fed animals. Elliott and Topps (1964) and Moore and King (1958) showed that an increase in ammonia concentration in the rumen was accompanied by a decrease in residual nitrogen.

The supplementation of hay with cassava flour significantly depressed the levels of  $\alpha$ -amino nitrogen in the rumen. This is in agreement with the results of Chalmers and Synge (1954), Annison (1956) and Leibholz (1969). For concentrate-based rations, the levels of  $\alpha$ -amino nitrogen increased with dietary nitrogen intake, percentage crude protein in the ration, and levels of ruminal total N and non-protein nitrogen. This is in agreement with the reports of Annison (1956) who also showed that though proteins were almost certainly converted into amino acids before degradation to ammonia, the concentration of free amino acids was usually low presumably because of their rapid uptake or degradation. There was a high correlation between ruminal ammonia and  $\alpha$ -amino nitrogen ( $r = 0.99$ ), and also between

Nonprotein nitrogen and  $\alpha$ -amino nitrogen ( $r = 0.95$ ). These results agree with those of Annison (1956) who showed that increases in ammonia concentration followed similar increases in the concentration of  $\alpha$ -amino nitrogen. He also showed that the increase in the concentration of free  $\alpha$ -amino N in the rumen immediately after feeding were largely due to the presence of free  $\alpha$ -amino N and labile amide N in the feeds.

The marked depression of ruminal ammonia observed when hay was supplemented with cassava flour was also observed in the case of blood urea nitrogen, and this is in agreement with the results of Lewis (1957) who found that changes in ruminal ammonia concentration resulted in similar changes in the blood urea levels. Increase in the concentration of blood urea was observed with increasing intake of dietary protein, and also with the increasing percentage of crude protein in rations B to F. Preston, Schnakenberg and Pfander (1965) obtained high correlation ( $r = 0.986$ ) between nitrogen intake per metabolic size and blood urea nitrogen. Similarly, Wallace, Knox and Hyder (1970) obtained 0.92, 0.92 and 0.77 as the correlation coefficients between blood urea and N intake, digestible N and retained N respectively. The value of  $r = 0.99$  also obtained in the present experiment between blood urea and ruminal ammonia N is high and shows that the regression equation could be used to estimate the blood urea levels at varying concentrations of ruminal ammonia nitrogen

for the rations used in the present experiment.

Preston et al. (1965) suggested that blood urea nitrogen levels could be used to assess protein utilization in lambs. Certain difficulties, however, make this almost impossible. From their results, they showed that blood urea nitrogen in excess of 10 mg/100 ml would indicate adequate protein intake in their ration. It is however, not correct to state that lower levels of blood urea nitrogen necessarily indicate poor nitrogen intake, for factors such as breed, age of animal and percentage of readily fermentable energy in the rations influence blood urea nitrogen levels. However, when the ration is defined, their suggestion could be useful. In the present report, even at the maximum N intake of 1.55 g/day/w<sub>kg</sub><sup>0.734</sup>, the level of blood urea nitrogen was still relatively low (4.9 mg/100 ml). Blood urea levels can be used to assess utilization of dietary protein especially of herbage. High protein herbages are likely to give rise to high levels of ruminal ammonia and subsequently high blood urea levels, which in turn would increase urinary urea excretion (Coccimano and Leng, 1967). The low values of blood urea nitrogen in the present report would then be interpreted to mean that the dietary N were being efficiently utilized. This is supported by very low urinary excretion even on the ration of highest concentration of crude protein. The low levels of blood urea can also show

that an appreciable amount of it was being recycled to the rumen and utilized there.

The results of Weller et al., (1958) and Freitag et al., (1970) showed that appreciable amount of nitrogen presented at the abomasum is of microbial origin. The values ranged from 74% by Weller et al., (1958) with a ration of hay to 99% by Freitag et al., (1970) with urea-based ration. It is therefore essential to know the amino acid composition of microbial protein especially as dietary protein is being replaced by non-protein compounds in ruminant nutrition. The biological value of ruminal bacteria and protozoa were 81 and 80% respectively and true digestibility were 74 and 91% for ruminal bacteria and protozoa respectively (McNaught et al., 1954).

The present report has shown that of the essential amino acids determined, histidine and methionine were present in very low concentration in bacterial protein. This is similar to the report of Bergen, et al., (1968) who also obtained low levels of these two amino acids. However, the value of 1.95 g/100 g amino acids obtained by these investigators for histidine is much higher than the present value of 0.79g/100g amino acids obtained in this experiment. The lysine value of 12.60g/100g amino acid obtained in the present studies is <sup>in</sup>very good agreement with that determined by Abde King and Engel (1964) but much higher than that reported by Bergen et al., (1968). The concentrations of tyrosine and phenylalanine

reported in the present report were lower than those of Bergen et al. (1968) and Abdo et al. (1964).

Similarly for ruminal protozoal protein, the amino acids present in lowest concentration are methionine (0.37g/100g AA) and histidine (1.05 g/100g Amino acids) and were both lower than the result of Bergen et al (1964). From these results, it appears that histidine and methionine present in least concentration might limit the utilization of microbial protein.

The amino acid composition of microbial protein can only give an estimation of limiting amino acids but it can not per se be assumed to limit the efficient utilization of dietary protein. Bergen et al. (1968a) therefore used the plasma amino acid score (PAA-S) method of McLaughlan (1964) and the restricted feeding regimen of rats with 10% protein rations to determine the limiting amino acid of microbial protein. They found that for rumen protozoal protein, histidine was the limiting amino acid. The plasma levels of free histidine in rats fed protozoal protein-based diets for ten days were extremely low, indicating that this acid was most limiting. They found that the limiting amino acid in bacterial protein was cystine, whereas arginine and histidine were the next two least available amino acids. Purser (1970) showed that pepsin was ineffective in releasing arginine from protozoal and bacterial proteins, and this could account for its low concentration in the blood plasma of rats fed microbial proteins.

Land and Virtanen (1959) using labelled ammonium nitrate ( $^{15}\text{NH}_4\text{NO}_3$ ) as major source of nitrogen for lactating goats observed that histidine was very weakly labelled of the amino acids of milk. They suggested that this may be due to the incapability of the ruminal bacteria to synthesize the imidazole ring. Cystine was also weakly labelled. Loosli and Harris (1945) suggested that the low level of methionine in microbial protein may be due to slow rate of synthesis in the rumen. From these results, it is likely that the limiting amino acids of microbial protein are histidine, cystine, methionine, and arginine.

Though the present investigations are comparable to those of Bergen *et al.* (1968), it must be emphasized that the method of preparation of bacterial and protozoal specimens may have brought about the differences observed in the results.



## CHAPTER THREE

### 3. ISOTOPIC STUDIES OF NITROGEN METABOLISM IN THE WEST AFRICAN DWARF WETHER SHEEP

#### 3.1 INTRODUCTION

The feeding of non-protein nitrogen (NPN) supplements to ruminants is based on the knowledge that ammonia is the major end-product of the degradation not only of the NPN but also of proteins in the rumen (McDonald, 1948). Ruminants have been maintained on diets in which the only source of N was either ammonium salts or urea (Loosli *et al.*, 1949; Virtanen, 1966), indicating that all the essential amino acids normally required by non-ruminants can be synthesized from ammonia by the ruminal micro-organisms.

Isotopic methods have been used to determine the rate of production of ruminal ammonia, blood urea and bacterial and protozoal nitrogen in the sheep (Pilgrim *et al.*, 1970; Mathison and Milligan, 1970; and Nolan and Leng, 1972).

In the present report, [ $^{15}\text{N}$ ] ammonium chloride and [ $^{15}\text{N}$ ] urea have been used to examine the rate of entry of ammonia into the ruminal ammonia pool, and urea into the body urea pool in sheep respectively. Estimates of the contribution of ruminal ammonia N to plasma urea and of plasma urea to ruminal ammonia pool, as well as utilization of ruminal ammonia by ruminal bacteria and protozoa were made. The utilization of infused ammonia for production of milk protein was also examined.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Animals and Their Management

Four adult West African Dwarf wether sheep, 2 - 2.5 year old and weighing 19 - 35 kg, each fitted with a permanent rumen cannula, in addition to two intact lactating sheep of the same breed, were used in these studies. The animals were individually housed in metabolism cages (Oyenuga, 1961). Five days before administration of isotope, the wethers were given their daily rations in equal portion at hourly intervals.

### 3.2.2 Diets:

Ration A consisted of a high quality Cynodon nlemfuensis/Centrosema pubescens hay (15.3% crude protein) and 300g of this basal ration were given to each animal. Ration F consisted of 300g of hay and 150g of a groundnut - based concentrate <sup>C</sup><sub>5</sub> (17.5% crude protein), and this was also supplied at hourly intervals, except for the lactating sheep where all the feed was supplied once. The groundnut cake-based concentrate is as already given in Chapter 2, but the chemical composition of the basal hay is shown in Table 3.1.

TABLE 3.1

Chemical Composition of the Basal Hay Fed to the West African Dwarf Sheep

	%
Crude Protein (CP)	15.3
Crude Fibre (CF)	27.5
Ether extracts (EE)	1.2
Nitrogen-free extracts (NFE)	50.5
Ash	5.5
Total	100.0

### 3.2.3 Plan of Experiment

On the day of the experiment, two sheep (Nos 210 and 273) received an aqueous solution (100ml) of  $\left[^{15}\text{N}\right]$  ammonium chloride (250mg, 99% enriched with  $^{15}\text{N}$ ) as a single infusion into the rumen. The other two sheep (Nos 186 and 259) were given a single infusion of  $\left[^{15}\text{N}\right]$  urea (250 mg, 95% enriched with  $^{15}\text{N}$ ) into the blood. The two lactating sheep (Nos. 72 and 90) each received an aqueous solution (100 ml) of  $\left[^{15}\text{N}\right]$  ammonium chloride (200mg, 99% enriched with  $^{15}\text{N}$ ). The solution was administered orally in two equal portions to each sheep at 8.00 a.m. and 2.00 p.m.

### 3.2.4 Collection of Faeces, Urine, Blood, Rumen and Milk Samples:

Samples of 10 ml ruminal fluid were taken at two - hourly intervals and immediately transferred into a deep-freezer at  $-5^{\circ}\text{C}$ . Five samples of ruminal fluid were taken during the ten hour sampling period.

Samples of 15ml of blood were placed in heparinized centrifuge tubes and centrifuged at 2000 rpm for 20 minutes. The plasma was stored in a deep freezer at  $-5^{\circ}\text{C}$ .

Urine from the wethers was collected in a collecting bottle containing 5ml of 10% mercuric chloride solution to prevent microbial breakdown of the nitrogenous components of the urine. Urine from the ewes was collected by using bladder catheters and also stored at  $-5^{\circ}\text{C}$ .

Faeces were collected from the wethers by means of harnesses to which were attached collection bags. Faeces from the ewes were collected from the cage floor at 8.00 a.m. in the morning.

The ewes were hand-milked at 8.00 a.m. The milk samples were stored in a deep - freezer at  $-5^{\circ}\text{C}$ , until required for analysis.

### 3.2.5 Separation of Bacteria and Protozoa in the rumen Liquor

Bacteria and protozoa of ruminal fluid were separated by the differential centrifugation method of Blackburn and Hobson (1960).

### 3.2.6 Analytical Procedures

The preparation of ruminal ammonia and blood urea for isotopic analysis was according to Nolan and Leng (1972), except that distillation was carried out in Markhams's (1942) distillation apparatus. Frothing of samples was prevented by addition of sec-octyl alcohol as the anti-foamant.

Nitrogen in the urine, faeces, milk and microbial samples was determined by the semi-micro kjeldahl procedures with NaOH used as alkaline reagent during steam distillation.

Samples were analyzed for enrichment of  $^{15}\text{N}$  by means of the mass spectrometer at IAEA, Vienna.

### 3.2.7 Theory

Estimation of pool size, urea space and entry rate was by the method of Zilversmith (1960).

$$\text{Pool size (P)} = a \begin{bmatrix} e_1 & -1 \\ e_2 & -1 \end{bmatrix}$$

where,  $a$  = g of labelled material injected or infused,

$e_1$  = atom per cent excess of added material,

$e_2$  = atom per cent excess of isolated material.

$$\text{Urea space} = \frac{\text{Pool size (P)}}{\text{Concentration of urea (g/l)}}$$

$$\text{Entry rate (g)} = \frac{P}{t_{1/2} \times 1.44}$$

where  $t_{1/2}$  is the time for half the enrichment to be lost from the sampled pool.

## 3.3 RESULTS

The recovery of  $^{15}\text{N}$  administered as ammonium chloride into the rumen or as urea into the blood stream is shown in Table 3.2. Low recovery of  $^{15}\text{N}$  injected into the rumen occurred in the urine, and the label was not recovered in the faeces of the four wethers. The result showed that 5.2% and 6.9% of administered dose of  $^{15}\text{N}$  were recovered in the urine of the wethers, and the values for the ewes were 4.4% and 4.8%. Of this amount recovered, 63.3% and 73.9% were recovered during the first 24 hours in the wethers and ewes respectively.

The mean recovery of  $^{15}\text{N}$  from the urine of the wethers after intravenous administration of  $[^{15}\text{N}]$  urea was 30.5%, and 89.8% of this was recovered during the first 24 hours of urination. None of the injected  $^{15}\text{N}$  was recovered in the faeces of the wethers.

The mean recovery of  $^{15}\text{N}$  in the faeces of the ewes was 9.1%, and 43.9% of this was recovered during the first 24 hours of administration.

The result showed that about 3.1% of administered dose of  $^{15}\text{N}$  was recovered in the milk protein, and of this 41.9% was recovered during the first 24 hours after administration. The total amount of isotope recovered in the urine, faeces, and milk of the ewes was about 17.2% of administered dose.

TABLE 3.2

The Recovery of  $^{15}\text{N}$  (Administered Either as Ammonium Chloride into the Rumen or Urea into the Blood) in the Faeces, Urine and Milk of Dwarf Sheep

Animal No.	Live Weight	Nitrogen Intake (g/day)	$^{15}\text{N}$ Administered (mg)	$^{15}\text{N}$ Recovered in Urine (% Administered)		$^{15}\text{N}$ recovered in Faeces (% Administered)		$^{15}\text{N}$ Recovered in milk (as % Administered)		TOTAL $^{15}\text{N}$ recovered (as % administered)
				First 24 hours	Total %	First 24 hours	Total %	First 24 hours	Total	
210	21.3	6.2	65.5	4.2	6.9	0	0	-	-	6.9
273	25.4	10.1	65.5	3.3	5.2	0	0	-	-	5.2
72	28.6	10.1	52.4	2.9	4.4	6.5	8.5	1.82	3.93	16.7
90	19.5	10.1	52.4	3.9	4.2	1.4	9.6	0.79	3.17	17.6
186	27.6	6.2	116.7	30.8	32.3	0	0	-	-	32.3
259	35.0	10.1	116.7	25.9	28.7	0	0	-	-	28.7

## KEY:

0 = Enrichment with  $^{15}\text{N}$  was zero atom % excess.  
 1 = Wethers used (No milk).

The enrichment of ammonia N, blood urea N, bacterial N and protozoal N with time after a single injection of  $[^{15}\text{N}]$  ammonium chloride into the rumen of sheep Nos. 210 and 273 is shown in Figures 3.1 and 3.2 respectively. The ratio of the area under the appearance curve for plasma  $[^{15}\text{N}]$  urea, bacterial  $[^{15}\text{N}]$  and protozoal  $[^{15}\text{N}]$  to the area under the disappearance curve for  $[^{15}\text{N}]$  ammonia, gave the proportion of urea N, bacterial N and protozoal N respectively contributed by ruminal ammonia (Table 3.3).

The results showed that ruminal ammonia N contributed 14.4% of plasma urea N in sheep fed on hay, while the corresponding value for that on hay/concentrate was 5.2%. In the sheep fed on hay, 33.2% of bacterial N and 19.0% of protozoal N were derived from ruminal ammonia N in the period under investigation. Ruminal ammonia N contributed 25.9% and 14.8% of bacterial N and protozoal N respectively in animals fed hay/concentrate ration. The enrichment of the protozoal N with  $^{15}\text{N}$  was 57.2% of that of bacterial N in the sheep fed on hay, and hay/concentrate rations.

The enrichment of blood urea N and ruminal ammonia N with time after a single injection of  $[^{15}\text{N}]$  urea into the blood of the sheep Nos. 187 and 259 is shown in figures 3.3 and 3.4 respectively. The ratio of the area under the appearance



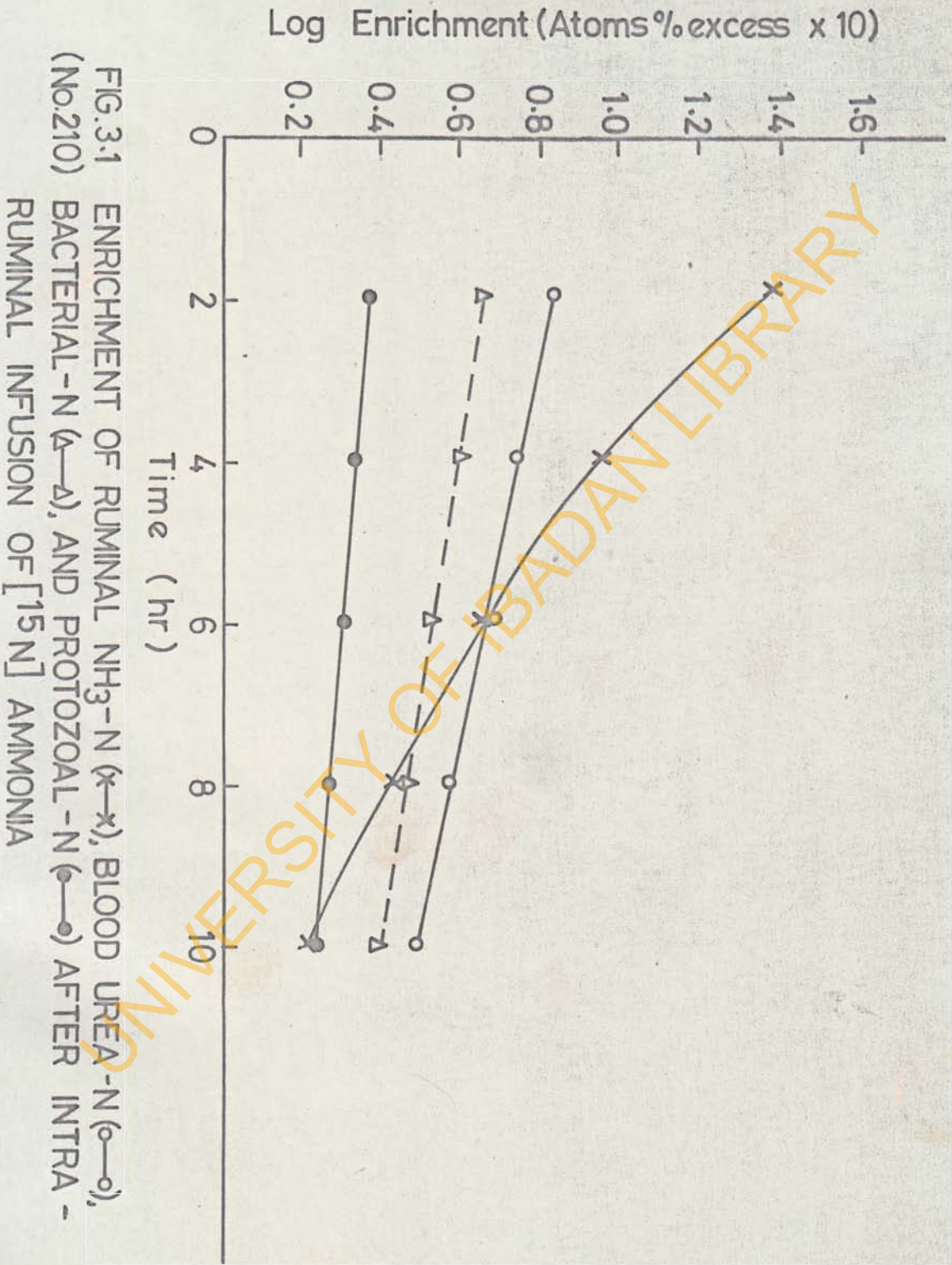


FIG.3.1 ENRICHMENT OF RUMINAL  $\text{NH}_3\text{-N}$  (×—×), BLOOD UREA-N (○—○), BACTERIAL-N (△—△), AND PROTOZOAL-N (◻—◻) AFTER INTRA-RUMINAL INFUSION OF  $^{15}\text{N}$  AMMONIA

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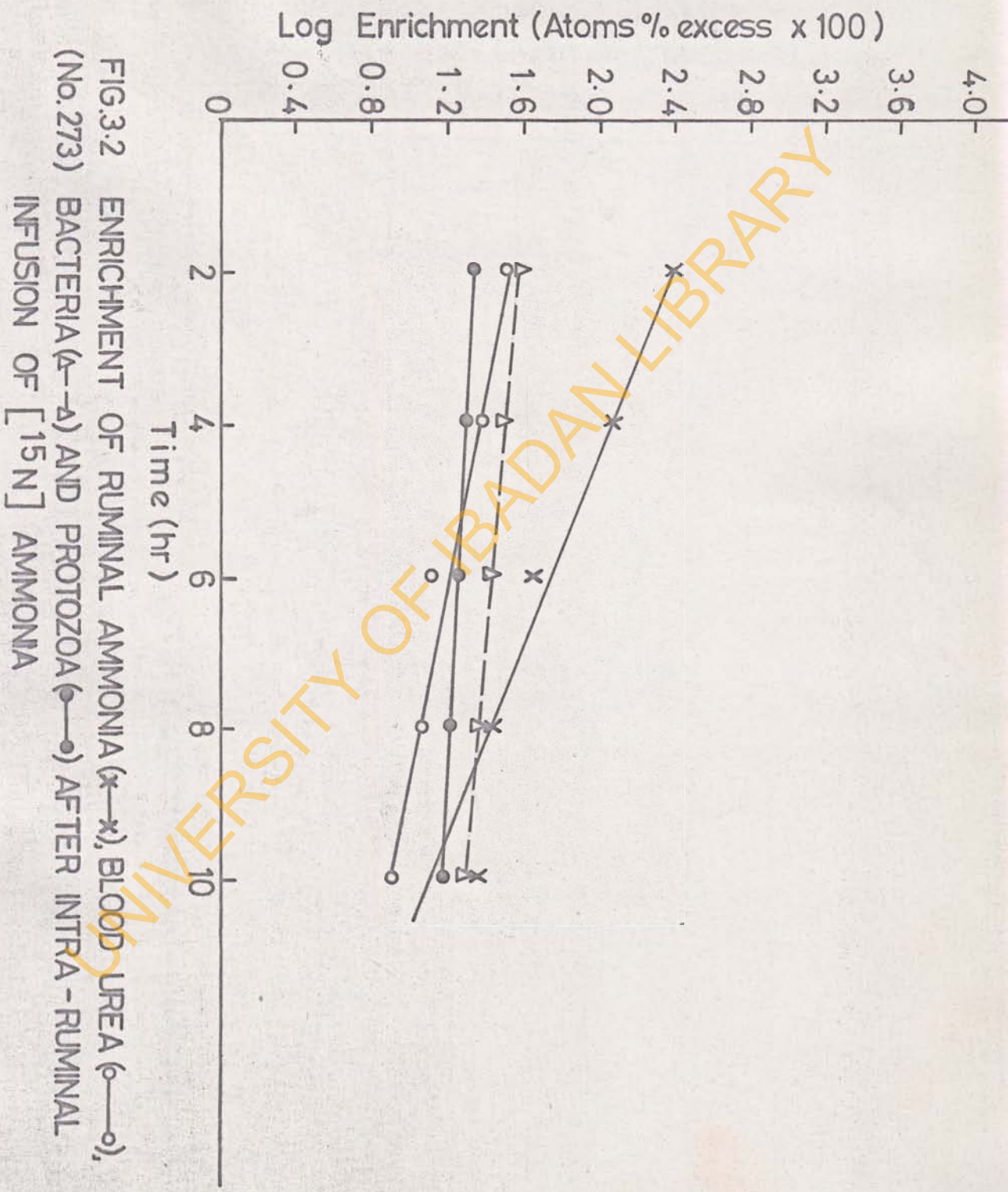


FIG.3.2 ENRICHMENT OF RUMINAL AMMONIA (x-x), BLOOD UREA (○-○),  
 (No. 273) BACTERIA (Δ-Δ) AND PROTOZOA (○-○) AFTER INTRA - RUMINAL  
 INFUSION OF [15N] AMMONIA

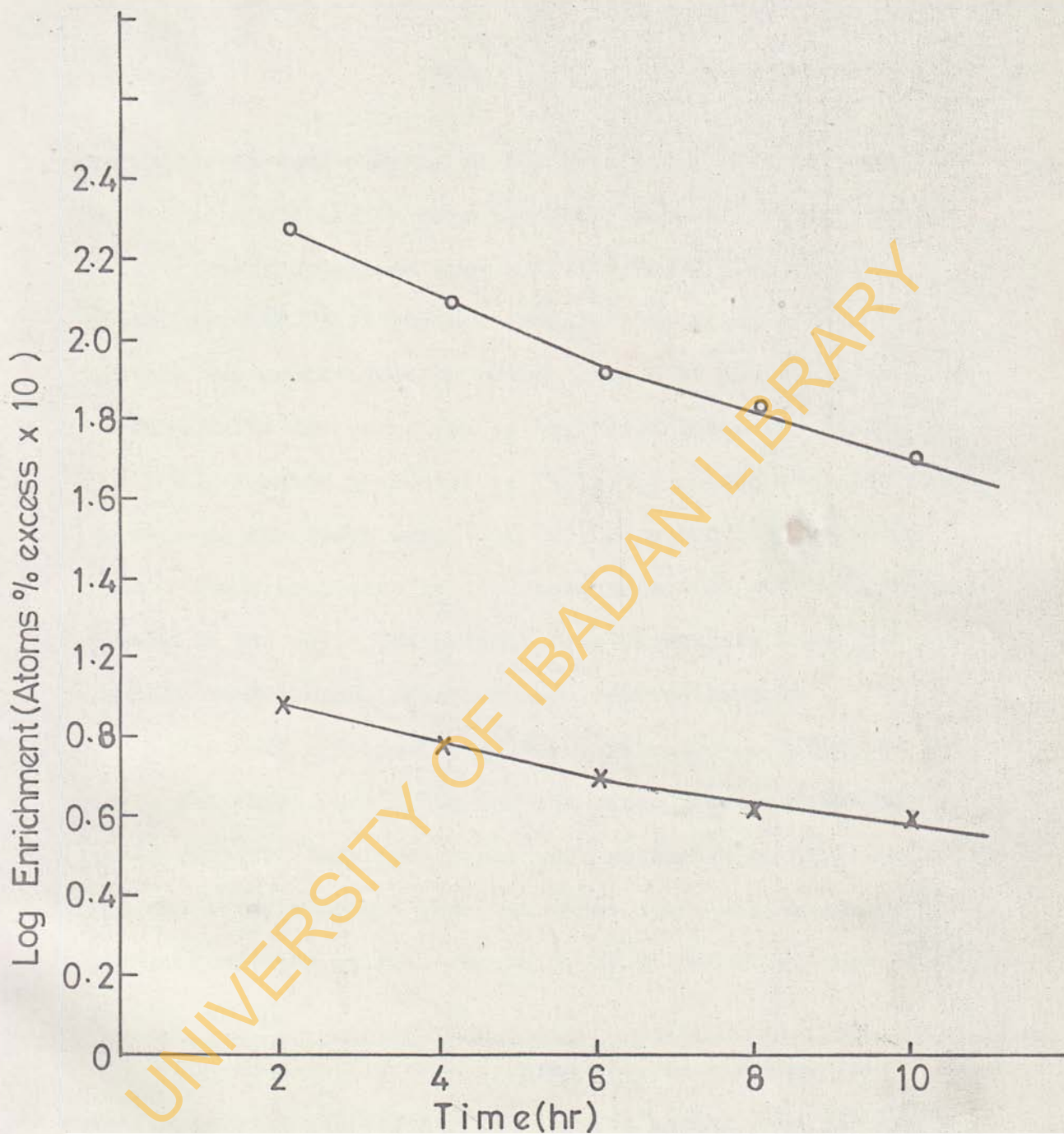


FIG. 3.4 ENRICHMENT OF BLOOD UREA (o—o), AND RUMINAL AMMONIA (x—x) AFTER INTRAVENOUS INJECTION OF  $[^{15}\text{N}]$  UREA

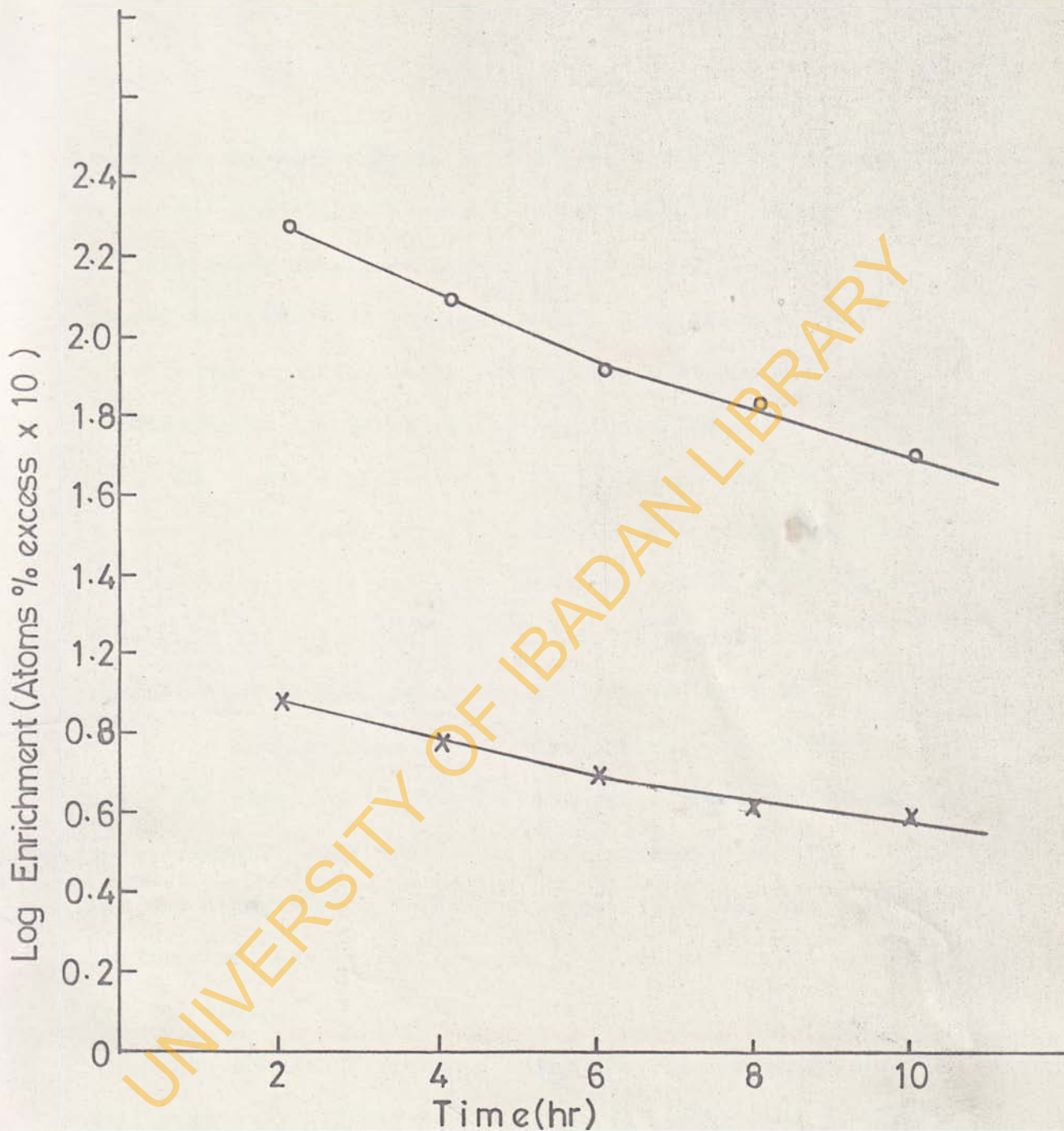


FIG. 3.4 ENRICHMENT OF BLOOD UREA (○—○), AND RUMINAL AMMONIA (x—x) AFTER INTRAVENOUS INJECTION OF  $[^{15}\text{N}]$  UREA (No.259)

curve for ruminal ammonia to the area under the disappearance curve for blood urea N gave the proportion of ruminal ammonia N contributed by plasma urea N (Table 3.3). The results showed that 47.2% of ruminal ammonia - N of hay - fed animals was contributed by plasma urea - N, and the corresponding value for sheep fed on hay/concentrate was 15.2%.

The results presented in Table 3.3 showed that the fluid volumes of the rumen were 5.1L and 6.9 L for the two sheep used. The N pool size in the rumen were 1.03 and 1.25g respectively. In the hay - fed animal, 8.6g of ammonia N entered the ruminal ammonia pool daily and was removed from it.

The body urea pool size were estimated as 3.66g for the hay - fed sheep, and 3.84g for the sheep given concentrate - based ration. Body urea space were estimated as 17.4 and 21.16L for the animals used. When body urea space was expressed as per cent of body weight, values of 62.9% and 62.2% were obtained for the sheep.

The amount of urea - N which entered the blood was estimated as 10.1g/day for the hay-fed animal, and 9.4 g/day for the animal on the concentrate - based ration. The amount of urea - N excreted per day were 2.8g and 4.7g for these animals (Table 3.3). The values obtained when urea excretion (g/day) is subtracted from total entry (g/day) were values of urea degraded in the alimentary tract daily, and is the

TABLE 3.3

Ammonia and Urea Metabolism in the West African Dwarf Sheep  
Estimated by Using Single Infusion of ( $^{15}\text{N}$ ) Ammonium Chloride  
and Single Injection of ( $^{15}\text{N}$ ) Urea into the Rumen and Blood  
Respectively

	A M M O N I A		U R E A	
	No. 210 (ration A)	No. 273 (ration F)	No. 186 (ration A)	No. 269 (ration F)
Live weight (kg)	21.3	25.4	27.6	35.0
N intake (g/day)	6.2	10.1	6.2	10.1
Fluid volume (l)	5.1	6.9	-	-
Ammonia concentration (mg N/100 ml)	20.0	18.0	-	-
Plasma urea concentration (mg N/100ml)	-	-	21.1	18.2
Pool size (g)	1.03	1.25	3.66	3.84
Total entry (g N/day)	8.64	10.32	10.1	9.4
Urea excretion (g N/day)	-	-	2.8	4.7
Urea degradation (g N/day)	-	-	7.3	4.7
Body urea space (l)	-	-	17.4	21.2
Body urea space (% body weight)	-	-	62.9	62.2
Microbial N from ruminal $\text{NH}_3\text{-N}$ (%)				
(a) Bacteria	33.2	25.9	-	-
(b) Protozoa	19.0	14.8	-	-
Plasma urea from ruminal ammonia (%)	-	-	14.4	5.2
Ruminal ammonia contributed by plasma urea (%)	47.2	15.2	-	-

estimate of blood urea transferred to the digestive system. The amount of urea N from blood degraded in the digestive system were 7.3 g/day, and 4.7 g/day for the animals on the basal hay and the concentrate - based ration respectively. These were **72.3%** and **30.0%** of total urea entry per day, for the sheep maintained on the basal hay and the concentrate - based ration respectively.

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## 3.4 DISCUSSION

The validity of measuring urea and ammonia kinetics using a single dose of  $^{15}\text{N}$  in the form of urea or ammonium salt is based on the assumption that the system described is in "steady state", i.e. the compartment sizes and turn-over rates remained constant during the experimental period. Under the hourly feeding conditions used, fairly constant concentrations of ammonia in ruminal fluid and urea in plasma were obtained, indicating that these conditions were largely fulfilled.

The values of 5.2% and 6.9% as the per cent of injected ammonium  $^{15}\text{N}$  recovered in the urine of the wethers, and 4.4% and 4.8% in the ewes obtained in the present experiments were very low compared with values of 38.6% observed by Piva and Silva (1968). Mathison and Milligan (1971), Nolan and Leng (1972) have observed very low recovery of  $^{15}\text{N}$  in the urine of sheep and have suggested that absorbed ammonia might not have been converted into urea in substantial amounts, and that the net retention of labelled N within the tissues of the sheep may not only indicate the extent of net utilization of ammonia, but could reflect exchange within the animal body as well. Nolan and Leng (1972) suggested that some of the absorbed ammonia could have entered nitrogenous compounds (such as amides or non-essential amino acids in the intestinal wall



or liver), which are then incorporated into slowly equilibrating pools of N in body protein. This is consistent with the reports of Piva and Silva (1968) who showed that administered dose of  $^{15}\text{N}$  was incorporated into the muscle protein of the sheep.

From the present report, 30.5% of  $^{15}\text{N}$  administered as [ $^{15}\text{N}$ ] urea through the blood was recovered in the urine, which suggest that administered urea was well utilized by the sheep. Mugerwa and Conrad (1971) showed that from 54 to 90% of injected urea was retained by the sheep. The present report with value of 69.5% retention of injected urea could be taken to mean that injected urea was synthesized into ruminal microbial protein which was then digested and the products utilized for synthesis of tissue proteins of the sheep. However, the percentage of injected urea retained would be influenced by dietary nitrogen intake, it would be high at low N intake, and low at high N intake (Coccimano and Leng, 1967; Mugerwa and Conrad, 1971).

The present report showed that 9.1% of  $^{15}\text{N}$  administered to the lactating sheep was recovered in the faeces. This appears to be in good agreement with the value of 7.9% obtained by Piva and Silva (1968).

The fact that  $^{15}\text{N}$  was not recovered in the faeces of the wethers is very difficult to explain. This may be due to the fact that whereas it was applied in two doses to the ewes, it was applied only in one dose to the wethers, and the loss by absorption from the rumen would be less with the ewes than with the wethers, and a higher proportion of  $^{15}\text{N}$  would be incorporated into microbial protein in the ewes than in the wethers.

The fact that absorbed ammonia may be utilized by a pathway other than the direct conversion into urea was demonstrated by the recovery of injected  $^{15}\text{N}$  in milk protein. In the present report, 3.1% of administered dose was recovered in milk protein and this is lower than the value of 5.6% obtained by Piva and Silva (1968). However, Land and Virtanen (1959) showed that the proportion of ammonia used for milk protein synthesis would be low with liberal protein feeding, and this may explain the observed low value in the present investigation. The total recovery of  $^{15}\text{N}$  in the ewes was 17.2% which showed that 82.8% was retained in the tissues.

The result of the present investigation showed that ruminal ammonia contributed 14.4 and 5.2% of the plasma urea of sheep fed on the basal hay and concentrate - based rations respectively ten hours after infusion of  $^{15}\text{N}$  into the rumen.

Nolan and Leng (1972) obtained the values of 11% and 45% three hours after infusion and at time infinity respectively.

The percentage of bacterial N derived from ruminal ammonia N were 33.2% and 25.9% for sheep fed hay and the concentrate - based ration respectively, ten hours after injection of  $^{15}\text{N}$ . Mathison and Milligan (1971) obtained the value of 50 - 65% as percentage of bacterial N derived from ruminal ammonia N after 144 hours of continuous infusion of  $^{15}\text{N}$  into the rumen.

The percentage of protozoal N derived from ruminal ammonia were 19.0% and 14.8% for sheep fed on hay and concentrate - based ration respectively. The values obtained by Mathison and Milligan (1971) using continuous infusion were 31 - 55% after 144 hours of infusion. The present report with values of 19.0% and 14.8% after 10 hours showed that the ruminal protozoa were utilizing ruminal ammonia at comparably high rate. The enrichment of the protozoal fraction with  $^{15}\text{N}$  was 57.2% of that of the bacterial fraction; the value obtained by Mathison and Milligan (1971) was 56 - 96%. These authors observed that as the concentration of ruminal ammonia increased, there was a decrease in the proportion of ammonia utilized by ruminal bacteria. In the present investigation, ruminal ammonia levels were high and this might contribute to the less

than maximum conversion of ruminal ammonia N into bacterial protein.

An appreciable quantity of blood urea was being taken to the rumen either via saliva or ruminal epithelium. This is shown by the enrichment of ruminal ammonia with  $^{15}\text{N}$  after intravenous injection of  $[\text{}^{15}\text{N}]$  urea. The result showed that ten hours after injection, 47.2% and 15.2% of ruminal ammonia N were derived from blood urea in sheep fed on hay and the concentrate - based rations respectively. These values are higher than the value of 12% obtained by Nolan and Leng (1972) and would appear that a larger quantity of blood urea was entering the rumen in the animals used for the present report.

The total entry rate of ammonia N into the rumen were 8.6g and 10.3g/day in sheep fed on hay and protein concentrate - based rations respectively. The ammonia is removed either by absorption through the ruminal epithelium, or by incorporation into microbial N or by loss through the digestive tract. Nolan and Leng (1972) obtained values 14.2 - 17.2g per day for sheep maintained on high quality hay.

The body urea N pool size obtained in the present investigation were 3.7g and 3.8g for sheep maintained on hay and the protein rations respectively. These are in good agreement with the values of 3.6 to 4.4g obtained by Nolan and Leng (1972). Coccimano and Leng (1967) obtained

values of urea pool size ranging from 0.55 to 12.90g and showed that urea pool size increased with plasma urea concentration.

The values of urea space obtained in the present investigation, were 17.41 and 21.21, and these are in good agreement with the value of 18.7L obtained by Nolan and Leng (1972). Coccimano and Leng (1967) obtained values ranging from 6.3 to 27.0L. Urea space as per cent body weight were 62.9% and 62.2% for sheep fed on hay and mixed ration respectively, and these are within the range of the values of 55 - 66% obtained by Nolan and Leng (1972).

The difference between total entry rate and total excretion rate of urea gave an estimate of the amount of urea degraded in the intestinal tract. The values were 7.3g/day and 4.7g/day in sheep fed on hay and the protein ration respectively, and urea degraded in the digestive tract as percentage of urea entering the body urea pool were 72.3% and 56.6% for sheep fed on hay and protein rations respectively. Coccimano and Leng (1967) obtained values ranging from 1.6 to 12.9g/day as urea degraded, and showed that the percentage of urea entering the body pool that is degraded decreased as dietary N increased, and this shows that in animals maintained on a low level of N, an appreciable amount of N can be supplied by blood urea. Nolan and Leng (1972) obtained the values 5.2

to 8.4gN/day as urea degradation rate in sheep maintained on high quality hay.

The fact that much N can be brought to the digestive tract of the ruminant in the form of urea via ~~saliva or~~ ruminal and intestinal epithelia is of great importance to domestic ruminants in the tropics, which may at times have access only to roughage of 3 - 5% crude protein content at the dry season of the year. The contribution of this blood urea would go a long way to making the ruminant livestock survive adverse climatic conditions particularly in the arid tropics where protein supply is at its lowest ebb.

## CHAPTER FOUR

### 4. THE INTAKE AND DIGESTIBILITY OF DRY MATTER AND NITROGEN METABOLISM IN THE WEST AFRICAN DWARF WETHER SHEEP MAINTAINED ON HAY AND CONCENTRATE SUPPLEMENTS.

#### 4. 1: INTRODUCTION

Even though the potential for meat production by the West African dwarf sheep is realised, there has not been much investigation of the way in which this breed utilizes local forage and concentrates. As a result, the nutrient requirements for this class of livestock are entirely lacking.

In the present report, the intake and digestibility of dry matter (DM) and the metabolism of nitrogen (N) of basal hay and concentrate supplements fed to the West African dwarf wether sheep were examined. Estimates of the crude protein requirement for maintenance as well as the metabolic faecal and endogenous urinary N were also made.

#### 4.2: MATERIALS AND METHODS.

The details of diets, animals and their management, plan of experiment, collection of faeces and urine, as well as analytical procedure are as previously described in Chapter 2.

## 4.3: RESULTS

The results of the digestibility of DM and N obtained with fistulated and intact sheep are presented in Table 4.1. The results for fistulated animals were compared with those of intact animals in order to show if fistulation affected the digestibility of DM and N. The results show that fistulation had no effect on digestibility of DM and N ( $P > 0.05$ ) and that digestive processes in fistulated sheep closely approximated those in intact sheep; therefore the results of the two sets of animals were pooled for subsequent use in the present investigation.

The mean DM intake, digestibility and N metabolism for the West African dwarf wether sheep maintained on basal hay and concentrate supplements are summarized in Table 4.2.

4.3.1 The Dry matter intake.

Supplementation of hay with concentrate C<sub>1</sub> had not significantly ( $P > 0.05$ ) increased daily DM intake with the mean DM intake of  $49.4 \pm 7.8$  g and  $62.4 \pm 5.3$  g DM intake per  $W_{kg}^{0.734}$  for rations A and B respectively. There were no significant increases in DM intake in Trial 2 ( $P > 0.05$ ), with the means ranging from  $61.2 \pm 3.8$  for rations C to  $72.6 \pm 3.3$  g per kilogram metabolic size per day for ration F.

The DM intake increased with increasing levels of dietary crude protein and also with nitrogen intake. The DM intake was not affected by the digestibility of DM even though there was some trends



TABLE 4.1

Effect of ruminal fistulation of the West African Dwarf Wether sheep on digestibility of dry matter and nitrogen contents of basal hay and concentrate supplements

	T R E A T M E N T S											
	A		B		C		D		E		F	
	Fistulated	Intact	Fistulated	Intact	Fistulated	Intact	Fistulated	Intact	Fistulated	Intact	Fistulate	Intact
DIGESTIBILITY OF DRY MATTER (PERCENT)	57.0	55.4	72.7	64.5	75.2	73.3	67.8	69.3	69.4	65.2	66.9	76.8
	57.3	53.6	68.5	72.2	79.4	78.8	70.3	71.9	80.2	72.5	75.0	77.2
	57.9	56.7	70.8	72.0	75.9	71.9	74.1	79.0	73.9	78.4	76.4	77.5
	56.5	57.3	74.7	75.8	80.8	74.1	76.1	76.3	77.9	76.7	76.4	75.8
	MEAN	57.2±0.3	55.7±0.7	71.7±1.2	71.1±2.3	77.8±1.5	74.1±1.3	74.1±2.1	75.4±1.5	73.2±2.5	73.2±2.5	73.7±1.7
DIGESTIBILITY OF NITROGEN (PERCENT)	56.7	57.1	63.4	63.6	59.7	58.6	62.5	58.3	57.4	61.4	72.0	72.4
	53.2	56.4	62.0	49.5	51.0	64.0	67.1	58.7	71.6	65.6	73.4	69.2
	54.9	49.7	56.1	63.8	55.6	-	71.0	73.1	62.4	73.5	70.7	74.2
	59.4	59.7	65.8	65.2	59.0	56.8	65.9	60.7	69.5	70.4	70.2	67.5
	MEAN	56.0±1.7	55.7±2.0	61.8±2.0	60.5±3.3	56.3±1.9	59.8±1.8	66.6±1.8	62.7±3.0	65.2±3.0	67.7±2.5	71.6±0.6

MEAN DIFFERENCES NOT SIGNIFICANT AT 5% LEVEL, BETWEEN FISTULATED AND INTACT ANIMALS IN THE PARAMETERS CONSIDERED.

TABLE 4.2

Dry matter intake, digestibility and N metabolism for the West African dwarf wether sheep maintained on basal hay and concentrate supplements.

	T R E A T M E N T S					
	Trial 1			Trial 2		
	A	B	C	D	E	F
INTAKE OF DRY MATTER (g/day/ $W_{kg}^{0.734}$ )	$x$ 49.4 ± 7.6	$x$ 62.4 ± 5.3	$a$ 61.2 ± 3.8	$a$ 67.8 ± 7.8	$b$ 68.9 ± 3.0	$a$ 72.6 ± 3.3
INTAKE OF NITROGEN (g/day/ $W_{kg}^{0.734}$ )	$x$ 0.62 ± 0.27	$x$ 0.49 ± 0.11	$a$ 0.84 ± 0.07	$a$ 0.91 ± 0.28	$b$ 1.28 ± 0.18	$b$ 1.55 ± 0.17
DIGESTIBILITY OF DRY MATTER OF RATION (%)	$x$ 56.5 ± 1.0	$y$ 71.4 ± 1.2	$a$ 76.5 ± 1.7	$a$ 73.1 ± 1.4	$b$ 74.2 ± 2.4	$b$ 75.1 ± 1.5
DIGESTIBILITY OF DRY MATTER OF CONCENTRATE (%)	-	$x$ 85.5 ± 4.6	$a$ 90.3 ± 2.2	$b$ 83.0 ± 1.1	$b$ 83.8 ± 1.8	$c$ 87.1 ± 2.8
DIGESTIBILITY OF NITROGEN OF RATION (%)	$x$ 55.9 ± 1.0	$x$ 61.2 ± 1.7	$a$ 63.3 ± 0.6	$b$ 69.4 ± 1.6	$b$ 70.0 ± 1.8	$c$ 76.3 ± 1.8
DIGESTIBILITY OF NITROGEN OF CONCENTRATES (%)	-	$x$ 75.7 ± 5.9	$a$ 68.7 ± 1.9	$b$ 78.6 ± 2.4	$b$ 80.6 ± 1.3	$c$ 88.6 ± 2.1
NITROGEN DIGESTED. (g/day/ $W_{kg}^{0.734}$ )	$x$ 0.35 ± 0.05	$x$ 0.30 ± 0.03	$a$ 0.54 ± 0.03	$ab$ 0.63 ± 0.03	$bo$ 0.91 ± 0.03	$c$ 1.20 ± 0.02
NITROGEN RETAINED. (g/day/ $W_{kg}^{0.734}$ )	$x$ 0.29 ± 0.05	$x$ 0.20 ± 0.02	$a$ 0.48 ± 0.04	$a$ 0.59 ± 0.09	$bc$ 0.86 ± 0.08	$c$ 1.11 ± 0.09
NITROGEN RETENTION (%)	$x$ 42.3 ± 2.3	$y$ 51.7 ± 1.7	$a$ 57.5 ± 1.6	$ab$ 63.6 ± 2.7	$b$ 66.5 ± 2.3	$b$ 69.5 ± 1.6

VALUES BEARING SAME SUPERSCRIPT IN THE ROW ARE NOT SIGNIFICANTLY DIFFERENT ( $P > 0.05$ )

TABLE 4.30

The regression equations showing relationships between nutrient utilization in the West African dwarf sheep maintained on hay and concentrate supplements.

Nos	Y	X	REGRESSION EQUATION	r
4.1	DMI	MS	$Y = 0.28 + (0.040 \pm 0.002)X$	0.50**
4.4	log FI	log W	$Y = -1.053 + (0.668 \pm 0.026)X$	0.97**
4.6	DMD	%C-R	$Y = 48.95 + 0.39X$	0.72*
4.7	DP	log% CP	$Y = 12.15 + (56.06 \pm 5.92)X$	0.91**
4.8	DN <sub>p</sub>	C <sub>p</sub> -C	$Y = 62.91 + 1.43X$	0.97**
4.9	DN	$C_p - C = X_1$ $\%C-R = X_2$	$Y = 51.67 + 1.33X_1 - 0.027X_2$	
4.10	DN/kg	NI/kg	$Y = -3.45 + (0.896 \pm 0.025)X$	0.99**
4.11	DC <sub>p</sub>	%CP	$Y = -1.86 + (0.862 \pm 0.020)X$	0.86**
4.12	ND	NI	$Y = 0.163 + (0.863 \pm 0.045)X$	0.99**
4.13	NR	ND	$Y = -0.014 + (0.939 \pm 0.029)X$	0.99**

DMI - Dry Matter Intake,

kg/day

MS - Metabolic Size,

$W^{0.734}$   
kg

FI - Feed Intake,

kg

W - Live-weight of animal,

kg

DMD - Dry matter Digestibility,

per cent

%C-R = Percent Concentrate in ration

DP - Percent Digestibility of Crude Protein of the ration

%CP - Percent Crude Protein in ration.

DN<sub>p</sub> - Percent Digestibility of Nitrogen of concentrate

C<sub>p</sub>-C = Percent Crude Protein in concentrate.

DN/kg - Nitrogen Intake/kg Dry Matter Intake.

DC<sub>p</sub> - Digestible Crude Protein, g

ND - Nitrogen Digested,  $g/day/W^{0.734}$   
kg

NI - Nitrogen Intake,  $g/day/W^{0.734}$   
kg

NR - Nitrogen Retained,  $g/day/W^{0.734}$   
kg

\* Correlation coefficient significant at  $(P < 0.05)$

\*\* Correlation coefficient significant at  $(P < 0.01)$

TABLE 4.3.2

The regression equations showing relationships between nutrient utilization in the West African dwarf sheep maintained on basal hay and concentrate supplements.

Nos	Y	X	REGRESSION EQUATION	r
4.14	NB	NI	$Y = -0.32 + (0.91 \pm 0.006)X$	0.91**
4.15	NB	NI	$Y = -0.20 + (0.83 \pm 0.03)X$	0.98**
4.16	NB	NI	$Y = -0.23 + (0.82 \pm 0.02)X$	0.98**
4.17	NB	NI	$Y = -0.07 + (0.75 \pm 0.02)X$	0.97**
4.18	NB	NI	$Y = -0.07 + (0.73 \pm 0.01)X$	0.99**
4.19	NB	NI	$Y = -0.13 + (0.77 \pm 0.01)X$	0.98**
4.20	FN	NI	$Y = 3.13 + (0.16 \pm 0.02)X$	0.62**
4.21	FN	CP-R	$Y = 3.68 + (0.14 \pm 0.04)X$	0.75*
4.22	UN	NI	$Y = -0.0238 + (0.0096 \pm 0.0011)X$	0.79*
4.23	+FN	NI	$Y = 1.50 + (0.24 \pm 0.06)X$	0.17

NB - Nitrogen Balance,  $g/day/W_{kg}^{0.734}$

NI - Nitrogen Intake,  $g/day/W_{kg}^{0.734}$

FN - Faecal Nitrogen, g/kg Dry Matter Intake

UN - Urinary Nitrogen,  $g/day W_{kg}^{0.734}$

NI\* - Nitrogen Intake, g/day

+ - MFN estimation for ration B.

\* - Correlation coefficient significant ( $P < 0.05$ )

\*\* - Correlation coefficient highly significant ( $P < 0.01$ )

with rations D, E and F of increasing DM intake with increasing DM digestibility.

The effect of supplementation of hay with the concentrates is shown in Table 4.4. The mean DM intake of hay was  $49.3 \pm 7.8$  g/day/ $W_{kg}^{0.734}$ . When the hay was supplemented with concentrate  $C_1$ , which consisted of mainly cassava flour, intake of hay fell to  $39.9$  g/ $W_{kg}^{0.734}$ , while  $22.4$  g/ $W_{kg}^{0.734}$  of concentrate  $C_1$  was consumed. Table 4.4 shows that in Trial 2 with the protein concentrates, there were decreases in the intake of hay and increases in the intake of concentrates. Thus, 100 g of concentrate  $C_1$  replaced 42.0 g of hay while 100g of concentrates  $C_2 - C_5$  replaced 66.8 g, 53.0 g, 54.6g and 49.0 g of hay respectively the mean being  $53.1 \pm 8.0$ . Thus, 100 g of concentrates replaced 53.1 g of hay during the voluntary intake of hay/concentrate rations by the sheep. From Table 4.4 it is seen that the intake of concentrates increased with increasing levels of crude protein in the concentrates but the consumption of hay was almost equal for rations C, D, E and F which contained crude protein. Less of concentrate  $C_1$  than  $C_2 - C_5$  was taken by the sheep.

The proportion of DM consumed which is concentrate increased with increasing crude protein in the concentrate, being 58.5% for ration C and 62.9% for ration F, but 35.9 for ration B. For rations C to F, the means differences of DM taken as concentrates were not significantly different ( $P > 0.05$ ).

It is thus seen from this study that while supplementation of hay (7.7% crude protein) with concentrates increased the total DM

TABLE 4.4

Effect of supplementing basal hay with concentrates on  
Intake of hay fed to the West African dwarf sheep

	Trial 1		Trial 2			
	A	B	C	D	E	F
TOTAL DRY MATTER INTAKE g/day/ $\frac{0.734}{\text{kg}}$	49.3	62.3	61.2	67.8	68.9	72.6
INTAKE OF HAY g/day/ $\frac{0.734}{\text{kg}}$	49.3	39.9	25.4	28.4	25.7	26.9
INTAKE OF CONCENTRATE g/day/ $\frac{0.734}{\text{kg}}$	-	22.4	35.8	39.8	43.2	45.7
INTAKE OF CONCENTRATE AS PERCENT TOTAL INTAKE	-	35.9	58.5	58.2	62.7	62.9
INTAKE OF HAY AS PERCENT TOTAL INTAKE	100	64.1	41.5	41.8	37.3	37.1
AMOUNT OF HAY REPLACED BY 100g OF CONCENTRATE DRY MATTER	-	42.0	66.8	53.0	54.6	49.0

intake, consumption of hay was reduced, and this reduction was greater for concentrates with higher levels of crude protein than for those of low crude protein content. At the same time, the level of consumption of concentrates increased with increasing levels of crude protein in the concentrates.

The following regression equation relating DM intake (Y) kg/day to the metabolic size of the sheep (X), was obtained:

$$Y = 0.20 + (0.040 \pm 0.002) X, \dots\dots\dots (4.1)$$

$$(r = 0.50, \quad P < 0.01)$$

The correlation coefficient was highly significant ( $P < 0.01$ ). This equation can be used to estimate the amount of feed required for the sheep. For example, the live weight of sheep No. 263 was 23.6 kg in the second trial and this is about 10.0 kg when converted to the metabolic size. The animal then requires 0.68 kg DM according to the regression equation. This value is approximately 3% of the live weight of the sheep.

It was found that the equation of the type

$$C = aW^b \dots\dots\dots (4.2)$$

where C is DM consumption in kilogram, and W is the live weight in kilogram, 'a' and 'b' being constants, could be used to estimate intake of DM by the sheep. Regression of log C over log W gives 'b' as the regression coefficient and 'a' as the constant. The equation above can be re-written as

$$\log C = \log a + b \log W \quad \dots\dots(4.3a)$$

$$\text{or } Y = A + b \log X \quad \dots\dots\dots(4.3b)$$

The equation of regression is

$$Y = (0.668 \pm 0.26) X - 1.053 \quad \dots\dots\dots(4.4)$$

The antilog of the constant gives a value of 0.0885. Thus the equation is

$$Y = 0.0885W^{0.668} \quad \dots\dots\dots(4.5)$$

$$(r = 0.91, P < 0.05)$$

The equation shows that DM consumption varies not with the weight but with the metabolic size of the animal ( $W_{kg}^{0.668}$ ). This approximates to 0.67, the exponent relating body weight to surface area.

#### 4.3.2 Dietary N intake:

The mean N intake when expressed on a metabolic body size basis ( $W_{kg}^{0.734}$ , where, W was the mean live weight over the period) were not significant for rations A and B in Trial 1, but highly significant ( $P < 0.01$ ) for rations C, D, E and F in Trial 2. Intake of N increased with increasing levels of dietary crude protein, ranging from  $0.49 \pm 0.11 \text{ g/W}_{kg}^{0.734}$  in ration B to  $1.55 \pm 0.17 \text{ g/W}_{kg}^{0.734}$  in ration F. The mean differences were not significant for animals or periods ( $P > 0.05$ ). Supplementation of basal hay with cassava did not significantly ( $P > 0.05$ ) reduce N intake. In Trial 2, N intake increased with increasing digestibility of the dietary N though not significantly ( $P > 0.05$ ).



#### 4.3.3 The digestibility of dry matter:

The supplementation of hay with cassava flour significantly ( $P < 0.05$ ) increased the DM digestibility of the ration. The DM digestibility for ration A was  $56.5 \pm 1.0\%$ , and was  $71.4 \pm 1.2\%$  for ration B. Mean differences were not significant ( $P > 0.05$ ) for the rations used in Trial 2. There was a slight increase in DM digestibility with increasing levels of dietary crude protein especially with rations D, E and F. From Table 4.2 it is seen that the DM digestibility of ration B containing a concentrate of little crude protein content was just as high as those for the rations containing substantial amount of crude protein in the concentrates.

The DM digestibility (Y) is related to the percent concentrate in the ration (X) by the equation .

$$Y = 0.39 X + 48.9$$

$$(r = 0.72, P < 0.05) \dots\dots\dots(4.6)$$

This shows that DM digestibility of these rations increased with increasing percentage of concentrate present in the rations. From this regression equation, the digestibility of the ration when no concentrate is present is 48.9%. This value is lower than 56.5% obtained for DM digestibility of hay (ration A). On the other hand, when there is no hay in the ration, the digestibility of the concentrates alone is about 88%. This value agrees very well with 86.0% obtained for the DM digestibility of the concentrates alone.

Similarly, it can be shown that when the ration contains 63% concentrate (Table 4.3 ration F), the DM digestibility is 73.5 and this is very

close to 75.1 obtained for DM digestibility of ration F (Table 4.2). The mean differences of DM digestibility were not significant within the experimental animals. ( $P > 0.05$ ).

#### 4.3.4 The digestibility of N.

The mean differences were highly significant ( $P < 0.01$ ) for the treatments in Trial 2. The digestibility values ranged from  $63.3 \pm 0.6$  for ration C to  $76.3 \pm 1.8$  % for ration F. There was a linear increase in digestibility with increasing crude protein intake and percent dietary crude protein, and this relationship (with  $r = 0.91$ ) was represented by the equation

$$Y = (56.06 + 5.92) \log X + 12.15 \dots\dots\dots(4.7)$$

where, Y is the percent digestibility of crude protein of the ration and X is the percent crude protein of the ration. The relationship was also represented by the equation

$$Y = 62.91 + 1.43 X \dots\dots\dots(4.8)$$

$$(r = 0.97)$$

where, Y is the percent digestibility of crude protein of concentrate and X is the percent crude protein of the concentrate.

The digestibility of crude protein of the ration is not only influenced by the percent crude protein of the concentrate but also by the percentage of concentrate in the ration. This relationship was represented by the equation

$$Y = 51.67 + 1.33X_1 - 0.027X_2 \dots\dots\dots(4.9)$$

where, Y is the digestibility of dietary crude protein,  $X_1$  is the percent crude protein in the concentrate and  $X_2$  is the percent concentrate in the ration.

From this equation, the digestibility of dietary crude protein increases with increasing crude protein content of the concentrate but decreases with increasing level of concentrate in the ration, which may be due to increasing metabolic faecal N (MFN) that accompany high levels of concentrates in the rations.

Supplementation of hay with cassava flour did not significantly ( $P > 0.05$ ) increase the digestibility value for the N content of hay, the mean values being  $55.9 \pm 1.0$  and  $61.2 \pm 1.7$  % for rations A and B respectively.

#### The True Digestibility of N.

The true digestibility of rations C, D, E and F used in Trial 2 has been determined by the regression methods and also by the Detergent method (Mason, 1969). The regression of digestible nitrogen per kg DM intake (Y) on N intake per kg DM intake (X) gives the regression equation:

$$Y = (0.896 \pm 0.025)X - 3.45 \dots\dots\dots(4.10)$$

$$(r = 0.99)$$

The coefficient of X which is 0.896 gives the estimate of true digestibility of the rations. Thus, true digestibility of nitrogen in the rations is 89.6 %. Regression of digestible crude

protein (Y) on the percentage crude protein of the ration (X) gives a regression equation:

$$Y = (0.862 \pm 0.020) X - 1.86 \dots\dots\dots(4.11)$$

$$(r = 0.86)$$

The coefficient of X gives an estimate of true digestibility and this is 0.862 or 86.2 %. When the value of the metabolic faecal nitrogen (MFN) is subtracted from the total faecal nitrogen, the faecal nitrogen of dietary value was obtained.

In table 4.5 is given the values of true digestibility for the experimental rations. For ration C, the total faecal nitrogen per kilogram DM intake was  $4.75 \pm 0.68$ ; and metabolic faecal nitrogen was 65.89 % of this. Since the apparent digestibility of ration C was 63.3 %, the true digestibility value is given as

$$63.3 + 65.89 \% \text{ of } [100 - 63.3]$$

$$\text{or } 63.3 + (0.6589 \times 36.7)$$

and this gives the true digestibility to be 87.5 %. When calculated in this way, the true digestibility of rations C, D, E and F are 87.5, 92.9, 88.2 and 90.4 respectively and the mean value is  $89.7 \pm 2.1$ .

The value of true digestibility was also determined using the Detergent method. These values are given in Table 4.6. It is possible to determine true digestibility since the proportion of faecal nitrogen derived from non-dietary origin is known. For instance, for sheep No. 186, the apparent digestibility of nitrogen

TABLE 4.5

True digestibility, biological value and net protein utilization values for the West African dwarf wether sheep maintained on basal hay and concentrate supplements.

Periods	RATION	TOTAL FAECAL NITROGEN (g/kg DM intake) TFN	METABOLIC FAECAL NITROGEN (g/kg DM intake) MFN	% MFN TFN	APPARENT DIGESTIBILITY %	TRUE DIGESTIBILITY %	BIOLOGICAL VALUE	NET PROTEIN UTILIZATION (TDX BV) NPU
1	C	4.75 ± 0.68	3.13	65.89	63.3	87.5	96.3	84.3
2	D	4.07 ± 1.02	3.13	76.90	69.4	92.9	96.0	89.2
3	E	5.27 ± 0.39	3.13	59.29	71.0	88.2	98.9	87.2
4	F	5.25 ± 0.50	3.13	59.62	76.3	90.4	92.6	83.7
MEAN		4.83 ± 0.48		65.42 ± 7.12		89.7 ± 2.1	95.9 ± 2.2	86.9 ± 2.2
	A	5.51 ± 0.14	3.13	56.80	55.9	80.9	85.7	69.3
	B	3.06 ± 0.35	1.50	49.0	61.2	80.2	100.0	80.2
MEAN		4.28 ± 0.72		52.9 ± 3.9		80.5 ± 0.3	92.8 ± 7.1	74.7 ± 5.4

of ration C is 64.1 %. This means that of every 100 g of nitrogen that is consumed, 64.1 g are absorbed and 35.9 g are lost in the faeces. Since the non-dietary faecal nitrogen constitute 77.4% of faecal nitrogen, (Table 4.6), it means that  $0.774 \times 35.9$  g of the faecal nitrogen are of non-dietary origin i.e. 27.8 g. The true digestibility of ration C is therefore  $64.1 + 29.8$  or 91.9 %.

From these calculations, it would be seen that the true digestibility of rations A to F ranged from 89.3 % for ration A to 96.0 % for ration F, with the mean value of  $92.9 \pm 2.3$  %. The values of 89.6 % and 86.2% from regression equations and  $89.7 \pm 2.1$  and  $92.9 \pm 2.3$  obtained from non-dietary faecal nitrogen methods, would appear to be in very good agreement. The true digestibility of nitrogen of the rations may therefore be estimated as being between 86 and 93%.

#### 4.3.5 Absorbed N

Nitrogen digested per metabolic size, was not affected by supplementation of hay with concentrate C<sub>1</sub>, the mean values being  $0.35 \pm 0.05$  and  $0.30 \pm 0.03$  for rations A and B respectively. In Trial 2, differences between means were very highly significant ( $P < 0.001$ ) for treatments, the values for nitrogen digested ranging from  $0.54 \pm 0.03$  on ration C to  $1.20 \pm 0.02$  on ration F. Nitrogen digested per metabolic size increased with increasing levels of crude protein in the ration. It also increases with the nitrogen intake according to

the regression equation given:

$$Y = (0.863 + 0.045) X - 0.163, \dots\dots\dots(4.12)$$

$$(r = 0.99)$$

where,

Y is the nitrogen digested per metabolic size per day, and X is the nitrogen intake, also per metabolic size per day. The mean differences were not significant ( $P > 0.05$ ) within experimental animals. It is of interest to note that nitrogen digested in rations A and B were 0.35 and 0.30 respectively even though the concentrate in ration B contained very little or no digestible nitrogen.

#### 4.3.6 Retained N:

Nitrogen retained per  $W_{kg}^{0.734}$  was the same for the two rations, with the mean values of  $0.29 \pm 0.05$  and  $0.28 \pm 0.02$  for rations A and B respectively. In Trial 2, the mean differences were significant ( $P < 0.01$ ) in nitrogen retained, ranging from  $0.48 \pm 0.04$  for ration C to  $1.11 \pm 0.09$  for ration F. Nitrogen retained increased as nitrogen digested, and the following equation describes the relationship:

$$Y = (0.939 + 0.029) X - 0.014 \dots\dots\dots(4.13)$$

$$(r = 0.99)$$

where, Y is nitrogen retained per metabolic size and X is the N digested per metabolic size. The coefficient of X is 0.939 and this gives an index of biological value, and shows that the animals were definitely utilizing the digested N efficiently. There were also

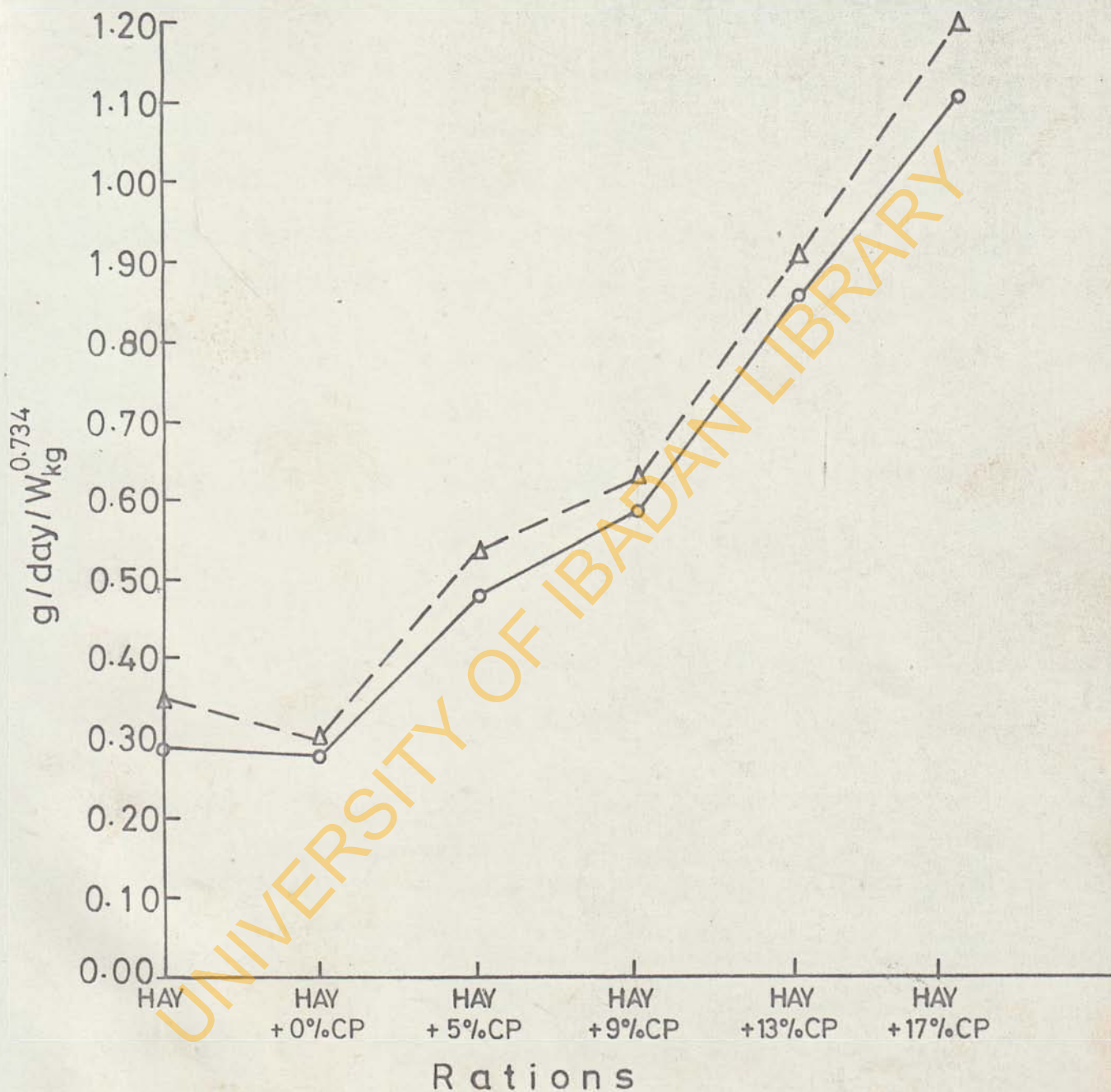


FIG.4.1 THE RELATIONSHIP BETWEEN ABSORBED N ( $\Delta$ - $\Delta$ ) AND RETAINED N ( $\circ$ - $\circ$ ) OF THE SHEEP MAINTAINED ON BASAL HAY AND CONCENTRATE SUPPLEMENTS



very high correlations between N retained, Y (per  $W_{kg}^{0.734}$ ) and N intake, X (per  $W_{kg}^{0.734}$ ) <sup>with</sup> which the r value being 0.91 in Trial 1, and 0.98, 0.98, 0.97 and 0.99 for periods 1, 2, 3 and 4 respectively of Trial 2. When the results of the four periods in Trial 2 were pooled, r value of 0.98 was obtained. Thus the values of retained N increased with increasing dietary N intake, and the relationship is represented by the following regression equations:

For Trial 1;

$$Y = -0.32 + (0.91 + 0.006) X, \dots\dots\dots(4.14)$$

$$(r = 0.91)$$

For Trial 2;

$$\text{Period 1, } Y = -0.20 + (0.83 \pm 0.03) X \dots\dots\dots(4.15)$$

$$\text{Period 2, } Y = -0.23 + (0.82 \pm 0.02) X \dots\dots\dots(4.16)$$

$$\text{Period 3, } Y = -0.07 + (0.75 \pm 0.02) X \dots\dots\dots 4.17$$

$$\text{Period 4, } Y = -0.07 + (0.73 \pm 0.01) X \dots\dots\dots(4.18)$$

$$\text{pooled value, } Y = -0.13 + (0.77 \pm 0.01) X \dots\dots\dots(4.19)$$

From these equations, the N intake at zero - N - balance were, for Trial 1,  $0.35 \text{ g/W}_{kg}^{0.734}$ , and 0.24, 0.24, 0.093 and 0.096 respectively for periods 1, 2, 3 and 4 of Trial 2.

#### 4.3.7 N retention (%)

The percent retention of N of ration B was significantly higher than that of ration A ( $P < 0.05$ ) with the mean value of  $51.7 \pm 1.7$  for ration B and  $42.3 \pm 2.3$  % for ration A. In Trial 2,

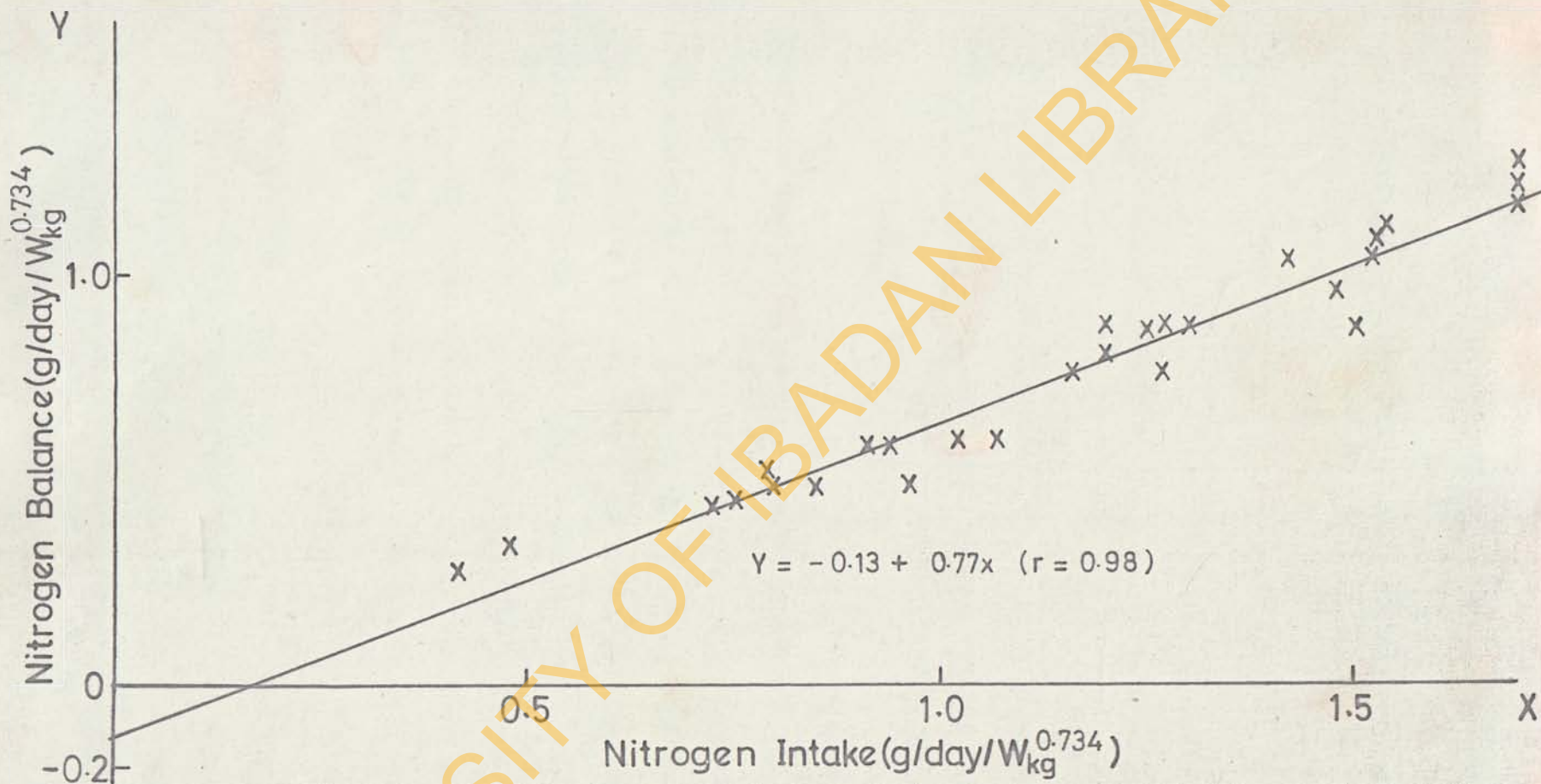


FIG.4.2 THE REGRESSION OF NITROGEN BALANCE ON NITROGEN INTAKE FOR THE SHEEP MAINTAINED ON HAY AND CONCENTRATE SUPPLEMENTS

the mean differences were significant ( $P < 0.05$ ) for treatments C to F in the N retention values, ranging from  $57.5 \pm 1.6$  for ration C to  $69.5 \pm 1.6\%$  for ration F. The N retention followed the same pattern as the digestibility values.

Nitrogen retention increased with increasing intake of N and also with increasing levels of dietary crude protein. The mean differences were not significant within animals ( $P > 0.05$ ).

It is seen by the closeness of the values of N digestibility and N retention that almost all the N digested was retained, with very little loss of N in urine. Two factors may be responsible for this, one is the inclusion of cassava flour, a readily fermentable carbohydrate, and the second is the fact that the animals were young and efficiently utilizing dietary N.

#### 4.3.8. The Metabolic faecal nitrogen (MFN)

Metabolic faecal nitrogen (MFN) which is the faecal N at zero N intake was obtained by two methods, the regression method and the detergent method (Mason, 1969).

The regression of faecal N (Y) in g/kg DM consumed on N intake (X), g/day, gave the following equation:

$$Y = 3.13 + (0.16 \pm 0.02) X \dots\dots\dots(4.20)$$

$$(r = 0.62, P < 0.01)$$

Faecal N at zero N intake is given by this equation as 3.13g/kg DM consumed.

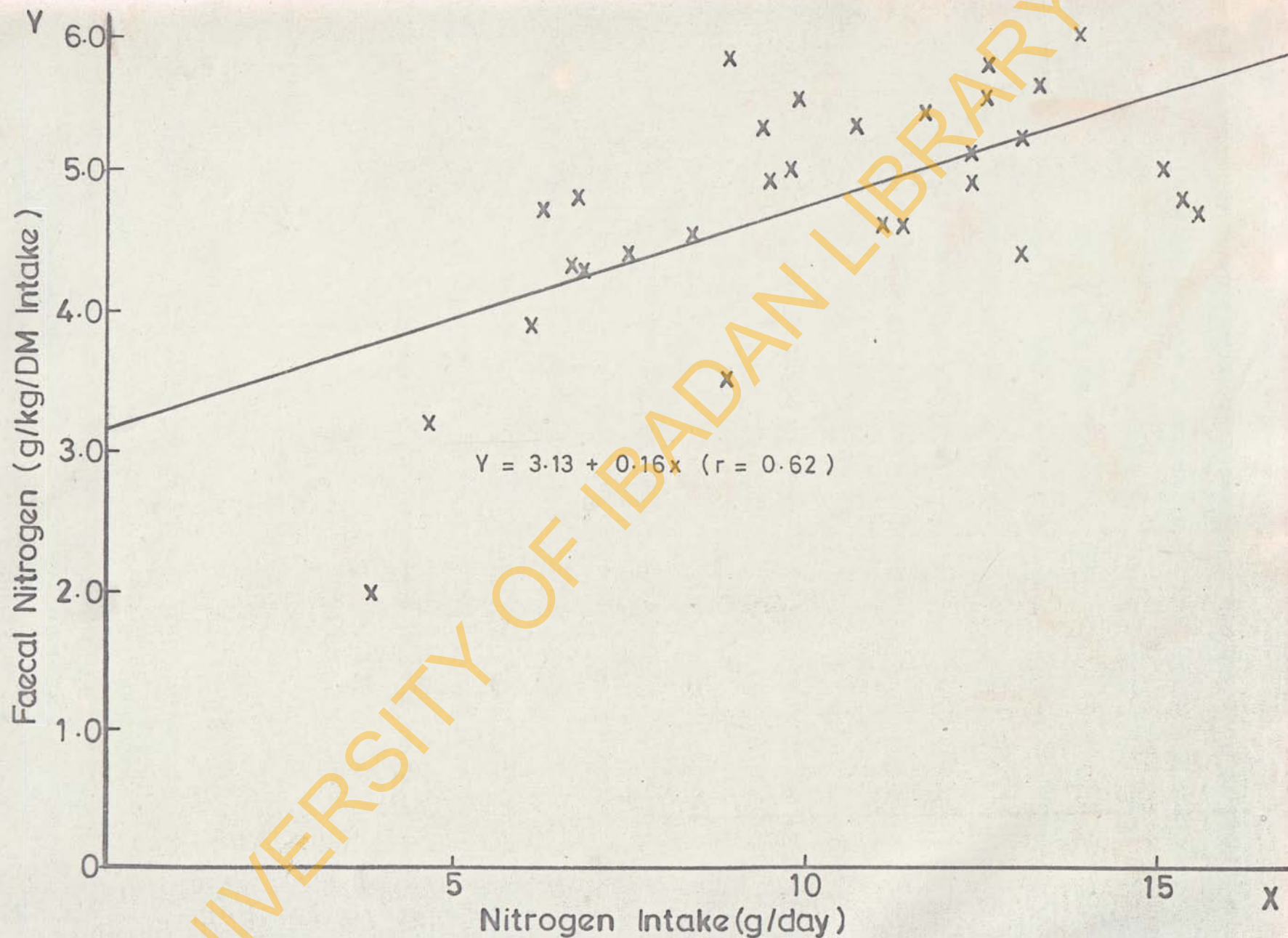


FIG.4.3 THE REGRESSION OF FAECAL NITROGEN ON NITROGEN INTAKE FOR THE SHEEP MAINTAINED ON HAY AND CONCENTRATE SUPPLEMENTS

When the faecal N (Y), expressed in g/kg DM consumed was regressed on percent crude protein in the ration (X), the following equation was obtained:

$$Y = 3.68 + (0.14 \pm 0.04) X \dots\dots\dots(4.21)$$

$$(r = 0.75, P < 0.05).$$

which gives the value of MFN as 3.68 g/kg DM consumed.

Regression of digestible N per kg DM intake (Y) on N intake per kg DM intake (X) gave equation 4.10 which gives the value of the MFN as 3.45 g/kg DM consumed. Similarly, regression of digestible crude protein (Y) on the percent crude protein of the ration (X) gave equation 4.11. The value of MFN from the equation is 1.86 g crude protein per 100 g DM consumed or 2.98g MFN/kg DM consumed.

The values of MFN obtained from regression equations are 3.13, 3.68, 3.45 and 2.98 g MFN per kg DM consumed, giving a mean value of  $3.31 \pm 0.16$ g MFN/kg DM consumed.

It is possible to estimate the value of MFN by the detergent method (Mason, 1969), as stated in Table 4.6. A knowledge of the faecal N per day, DM intake per day and the percent of faecal N that is non-dietary could allow the estimation of MFN. For example, for ration C (Table 4.6), the faecal N per day was 2640 mg and 77.4% of it was of non-dietary origin, that is 2080 mg is the MFN. The DM intake was 608.1 g per day. Therefore MFN is 2080 mg per 608.1 g DM consumed or 340.20 mg/100g DM consumed. The mean value of MFN from

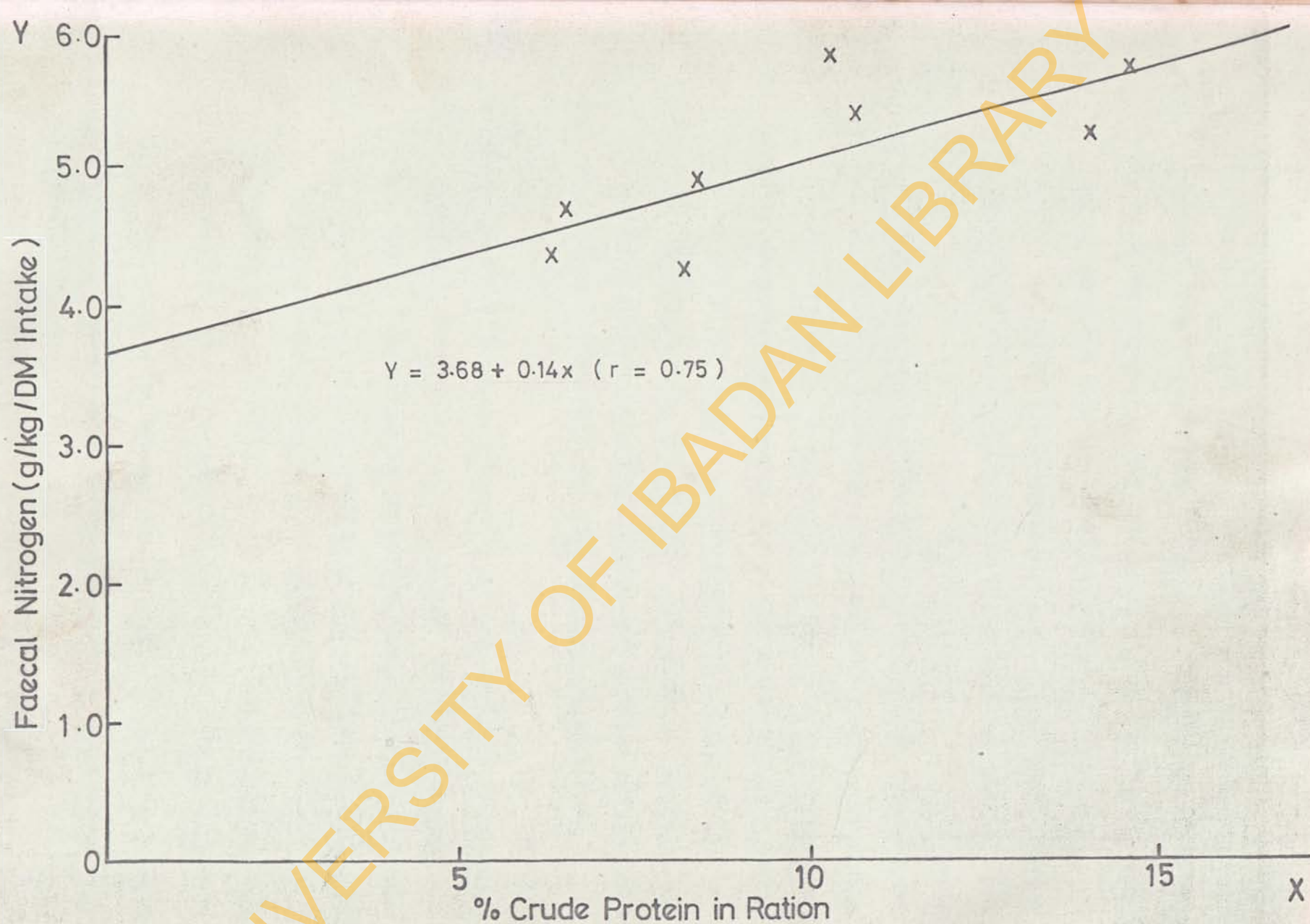


FIG.4.4 REGRESSION OF FAECAL -N ON CRUDE PROTEIN CONTENTS OF BASAL HAY AND CONCENTRATE SUPPLEMENTS FEED TO THE WEST AFRICAN DWARF WETHER SHEEP

Table 4.6 was  $311.94 \pm 28.6$  ng/100g DM consumed or 3.12 g/kg DM consumed. If the lowest two values (158.5 and 215.4) are excluded from the calculation of the mean, the MFN is 3.75 g/kg DM consumed.

The value of the MFN obtained by the detergent method is in very good agreement with those obtained from the regression equations and this indicates that the values of the MFN actually lie between 3.0 and 3.7 g/kg DM consumed for West African dwarf wether sheep weighing 17 to 27 kg and maintained on hay/concentrate rations.

The percentage of undigested nitrogen, microbial nitrogen, water-soluble nitrogen and non-dietary nitrogen in sheep faeces are also given in Table 4.6. About 1 g of faeces from each animal was analyzed for the components of the faeces. Table 4.6 shows that total nitrogen per unit weight of faeces is influenced by the content of nitrogen of the feeds, for while 1 g of faeces from animals on ration A contained about 12.3 mg of nitrogen, the corresponding amount of nitrogen in 1 g of faeces from animals on ration F was 22.4 mg (Table 4.6).

This trend of increasing faecal nitrogen per g of faeces occurred from ration A to ration F except for ration D.

The value of undigested dietary nitrogen recovered in the faeces ranged from 17.6 % for ration F to 24.3 % for ration A, the mean being  $21.1 \pm 3.8$ . The value of undigested dietary nitrogen (UDN) for ration F, 17.6 %, is lower than 24.3 % obtained for the

The Percentage of the Undigested N, Microbial N, Water Soluble N and Non-dietary Faecal N in the Faeces of the West African dwarf wether sheep maintained on hay and concentrates

SHEEP NOS.	RATIONS	TOTAL FAECAL N	UNDIGESTED DIETARY N	MICROBIAL & ENDOGENOUS N (MEN)	WATER - SOLUBLE N	NON-DIETARY FAECAL N (NDFN)	MEN NDFN	APPARENT DIGESTIBILITY %	TRUE DIGESTIBILITY %	FAECAL ng/day	DRY-MATTER INTAKE g/day	METABOLIC FAECAL N ng/day	N F N mg/100g DM INTAKE
186 268	C	100% (15.8mg)	22.6 (3.6)	61.8 (9.6)	15.6 (12.2)	77.4	0.80	64.1	91.9	2640	608.1	2080	340.20
173 263	D	100% (14.3)	18.9 (2.8)	64.5 (9.2)	16.6 (2.4)	81.1 (11.6)	0.80	70.1	94.3	1690	423.1	1360	321.22
184 301	E	100% (17.1)	19.9 (3.4)	63.9 (11.0)	16.2 (2.8)	80.1 (13.8)	0.79	72.8	94.6	2655.8	506.0	2100	416.29
179 259	F	100% (22.4)	17.6 (3.9)	62.1 (15.0)	15.3 (3.4)	82.4 (18.5)	0.82	77.0	96.0	1190	629.3	980	158.45
173 259	A	100% (12.3)	24.3 (3.0)	54.4 (6.7)	21.4 (2.6)	75.7 (9.3)	0.72	55.7	89.3	2460	443.4	1865	420.09
259 173	B	100% (14.0)	23.6 (3.3)	57.9 (8.0)	18.5 (2.6)	76.4 (10.7)	0.76	63.6	91.4	1495	529.6	1140	215.41
MEAN	N	100	21.1	61.6	17.3	78.9	0.78		92.9				311.94
STD	ERROR		=3.8	=6.3	=3.4	=2.5	=0.05		2.3				28.6



basal hay ration. This closeness in value suggests that the much lower digestibility of nitrogen of ration A than ration F is not so much due to its indigestibility but to greater MFN in ration A than in ration F. In the estimation of UDN, there is the tendency of increasing percentage of UDN with decreasing level of crude protein in the ration and the relationship is illustrated as follows:

Rations:	A	B	C	D	E	F
UDN	24.3	23.6	22.6	18.9	19.9	17.6

From this illustration, it may be inferred that not only the apparent digestibility of crude protein but also the true digestibility increased with increasing levels of dietary crude protein.

The percentage faecal nitrogen derived from microbial and endogenous nitrogen (MEN) ranged from 54.4 for ration A to 64.5 % for ration D, with the mean value of  $61.6 \pm 6.3\%$ . The value of MEN seemed to increase with increasing levels of dietary crude protein up to ration D.

Water soluble nitrogen (WSN) accounted for  $17.3 \pm 3.4\%$  of faecal nitrogen, the value being highest for animals on ration A, 21.4 % and lowest for animals on ration F, 15.3 %. The trend observed here is that of decreasing WSN with increasing levels of dietary nitrogen. The WSN may be amines, amides, some amino acids, ammonia, but mainly digestive juices.

Non-dietary faecal nitrogen (NDFN) is obtained by adding the values of microbial and endogenous nitrogen (MEN) and water-soluble

nitrogen (WSN), This is the faecal nitrogen of non-dietary origin. It is apparent that the terms Non-dietary faecal nitrogen (NDFN) and Metabolic faecal nitrogen (MFN) are synonymous. The former term (NDFN) is preferred since there is a tendency to regard MFN as a simple entity representing nitrogen of endogenous origin.

The values of NDFN obtained ranged from 75.7 % for ration A to 82.4 % for ration F. The trend observed here is that of increasing concentration of NDFN with increasing dietary crude protein levels.

The trend followed the same pattern as that of MEN: The mean value of NDFN was  $78.9 \pm 2.5$  %.

The percentage of NDFN that is of MEN origin ranged from 72. % for ration A to 82.4 % for ration F, with the mean value of  $78. \pm 5$  %. The trend observed here is the increase in the percentage NDFN derived from MEN from ration A to ration F.

From the results obtained, it may be concluded that UDN, MEN and WSN accounted for  $21. 1 \pm 3.8$  %,  $61.6 \pm 6.3$  % and  $17.3 \pm 3.4$  % of the faeces of sheep maintained on basal hay and concentrate rations.

#### 4.3.9 Endogenous urinary nitrogen (EUN):

Endogenous urinary nitrogen (EUN) is nitrogen excreted in urine at zero N intake. The value of EUN was obtained by regression of urinary N ( $\text{g/day/W}_{\text{kg}}^{0.734}$ ), Y on N intake (g/day), X. The relationship is given by the following equation:

$$Y = (0.0096 \pm 0.0011) X - 0.0238 \dots\dots\dots(4.22)$$

$$(r = 0.79, P < 0.05)$$

From this equation, the urinary N at zero N intake is the EUN giving a value of  $0.0238 \text{ g/day/W}_{\text{kg}}^{0.734}$ .

#### 4.3.10 The Biological value of the rations:

The true digestibility (TD), Biological value (BV) and Net protein utilization (NPU) values for the rations are presented in Table 4.5.

The BV was determined using the MFN value of  $3.13 \text{ g/kg DM}$  consumed, and  $0.0238 \text{ g/day/W}_{\text{kg}}^{0.734}$  as EUN. The equation used is:

$$\frac{\text{NI} - (\text{FN} - \text{MFN}) - (\text{UN} - \text{EUN})}{\text{NI} - (\text{FN} - \text{MFN})} = \text{BV} \dots\dots\dots(4.23_a)$$

where, NI is the N intake, FN is the faecal N, UN is the urinary N, EUN is endogenous urinary N, and BV is the biological value of the ration.

The BV for the basal hay ration was 85.7, while the BV for the protein-based rations ranged from 92.6 % for ration F to 98.9 % for ration E with the mean BV of  $95.9 \pm 2.2$  %. Maximum BV, (100.0%) was obtained with ration B, and minimum BV (85.7%) was obtained for the basal hay. The BV were also high for rations C (96.3 %) and D (96.0 %), these containing 6.5 and 8.5 % crude protein respectively.

From the BV determinations, it is seen that the protein of hay and mixed rations are very well utilized by the growing sheep.

It also shows that maximum BV was obtained with ration B which supplied the least amount of crude protein ( $0.49\text{g/day/Wkg}^{0.734}$ ). The BV of the basal hay (85.7 %) is almost as high as those of the mixed rations.

#### 4.3.11 The coefficient of net utilization of dietary proteins (NPU).

The coefficient of net utilization of dietary protein is the product of true digestibility (TD) and the biological value (BV) and is also known as the net protein utilization (NPU). From Table 4.5 the coefficient of net utilization was calculated as:

$$\frac{\text{TD} \times \text{BV}}{100} \dots\dots\dots (4.23_b)$$

The value for ration B was 80.2 % and the value for basal hay was 69.3, but the values ranged from 83.7 to 89.2 for the protein-based rations. The ration with the highest level of crude protein (ration F, 14 % crude protein) had the lowest value for the coefficient of net utilization of the protein-based rations. The mean value for protein-based rations was  $86.1 \pm 2.2$  %.

#### 4.3.12 The protein requirement for the maintenance of West African dwarf wether sheep.

The digestible crude protein (DCP) required for maintenance is the amount of digestible crude protein intake required to keep the animal in zero N - balance or N equilibrium.

The regression equations obtained when N - balance,  $\text{g/day/W}_{\text{kg}}^{0.734}$ , was regressed on N intake,  $\text{g/day/W}_{\text{kg}}^{0.734}$ , are given in Table 4.7.

The N intake at zero N-balance in Trial 1, with rations A and B was  $0.35 \text{ g/day/W}_{\text{kg}}^{0.734}$ , the crude protein intake at zero N balance was  $2.20 \text{ g/day/W}_{\text{kg}}^{0.734}$ . The mean digestibility of rations A and B was 58.5 %, and the DCP required for maintenance was estimated as  $1.28 \text{ g/day/W}_{\text{kg}}^{0.734}$  or  $1.73 \text{ g/day/kg}$  live weight.

In Trial 2, N intake at zero N balance were 0.24, 0.28, 0.093 and  $0.096 \text{ g/day/W}_{\text{kg}}^{0.734}$  for period 1 to 4, which are equivalent to 1.51, 1.75, 0.58 and  $0.59 \text{ g crude protein/day/W}_{\text{kg}}^{0.734}$ . Since the mean digestibility coefficient of ration C, D, E and F was 70 %, the DCP required for maintenance were estimated as 1.06, 1.23, 0.41 and 0.41 in periods 1, 2, 3 and 4 respectively of Trial 2. When these are expressed per kg live weight, the values are 1.44, 1.70, 0.56 and  $0.56 \text{ g/day/kg}$  live weight for periods 1, 2, 3 and 4 respectively. There was a sharp decrease ( $P < 0.05$ ) in the value of the DCP required for maintenance in the 3rd and 4th period of Trial 2, therefore the mean value for periods 1 and 2, and also of periods 3 and 4 were used. The mean estimate of DCP required for maintenance in periods 1 and 2 of Trial 2 is  $1.15 \pm 0.08 \text{ g/day/W}_{\text{kg}}^{0.734}$  or  $1.57 \pm 0.13 \text{ g/day/kg}$  live weight, while the mean value for periods 3 and 4 is  $0.41 \text{ g/day/W}_{\text{kg}}^{0.734}$  or  $0.56 \text{ g/day/kg}$  live weight.

The regression equation obtained when the four periods of Trial 2 were pooled gave an estimate of the mean DCP requirement for

TABLE 4.7

Digestible crude protein requirement of the West African dwarf sheep maintained on basal hay with concentrate supplements

TRIAL	REGRESSION	NITROGEN INTAKE AT ZERO N - BALANCE (g/day/ $W_{kg}^{0.734}$ )	CRUDE PROTEIN INTAKE AT ZERO N BALANCE (g/day/ $W_{kg}^{0.734}$ )	DIGESTIBLE CRUDE PROTEIN REQUIREMENT FOR MAINTENANCE (g/day/ $W_{kg}^{0.734}$ )	DIGESTIBLE CRUDE PROTEIN REQUIREMENT FOR MAINTENANCE (g/day/kg Live Weight)
1	$Y = -0.32 + 0.91X$	0.35	2.20	1.28	1.73
2	(i) $Y = -0.20 + 0.83X$	0.24	1.51	1.06	1.44
	(ii) $Y = -0.23 + 0.82X$	0.28	1.75	1.23	1.70
MEAN		0.26	1.63	1.15	1.57
	(iii) $Y = -0.07 + 0.75X$	0.093	0.58	0.41	0.56
	(iv) $Y = -0.07 + 0.73X$	0.096	0.59	0.41	0.56
MEAN		0.095	0.59	0.41	0.56
I-IV	$Y = -0.13 + 0.77X$	0.170	1.06	0.74	1.01

maintenance as  $0.74 \text{ g/day/W}_{\text{kg}}^{0.734}$  or  $1.01 \text{ g/day/kg}$  live weight.

From the results, it is obvious that the DCP requirement for maintenance was high in Trial 1 and in the first two periods of Trial 2 but sharply declined during the 3rd and 4th periods of Trial 2. The stage of growth of the sheep will therefore indicate the DCP requirement for maintenance. A value of  $0.74 \text{ g/day/W}_{\text{kg}}^{0.734}$  may be taken as the mean DCP requirement for maintenance over the experimental period.

The factorial method of the Agricultural Research council (1965) was also used to estimate the digestible crude protein (DCP) requirement for maintenance and growth (Table 4.8). The equations used were:

$$\text{DCP requirement for maintenance and growth (g/day)} \\ = 6.25 \left[ (\text{UE} + \text{G}) \frac{100}{\text{BV}} + \text{MF} \left( \frac{100}{\text{BV}} - 1 \right) \right] \dots\dots\dots(4.24)$$

where UE = Urinary endogenous loss (g/day)

G = Retention of nitrogen in live weight gain, estimated as 2.5 % of gain for sheep.

BV = Biological value of protein

MF = Metabolic faecal nitrogen, estimated as  $kD \text{ g/day}$ , where D is dry matter intake in kg/day and k is the metabolic faecal nitrogen, g N/kg Dry matter intake.

The estimates of the DCP requirement for maintenance and growth are summarized in Table 4.8. The values of urinary endogenous

TABLE 4.8

The estimate of the digestible crude protein requirement for maintenance and growth of the West African dwarf wether sheep maintained on hay and concentrate supplements by the factorial method.

TRIALS	Initial Weight of animals (kg)	Final weight of animals (kg)	Mean weight of animals (kg)	Live weight change g/day	Mean weight of animals $(W_{0.734})$ (kg)	Mean Dry matter intake (kg/day)	Endogenous Urinary N (g/day)	Metabolic Faecal (g/day)	Mean Biological value of rations	Digestible crude protein requirement for maintenance (g/day)	Digestible crude protein requirement for maintenance $(g/day/W_{0.734} \text{ kg})$	Digestible crude protein requirement for maintenance and growth (g/day)	Digestible crude protein requirement for growth (g/day)
1	19.5 $\pm$ 1.3	19.6 $\pm$ 1.4	19.5	2.5	8.9	0.48	0.21	1.50	92.8	2.17	0.24	2.57	0.40
2	19.6 $\pm$ 1.4	20.5 $\pm$ 1.3	20.0	11.3	9.0	0.59	0.21	1.84	95.9	1.93	0.20	3.58	1.75
MEAN						0.53 $\pm$ 0.05	0.21	1.67 $\pm$ 0.17	94.3 $\pm$ 1.5	2.00 $\pm$ 0.17	0.22 $\pm$ 0.02	3.07 $\pm$ 0.50	1.08 $\pm$ 0.62



loss and metabolic faecal nitrogen used were  $0.0238\text{g/day/W}_{\text{kg}}^{0.734}$  and  $3.13\text{g/kg}$  Dry matter intake respectively. The values of DCP requirement for maintenance were 2.17 and  $1.83\text{g/day/sheep}$ , or 0.24 and  $0.20\text{g/day/W}_{\text{kg}}^{0.734}$  for trials 1 and 2 respectively, with a mean of  $2.00\text{g DCP per day/sheep}$  or  $0.22\text{g/day/W}_{\text{kg}}^{0.734}$ . The mean DCP requirement for maintenance, obtained by the factorial method is much lower than the value of  $0.74\text{g/day/W}_{\text{kg}}^{0.734}$  obtained by the nitrogen balance method. There was no substantial gain in weight in trial 1 but estimate based on trial 2 gave the value of DCP requirement for growth as  $1.75\text{g/day per sheep}$  or  $0.19\text{g/day/W}_{\text{kg}}^{0.734}$ .

#### 4.4 DISCUSSION

The results of comparison of digestibility in fistulated and intact sheep showed no significant differences in the digestibility of dry matter and protein contents of the feeds. This is in agreement with the reports of Dorori and Loosli (1959) and Hayes, Little and Mitchell (1964). Hayes et al. (1964) used all hay, all concentrates and equal parts of hay and concentrate in rumen fistulated and intact steers. Lesperance and Bohman (1963) reported that rumen fistulation significantly lowered the apparent digestibility of DM, crude fibre, gross energy and organic matter in four rations. Connor, Bohman, Lesperance and Kinsinger (1963) found similar results for Dry matter, crude protein, and organic matter in forages but

Ridley, Lesperance, Jensen and Bohman (1963) concluded that rumen fistulation did not affect pasture digestibility. Periodic removal of rumen contents from the fistulated animals may have affected digestion in these pasture trials. When the animals have just been fed the rumen is distended and if the cannula is not well placed, there is a tendency for loss of ruminal digesta. In the present report, the animals were kept in the cage, and the cannulae were permanently fixed. The problem of leakage of digesta from the rumen did not occur. Moreover, except on the animals maintained on hay, the ruminal digesta was not fluid but a little semi solid which would also prevent leakage from the animals. The investigators mentioned have all used fistulated steers. There has been no report for sheep but it has always been assumed that it would resemble that of the steer. The present report using fistulated West African Dwarf sheep confirms that rumen fistulation particularly if permanently fixed does not have any deleterious effects on the digestibility and metabolism of dry matter and crude protein and on the general well being of the animals.

The mean value of DM intake of the sheep in the present report was  $49.4 \pm 7.8 \text{ g/day/W}_{\text{kg}}^{0.734}$  for hay (7.7 % crude protein). This value is lower than  $54.6 \text{ g/day/W}_{\text{kg}}^{0.734}$  obtained for lambs of similar live weight by Elliott and Topps (1963) but similar to the value of  $49.2 \text{ g/day/W}_{\text{kg}}^{0.734}$  obtained for sheep maintained on Dactylis glomerata hay (crude protein 6.7 %) by Crabtree and Williams (1971).

The supplementation of hay with cassava flour had increased total dry matter intake though not significantly ( $P > 0.05$ ), and this agrees with the reports of Elliott and Topps (1963) and of Crabtree and Williams (1971) that total DM and digestible energy intake increased when hay was supplemented with energy - rich concentrates. Dry matter intake in Trial 2 increased with increasing levels of dietary crude protein even though this was not significant ( $P > 0.05$ ). Elliott and Topps (1963) showed that voluntary intake of low protein feeds by sheep is closely related to the nitrogen content of the feed.

In the present experiment, there were slight increases in DM intake with increasing DM digestibility of rations D, E and F. This relationship was not significant but agrees with the reports of Elliott and Topps (1963) who obtained a low correlation ( $r = 0.273$ ,  $P > 0.05$ ) between DM intake and digestibility. The value of  $72.6 \pm 3.3$  g/day/ $W_{kg}^{0.734}$  obtained in this report as intake for ration F is close to  $74.5$  g/day/ $W_{kg}^{0.734}$  obtained for a similar ration for sheep by Elliott and Topps (1963).

The supplementation of hay with concentrate  $C_1$  (ration B) mainly cassava flour has resulted in the decrease in hay DM intake. When the concentrates containing crude protein were fed in Trial 2, the intake of hay DM fell progressively as concentrate DM intake increased. This seems to contradict the findings of Blaxter and Wainman (1963) that appetite of sheep for hays of low protein content is increased by supplementation with concentrate foods. Crabtree and

Williams (1971) found with hay of a higher digestibility of energy (60.7 %), that concentrate feeding reduced hay intake. In the present report, when the basal hay was supplemented with concentrates, 100g DM of C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> and C<sub>5</sub> replaced 42.0, 66.8, 53.0, 54.6 and 49.0 g respectively of hay DM., the mean value being 53.1 ± 8.0.

Blaxter, Wainman and Wilson (1961) showed that when concentrates were added to high quality fodder, the DM consumption of fodder fell by slightly less than the amount of DM consumed as concentrates, for instance, that 100g DM of concentrate replaced 79 g of fodder. They showed, however, that when concentrates were added to the ration of poor quality fodder, 100 g of concentrate DM replaced 47 g of poor quality hay.

The value of 53.1 g obtained in the present investigation is slightly higher than 47 g obtained by Blaxter *et al.* (1961).

The intake of concentrate DM increased with increasing levels of crude protein in the concentrate. Similarly intake of concentrate as percentage total DM intake also increased with increasing crude protein content of the concentrate. The amount of DM intake by the sheep in the present investigation was estimated as 3% of body weight or about 6 % of the metabolic weight, a value used in several feeding experiments (Bergen *et al.*, 1968).

When equation 4.2,

$$C = aW^b$$

is used to estimate DM intake, 'b' was obtained as 0.668 in the present report. This shows that DM consumption of these rations is related to the live weight of the sheep raised to the power of 0.668. Blaxter et al. (1961) showed that DM consumption is influenced by the nature of the ration; consequently, the value of the exponent 'b' will also be influenced by the ration. Blaxter et al. (1961) obtained 0.70 as the value of 'b' for rations of hay.

The present report with the value of  $0.668 \pm 0.026$  is not different from 0.66, the exponent which relates body weight to body surface area, and slightly lower than 0.734, also the exponent relating basal energy metabolism to body weight (Bredy, 1945). The indication in the present report of a value of 'b' similar to 0.67 which confirms the reports of other investigators may perhaps be attributed to the necessity for the sheep under tropical conditions to maintain homeothermy by heat loss through the body surface and hence to a closer relationship between metabolism (in this case as indicated by intake) and body surface, than between intake and body weight (Butterworth, 1966).

The West African dwarf wether sheep used consumed slightly less nitrogen in ration B (basal hay + Supplement C<sub>1</sub>) than in ration A (basal hay); concentrate C<sub>1</sub> contained little protein (1.57%) but the concentrate served mainly as a source of energy. Since intake of this concentrate did not improve the intake of hay, the

major source of dietary nitrogen, it is expected that reduction in hay intake with supplementation with concentrate C<sub>1</sub> will lead to some reduction in total intake of nitrogen. In Trial 2, nitrogen intake increased with increasing level of crude protein in the concentrate, which is in agreement with the findings of Robinson and Forbes (1970) who used weaned lambs. From this, it is seen that lambs are likely to take low amount of nitrogen in a ration of low crude protein content, which may not be sufficient to maintain them in nitrogen equilibrium. In the present investigation, all the animals were in positive nitrogen balance indicating that nitrogen intake was adequate at least for maintenance since the animals did not put on much weight. The fact that supplementation of hay (7.7 % crude protein) with energy did not significantly reduce total nitrogen intake offers an advantage in animal feeding. In places where the crude protein level in herbage is just adequate (about 8 %), supplementation of herbage with energy while removing the limitation to animal growth due to energy, would not introduce shortage of crude protein. It is likely that the animals would consume sufficient energy for their requirement but not too much as to significantly reduce their intake of herbage, the major source of crude protein. This situation may not, however, apply if the crude protein content of herbage is very low (about 4%). In this case, nitrogen intake may be so low as to make nitrogen limiting to the growth of the animals.

Robinson and Forbes (1970) showed with lambs fed on soya bean meal that the nitrogen intake per metabolic size were 0.53, 1.03, 1.60 and 1.81 g when the rations contained 7.3%, 13.3%, 19.8% and 25.1% crude protein respectively which, in the present studies, the values of 0.84, 0.91, 1.28 and 1.55 g per  $W_{kg}^{0.734}$  were obtained.

The DM digestibility of ration B was significantly ( $P < 0.01$ ) higher than that of ration A. This is in agreement with the results of Crabtree and Williams (1971) who showed higher DM digestibility of mixed rations than for hay alone. The DM digestibility of rations B to F were high, no doubt, due to the presence of concentrate supplements. The supplementation of hay of low DM digestibility with a concentrate of high DM digestibility is expected to lead to higher DM digestibility of the mixed ration. There was no marked effect of level of dietary crude protein on DM digestibility even though slight DM digestibility increases were observed with rations D, E and F. Robinson and Forbes (1970) had observed linear increase in DM digestibility with increasing crude protein intake.

In the present report, there was a slight increase in DM digestibility with increasing DM intake especially for rations D, E and F. The present report that DM digestibility of a ration increases with increasing proportion of concentrate in the ration, is in agreement with the findings of Crabtree and Williams (1971).

The lower digestibility of DM in period 1 than periods 2 to

4 of Trial 2 reflects the higher intake of highly digestible concentrate fraction during periods 2 to 4 than in period 1 (Robinson and Forbes (1970)).

Similarly, supplementation of the basal hay (7.7% crude protein) with the concentrate (C<sub>1</sub>) did not significantly affect its digestibility. Campbell, Shorroff and Ishizaki (1969) found that supplementation of Kikuyu grass (Pennisetum clandestinum), containing 5.5% crude protein, with energy decreased its digestibility of nitrogen, and they concluded that the depression of apparent crude protein digestibility resulted from increased metabolic faecal nitrogen. Fick, Ammerman, Gowan, Loggins and Cornell (1973) reported improved digestibility of nitrogen of poor quality grass (3.28% crude protein) with supplementation with energy in the form of corn meal, sucrose and starch, when energy-rich concentrates formed about 25% of the rations but that at higher levels of supplementation, energy did not affect digestibility of nitrogen contained in hay.

The findings that supplementation of hay with energy-rich concentrates lead to increased metabolic faecal nitrogen would seem to depend on the quantities of the concentrates added to the basal forage and the quality of the forage. In ration B, cassava flour comprised about 33% of the ration and it is not likely that at that level of supplementation metabolic faecal nitrogen had assumed any prominence. In fact, the slight increase in digestibility



coefficient of hay nitrogen with energy supplementation in the present report may be due to rapid multiplication of ruminal micro-organisms and hence slightly better digestibility of the hay. It is however likely that with very poor quality hay, supplementation with energy would lead to increased metabolic faecal nitrogen mainly of microbial origin.

In Trial 2, the digestibility of dietary nitrogen increased with increasing levels of dietary crude protein, and also with crude protein intake. This is in agreement with the reports of Robinson and Forbes (1970) and Andrews and Ørskov (1970a) who also reported increased digestibility of dietary crude protein with increasing levels of crude protein in the rations, and also with increasing intake of crude protein. This indicates decreasing quantitative importance of the metabolic faecal nitrogen with increasing crude protein content of the ration.

The regression equation relating digestibility coefficient of nitrogen and percent crude protein in the rations (Eq. 4.7) would give higher values for corresponding dietary crude protein, than that of French, Glover and Duthie (1957) shown as follow:

$$Y = 73.7 \log X - 19.8 \quad \dots\dots\dots(4.25)$$

$$Y = 70.3 \log X - 14.9 \quad \dots\dots\dots(4.26)$$

for mixed feed and herbage plus mixed feed respectively. Because of the costs and labour of conducting classical digestibility experiments, attempts have been made to derive correlations between

digestible nutrients available to the animal and the crude dietary components from which they are derived. Such regression equation as given in the present studies has several practical applications, one being the determination of the average digestibility of crude protein of rapidly growing grasses and herbage at different stages of development, another being the evaluation of average crude protein digestibility of single or compound feeds especially where adequate facilities are not available. It can also be used for computing maintenance and production rations. The digestibility coefficient of nitrogen is not dependent only on the crude protein content of concentrate but also on the percentage of concentrate in the ration. It has already been shown in Equation 4.9 that total digestibility of a ration increases with increase in percentage crude protein of supplemental concentrate but tends to decrease as the proportion of concentrate increases. The tendency for digestibility of nitrogen to decrease as percentage of concentrate increases is due to increased metabolic faecal losses that accompany supplementation with concentrate especially at low nitrogen intake.

The lower digestibility of nitrogen in period 1 than in periods 2, 3 and 4 may be due to lower intake of the highly digestible concentrate fraction of the ration in period 1, compared with periods 2 to 4.

The values of true digestibility obtained by regression and detergent methods are in very good agreement showing that the true

digestibility of dietary nitrogen is between 86 and 92%. The value obtained from regression equation were between 86 and 89% and those from the detergent method were from 89 to 96%. The higher value obtained from the detergent method is as expected in view of the possibility of extracting from the faeces other nitrogenous materials which are neither microbial nor endogenous, but dietary, and this would tend to increase the non-dietary faecal nitrogen and hence over-estimate the true digestibility. This may be particularly so in the case of water-soluble nitrogen. Some of the water-soluble nitrogen might in fact be of dietary origin but are assumed to be included in the non-dietary faecal fraction.

Mason (1969) has shown that the assumption that the process of digestion in the animal does not affect the extractability of the undigested dietary nitrogen in the faeces samples may not be strictly true. He used the detergent method and obtained the true digestibility values of 91 - 92% for ryegrass hay, and 98 - 99% for soya bean meal-based ration and reported that the values were probably higher than the true values because some undigested dietary pigments were extracted by the procedures employed.

The true digestibility value of  $92.9 \pm 2.3$  obtained in the present experiment is very good agreement with Mason's (1969) values of 91 to 92% and Singh and Mahadevan's (1970) value of  $93.4 \pm 1.9\%$ .

The amount of N absorbed by the sheep did not differ with rations A and B, but increased with increasing intake of dietary

crude protein. This is expected since the digestibility of the rations increased with increasing intake of nitrogen. The increase in N absorbed with increasing N intake agrees with the reports of Stobo and Roy (1973). Since the value of the metabolic faecal N (MEN) is relatively constant per unit DM intake, it is expected that the proportion of N absorbed will increase with increasing dietary crude protein intake.

The N retained per metabolic size ( $W_{kg}^{0.734}$ ) increased linearly with increasing N intake and also with absorbed N. About 93.9% of absorbed N was retained by the sheep. This is to be expected since the sheep were young and laying down tissues by utilizing absorbed N. The slope of the regression equation of retained N with absorbed N is  $0.939 \pm 0.029$ , and has been termed 'the Nitrogen balance index of absorbed N' (Allison, 1965) as it demonstrates the rate at which absorbed N fills the protein stores of the animal body; consequently, this index is a function of the biological value of the dietary N. The high N-balance index for the N content of the rations C, D, E and F indicates the very high efficiency with which these animals utilized the protein of the concentrate-based rations.

The N balance index value of 0.939 was higher than the value obtained for casein (0.65), and for urea (0.83) but lower than the value of 1.05 obtained for gluten by Deif, El-Shazly and Abou Alkada (1968).

The value of 0.77 obtained as the N balance index of intake N in the present studies was higher than 0.69 obtained for casein but less than 1.02 and 0.97 obtained for gluten and urea respectively by Doif, El-Shazly and Abou Alkada (1968), again tending to indicate very efficient utilization of the N contents of the rations.

It is obvious from Table 4.2 that at the highest level of N intake, N retained was still increasing and the value of  $1.11 \pm 0.09$  g/day/ $W_{kg}^{0.734}$  could not be the maximum N retainable by the sheep. Black, Pearce and Tribe (1973) obtained the maximum value of N retained per  $W_{kg}^{0.734}$  for lambs weighing 20.8 kg as  $1.05$  g/day/ $W_{kg}^{0.734}$ . It is likely that, given rations higher in crude protein, the value of  $1.11$  g/day/ $W_{kg}^{0.734}$  obtained in the present investigation might be exceeded. Black *et al.* (1973) also obtained the value of  $1.46$  g/day/ $W_{kg}^{0.734}$ .

Since the weight range of the sheep used in the present report was 15 - 26 kg, the maximum N retained is more likely to be closer to 1.05 than to 1.46 g/day/ $W_{kg}^{0.734}$ . Therefore the value of  $1.11$  g/day/ $W_{kg}^{0.734}$  obtained in the present experiment would appear to have reached the maximum N retention by this group of animals with mature weight of 20 kg. The fact that the mean differences within these animals were not significant ( $P > 0.05$ ) indicates that N retained was almost the same within the weight range 15 - 26 kg of the animals used in the present experiment.

The percent N retention increased as the N intake and digestibility of the ration. N retention was also influenced by the stage of

growth of the animals. In the present report, young dwarf sheep at their early maturity were used and nitrogen retention values were high, which shows that dietary N was being utilized in the formation of tissues. Stobo and Roy (1973) reported curvilinear increase in N retention with N intake and this is in agreement with the report of Robinson and Forbes (1970).

The supplementation of hay with concentrate (C<sub>1</sub>) mainly cassava flour, a readily digestible form of energy, increased N retention. This is in agreement with the report of Pick *et al.* (1973) that supplemental energy improved utilization of low quality hay. In the present experiment, supplementation of hay with concentrate (C<sub>1</sub>) decreased urinary N and hence increased N retention values.

The values of metabolic faecal N (MFN) obtained in the present report for lambs varied from 2.98 to 3.68 g/kg DM consumed. This is lower than the value of 5.0 g/kg DM consumed often quoted for ruminants (Maynard and Loosli, 1962). The values are however comparable to that of Deif *et al.* (1968) who reported MFN value of 3.58 g/kg DM consumed, and also to that of Elliott and Topps (1963), who obtained 3.66 gN/kg DM consumed but higher than those of Black *et al.* (1973), Walker and Fouchney (1964a) and Lofgreen and Kleiber (1953) who reported MFN values of 2.04, 2.90 and 2.70 g/kg DM intake respectively. These investigators with the lower MFN values used young lambs or calves maintained on liquid diets.

A wide variation had been obtained in MFN values by some investigators. The estimate of Robinson and Forbes (1970) was

6.5 g/kg DM intake and is higher than 5.0 g/kg DM intake while Mason (1969) using the detergent method obtained a range of 4.4 to 7.2 g/kg DM intake for the MFN values. From this, it is evident that a number of factors influence excretion of metabolic faecal nitrogen. The composition of feeds influences excretion of metabolic faecal nitrogen. Most of the investigators who obtained low values for MFN used highly digestible feed like solid or liquid milk (Black et al., 1973) and those who obtained higher values used highly fibrous rations (Mason, 1969). Schneider (1935) showed that the excretion of MFN was influenced by body size as well as by the level of feed intake, MFN tending to increase with increasing live weight of animal. Blaxter and Wood (1951) showed that MFN excretion increased on rations of low digestibility. Lofgreen and Kleiber (1953), using the N:P<sup>32</sup> ratio to determine MFN in young calves showed that MFN increased with increasing calf weights. Walker and Faichney (1964a) obtained a mean of 2.90 MFN/kg DM intake using protein-free diets. However, when nitrogen-free diets are given to the ruminant animals, their protein metabolism is substantially altered (Waterlow, 1968) and the results obtained may not apply under normal feeding conditions.

Mason (1969) showed that the proportion of nitrogenous contents of faeces is influenced by the type of the rations. He found that when animals were maintained on rations of hay or other roughages, the percent of undigested dietary N (UDN) ranged

from 11 - 28 %, increasing from 11 % for dry grass to 28 % for oat straw. The estimated mean value of  $21.7 \pm 3.8$  obtained in the present studies for hay/concentrate rations are well within the range obtained by Mason (1969). Since the growth of ruminal micro-organisms is enhanced by the presence of readily fermentable carbohydrates, it is expected that the microbial and endogenous fraction (MEN) would respond to the levels of energy in the ration. The results of Mason (1969) showed higher percentage of MEN on barley ration than on dried grass, 73 % and 60 % respectively. The MEN fraction consists of bacteria, protozoa and digestive juices and other N of endogenous origin.

Water-soluble nitrogen is expected to be higher when rations which are highly soluble are fed to animals. Thus the highest percentage of WSN (56 %) was obtained by Mason (1969) on a ration containing much glucose and some urea, a synthetic diet the components of which are soluble. For conventional rations of hay and concentrates, the estimates of Mason (1969) ranged from 16 - 28 %. The values obtained in present experiment ranged from 15.3 to 21.4 % with a mean value of  $17.3 \pm 3.4$  % and are well within range of Mason's (1969) determinations. The groundnut meal used, though soluble in ruminal liquor, could easily and rapidly be incorporated into microbial cell components and therefore little of it will appear as part of WSN fraction.



The mean value of Non-dietary faecal nitrogen (NDFN) obtained in this present investigation was  $78.9 \pm 2.5 \%$  and is also well within the range  $72 - 97 \%$  obtained by Mason (1969), who also showed that this fraction increased with increasing proportion of readily fermentable carbohydrates in the ration, lowest for oat straw (72%) and highest for barley diets (97%).

The percentage MEN in NDFN obtained in the present investigation ranged from 72% to 82.4% and the mean value of  $78 \pm 5 \%$  also falls within the range,  $66 - 81 \%$ , obtained by Mason (1969) using conventional rations. The results obtained in the present report are all comparable with that of Mason (1969) and show that for conventional type of rations, the proportions of UDN, MEN and WSN do not differ much from other reported values. It must be borne in mind that when detergents are used in extraction, some undigested dietary fractions such as pigment could be extracted (Mason, 1969). This would tend to lower the estimate of UDN and to increase that of NDFN.

The estimate of endogenous urinary nitrogen (EUN) obtained in this report as  $0.0238 \text{ g/day}/\text{W}_{\text{kg}}^{0.734}$  is lower than the values of  $0.170 \text{ g/day}/\text{W}_{\text{kg}}^{0.734}$  obtained by Walker and Faichney (1964) by giving protein-free diets, and the value of  $0.056 \text{ g/day}/\text{W}_{\text{kg}}^{0.734}$  obtained by Black *et al.* (1973) using lambs with live weights ranging from 7.8 to 30 kg. It is also less than  $0.038 \text{ g/day}/\text{W}_{\text{kg}}^{0.734}$  obtained by Singh and Mahadevan (1970) using adult rams. The value of

endogenous urinary nitrogen (EUN) may not be constant under all conditions. The results of Ashworth and Cowgill (1938) suggest that in rats, the amount of endogenous urinary nitrogen per  $\frac{0.734}{W_{kg}}$  rises slightly as live weight increase, but there is insufficient published information to identify a similar trend in lambs. The low value of EUN obtained in this work for the West African dwarf sheep may be an adaptation to subsistence on low quality forage, the condition prevalent in the tropics where natural grass species rapidly decline in nutritional value (Oyenuga, 1957). Mugerwa and Conrad (1971) have shown that urinary urea excretion is indicated by the levels of blood urea. It may be that in West African dwarf sheep, blood urea nitrogen is preserved by re-cycling into the rumen thus decreasing the possibility of much loss via the urine. The very low loss of nitrogen via urine may be given in support of this suggestion.

The biological value of ration B, 100%, was higher than that of basal hay (85.7%), while the biological value of protein-based rations was  $95.9 \pm 2.2$  or  $0.959 \pm 0.022$  and these high values show that the dietary nitrogen was being well utilized by the lambs. The value is higher than 0.83 and 0.65 obtained for urea and casein respectively by Deif et al. (1968). The high biological value of the ration may be due to very low excretion of urinary nitrogen, and this may in turn be caused by the presence of readily fermentable carbohydrate in the form of cassava flour in the rumen depressing

ruminal ammonia production, blood urea and urinary urea levels. The sheep at this stage of growth were retaining a lot of nitrogen for tissue protein synthesis. The fact that maximum Biological value with the rations was attained on a ration supplying the least amount of crude protein and which then declined suggests that maximum BV is obtained at minimum dietary N intake. The Biological value obtained in the present report is higher than that obtained by Singh and Mahadevan (1970), a mean of  $86.9 \pm 8.68\%$ . It is also higher than the value of 0.851 or 85.1% obtained by Stobo and Roy (1973) who also fed groundnut meal-based rations to ruminant calves.

The Thomas-Mitchell concept of biological value as a practical measure of the quality of a protein has two serious limitations. The first is the difficulty of obtaining a measure of the endogenous urinary, and to a less extent, the metabolic faecal nitrogen. In practice, the former is sometimes calculated on the basis of body size according to the equation

$$\text{mg EUN} = 146 W^{0.75} \dots\dots\dots (4.27.)$$

If the values for EUN are to be determined experimentally, it is necessary to prepare a nitrogen-free diet of the type used by Walker and Faichney (1964a).

The second difficulty arises from the fact that the levels of nitrogen fed modifies the calculated Biological value independently

of amino acid balance, because desmination appears to proceed somewhat according to the law of mass action (Crampton, 1956). Thus to obtain maximum Biological value, there must be a minimum of protein furnished. This is shown by the fact that ration B which supplied the least amount of N had the highest biological value (100%). This automatically means that production rations, including those for growth, where liberal protein feeding is necessary for maximum performance, show low biological values as compared to maintenance rations. Hence, biological values of individual feeds will change according to the rations in which they are used. Biological values are constants only if the protein is used entirely for maintenance. In order to compare such figures for different feeds, they must have been determined at the same protein levels of intake, and to standardise this such rations are often adjusted to 10% protein. Errors of the estimate of Biological value can be reduced by using the values of EUN and MFN determined during the experiment. It would be wrong to use equation 4.27 to estimate EUN since the value obtained would be higher or lower than those of other investigators. Errors due to overestimation or underestimation of MFN and EUN were minimised in the present investigation. The finding of Barnes, Bates and Maack (1946) showed that age, class of animal and the production involved would tend to influence the effective apparent biological value of a protein in addition to amino acid balance.

Net protein utilization (NPU) takes into account the response of the animal to the dietary protein intake. The estimate reported here of  $86.1 \pm 2.2$  % is higher than the estimated value of  $79.6 \pm 2.47$  reported by Singh and Mahadevan (1970). This difference in the values of Net protein Utilization (NPU) obtained in the present report and that of Singh and Mahadevan (1970) is due to a higher estimate of Biological value obtained in the present work ( $95.9 \pm 2.2$ ) than that reported by these investigators ( $86.9 \pm 3.68$ ).

In determining the requirement of crude protein by the sheep, some assumptions have been made, and these have been summarised by Black *et al.* (1973) as follows (a) that measurement of nitrogen balance gives an accurate estimation of the nitrogen retained by the lambs (b) that the inevitable losses of nitrogen in urine and faeces can be determined by an extrapolation to zero protein intake of the results from animals given a wide range of protein intakes, and that (c) the inevitable losses of nitrogen per metabolic size, (d) the energy requirements for maintenance per metabolic size and (e) the efficiencies of utilization of metabolizable energy for maintenance and for production are constant over the range in live weight and diets covered by the experiment.

With careful experimentation the common losses of N due to errors in urine collection and failure to consider dermal losses in sheep range from only 1.2 to 2.6 % of the faecal and urinary output of N (Martin, 1966). Moreover, because N loss from the integument is

a result of metabolism and is inevitable, it should be included in an estimation of protein requirements. The endogenous losses of N in urine and faeces obtained by using N-free diets are usually higher than when ample supply of N is included in rations. However, the fact that values obtained by investigators using similar animals and rations agree closely, showed the reliability of extrapolation techniques.

When animals with a narrow weight range are used, differences in the values of EUN, MFN, and energy required for maintenance are not likely to be great.

The value reported here of digestible protein requirement for maintenance in periods 1 and 2 of Trial 2 was  $1.15 \text{ g/day/W}_{\text{kg}}^{0.734}$  or  $1.57 \text{ g/day/W}_{\text{kg}}^{0.734}$  live weight, and for periods 3 and 4, the value was  $0.41 \text{ g/day/W}_{\text{kg}}^{0.734}$  or  $0.56 \text{ g/day/kg}$  live weight. A very sharp decline during periods 3 and 4 was obvious. This observation shows that protein requirements of growing animals change rapidly with age or live weight and agrees with the usual observations of some investigators (Black *et al.*, 1973). The mean value for the experimental period was  $0.74 \text{ g/day/W}_{\text{kg}}^{0.734}$  or  $1.01 \text{ g/day/kg}$  live weight. Black *et al.* (1973) found that the endogenous N losses in lambs of 20 kg live weight was  $0.20 \text{ g digestible nitrogen/day/W}_{\text{kg}}^{0.734}$  or  $1.250 \text{ g digestible crude protein per metabolic size per day}$ . The value of 1.250 is similar to 1.50 obtained in this present work during periods 1 and 2 but higher

than  $0.74 \text{ g/day/kg } W^{0.73}$ , the mean value for periods 1 to 4.

The present report with value of 1.150 is also similar to the value of  $1.16 \text{ g/day/W}_{\text{kg}}^{0.734}$  obtained by Robinson and Forbes (1966) using non-pregnant ewes, slightly higher than the values of 0.875 and  $0.893 \text{ g/daykg } W^{0.73}$  obtained by Singh and Mahadevan (1970) using adult rams, maintained on groundnut meal-based rations, but very much lower than  $3.6 \text{ g/day/kg } W^{0.73}$  recommended by Brody (1945).

The present reported value is about 33% of Brody's recommendation. Elliott and Topps (1964) have shown that the proportion of roughage to concentrate in the ration influenced maintenance requirement of sheep. They showed that the maintenance requirement increase with increasing roughage to concentrate in the ration.

Elliott and Topps (1964) showed that the generally accepted standards for digestible N for maintenance appear to be excessive by a factor of 3 when applied to African cattle and sheep given diets adequate in energy. The present value of requirement is about 33% of Brody's recommendation. This is therefore in agreement with the observation of Elliott and Topps (1964). The high efficiency of N utilization of the rations fed to the West African dwarf wether sheep is apparently associated with low endogenous losses and also high biological values of the protein. The rations used contained cassava flour, a readily fermentable source of energy and this reduced N losses due to deamination of protein in the rumen and subsequent loss of N in urine. The low protein requirement for the sheep used

may also be due to the fact that an appreciable amount of nitrogen of urea was recycled to the rumen from the blood, and this would be efficiently fixed into ruminal microbial protein in the presence of readily fermentable sources of energy. Since in the sheep used, about 60% of dry matter consumed was in the form of highly fermentable cassava flour, it is expected that any re-cycled urea would be well utilized.

Resenthal and Allison (1951) have noted in monogastric animals, the protein-sparing action of energy-rich feeds. Elliott and Topps (1963) suggested that the same or similar mechanism may be present in the ruminant, quite apart from the improvement in protein quality brought about by ruminal micro-organisms. They also reported that African cattle may through natural selection in a protein-deficient environment, have evolved some physiological process for conserving N under such stress conditions. The same is probably true of sheep and other ruminants under humid tropical environments.



## CHAPTER FIVE

### 5. DIGESTION AT DIFFERENT SITES OF THE ALIMENTARY TRACT OF THE WEST AFRICAN DWARF SHEEP

#### 5.1

#### I N T R O D U C T I O N

The digestion of feeds in the sheep may be divided into three stages, namely the fermentative processes that occur in the first three parts of the stomach (rumen, reticulum and omasum), hydrolytic digestion in the abomasum and small intestine and finally a secondary fermentative stage in the large intestine.

The present report deals with sheep maintained on Cynodon nlemfuensis/Centrosema pubescens hay with or without protein and cassava flour supplements. The aim of the experiment was to investigate the suitability of using chromic oxide-impregnated paper to determine the extent of digestion of feed in the different parts of the digestive tract of the West African Dwarf wether sheep.

#### 5.2

#### MATERIALS AND METHODS

##### 5.2.1 Animals and their Management

Twelve West African dwarf wether sheep, 8 - 13 months old and weighing 13 to 20kg were used in this experiment. Each sheep was kept in a metabolism cage (Oyenuga, 1961). The animals were usually fed at 8.00 a.m. everyday. The residues which might be left over were collected, weighed and

stored for chemical analysis (in order to determine nutrient intake). The animals had free access to salt licks and were weighed at the beginning of the experiment and just before they were slaughtered.

#### 5.2.2 Experimental Rations

The experimental rations used in this study are the same as those used in Chapter two, (Table 2.1).

#### 5.2.3 Plan of Experiment

The twelve wether sheep were randomised into six groups of two animals in each group, and to each group was assigned one of the six experimental rations. Four grams of chromic oxide - impregnated papers were given orally to each of the sheep with the aid of a balling gun just before feeds were offered every morning. There was a 14 - day preliminary period and a 6 - day collection period during which faecal samples were collected.

#### 5.2.4 Collection of Faeces

A day prior to collection, the animals were fitted with harnesses to which were attached collection bags. A polythene bag was placed in each collection bag to allow for easy collection of faeces. The bags were emptied daily just before the morning feeding. Faeces were dried to a constant weight in a forced - draught oven at 70°C for 48 hours. The daily dried faeces were bulked for each animal, milled, and stored in air-tight glass bottles until required for analysis.

### 5.2.5 Collection of Digesta from the Different sites of the digestive tract.

After the faecal collection period, the animals were slaughtered, four in a day at 8.00 a.m. The viscera were removed as quickly as possible and ligatures were placed in the following places; the reticulo-omasal junction, the omaso-abomasal junction, the pylorus, the proximal small intestine (the first metre), the distal small intestine (the last metre), the ileo - caecal junction, and the rectum.

Digesta were removed from the sections, weighed and mixed, and an aliquot was taken and freeze-dried at  $-20^{\circ}\text{C}$  for 6 days. The digesta samples were milled and stored in air - tight sample bottles until required for analysis.

### 5.2.6 Analytical Procedure

AOAC (1970) method was used to determine total N and ash in the digesta using Markham (1942) semi-micro Kjeldahl apparatus for N determination.

Chromic oxide in the digesta samples was determined by the method of Williams, Davie and Iismaa (1962) using Perkin - Elmer Atomic absorption spectrophotometer (Model 290).

### 5.2.7 Estimation of Digestibility in the different sites of digestive system.

Omasal samples were used to estimate digestion in the reticulo-rumen and omasum; abomasal samples were used to estimate

digestion in the stomach (rumen, reticulum, omasum and abomasum). Digesta sample from the terminal ileum was used to estimate digestion in the small intestine. Rectal samples were used to estimate total digestibility (Holmes et al., 1970).

### 5.3

## R E S U L T S

### 5.3.1 Total Digestibility of Dry Matter and Nitrogen

The comparison of the total collection and chromic oxide methods for the determination of dry matter and N digestibilities is shown in Table 5.1.

The dry matter digestibility of the rations by the chromic oxide method ranged from 53.3% with the basal hay, to 80.6% for ration F. The DM digestibility of the concentrate-supplemented rations were significantly higher than that of the basal hay ( $P < 0.01$ ). There seemed to be increases in DM digestibility with increasing crude protein content of the ration, especially with rations E and F. The DM digestibility as determined by the total collection method ranged from 54.2% for the basal hay to 82.0% for ration F. The mean differences of DM digestibility by the chromic oxide and total collection methods were not significant ( $P > 0.05$ ).

The digestibility of N by the chromic oxide method ranged from 37.2% with basal hay to 74.8% with ration F. The digestibility of N increased with increasing N intake and also with

TABLE 5.1

A Comparison of the Total Collection and Chromic Oxide Methods<sup>+</sup>  
For the Determination of Dry Matter and N Digestibilities with  
the West African Dwarf sheep Maintained on Hay and Concentrate  
Supplements

Ration	Nutrient	Nutrient Digestibility %	
		Chromic Oxide Method	Total Collection Method
A	Dry matter	53.3	52.4
	Nitrogen	37.3	40.7
B	Dry matter	73.2	76.1
	Nitrogen	31.3	40.6
C	Dry matter	71.2	75.9
	Nitrogen	44.2	52.9
D	Dry matter	71.1	76.2
	Nitrogen	59.4	66.8
E	Dry matter	79.5	79.9
	Nitrogen	66.0	70.0
F	Dry matter	80.6	82.0
	Nitrogen	74.8	78.7

<sup>+</sup> Mean differences of digestibility values by the two methods not significant at ( $P > 0.05$ ).

increase in the crude protein content of the rations. The value of the digestibility of N by the total collection method ranged from 40.7% with basal hay to 78.7% with ration F, and this also shows that digestibility of N increased with level of dietary crude protein. The values of N digestibility by the total collection method were consistently higher than the values obtained by the chromic oxide method; however, mean differences were not significant ( $P > 0.05$ ).

### 5.3.2 Chromic Oxide Recovery

The chromic oxide recovery for the experimental animals is shown in Table 5.2. The value ranged from 84.8 to 104.5% with a mean value of  $95.0 \pm 1.7\%$ . Three of the experimental animals had a recovery of chromic oxide less than 90%, and three had a recovery greater than 100%.

### 5.3.3 Digestibility of dry matter at different sites of the digestive tract.

The digestibility of dry matter at different sites of the digestive tract is shown in Tables 5.3.1 and 5.3.2 and total digestibility of the ration is obtained by **adding** together the digestibility in all the sections of the digestive tract (Table 5.3.1). Table 5.3.1 shows that 44.6% out of a total digestibility of 53.3% for dry matter, took place in the reticulo-rumen plus omasum with basal hay, and 46.6% of a total digestibility of 73.2% took place in the reticulo-rumen

TABLE 5.2

Chromic Oxide Recovery for the West African Dwarf Wether Sheep  
Maintained on Basal Hay and Concentrate Supplements

Sheep No.	CR Intake (g)	Faecal CR (g)	% Recovery
314	5.28	5.02	95.08
336	5.28	5.12	96.97
343	5.28	4.66	88.26
399	5.28	4.48	84.85
499	5.28	5.38	101.89
510	5.28	4.98	94.32
320	5.28	4.62	87.50
513	5.28	5.22	98.86
518	5.28	5.32	100.76
484	5.28	5.52	104.55
358	5.28	4.96	93.94
570	5.28	4.92	93.18

Mean

95.01  $\pm$  1.74

TABLE 5.3.1

The percentage digestibility of dry matter taking place in the sections of the digestive tract of the West African dwarf wether sheep maintained on hay and concentrate supplements, using the chromic oxide ratio

Ration	% Digesti- bility in the reticu- lo-rume- omasum	% Digesti- bility in the abomasum	% Digesti- bility in the stomach	% Digesti- bility in the small in- testine	% Digesti- bility in the Caecum and colon	Total Digesti- bility in digestive tract
A	44.6	13.3	57.9	0	-4.5	53.3
B	46.6	4.0	50.6	16.7	6.0	73.2
C	43.2	0.1	43.3	3.1	19.8	71.2
D	65.8	11.0	54.8	-3.7	20.0	71.1
E	48.6	-8.0	40.6	16.2	22.7	79.5
F	47.5	7.5	55.0	9.0	16.6	80.6
Mean	49.4	1.0	50.4	7.7	13.4	71.9
SE	3.4	3.8	2.9	3.5	4.3	4.0



TABLE 5.3.2

The percentage digestible dry matter taking place in the sections of the digestive tract of the West African dwarf wether sheep Maintained on hay and concentrate supplements, using chromic Oxide ratio

Ration	% Digestibility in the reticulo- rume+ Omasum	% Digestibility in the abomasum	% Digestibility in the stomach	% Digestibility in the small intestine	% Digestibility in the caecum and colon
A	83.6	24.9	108.5	0	-8.5
B	65.5	4.0	69.5	22.9	7.6
C	60.7	0.1	60.8	11.4	22.8
D	91.0	-14.4	76.6	-5.4	28.8
E	61.1	-10.1	50.0	20.4	28.6
F	59.0	9.3	68.3	11.2	20.5
Mean	70.2	2.3	72.5	10.1	17.4
SE	5.6	5.8	8.1	4.5	5.9

plus omasum with ration B. The corresponding values for rations C, D, E and F were 43.2, 65.8, 48.6 and 47.5% out of total digestibility of 71.2, 71.1, 79.5 and 80.6% respectively. Table 5.3.2 shows the percentage of digestible dry matter taking place in the various sections of the digestive tract. The values in table 5.3.2 are obtained from Table 5.3.1 by dividing each value in Table 5.3.1 by the total digestibility value, for example, the percentage of digestible dry matter taking place in the reticulo-rumen and omasum with ration A is calculated as:

$$\frac{44.6}{53.3} \times 100 = 83.6\% \dots\dots\dots 5.1$$

The results show that a mean of  $72.5 \pm 8.1\%$  of digestible dry matter was obtained in the stomach. In the small intestine, the value was  $10.1 \pm 4.5\%$ , and a mean of  $17.4 \pm 5.9\%$  took place in the caecum plus colon.

#### 5.3.4 Digestibility of Organic Matter in the Various Sections of the Digestive Tract.

The digestibility of organic matter is shown in Table 5.4.1 and 5.4.2. Table 5.4.1 shows that 44.6% of a total organic matter digestibility of 54.4% took place in the reticulo-rumen plus omasum with basal hay. The corresponding values were 48.4, 42.7, 65.6, 49.7 and 49.0% of total digestibility coefficients of 74.5, 72.3, 73.8, 80.6 and 82.0% with rations B, C, D, E and F respectively.

TABLE 5.4.1

The percentage digestibility of organic matter taking place in the Sections of the digestive tract of the West African dwarf wether sheep maintained on hay and concentrate supplements, using the

Chromic Oxide ratio

Ration	% Digestibility in the reticulo-rumen + Omasum	% Digestibility in the abomasum	% Digestibility in the stomach	% Digestibility in the small intestine	% Digestibility in the caecum plus colon	Total Digestibility in the digestive tract
A	44.6	13.7	58.3	0.9	-4.8	54.4
B	48.4	2.4	50.8	18.4	6.1	75.3
C	42.7	0.3	43.0	10.6	18.7	72.3
D	65.6	-7.3	58.3	-3.5	19.1	73.9
E	49.7	-7.4	42.3	18.1	20.2	80.6
F	49.0	8.9	57.9	8.4	15.7	82.0
Mean	50.0	1.8	51.8	8.8	12.4	73.0
SE	3.3	3.5	3.1	3.5	4.0	4.0

TABLE 5.4.2

The percentage digestible organic matter taking place in the Sections of the digestive tract of the West African dwarf wether sheep maintained on hay and concentrate supplements using the chromic oxide ratio

Ration	% Digestibility in the reticulo-rumen+omasum	% Digestibility in the abomasum	% Digestibility in the stomach	% Digestibility in the small intestine	% Digestibility in the caecum and colon
A	82.0	25.2	107.2	1.6	-8.8
B	65.6	2.2	67.8	24.3	7.9
C	59.1	0.3	59.4	14.7	25.9
D	87.0	-8.7	78.3	-4.9	26.6
E	61.6	-9.1	52.5	22.4	25.1
F	59.8	10.8	70.6	10.2	19.2
Mean	69.2	3.5	72.7	11.4	15.9
SE	5.0	5.3	7.8	4.7	5.7

Table 5.4.2 shows that 82.0% of the total digestible O.M took place in the reticulo-rumen plus omasum with basal hay and the mean value with all the rations is  $69.2 \pm 5.0\%$ . Similarly,  $3.5 \pm 5.3\%$  of digestible OM was obtained for the abomasum,  $11.4 \pm 4.7\%$  for the small intestine and  $16.0 \pm 5.7\%$  for the large intestine.

#### 5.3.5 Nitrogen Intake, Distribution and Absorption in the Various Sections of the Digestive tract of the Sheep

Table 5.5 shows the intake, distribution and absorption of N in the stomach and intestine of the sheep. More N was recovered at the omasum than the total N intake. The values of N absorbed in the proximal small intestine were negative and this indicates that large amount of secretion of nitrogenous substances took place there. At the distal small intestine the absorption was much. The sum of N absorbed in the proximal and distal small intestine is the estimate of the net absorption of N in the small intestine, for instance, - 4.19 g/day of N was absorbed at proximal small intestine (4.19 g/day was secreted), and 4.99 g/day was absorbed at the distal small intestine, therefore net absorption in the small intestine per day is  $4.99 + (-4.19\text{g})$  or 0.80g N per day for the sheep maintained on ration A (Table 5.5). The nitrogen absorbed in the small intestine as percentage of N intake was  $61.6 \pm 22.6$ , and N

TABLE 5.5

Nitrogen Intake, Distribution and Absorption at Different Sites of the Alimentary Canal  
of the West African Dwarf Sheep Maintained on Hay and Concentrate Supplements

Ration	N Intake (g/day)	N Passing through omasum per day (g/day)	N Flowing through abomasum (g/day)	N Flowing through the proximate small intestine (g/day)	N Flowing through the distal small intestine (g/day)	N Flowing through the return (g/day)	N Absorbed in the reticulo-rume plus omasum (g/day)	N Absorbed in the abomasum (g/day)	N Absorbed in the proximal small intestine (g/day) (X)	N absorbed in the distal small intestine (g/day) (Y)	N Absorbed in the whole small intestine (g/day) (X+Y)	N Absorbed in the large intestine (g/day)	N Absorbed in the small intestine as per cent intake	N Absorbed in the small intestine as per cent of N passing through the abomasum per day
A	5.59	3.88	2.41	6.60	1.61	2.69	1.71	1.47	-6.9	4.99	0.80	-1.08	14.3	33.2
B	3.61	4.64	4.68	7.89	1.78	2.65	-1.02	-0.04	-3.20	6.12	2.12	-0.82	80.9	62.3
C	3.62	3.72	3.40	8.36	1.75	2.04	-0.78	0.33	-4.97	6.61	1.64	-0.29	45.3	48.2
D	4.84	3.92	4.00	7.04	2.53	2.09	0.48	-0.07	-3.05	4.51	1.46	0.44	30.2	36.5
E	3.24	4.16	6.35	17.64	1.00	1.11	-0.92	-2.19	-11.29	16.64	5.35	-0.11	165.1	84.3
F	12.03	12.07	7.27	6.67	3.22	3.09	-0.04	4.80	0.60	3.45	4.05	0.13	33.7	55.7
Mean													61.6	53.4
SE												±	22.6	± 7.7

absorbed in the small intestine as percentage of N passing through the abomasum was  $53.4 \pm 7.7\%$ .

The correlation between N intake (X), in g/day, and the gain of N at abomasum (Y), g/day, was high, negative and significant ( $P < 0.05$ ). The regression equation is as follows:

$$Y = 0.76 x + 3.53 \dots\dots\dots 5.2$$

$$(r = -0.86).$$

From the equation, it can be shown that at zero N intake, 3.53 g/day of N could still flow into the abomasum from the reticulo-rumen and omasum. It can also be shown that at the N intake of 4.64 g/day, there would be no net gain of N from the rumen. At the intake of N higher than 4.64g per day, a net loss of N would be expected to occur in the rumen. The fact that the correlation was negative showed that a net gain of N in the stomach occurred only at low levels of N intake.

#### 5.4

#### D I S C U S S I O N

The rations used in the present investigation were similar to that used for the experiments reported in Chapters 2 and 4. The values of the dry matter digestibility obtained in this report were therefore similar to the values obtained in Chapter 4 (Table 4.2); it shows that there has been no appreciable variation in the composition of the rations.

In the present report, increasing digestibility coefficient of N was reported, with increasing levels of dietary crude protein; which is in agreement with the results of Andrews and Orskov (1970a). The digestibility coefficients of dry matter and N by the total collection and indicator methods showed no significant differences ( $P > 0.05$ ), and this shows the reliability of the chromic oxide paper method for determining total digestibility in the digestive tract. It must be borne in mind that if faecal samples were taken as often as possible, the differences in the digestibility values might be eliminated, and the fact that in the present report, the sample was taken once could have led to the little differences obtained since one sample might not be truly representative of faecal excretion for the day.

The mean percentage recovery of chromic oxide for the animals was  $95.0 \pm 1.7\%$ . Virtually complete recovery of chromic oxide in the faeces of sheep has been reported by McRae and Armstrong (1969), using chromic oxide - impregnated paper, by Putnam *et al.*, (1958) using chromic oxide in gelatin capsules and fed to cows, by Cowlshaw and Alder (1963) using both chromic oxide - impregnated paper and the oxide with oil as the carrier. However, certain investigators have reported incomplete recovery of chromic oxide. Johnson *et al.*, (1964)



found 101.8% recovery with chromic oxide - impregnated paper but only 93.3% recovery when the powder was given. Figden and Brisson (1956) obtained recoveries in the faeces of 101%, 94% and 87% of the administered dose in three separate, 4 - day trials each with four sheep, and Deinum, Immink and Dejis (1962) obtained recoveries of 97.5%, 98.6% and 98.4% in experiments with cows given 50g of chromic oxide - impregnated paper daily and found traces of the oxide in the liver, lymph glands and kidneys. They suggested that some absorption of the marker might have occurred. The value of  $95.1 \pm 1.7\%$  obtained in the present report may therefore be taken to lie within the range obtained by several investigators. Estimates of chromic oxide recovery in the faeces would normally be less than 100% because most sources of error lead to losses of chromic oxide. Loss of faeces from the collection bag have been noted by several investigators including Carter, Bolin and Erickson (1960) and Scaut (1961). Losses may occur if the bags are not emptied often enough or if the harness is not correctly adjusted, particularly if the faeces have a high water content ( $< 12\%$  dry matter). Some of the faeces may stick to the bag. In the experiment reported, a polythene bag was fitted into the collection bag to prevent faecal loss. Carter et al. (1960) and Scaut (1961) found that 5 - 7 days were sufficient for preliminary period. Bruce, Goodal, Kay,

Phillipson and Vowles (1966) showed that excretion of chromic oxide impregnated on to paper was irregular but fully recovered.

In the present report 72.5% of digestible dry matter and 72.6% of digestible organic matter digestion took place in the stomach. These values are higher than the values of 43% obtained for hay by Balch (1957), and 60% obtained for straw by Badawy *et al.* (1958) but similar to the value of 71.3% obtained by Holmes *et al.* (1970); however, these authors used lignin as indigestible marker. Drennan *et al.* (1970) using chromic oxide powder to estimate digestion in the stomach, obtained values ranging from 7 to 36%. They suggested that the low values might be due to rapid or uneven passage of marker from the rumen. The present report shows that the digestibility values given by chromic oxide - impregnated paper are consistent.

Most of the investigators who have determined digestibility of nutrients in the digestive tract of ruminants with chromic oxide as indicator have also used animals with re-entrant cannulae in the abomasum and terminal ileum. The results reported in the present study also agree with their reports. Thus, Hogan and Phillipson (1960) found that of the total dry matter digested in the sheep, 70% took place in the stomach, 11% in the small intestine and 19% in the large intestine. These values appear to be in very good agreement with the present report with values of  $72.5 \pm 8.1\%$ ,  $10.1 \pm 4.5\%$  and  $17.4 \pm 5.9\%$  for

the stomach, small intestine and large intestine respectively. Topps, Kay and Goodal (1968) showed that for animals fed with hay, 67% of the digestible dry matter disappear in the stomach, 22% in the small intestine and 11% in the large intestine whereas for animals taking concentrate-based rations, the digestibility coefficients were 69%, 17%, and 14% in the stomach, small intestine and large intestine respectively. The present results showed that of the total organic matter digested, 72.6% took place in the stomach, 11.4% took place in small intestine, and 16% took place in the large intestine. Bruce et al., (1966) reported values of 68%, 20% and 12% of organic matter digested as taking place in stomach, small intestine and large intestine respectively. Hogan, Connell and Mills (1972) reported a value of 74% of the total digestibility of organic matter as occurring in the stomach when hay was fed to sheep. Ørskov, Fraser, McDonald and Smart (1974) also reported that 60% of the dietary organic matter disappeared in the rumen, 32% in the small intestine and 8% in the large intestine.

In the present report, there was a net gain of nitrogen in the stomach of the sheep receiving low levels of nitrogen and a net loss in some receiving higher levels of nitrogen. There was a negative correlation ( $r = -0.86$ ) between the

nitrogen intake and the gain of nitrogen in the stomach. This relationship indicates that at the zero nitrogen intake up to 3.53g of nitrogen per day could still flow to the abomasum undoubtedly from microbial and salivary sources and other metabolic excretory nitrogen sources. Also from the regression equation, no net gain of nitrogen would take place at the intake of 4.64g of nitrogen per day. This is in agreement with the reports of Gray, Pilgrim and Weller (1958), Clarke, Ellinger and Phillipson (1966), Nicholson and Sutton (1969), McRae (1970), Harris and Phillipson (1962), Hogan and Weston (1967), Topps et al., (1968), Kay, McLeod and Pavlicevic (1972) and Ørskov et al., (1974). These investigators reported that when sheep were consuming low dietary N more total N passed to the duodenum than were eaten daily in the feeds, whereas when these animals were consuming high dietary N, less total N passed to the duodenum than in the feeds. Gray et al., (1958) calculated that there was an apparent gain of N in the stomach when sheep consumed feeds containing less than 5g nitrogen daily. This is in agreement with the present report with value of 4.64g N per day. Harris and Phillipson (1962) reported 50% more N per day, passing the abomasum of sheep than present in rations of low N.

The present results showed that a large quantity of nitrogen was secreted at the anterior small intestine and absorbed at the posterior portion. Badawy *et al.* (1958) found that increase in the N was considerable at the proximal half of the small intestine while absorption took place in the terminal half. Ben-Ghedalia, Tagari and Bondi (1974) reported that in the sections of the intestine from 1 - 15m posterior to the pylorus, the amounts of water, dry matter and total N decreased gradually as a result of absorption through the intestinal wall, that the region 7 - 15m from pylorus was more active with respect to the absorption of N whereas water, and dry matter were absorbed to a greater extent in the region 1 to 7m from the pylorus. The only part of the intestine in which substantial increases in water, dry matter and total N were found was the section immediately distal to the pylorus and these increases were caused by the inflow of bile, parcreatic and duodenal juices. These investigators found net increases beyond the entry of the common bile duct to be 2.7g protein - N and 2.0g non-protein - N per day, and that only very small changes occurred after 15m distant from the pylorus. From the present studies, the N absorbed in the small intestine as percentage of N intake was  $61.6 \pm 22.6\%$  and when expressed as percentage of N passing through the abomasum, the value was  $53.4 \pm 7.7\%$ .

Even though there were individual differences between animals due to difficulty of sampling in the present experiment, the results obtained are in reasonable agreement with those obtained with re-entrant cannulation for the purpose of partitioning digestibility in the various sections of the digestive tract. The general weakness of slaughter technique is that samples can only be obtained once from slaughtered animals, and since the samples might not be truly representative of the digesta flowing through the organ, considerable error due to sampling could be introduced if care were not exercised during the sampling. The great advantage of using animals with re-entrant cannulae over slaughter technique is that the former animals live long enough for repeated samplings to be carried out but the present results showed that reliable results could be obtained using slaughter techniques.

## SUMMARY OF CONCLUSION

The levels of ruminal metabolites of nitrogenous origin obtained in the present investigation were similar to the levels obtained by other investigators using similar rations, and this shows that the metabolism of forage and concentrate supplements in the West African dwarf sheep was similar to that of other breeds of sheep.

The composition of the rations markedly influenced the concentrations of metabolites in the rumen. There was a tendency for total N, protein N, and non-protein N to increase with increasing levels of crude protein in the ration. Ruminal ammonia levels were low with all the rations used and this indicates that not much dietary N would be lost in the urine. Supplementation of the forage with cassava flour greatly depressed ruminal ammonia levels and this enables the rumen micro-organisms to convert ammonia - N into microbial protein. In the present study, low levels of ruminal ammonia could be interpreted to mean that dietary N was being very efficiently utilized. The levels of blood urea were low and increased as ruminal ammonia. It is known that urinary N excretion increases with increasing blood urea N. However, the low levels of blood urea obtained in the present investigation was associated with low urinary N excretion, and high nitrogen retention.

The ruminal micro-organisms have very low concentration of methionine and histidine, and high levels of lysine and leucine. Rations high in non-protein nitrogen, for example, urea-based rations may require methionine and histidine for efficient utilization by the micro-organisms.

The results obtained when  $[^{15}\text{N}]$  ammonium chloride and  $[^{15}\text{N}]$  urea were used to study the kinetics of ammonia and urea metabolism, were in very good agreement with those of other investigators (Coccimano and Leng, 1967; Mugerwa and Conrad, 1971; Nolan and Leng, 1972). The results however, showed that the West African dwarf sheep was able to recycle a substantial amount of blood urea to the digestive tract, which may be an adaptation for existence in areas where dietary N supply is often inadequate.

The results obtained in the present report shows that fistulation has no effect on the digestibility of dry matter and nitrogen by the sheep. The intake of dry matter by the West African dwarf sheep was similar to that of other breeds of sheep when the results are expressed on the metabolic size basis. Dry matter intake was related to the body weight raised to the power of 0.668 ( $W_{\text{kg}}^{0.668}$ ). The value of 0.668 is similar to 0.66, the exponent which relates body weight to body surface area, and this suggests that in the West African



dwarf sheep, metabolism is related more to body area than to body weight. Butterworth (1966) suggested that this might be due to the necessity for the sheep under tropical conditions to maintain homoeothermy by heat loss through the body surface.

The sheep used in the present investigation were utilizing dietary N very efficiently. Urinary N levels were low and N retention values were high especially with the concentrate - based rations, showing that dietary N is best utilized in the presence of readily fermentable sources of energy. In the present investigation, the readily fermentable source of energy was in the form of cassava flour.

The metabolic faecal nitrogen (MFN) values obtained in the present report ranged from 3.0 to 3.7g N/kg dry matter intake which is lower than the value of 5.0g/kg DM intake often quoted for ruminants (Maynard and Loosli, 1969) but is comparable to the values of 3 to 3.7 g/kg DM intake obtained by several investigators (Elliott and Topps, 1963; Deif *et al.*, 1968). However, the nature of the diets influence metabolic faecal N excretion (Mason, 1969). Faecal analysis showed that for conventional type of ration, the percentage of non-dietary faecal nitrogen (NDFN), microbial and endogenous nitrogen (MEN), and water-soluble nitrogen (WSN) do not vary appreciably.

The low endogenous urinary N value ( $0.0238 \text{ g/day/w}_{\text{kg}}^{0.734}$ ) obtained in the present report may be an adaptation of the tropical breeds of sheep for survival on forages of low N content.

The biological values of the rations used were very high, showing that the sheep were utilizing dietary N efficiently. The value of  $95.9 \pm 2.2\%$  obtained with protein-based rations is higher than the values 85 - 87% obtained for groundnut meal - based rations by Singh and Mahadevan (1970), and Stobo and Roy (1973).

The value of the crude protein requirement for maintenance obtained in the present investigation, by the N - balance method was about 33% of Brody's (1945) recommended value for a sheep of similar live weight. Elliott and Topps (1964) had observed that the generally accepted standards for digestible N for maintenance appear to be excessive by a factor of 3 when applied to African cattle and sheep given diets adequate in energy. The value of digestible crude protein required for maintenance, obtained by the factorial method was much lower than the value obtained by N balance method. The low crude protein requirement for maintenance of the West African dwarf sheep may be an adaptation to life in areas where crude protein supply is often inadequate.

The present report shows that chromic oxide is well recovered in the faeces but it is not very evenly distributed in the digesta. The digestibility coefficient obtained could be subject to some error due to this uneven distribution. The variation in the concentration of chromic oxide between periods was reported by McRae (1970). The results obtained in the present report have shown that the mean values of digestibility coefficients obtained are better and more consistent than those of the investigators who used powdered chromic oxide in the rations, and this shows that chromic oxide - impregnated paper could be used to give correct estimates of digestibility at different sites of the alimentary tract of the sheep. The present reports is also in reasonable agreement with the reports of investigators who used re-entrant cannulation techniques.

From the present reports, it is concluded that the West African dwarf sheep is well adapted to survival under low protein intake, and is able to utilize dietary nutrients very efficiently.

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A P P E N D I X

TABLE A1

NITROGEN INTAKE, DIGESTIBILITY AND UTILIZATION IN THE WEST  
AFRICAN DWARF SHEEP.

RATION - A ANIMAL NO.	N INTAKE (g/day)	FAECAL-N (g/day)	URINARY-N (g/day)	DIGESTED-N (g/day)	RETAINED-N (g/day)	% DIGESTI- BILITY	% RETE- TION	FAECAL - N g/kg D.M. CONSUMED
179	6.35	2.72	1.07	3.63	2.56	57.14	40.31	5.35
259	8.88	3.84	0.75	5.04	4.29	56.69	47.18	5.40
268	5.29	2.31	0.57	2.98	2.41	56.36	45.56	5.45
186	9.07	4.25	0.74	4.82	4.08	53.18	44.98	5.85
263	2.18	1.10	0.42	1.08	0.66	49.73	30.28	6.30
173	5.79	2.61	0.45	3.18	2.73	54.89	47.15	5.63
301	2.65	1.07	0.49	1.58	1.09	59.69	41.13	5.04
184	2.91	1.18	-	1.73	-	59.41	-	5.07

A

HAY

TABLE 2

NITROGEN INTAKE, DIGESTIBILITY AND UTILIZATION IN THE WEST  
AFRICAN DWARF SHEEP.

ANIMAL	INTAKE-N (g/day)	FAECAL-N (g/day)	URINARY-N (g/day)	DIGESTED-N (g/day)	RETAINED-N (g/day)	% DIGESTI- BILITY	% RETEN- TION	FAECAL-N g/kg D.M. CONSUMED
179	3.98	1.45	0.04	2.53	2.49	63.57	62.56	2.84
259	3.83	1.40	0.11	2.43	2.32	63.45	60.57	2.61
268	2.83	1.43	0.05	1.40	1.35	49.47	47.70	3.50
186	4.53	1.72	0.05	2.81	2.76	62.03	60.93	3.21
263	4.39	1.59	0.22	2.80	2.58	63.78	58.77	3.04
173	4.15	1.82	0.14	2.33	2.19	56.14	52.77	3.64
301	4.05	1.41	0.13	2.64	2.51	65.19	62.00	2.07
184	5.62	1.92	0.83	3.70	2.87	65.84	51.07	3.53

B

TABLE 3

NITROGEN INTAKE, DIGESTIBILITY AND UTILIZATION IN THE WEST  
AFRICAN DWARF SHEEP.

TRIAL 2  
PERIOD 1

RATION	I.D. NO ANIMAL	INTAKE-N (g/day)	FAECAL-N (g/day)	URINARY-N (g/day)	DIGESTED-N (g/day)	RETAINED-N (g/day)	% DIGESTI- BILITY	% RETEN- TION	FAECAL-N g/kg.D.M
C	179	5.98	2.28	0.20	3.70	3.50	61.87	58.52	4.70
C	259	7.41	2.92	0.07	4.49	4.42	60.59	59.65	4.38
D	268	9.41	3.67	0.25	5.74	5.49	61.00	58.34	4.90
D	186	6.71	2.33	0.09	4.38	4.29	65.27	63.93	4.28
E	263	8.79	3.17	0.22	5.62	5.40	63.93	61.43	5.82
E	173	11.59	3.84	1.10	7.75	6.65	66.87	57.38	5.37
F	301	12.28	3.22	0.18	9.06	8.88	73.78	72.31	5.22
F	184	12.49	3.22	0.27	9.27	9.00	74.22	72.06	5.74

5.05±0.55

TABLE 4

NITROGEN INTAKE, DIGESTIBILITY AND UTILIZATION IN THE WEST  
AFRICAN DWARF SHEEP.

TRIAL 2  
PERIOD 2

RATION	I.D. NO. ANIMAL	INTAKE-N (g/day)	FAECAL-N (g/day)	URINARY-N (g/day)	DIGESTED-N (g/day)	RETAINED-N (g/day)	% DIGESTI- BILITY	% RETEN- TION	FAECAL-N g/kg D.M
D	179	10.55	3.47	0.89	7.08	6.19	67.11	58.67	5.30
D	259	11.31	3.19	0.55	8.12	7.58	71.79	67.02	4.57
E	268	13.19	3.61	0.93	9.58	8.65	72.63	65.58	5.58
E	186	12.34	2.82	0.66	9.52	8.86	77.15	71.80	4.88
F	263	13.00	2.67	1.33	10.33	9.00	79.46	69.23	4.36
F	173	13.07	3.48	1.76	9.59	7.83	73.37	59.91	5.24
C	301	6.00	2.04	0.12	3.96	3.96	3.84	66.00	3.93
C	184	7.10	2.37	1.11	4.73	3.62	66.62	51.00	6.12

5.00±0.66

TABLE 5

NITROGEN INTAKE, DIGESTIBILITY AND UTILIZATION IN THE WEST  
AFRICAN DWARF SHEEP.

TRIAL 2  
PERIOD 3

RATION	I.D. NO ANIMAL	INTAKE-N (g/day)	FAECAL-N (g/day)	URINARY-N (g/day)	DIGESTED-N (g/day)	RETAINED-N (g/day)	% DIGESTI- BILITY	% RETEN- TION	FAECAL-N g/kg D.M CONSUMED
E	179	11.14	2.75	0.20	8.39	8.19	75.31	73.52	4.55
E	259	12.65	4.01	0.74	8.64	7.90	68.30	62.45	5.48
F	268	15.53	3.41	0.59	12.12	11.53	78.04	74.24	5.72
F	186	15.09	3.41	1.00	11.68	10.68	77.40	70.77	4.95
C	263								
C	173	9.34	3.61	0.55	5.73	5.18	61.35	55.46	5.30
B	301	8.76	2.13	0.22	6.63	6.41	75.68	73.17	3.53
B	184	3.83	1.00	0.11	2.83	2.72	73.89	71.01	1.96

4.50±1.16



TABLE 6

NITROGEN INTAKE, DIGESTIBILITY AND UTILIZATION IN THE WEST  
AFRICAN DWARF SHEEP.

TRIAL 2  
PERIOD 4

RATION	I.D. NO. ANIMAL	INTAKE (g/day)	FAECAL-N (g/day)	URINARY-N (g/day)	DIGESTED-N (g/day)	RETAINED-N (g/day)	% DIGESTI- BILITY	% RETEN- TION	FAECAL-N g/kg D.M. CONSUMED
F	179	13.84	3.34	1.18	10.50	9.32	75.87	67.34	5.95
F	259	15.27	3.35	1.20	11.92	10.72	78.06	70.20	4.80
C	268	8.29	3.18	0.41	5.11	4.70	61.64	56.69	4.53
C	186	6.59	2.20	0.51	4.39	3.88	66.62	58.88	4.28
D	263	6.72	2.02	0.63	4.70	4.07	69.94	60.57	4.75
D	173	4.59	1.36	0.20	3.23	3.03	70.37	66.01	3.23
E	301	9.74	2.64	0.24	7.10	6.86	72.90	70.45	5.05
E	184	9.81	2.67	0.32	7.14	6.82	72.78	69.52	5.46

4.76±0.76

TABLE 7

NITROGEN INTAKE, DIGESTIBILITY AND UTILIZATION IN THE WEST  
AFRICAN DWARF SHEEP.

RATION A<sub>2</sub>

ANIMAL NO.	INTAKE-N (g/day)	FAECAL-N (g/day)	URINARY-N (g/day)	DIGESTED-N (g/day)	RETAINED-N (g/day)	% DIGESTIBILITY	% RETENTION
179	3.19	1.49	0.75	1.70	0.95	53.43	29.78
259	5.10	2.61	1.30	2.49	1.19	48.74	23.33
268	4.17	1.90	0.22	2.27	2.05	54.49	49.16
186	4.79	1.93	1.10	2.86	1.76	59.73	36.74
263	3.19	1.23	-	1.96	-	61.54	-
173	4.79	2.15	0.22	2.64	2.42	55.13	50.52
301	3.19	1.44	0.49	1.75	1.26	55.00	39.50
184	2.55	1.12	0.30	1.43	1.13	55.92	44.31

A

TABLE 8

RATION A

RUMEN AND BLOOD METABOLITES OF THE WEST AFRICAN DWARF SHEEP

I D NOS.	RUMEN AMMONIA mg/100 ml			BLOOD UREA mg/100 ml	PROTEIN-N mg/100 ml			NON-PROTEIN-N mg/100 ml			NON-AMMONIA NON-PROTEIN-N			TOTAL-N mg/100 ml
	1 HR	2 HRS	3 HRS		1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	
179														
259	4.8	4.5	4.3	6.9	32.0	28.0	25.0	8.5	7.0	7.0	3.7	2.5	2.7	35.8
	5.8	4.0	4.0	5.6										
	3.7	2.6	2.5	4.0										
268														
186	4.6	4.2	4.3	7.5	36.0	32.0	36.0	6.0	7.0	7.5	1.4	2.8	2.7	41.5
	4.3	4.3	4.4	4.8										
	5.6	4.5	4.0	4.2										
173	4.6	4.0	4.0	3.8	39.6	42.0	16.8	43.2	11.2	5.6	38.6	12.8	1.6	61.6
	5.4	5.4	6.0	4.8										
	4.3	3.5	3.5	4.0										
301														
184	6.8	4.6	4.4	8.0	22.4	29.2	14.0	14.0	14.0	5.6	7.2	13.4	1.2	33.1
	8.0	10.8	4.0	8.0										
	6.8	6.0	3.5	2.0										

TABLE 9

RATION B

RUMEN AND BLOOD METABOLITES OF THE WEST AFRICAN DWARF SHEEP

I D NOS	RUMEN AMMONIA (mg/100 ml)			BLOOD UREA mg/100 ml	PROTEIN-N (mg/100 ml)			NON-PROTEIN-N (mg/100 ml)			NON-PROTEIN NON-AMMONIA-N (mg/100 ml)			TOTAL-N
	1 HR	2 HRS	3 HRS		1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	
179														
	0.1	0.1	0.2	1.1	36.4	8.4	8.4	5.6	5.6	2.8	5.5	5.5	2.6	22.4
259	1.1	0.7	0.7	1.2										
	0.6	0.3	0.3	1.4										
268														
	0.6	0.6	0.6	0.5	25.2	25.2	14.0	16.8	8.4	5.6	16.2	7.8	5.0	31.7
186	1.0	1.1	1.1	1.2										
	0.6	0.6	0.5	1.2										
263														
	0.6	0.7	0.6	0.9	16.8	16.8	30.8	8.4	8.4	8.4	7.8	7.7	7.8	29.9
173	0.6	0.9	1.1	1.2										
	1.9	2.4	2.4	1.4										
301														
	4.0	2.4	2.4	2.0	33.6	30.6	14.0	11.2	8.4	8.4	7.2	6.0	6.0	35.5
184	4.0	1.1	2.0	1.2										
	1.5	2.8	2.4	3.1										

TABLE 10

RATION C.

RUMEN AND BLOOD METABOLITES OF THE WEST AFRICAN DWARF SHEEP

PER- IOD	I.D NOS	RUMEN AMMONIA (mg/100 ml.)			BLOOD UREA mg/100 ml	PROTEIN-N (mg/100 ml)			NON-PROTEIN-N (mg/100 ml)			NON-AMMONIA NON-PROTEIN			TOTAL-N (av.) mg/100 ml.
		1 HR	2 HRS	3 HRS		1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	
1	179	0.6	0.6	0.7	1.2	25.2	16.8	12.0	11.2	11.2	3.0	10.6	10.6	2.3	26.5
		0.6	0.6	0.5	0.9										
		0.7	0.5	0.6	0.9										
4	268														
4	186	1.8	2.8	2.6	2.3	53.2	38.0	22.3	15.8	13.4	9.6	14.0	10.6	7.0	50.8
		2.6	2.8	2.8	3.0										
		2.6	2.1	2.7											
3	263														
3	173	2.0	2.1	3.2	2.1	42.0	42.0	39.0	22.4	30.8	8.4	20.4	28.7	5.2	61.5
		3.2	2.2	1.6	2.5										
		2.8	1.3	1.2	1.3										
2	301														
2	184	1.6	2.4	1.2	1.5	36.8	28.0	22.4	14.0	11.2	8.4	12.4	9.8	7.2	40.3
		1.4	1.5	1.3	2.6										
		4.0	2.6	1.3	2.0										

TABLE 11

RATION D

RUMEN AND BLOOD METABOLITES OF THE WEST AFRICAN DWARF SHEEP

PER- IOD	I.D NOS.	RUMEN AMMONIA (mg/100 ml)			BLOOD UREA mg/100ml	PROTEIN-N (mg/100 ml)			NON-PROTEIN-N (mg/100 ml)			NON-AMMONIA NON-PROTEIN-N (mg/100 ml)			TOTAL (av.) mg/100 ml.
		1 HR	2 HRS	3 HRS		1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	
2	179														
2	259	5.2	3.6	2.4	3.6	30.8	50.4	50.4	11.2	8.4	8.4	6.8	6.9	7.4	53.2
		4.4	1.5	1.0	3.2										
		7.2	5.0	2.2	4.0										
1	268														
1	186	1.8	2.0	0.8	1.4	50.4	53.2	30.8	22.4	25.2	14.0	20.6	23.2	13.2	65.3
		0.8	0.7	0.8	0.9										
		0.9	1.0	1.2	1.0										
4	263														
4	173	4.8	1.8	1.8	3.4	58.8	53.2	50.4	28.4	23.5	10.6	23.6	21.7	8.8	79.0
		4.8	3.2	1.6	2.6										
		4.0	2.6	1.4	4.6										
3	301														
3	184	1.6	2.8	2.4	2.3	56.0	72.8	50.4	22.4	30.8	8.4	20.8	28.0	6.0	80.3
		2.2	1.6	1.8	2.3										
		1.8	1.5	1.4	2.1										

TABLE 12

RATION E

RUMEN AND BLOOD METABOLITES OF THE WEST AFRICAN DWARF SHEEP

PER- IOD	I.D NOS	RUMEN AMMONIA (mg/100 ml)			BLOOD UREA mg/100 ml	PROTEIN-N (mg/100 ml)			NON-PROTEIN-N (mg/100 ml)			NON-AMMONIA NON-PROTEIN-N (mg/100 ml)			TOTAL (av.) (mg/100 ml)
		1 HR	2 HRS	3 HRS		1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	
3	179														
3	259	5.8	4.2	4.0	3.8	75.6	52.5	38.4	25.6	29.3	10.8	19.8	25.1	6.8	77.4
		4.4	2.6	1.3	3.2										
		5.2	3.6	2.2	3.3										
2	268														
2	186	9.0	3.2	1.8	6.0	78.4	28.0	44.8	28.0	14.0	25.2	19.0	10.8	23.4	
		5.0	3.8	2.0	8.0										
		7.8	10.0	6.2	7.6										
1	263														
1	173	1.8	0.9	0.9	1.8	61.6	70.0	36.4	42.0	14.0	8.4	40.2	13.1	7.5	
	3.3	1.8	1.8	1.4											77.5
	4.2	3.6	3.2	1.0											
4	30.														
4	184	4.2	4.0	3.2	4.2	61.6	58.8	64.4	30.2	26.5	13.8	26.0	22.5	10.6	
		6.0	5.9	2.0	3.8										85.1
		3.6	3.8	1.6	3.2										

TABLE 13

RATION F

RUMEN AND BLOOD METABOLITES OF THE WEST AFRICAN DWARF SHEEP

PER- IOD	I.D NOS	RUMEN-NH <sub>3</sub> (mg/100 ml)			BLOOD UREA (mg/100 ml)	PROTEIN-N (mg/10 ml)			NON-PROTEIN-N (mg/100 ml)			NON-NH <sub>3</sub> NON-PROTEIN-N <sup>1</sup> N (mg/100 ml)			TOTAL (mg/100 ml.)
		1 HR	2 HRS	3 HRS		1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	1 HR	2 HRS	3 HRS	
4	179														
4	259	6.4	5.6	6.0	4.2	61.6	70.0	81.2	25.7	30.4	28.3	18.4	24.8	22.3	99.1
		6.2	2.2	1.6	4.6							19.3			
		6.0	3.4	2.8	5.2										
3	268														
3	186	6.8	6.6	4.8	4.6	42.0	42.0	106.0	22.4	42.0	98.0	15.6	33.4	93.2	117.5
		5.8	3.6	2.4	4.4								35.4		
		4.8	3.0	1.6	4.7										
2	263														
2	173	16.4	6.4	2.6	8.4	56.0	142.9	140.0	28.0	98.0	98.6	11.6	91.6	95.4	187.6
		5.2	4.8	3.8	7.2										
		8.2	5.3	2.4	6.8										
1	301														
1	184	1.7	1.7	1.6	1.9	72.8	67.2	67.2	28.0	25.2	25.2	26.3	23.5	23.6	95.2
		3.6	4.2	2.7	5.3										
		1.0	1.0	1.0	0.0										



TRIAL ONE  
PERIOD ONE

TABLE 14  
DRY MATTER INTAKE, DIGESTIBILITY AND N UTILIZATION IN WEST AFRICAN DWARF SHEEP

ANIMAL NO.	WEIGHT (kg)	RATION	N			DIGESTIBILITY %	RETENTION %	DRY MATTER	
			INTAKE g/day/W <sup>0.75</sup> <sub>kg</sub>	N-DIGESTED g/day/W <sup>0.75</sup> <sub>kg</sub>	N-RETAINED			INTAKE g/day/W <sup>0.75</sup> <sub>kg</sub>	DIGESTIBILITY %
179	15.88	A	0.85	0.48	0.35	57.1	40.3	67.5	55.4
259	21.77	A	0.94	0.53	0.45	56.7	47.2	74.9	57.0
268	19.96	A	0.60	0.34	0.27	56.4	45.6	47.6	53.6
186	19.50	A	1.04	0.56	0.47	53.2	45.0	83.0	57.3
263	22.68	B	0.48	0.31	0.28	63.8	58.8	57.0	72.0
173	26.31	B	0.39	0.21	0.20	56.1	52.8	46.0	70.8
301	13.15	B	0.58	0.37	0.35	65.2	62.0	96.5	75.8
184	17.69	B	0.69	0.45	0.36	65.8	51.1	66.7	74.7

TABLE 15

TRIAL ONE  
PERIOD TWO

DRY MATTER INTAKE, DIGESTIBILITY AND N UTILIZATION IN WEST AFRICAN DWARF SHEEP

ANIMAL NO.	WEIGHT (kg)	RATION	N-INTAKE N-DIGESTED N-RETAINED			DIGESTIBILITY %	RETENTION %	DRY MATTER DIGESTIBILITY	
			g/day/W <sup>0.75</sup> kg					INTAKE g/day/W <sup>0.75</sup> kg	DIGESTIBILITY %
179	15.88	B	0.53	0.33	0.33	62.6	62.6	67.8	64.5
259	21.77	B	0.40	0.25	0.24	63.4	60.6	56.6	72.7
268	19.96	B	0.31	0.16	0.15	49.5	47.7	45.9	72.2
186	19.05	B	0.52	0.33	0.33	62.0	62.0	62.4	68.5
263	20.86	A	0.23	0.12	0.07	49.7	30.3	17.9	56.7
173	26.31	A	0.53	0.29	0.25	54.9	47.1	42.6	57.9
301	14.51	A	0.41	0.24	0.17	59.7	41.1	32.4	57.3
184	17.69	A	0.36	0.21	-	59.4	-	28.6	56.5

TABLE 16

2ND TRIAL  
1ST PERIOD

DRY MATTER INTAKE, DIGESTIBILITY AND N UTILIZATION IN WEST AFRICAN DWARF SHEEP

ANIMAL NO.	WEIGHT (kg)	RATION	N-INTAKE	N-DIGESTED g/day/W <sup>0.73</sup> <sub>kg</sub>	N-RETAINED	DIGESTIBILITY %	RETENTION %	DRY-INTAKE g/day/W <sup>0.73</sup> <sub>kg</sub>	MATTER DIGESTIBILITY %
179	15.42	C	0.81	0.50	0.47	61.9	58.5	65.8	73.3
259	21.77	C	0.78	0.47	0.47	60.6	59.6	70.4	75.2
268	20.86	D	1.02	0.62	0.60	61.0	58.3	81.5	69.3
186	18.60	D	0.79	0.52	0.51	65.3	63.9	64.4	67.8
263	21.32	E	0.94	0.60	0.58	63.9	61.4	58.3	65.2
173	26.31	E	1.07	0.72	0.61	66.9	57.4	65.7	69.4
301	14.51	F	1.74	1.29	1.26	73.8	72.3	87.5	76.8
184	17.69	F	1.53	1.14	1.10	74.2	76.2	69.0	66.1

TABLE 17

2ND TRIAL  
2ND PERIOD

DRY MATTER INTAKE, DIGESTIBILITY AND N UTILIZATION IN WEST AFRICAN DWARF SHEEP

ANIMAL NO.	WEIGHT	RATION	N-INTAKE	N-DIGESTED g/day/W <sup>0.73</sup> kg	N-RETAINED	DIGESTI- BILITY %	RETENTION %	DRY MATTER INTAKE g/day/W <sup>0.73</sup> kg	MATTER DIGESTI- BILITY %
179	14.51	D	1.50	1.01	0.88	67.1	58.7	92.9	71.9
259	21.32	D	1.21	0.87	0.81	71.8	67.0	74.7	70.3
268	19.96	E	1.48	1.07	0.97	72.6	65.6	72.7	72.5
186	17.24	E	1.54	1.19	1.11	77.1	71.8	72.4	80.2
263	23.59	F	1.29	1.02	0.89	79.5	69.2	60.9	77.2
173	24.95	F	1.25	0.92	0.75	73.4	59.9	63.4	75.0
30.1	13.15	C	0.91	0.61	0.58	66.0	64.0	79.1	78.8
184	15.42	C	0.96	0.64	0.49	66.6	51.0	52.5	79.4

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TABLE 18

2ND TRIAL  
3RD PERIOD

DRY MATTER INTAKE, DIGESTIBILITY AND N UTILIZATION IN WEST AFRICAN DWARF SHEEP

ANIMAL NO.	WEIGHT (kg)	RATION	N-INTAKE	N-DIGESTED	N-RETAINED	DIGESTIBILITY %	RETENTION %	DRY INTAKE	MATTER DIGESTIBILITY %
				$\text{g/day/W}^{0.75}$ kg				$\text{g/day/W}^{0.75}$ kg	
179	16.78	E	1.42	1.07	1.04	75.3	73.5	77.0	78.4
259	22.68	E	1.29	0.88	0.81	68.3	62.4	74.9	73.9
268	20.41	F	1.72	1.34	1.27	78.0	74.2	80.0	77.5
186	19.05	F	1.76	1.36	1.24	77.4	70.8	80.1	76.4
263	22.22	C		0.34				36.7	71.9
173	26.76	C	0.85	0.52	0.48	61.3	55.5	71.7	75.9
301	16.33	D	1.19	0.89	0.87	75.7	73.2	81.9	79.0
184	17.24	D	0.48	0.35	0.34	73.9	71.0	63.9	74.1

TABLE 19

2ND TRIAL  
4TH PERIOD

DRY MATTER INTAKE, DIGESTIBILITY AND N UTILIZATION IN WEST AFRICAN DWARF SHEEP

ANIMAL NO.	WEIGHT (kg)	RATION	N-INTAKE	N-DIGESTED	N-RETAINED	DIGESTIBILITY	RETENTION	DRY-MATTER		BIOLOGICAL VALUE
				g/day/W <sup>0.73</sup> kg		%	%	INTAKE	DIGESTIBILITY	
				g/day/W <sup>0.73</sup> kg				g/day/W <sup>0.73</sup> kg	%	
179	17.24	F	1.73	1.31	1.17	75.9	67.3	70.3	75.8	91.92
259	23.59	F	1.52	1.18	1.03	78.1	70.2	69.4	76.4	93.19
268	22.68	C	0.85	0.52	0.48	61.6	56.7	71.8	74.1	97.54
186	19.50	C	0.75	0.50	0.44	66.6	58.9	58.9	80.8	95.00
263	21.32	D	0.72	0.50	0.44	69.9	60.6	45.6	76.3	93.20
173	26.31	D	0.42	0.29	0.28	70.4	66.0	38.7	76.1	98.70
301	16.33	E	1.27	0.92	0.89	72.9	70.4	68.1	76.7	99.31
184	16.78	E	1.25	0.91	0.87	72.8	69.5	62.4	77.9	98.50

TABLE 20

1ST TRIAL  
1st PERIOD

CONCENTRATION OF RUMEN AND BLOOD METABOLITES (mg/100ml) IN THE WEST AFRICAN DWARF SHEEP

ANIMAL NO.	RATION	TOTAL-N	PROTEIN-N	NON-PROTEIN-N	AMMONIA-N	NON-AMMONIA N	RESIDUAL N	α-AMINO-N (Umele/ml)	BLOOD UREA-N
259	A	35.8	28.3	7.5	4.51	31.3	3.0	3.1	6.9
186	A	41.5	34.6	6.9	4.4	37.1	2.5	3.6	7.5
173	B	29.9	21.5	8.4	0.6	29.3	7.8	1.8	0.9
184.	B	35.5	26.1	9.4	2.9	32.6	6.5	1.1	2.0
LST TRIAL      2ND PERIOD									
259	B	22.4	17.7	4.7	0.1	22.3	4.6	1.8	1.1
186	B	31.7	21.5	10.2	0.6	31.1	9.6	1.7	0.5
173	A	52.8	32.8	20.0	4.2	48.6	15.8	3.6	3.8
184	A	33.1	21.9	11.2	5.3	27.8	5.9	2.7	8.0

TABLE 21

2ND TRIAL  
PERIOD 1

CONCENTRATION OF RUMEN AND BLOOD METABOLITES (mg/100ml) IN THE WEST AFRICAN DWARF SHEEP

ANIMAL NO.	RATION	TOTAL-N	PROTEIN-N	NON-PROTEIN-N	AMMONIA-N	RESIDUAL-N	α-AMINO-N (u mole/ml)	BLOOD UREA N
259	C	26.5	18.0	8.5	0.6	7.8	1.45	1.0
186	D	65.3	44.8	20.5	1.5	19.0	3.35	1.1
173	E	77.5	56.0	21.5	1.2	20.3	5.50	1.4
184	F	95.2	69.1	26.1	1.7	24.5	8.80	2.7
2ND TRIAL PERIOD 2								
259	D	53.2	43.9	9.3	3.7	5.6	3.80	3.6
186	E	72.8	50.4	22.4	4.7	17.7	4.50	7.2
173	F	187.6	112.9	74.7	8.5	66.2	6.00	7.5



TABLE 22

2ND TRIAL CONCENTRATION OF RUMEN AND BLOOD METABOLITES (ng/100ml) IN THE WEST AFRICAN DWARF SHEEP PERIOD 3

ANIMAL NO.	RATION	TOTAL-N	PROTEIN-N	NON-PROTEIN-N	AMMONIA-N	RESIDUAL-N	α-AMINO-N (u mole/ml)	BLOOD UREA N
259	E	77.4	55.5	21.9	4.7	17.2	5.65	3.4
186	F	117.5	63.3	54.2	6.1	48.1	5.85	4.6
173	C	61.5	41.0	20.5	2.4	18.1	3.50	1.9
184	D	80.3	59.7	20.5	2.3	18.3	2.25	2.2
2ND TRIAL PERIOD 4								
259	F	99.1	70.9	28.2	6.0	22.1	4.50	4.7
186	C	50.8	37.8	13.0	2.4	10.5	1.50	2.7
173	D	74.9	54.1	20.8	2.8	18.0	2.70	3.5
184	E	85.1	61.6	23.5	3.8	19.7	3.50	3.7

TABLE 23

DRY MATTER INTAKE AND DIGESTIBILITY OF RATIONS BY THE  
WEST AFRICAN DWARF SHEEP

## HAY (I) RATION A

I.D NO. ANIMAL	INTAKE DM (g/day)	FAECAL DM (g/day)	% DIGESTIBILITY
179	508.0	226.8	55.35
259	710.6	305.4	57.02
268	423.4	196.6	53.57
186	725.8	310.0	57.29
263	174.6	75.6	56.70
173	463.4	195.0	57.92
301	212.4	90.7	57.30
184	232.8	101.3	56.49
<u>HAY II</u>			
179	255.2	102.1	59.99
259	408.2	141.8	65.26
268	333.4	153.1	54.08
186	383.3	131.5	65.69
263	255.2	88.5	65.32
173	383.3	139.5	63.61
301	255.2	79.4	68.89
184	204.1	64.6	68.35

TABLE 24

DRY MATTER INTAKE AND DIGESTIBILITY OF RATIONS BY THE  
WEST AFRICAN DWARF SHEEP

HAY AND CASSAVA. (RATION B)

ANIMAL NO.	INTAKE (g/day)	FAECAL (g/day)	% DIGESTIBILITY
179	510.3	181.4	64.45
259	536.4	146.3	72.73
268	408.2	113.4	72.22
186	536.4	169.0	68.49
263	522.8	146.3	72.02
173	500.1	146.3	70.75
301	680.4	164.4	75.84
184	543.2	137.2	74.74

TABLE 25

DRY MATTER INTAKE AND DIGESTIBILITY OF RATIONS BY THE  
WEST AFRICAN DWARF SHEEP

PERIOD 1

RATIONS	ID. NO. ANIMAL	g/day INTAKE	g/day FAECAL	% DIGESTIBILITY
C	179	484.8	129.5	73.29
C	259	667.2	165.4	75.21
D	268	748.4	229.6	69.32
D	186	544.3	175.3	67.79
E	263	544.3	189.5	65.19
E	173	714.4	218.3	69.44
F	301	616.6	143.2	76.78
F	184	561.3	143.2	66.08
<u>PERIOD II</u>				
D	179	654.9	184.3	71.86
D	259	697.4	207.4	70.26
E	268	646.4	177.7	72.51
E	186	578.3	114.3	80.23
F	263	612.4	139.9	77.16
F	173	663.4	165.8	75.00
C	301	518.8	110.1	78.78
C	184	387.0	79.8	79.36

TABLE 26

DRY MATTER INTAKE AND DIGESTIBILITY OF RATIONS BY THE  
WEST AFRICAN DWARF SHEEP

PERIOD III

RATIONS	ID. NO. ANIMAL	g/day INTAKE	g/day FAECAL	% DIGESTIBILITY
E	179	603.9	130.4	78.40
E	259	731.4	190.9	73.90
F	268	722.9	162.5	77.52
F	186	688.9	162.5	76.41
C	263	353.0	99.2	71.89
C	173	680.4	164.0	75.90
D	301	603.9	126.6	79.03
D	184	510.3	132.3	74.07
<u>PERIOD IV</u>				
F	179	561.3	136.1	75.76
F	259	697.4	164.4	76.42
C	268	701.7	181.4	74.14
C	186	514.6	98.8	80.80
D	263	425.2	100.6	76.33
D	173	421.0	100.6	76.09
E	301	523.0	121.9	76.69
E	184	489.0	108.2	77.87

TABLE 27

THE DIGESTIBILITY OF THE NITROGEN OF THE CONCENTRATE  
SUPPLEMENTS OF THE FEED BY THE WEST AFRICAN DWARF SHEEP

C O N C E N T R A T E S					
ANIMAL NO.	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
179	69.3	67.6	73.1	84.1	83.0
259	83.5	64.1	82.5	77.5	93.3
268	44.5	65.5	64.9	82.2	94.7
186	79.6	78.0	81.7	87.2	94.0
263	90.5	-	77.9	79.0	94.4
173	87.7	65.7	85.9	76.4	84.9
301	74.7	71.4	83.3	79.8	82.7
184	*	68.8	79.3	78.4	81.6
TOTAL	529.8	481.1	628.6	644.6	708.6
MEAN	75.7	68.7	78.6	80.6	88.6
SE	5.9	1.9	2.4	1.3	2.1

\* Value greater than 100%, excluded from estimation of mean.

TABLE 28

THE DIGESTIBILITY OF THE DRY MATTER OF THE CONCENTRATE SUPPLEMENTS OF THE FEED BY THE WEST AFRICAN DWARF SHEEP.

C O N C E N T R A T E S						
I.D. NOS.	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	
179	72.5	94.7	81.8	89.4	83.4	
259	*	91.0	79.6	87.3	88.5	
268	85.9	88.9	82.5	83.6	96.0	
186	90.6	97.7	82.5	83.6	96.0	
263	*	79.2	84.0	74.2	87.4	
173	93.1	87.9	83.9	78.5	85.5	
301	*	97.0	89.4	86.7	89.0	
184	*	86.2	80.5	86.9	70.9	
TOTAL	342.1	722.6	663.9	670.2	690.5	
MEAN	85.5	90.3	83.0	83.8	87.1	86.0±1.3
SE	4.6	2.2	1.1	1.8	2.8	

\* Values greater than 100%, excluded from estimation of mean.

TABLE 29

DRY MATTER INTAKE (g/day/W<sub>kg</sub><sup>0.73</sup>)

The Test of the Differences of the means of dry matter intake by sheep fed rations C, D, E and F.

A N I M A L S						
PERIOD	1	2	3	4	SUM	MEAN
1	C: 68.08	D: 72.96	E: 62.00	F: 78.20	281.24	70.3
2	D: 83.82	E: 72.54	F: 62.14	C: 65.79	284.29	71.1
3	E: 75.94	F: 80.04	C: 49.21	D: 72.40	277.59	69.4
4	F: 69.82	C: 61.85	D: 42.13	E: 65.23	239.03	59.8
SUM	297.66	287.39	215.48	281.62	1082.15	
MEAN	74.4	71.8	53.9	70.4		

SUMMARY BY TREATMENT

	C	D	E	F	
SUM	244.93	271.31	275.71	290.20	1082.15
MEAN	61.2	67.8	68.9	72.6	

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	1771.12			
PERIOD	3	336.54	112.18	5.44*	
ANIMALS	3	1043.45	347.82	16.88**	4.76
TREATMENT	3	267.43	89.14	4.32	
ERROR	6	123.70	20.61		

Differences of means significant for round ( $P < 0.05$ ) and animals ( $P < 0.001$ )



TABLE 30

DRY MATTER DIGESTIBILITY (%)

The Test of the differences of the means of dry matter digestibility of rations C, D, E and F by the sheep.

A N I M A L S						
PERIOD	1	2	3	4	SUM	MEAN
1	C: 74.2	D: 68.5	E: 67.3	F: 71.4	281.4	70.4
2	D: 71.1	E: 76.3	F: 76.1	C: 79.1	302.6	75.7
3	E: 76.1	F: 76.9	C: 75.9	D: 76.5	305.4	76.4
4	F: 76.1	C: 77.4	D: 76.2	E: 77.3	307.0	76.8
SUM	297.5	299.1	295.5	304.3	1196.4	
MEAN	74.4	74.8	73.9	76.1		

SUMMARY BY TREATMENT

	C	D	E	F	
SUM	306.6	292.3	297.0	300.5	1196.4
MEAN	76.7	73.1	74.2	75.1	

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	170.83			
PERIOD	3	106.91	35.64	7.90*	
ANIMALS	3	10.64	3.55	0.79	4.76
TREATMENT	3	27.21	9.07	2.01	
ERROR	6	26.07	4.51		

Differences of means significant for periods ( $P < 0.05$ )

TABLE 31  
INTAKE OF NITROGEN

The Test of the differences of the intake of nitrogen by sheep fed rations C, D, E and F (g/day/W<sup>0.73</sup><sub>kg</sub>)

A N I M A L S						
PERIOD	1	2	3	4	SUM	MEAN
1	C: 0.80	D: 0.90	E: 1.00	F: 1.63	4.33	1.08
2	D: 1.35	E: 1.51	F: 1.27	C: 0.93	5.06	1.26
3	E: 1.35	F: 1.74	C: 0.85	D: 0.83	4.77	1.19
4	F: 1.62	C: 0.80	D: 0.57	E: 1.26	4.25	1.06
SUM	5.12	4.95	3.69	4.65	18.41	
MEAN	1.28	1.24	0.92	1.16		

SUMMARY BY TREATMENT

	C	D	E	F	
SUM	3.38	3.65	5.12	6.26	18.41
MEAN	0.84	0.91	1.28	1.55	

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	1.95			
PERIOD	3	0.11	0.037		4.76
ANIMALS	3	0.31	0.103		
TREATMENT	3	1.36	0.453	16.01**	
ERROR	6	0.17	0.0283		

Differences of means highly significant for Treatment ( $P < 0.001$ )

TABLE 32

DIGESTIBILITY OF NITROGEN

The Test of the differences of the means of the % digestibility of rations C, D, E and F.

ANOVA

Sources	df	ss	Ms	F	Tab. F.05
TOTAL	15	489.98	-	-	-
PERIOD	3	88.42	29.47	6.15*	4.76
ANIMALS	3	27.38	9.13	1.91	-
TREATMENT	3	345.43	115.14	24.04**	-
ERROR	6	28.75	4.79	-	-

Differences of means significant for period ( $P < 0.05$ ) and Treatment ( $P < 0.001$ )

TABLE 33

DIGESTIBILITY OF CONCENTRATES: N.

The Testing of the differences of the means of the % digestibility of the nitrogen of concentrates 2, 3 and 5.

ANOVA

Sources	df	ss	Ms	F	Tab.F.05
TOTAL	15	1010.10	-	-	-
PERIOD	3	99.35	33.12	3.30	-
ANIMALS	3	21.45	7.15	0.71	4.76
TREATMENT	3	828.99	276.33	27.49**	-
ERROR	6	60.31	10.05	-	-

Differences of means very highly significant for Treatment ( $P < 0.001$ ).

TABLE 34

NITROGEN DIGESTED (g/day/W<sub>kg</sub><sup>0.734</sup>)

The Test of the differences of the means of N digested by sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab.f.05
TOTAL	15	1.41	-	-	-
PERIOD	3	0.09	0.03	1.76	4.76
ANIMALS	3	0.16	0.053	3.12	-
TREATMENT	3	1.06	0.353	20.76**	-
ERROR	6	0.10	0.017	-	-

Differences of means very highly significant for Treatment (P < 0.001)

TABLE 35

NITROGEN RETAINED (g/day/W<sub>kg</sub><sup>0.734</sup>)

The Test of the differences of the means of N retained by sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	ss	MS	F	Tab. F.05	
TOTAL	15	1.28	-	-	-	-
PERIOD	3	0.05	0.017	1.28	-	-
ANIMALS	3	0.19	0.063	4.74	4.76	-
TREATMENT	3	0.96	0.32	24.06**	-	-
ERROR	6	0.08	0.0133	-	-	-

Differences of means very highly significant (P < 0.001) for treatment.

TABLE 36

NITROGEN RETENTION (%)

The Test of the differences of the means of the % N retention by sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	563.78	-	-	-
PERIOD	3	61.97	20.66	1.59	-
ANIMALS	3	107.50	35.83	2.76	4.76
TREATMENT	3	316.37	105.46	8.12**	-
ERROR	6	77.94	12.99	-	-

Differences of means significant for Treatment - ( $P < 0.05$ )

TABLE 37

TOTAL RUMINAL NITROGEN (mg/100 ml).

The Test of the differences of the means of the total ruminal nitrogen of sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	20,231.95	-	-	-
PERIOD	3	1,128.66	376.22	0.85	-
ANIMALS	3	2,979.47	993.16	2.24	4.76
TREATMENT	3	13,460.04	4486.68**	10.10**	-
ERROR	6	2,663.78	443.96	-	-

Differences of means highly significant for Treatment ( $P < 0.01$ ).

TABLE 38

NON-AMMONIA NITROGEN (mg/100 ml).

The Test of the differences of the means of non-ammonia nitrogen in the rumen liquor of sheep fed rations C, D, E and F.

ANOVA

SOURCE	df	SS	MS	F	Tab. F.05
TOTAL	15	18,417.79	-	-	-
PERIOD	3	2,770.04	923.35	2.19	-
ANIMALS	3	826.16	275.39	0.65	4.76
TREATMENT	3	12,289.41	4,096.47	9.71*	-
ERROR	6	2,532.18	422.03	-	-

Differences of means highly significant for Treatment ( $P < 0.01$ ).

TABLE 39

RUMINAL PROTEIN NITROGEN (mg/100 ml).

The Test of the differences of the means of the ruminal protein nitrogen of sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	6686.12	-	-	-
PERIOD	3	318.02	106.01	0.72	-
ANIMALS	3	864.81	288.27	1.97	4.76
TREATMENT	3	4624.09	1541.36	10.52**	-
ERROR	6	879.19	146.53	-	-

Differences of means highly significant for Treatment ( $P < 0.01$ ).

TABLE 40

RUMINAL NON-PROTEIN NITROGEN (mg/100 ml)

The Test of the differences of the means of the ruminal non-protein nitrogen of sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	4258.80			
PERIOD	3	345.28	115.09	0.96	
ANIMALS	3	773.12	257.71	2.15	4.76
TREATMENT	3	2420.08	806.69	6.72*	
ERROR	6	720.32	120.05		

Differences of means significant for Treatment ( $P < 0.05$ )

TABLE 41

RUMINAL RESIDUAL NITROGEN (mg/100 ml)

The Test of the differences of the means of the ruminal residual nitrogen of sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	3411.70			
PERIOD	3	226.08	75.36	0.76	
ANIMALS	3	659.48	219.83	2.22	4.76
TREATMENT	3	1933.51	644.17	6.51*	
ERROR	6	593.63	98.94		

Differences of means significant for Treatment ( $P < 0.05$ ).

TABLE 42

RUMINAL AMMONIA-N (mg/100 ml)

The Testing of the differences of the means of ruminal ammonia N of sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	33.61			
ANIMALS	3	1.27	0.42	2.33	
PERIOD	3	12.49	4.16	23.11***	4.76
ERROR	3	1.06	0.18		

Differences between means very highly significant for both periods and Treatment ( $P < 0.001$ ).

TABLE 43

RUMINAL AMMONIA-N/TOTAL NITROGEN

Differences of the means of Ruminal Ammonia-N/Total N in the rumen of sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	43.59			
ANIMALS	3	11.72	3.91	15.04**	
PERIOD	3	28.37	9.46	36.38**	4.76
TREATMENT	3	1.95	0.65	2.50	
ERROR	6	1.55	0.26		

Differences of means very highly significant for both Animals and periods ( $P < 0.001$ ).



TABLE 44

α - AMINO NITROGEN OF RUMEN

LIQUOR: The Test of the differences of means of X-amino nitrogen (u-mole/ml) in the rumen of the sheep.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	55.67			
PERIOD	3	6.53	2.18	1.39	
ANIMALS	3	1.26	0.42	0.27	4.76
TREATMENT	3	38.43	12.81	8.16*	
ERROR	6	9.45	1.57		

Differences of means significant for Treatment ( $P < 0.05$ ).

TABLE 45

RESIDUAL N/NON-PROTEIN N.

The Test of the differences of the means of residual N/Non-protein N in the rumen of sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	782.82			
PERIOD	3	470.87	156.96	11.27**	
ANIMALS	3	68.45	22.82	1.64	4.76
TREATMENT	3	159.92	53.31	3.83	
ERROR	6	83.58	83.58	13.93	

Differences of means highly significant for period ( $P < 0.01$ ).

TABLE 46

PROTEIN-N/TOTAL RUMINAL-N

The Test of the differences of the means of Protein N/Total N in the rumen of sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	576.64			
PERIOD	3	90.99	30.33	0.95	
ANIMALS	3	144.92	48.31	1.51	4.76
TREATMENT	3	148.99	49.66	1.55	
ERROR	6	191.74	31.96		

Differences of means not significant for period, Animal or Treatment ( $P > 0.05$ ).

TABLE 47

BLOOD UREA-N (mg/100 ml)

The Testing of the differences of the means of the blood urea levels (mg/100 ml) of sheep fed rations C, D, E and F.

ANOVA

SOURCES	df	SS	MS	F	Tab. F.05
TOTAL	15	56.11			
ANIMALS	3	3.48	1.16	1.22	
PERIOD	3	25.64	8.55	9.00*	4.76
TREATMENT	3	21.27	7.09	7.46*	
ERROR	6	5.72	0.95		

Differences of means significant for both period and Treatment ( $P < 0.05$ ).

TABLE 48

DUNCAN MULTIPLE RANGE FOR TESTING DIFFERENCES OF MEANS

(I) Digestibility of nitrogen (%).

Treatment being compared	Range between means	No. of means	L S R
F and C	76.3 - 63.3 = 13.0	4	4.6
F and D	76.3 - 69.4 = 6.9	3	4.5
F and E	76.3 - 71.0 = 5.3	2	4.4
E and C	71.0 - 63.3 = 7.7	3	4.5
E and D	71.0 - 69.4 = 1.6	2	4.4
D and C	69.4 - 63.3 = 6.1	2	4.4
Treatments: F      E      D      C			
Mean	76.3    71.0    69.4    63.3		

(II) Nitrogen intake (d/day/W<sup>0.734</sup>)  
kg

F and C	1.55 - 0.84 = 0.71	4	0.35
F and D	1.55 - 0.91 = 0.64	3	0.35
F and E	1.55 - 1.28 = 0.27	2	0.34
E and C	1.28 - 0.84 = 0.44	3	0.35
E and D	1.28 - 0.91 = 0.37	2	0.34
D and C	0.91 - 0.84 = 0.07	2	0.34
Treatments:      F              E              D              C			
Means:	<u>1.55</u> <u>1.28</u> <u>0.91</u> <u>0.84</u>		

Means joined by the same under-line not significant ( $P > 0.05$ ).

TABLE 48 CONTD.

(III) Nitrogen digested (g/day/w<sup>0.734</sup><sub>kg</sub>)

Treatment being compared	Range between means	No. of means	L S R
F and C	1.20 - 0.54 = 0.66	4	0.28
F and D	1.20 - 0.63 = 0.57	3	0.27
F and E	1.20 - 0.91 = 0.29	2	0.26
E and C	0.91 - 0.54 = 0.37	3	0.27
E and D	0.91 - 0.63 = 0.28	2	0.26
D and C	0.63 - 0.54 = 0.09	2	0.26
Treatments:	F            E            D            C		
Means:	1.20        0.91 <u>0.63</u> 0.54		

(IV) Nitrogen retention (%)

F and C	69.5 - 57.5 = 12.0	4	7.6
F and D	69.5 - 63.6 = 5.9	3	7.5
F and E	69.5 - 66.5 = 3.0	2	7.2
E and C	66.5 - 57.5 = 9.0	3	7.5
E and D	66.5 - 57.5 = 2.9	2	7.2
D and C	63.6 - 57.5 = 6.1	2	7.2
Treatments:	F            E            D            C		
Means:	<u>69.5</u> <u>66.5</u> <u>63.6</u> 57.5		

Means joined by same under-line not significant ( $P > 0.05$ ).

TABLE 48 CONTD.

(V) Nitrogen retained ( $\text{g/day/W}_{\text{kg}}^{0.734}$ )

Treatments being compared	Range between means	No. of means	L S R
F and C	1.11 - 0.48 = 0.63	4	0.24
F and D	1.11 - 0.59 = 0.52	3	0.23
F and E	1.11 - 0.86 = 0.25	2	0.23
E and C	0.86 - 0.48 = 0.38	3	0.24
E and D	0.86 - 0.59 = 0.27	2	0.23
Treatments:	F            E            D            C		
Means:	1.11        0.86 <u>0.59</u> <u>0.48</u>		

(VI) Digestibility of the N of concentrates (%)

C <sub>5</sub> and C <sub>2</sub>	88.5 - 68.3 = 20.2	4	6.7
C <sub>5</sub> and C <sub>3</sub>	88.5 - 78.7 = 9.8	3	6.6
C <sub>5</sub> and C <sub>4</sub>	88.5 - 80.6 = 7.9	2	6.3
C <sub>4</sub> and C <sub>2</sub>	80.6 - 68.3 = 12.3	3	6.6
C <sub>4</sub> and C <sub>3</sub>	80.6 - 78.7 = 1.9	2	6.3
C <sub>3</sub> and C <sub>2</sub>	78.7 - 68.3 = 10.4	2	6.3
Treatments:	C <sub>5</sub> C <sub>4</sub> C <sub>3</sub> C <sub>2</sub>		
Means:	88.5 <u>80.6</u> <u>78.7</u> 68.3		

Means joined by same under-line not significant ( $P > 0.05$ )

(VII) Total ruminal N (mg/100 ml)

F and C	128.9 - 44.8 = 80.1	4	44.3
F and D	124.9 - 68.4 = 56.5	3	43.6
F and E	124.9 - 78.2 = 46.7	2	42.1
E and C	78.2 - 44.8 = 33.4	3	43.6
E and D	78.2 - 68.4 = 9.8	2	42.1
D and C	68.4 - 44.8 = 23.6	2	42.1
Treatments:	F            E            D            C		
Means:	124.9 <u>78.2</u> <u>68.4</u> <u>44.8</u>		

Means joined by same under-line not significant ( $P > 0.05$ )

TABLE 48 CONTD.

(VIII) Ruminal protein N (mg/100 ml).

Treatments being compared	Range between means	No. of means	L S R
F and C	79.1 - 31.5 = 47.6	4	25.4
F and D	79.1 - 50.6 = 28.5	3	25.0
F and E	79.1 - 55.9 = 23.2	2	24.2
E and C	55.9 - 31.5 = 24.4	3	25.0
E and D	55.9 - 50.6 = 5.3	2	24.2
D and C	50.6 - 31.5 = 21.1	2	24.2

Treatments:	F	E	D	C
Means:	79.1	55.9	50.6	31.5

(IX) Ruminal non-protein N (mg/100 ml).

F and C	45.8 - 13.3 = 32.5	4	23.0
F and D	45.8 - 17.8 = 28.0	3	22.6
F and E	45.8 - 22.3 = 23.5	2	21.9
E and C	22.3 - 13.3 = 9.0	3	22.6
E and D	22.3 - 17.8 = 4.5	2	21.9
D and C	17.8 - 13.3 = 4.5	2	21.9

Treatments:	F	E	D	C
Means:	45.8	22.3	17.8	13.3

Means joined by same under-line not significant ( $P > 0.05$ ).

TABLE 48 CONTD.

(X) Ruminal ammonia N (mg/100 ml)

Treatments being compared	Range between means	No. of means	L.S R
F and C	4.50 - 1.80 = 2.70	4	0.87
F and D	4.50 - 2.37 = 2.13	3	0.86
F and E	4.50 - 3.83 = 0.67	2	0.83
E and C	3.83 - 1.80 = 2.03	3	0.86
E and D	3.83 - 2.37 = 1.46	2	0.83
D and C	2.37 - 1.80 = 0.57	2	0.83

Treatments:	F	E	D	C
Means:	4.50	3.83	2.37	1.80

(XI) Blood urea N (mg/100 ml)

F and C	4.88 - 1.90 = 2.90	4	2.07
F and D	4.88 - 2.60 = 2.28	3	2.04
F and E	4.88 - 3.92 = 0.96	2	1.97
F and C	3.92 - 1.90 = 2.02	3	2.04
E and D	3.92 - 2.60 = 1.32	2	1.97
D and C	2.60 - 1.90 = 0.70	2	1.97

Treatments:	F	E	D	C
Means:	4.88	3.92	2.60	1.90

Means joined by same under-line not significant (P > 0.05).

TABLE 48 CONTD.

(XII) Ruminal residual N (mg/100 ml)

Treatments being compared	Range between means	No. of means	L S R
F and C	40.2 - 11.6 = 28.6	4	20.9
F and D	40.2 - 15.2 = 25.0	3	20.6
F and E	40.2 - 18.7 = 21.5	2	19.9
E and C	18.7 - 11.6 = 7.1	3	20.6
E and D	18.7 - 15.2 = 3.5	2	19.9
D and C	15.2 - 11.6 = 3.6	2	19.9

Treatments:	F	D	D	C
Means:	40.2	<u>18.7</u>	<u>15.2</u>	<u>11.6</u>

(XIII) - amino N (u mole/ml)

F and C	6.29 - 2.31 = 3.98	4	2.62
F and D	6.29 - 3.02 = 3.27	3	2.58
F and E	6.29 - 4.79 = 1.50	2	2.49
E and C	4.79 - 2.31 = 2.48	3	2.58
E and D	4.79 - 3.02 = 1.77	2	2.49
D and C	3.02 - 2.31 = 0.71	2	2.49

Treatments:	F	E	D	C
Means:	6.29	<u>4.79</u>	3.02	2.31

Means joined by same under-line not significant ( $P > 0.05$ ).



TABLE 49

DRY MATTER INTAKE, PER CENT CONCENTRATE IN RATION  
AND PER CENT. CRUDE PROTEIN OF THE RATIIONS

No.	Ratio	Total Intake (g/d)	Concentrate	% Concentrate	% C.P.
179	C	484.8	221.1	45.61	6.49
259	C	667.2	357.2	53.54	6.30
265	D	748.4	407.7	54.47	8.41
186	D	544.3	229.6	42.19	8.25
263	E	544.3	263.7	48.44	10.31
173	E	714.4	399.7	55.95	10.72
301	F	616.6	378.5	61.38	14.02
184	F	561.3	374.2	66.66	14.57
<u>2ND PERIOD</u>					
179	D	654.9	409.3	62.34	8.51
259	D	697.4	408.3	58.54	8.46
268	E	646.4	408.3	63.16	11.11
186	E	578.3	408.2	70.59	11.51
263	F	612.4	408.2	66.66	14.57
173	F	663.4	408.3	61.54	14.04
301	C	518.8	280.7	54.10	6.29
184	C	387.0	297.7	76.92	5.69

Per cent Concentrate in the Ration.

Mean values:

RATION	MEAN	VALUE
B	35.9 ±	14.1
C	58.5 ±	9.2
D	58.2 ±	12.8
E	62.7 ±	7.6
F	62.9 ±	5.0

TABLE 49

DRY MATTER INTAKE, PER CENT CONCENTRATE (CONTINUED)

Nos.	Ratio	Total Intake (g/d)	Concentrate	% Concentrate	% C.P.
<u>3RD PERIOD</u>					
179	E	603.9	408.3	67.61	11.35
259	E	731.4	408.2	55.81	10.72
268	F	722.9	408.2	56.47	13.52
186	F	688.9	408.2	59.26	13.80
263	C	353.0	238.2	67.47	5.94
173	C	680.4	408.2	60.00	6.14
301	D	603.9	408.3	67.61	8.58
510	D	374.3	374.2	73.33	8.66
<u>4TH PERIOD</u>					
179	F	561.3	408.2	72.72	15.18
259	F	697.4	408.3	58.54	13.73
268	C	701.7	408.2	58.18	6.19
186	C	514.6	268.0	52.07	6.34
263	D	425.2	306.1	72.00	8.63
173	D	421.0	148.8	35.35	8.15
301	E	523.0	344.4	65.85	11.25
184	E	489.0	344.4	70.43	11.51

% Concentrate in the Rations.

## RATION B

Animal Nos.	Ration	Total D.M. Intake (gm)	Concentrate	% Concentrate
179	B	510.3	272.1	653.33
259	B	536.4	134.1	25.00
268	B	408.2	236.5	57.94
186	B	536.4	178.8	33.33
263	B	522.8	180.5	34.52
173	B	500.1	183.0	36.59
301	B	680.4	249.0	36.59
184	B	543.2	54.7	10.07

TABLE 50

MEAN L/C LIVE WEIGHT OF THE WEST AFRICAN DWARF  
SHEEP DURING THE DIGESTION EXPERIMENTS

TRIAL ONE

Period	Animal Nos.	Weight (kg)	W <sup>0.734</sup> kg
1	179	15.88	7.53
	259	21.77	9.48
	268	19.96	8.89
	186	19.50	8.74
	263	22.68	9.76
	173	26.31	10.88
	301	13.15	6.56
	184	17.69	8.14
2	179	15.88	7.53
	259	21.77	9.48
	268	19.96	8.89
	186	19.05	8.60
	263	20.86	9.18
	173	26.31	10.88
	301	14.51	7.05
	184	17.69	8.14

TABLE 50

MEAN LIVEWEIGHT OF THE ANIMALS (KG)

TRIAL TWO

Period	Animal Nos.	Weight (Kg)	W <sup>0.734</sup> kg
1	179	15.42	7.37
	259	21.77	9.48
	268	20.86	9.18
	186	18.60	8.45
	263	21.32	9.33
	173	26.31	10.88
	301	14.51	7.05
	184	17.69	8.14
2	179	14.51	7.05
	259	21.32	9.33
	268	19.96	8.89
	186	17.24	7.99
	263	23.59	10.05
	173	24.95	10.47
	301	13.15	6.56
	184	15.42	7.37

TABLE 50

MEAN LIVE WEIGHT OF THE ANIMALS (Kg)

Period	Animal Nos.	Weight (W) Kg.	$W^{0.734}$ kg
3	179	16.78	7.84
	259	22.68	9.77
	268	20.41	9.04
	186	19.05	8.60
	263	22.22	9.62
	173	26.76	11.02
	184	17.24	7.99
4	179	17.24	7.99
	259	23.59	10.05
	268	22.68	9.77
	186	19.50	8.74
	263	21.32	9.33
	173	26.31	10.88
	301	16.33	7.68
	184	16.78	7.84

TABLE 51

INTAKE, OUTPUT AND APPARENT DIGESTIBILITY OF NITROGEN AND NITROGEN  
RETENTION OF SHEEP (9g/day) MAINTAINED ON BASAL HAY AND CONCENTRATE  
SUPPLEMENTS

Animal	Ration	Intake-N	Faecal-N g/day	Urinary-N	Digestibility	Retained-N	Milk-N
186	A	6.2	2.3	2.8	62.9	1.1	
210	A	6.2	1.9	1.6	69.3	2.7	
259	F	10.1	2.0	4.7	79.2	3.4	
273	F	10.1	2.5	4.4	75.2	3.2	
72	F	10.1	2.5	4.4	75.2	3.2	2.1
90	F	10.1	2.0	5.4	79.2	2.7	0.8

TABLE 52

ENRICHMENT (E) OF RUMINAL AMMONIA, BLOOD UREA,  
BACTERIA AND PROTOZOA AFTER ADMINISTRATION OF  
<sup>15</sup>N INTO THE RUMEN OR BLOOD OF WETHER SHEEP

	No 210	No 273	No 186	No 259
	ATOMS	% EXCESS	OF <sup>15</sup> N	
RUMINAL AMMONIA	2.377	2.473	0.322	0.075
	1.291	1.290	0.288	0.060
	0.442	0.440	0.261	0.050
	0.278	0.275	0.259	0.043
	0.234	0.235	0.196	0.041
BLOOD UREA	0.677	0.323	2.647	1.905
	0.568	0.251	1.939	1.269
	0.476	0.130	1.600	0.829
	0.386	0.128	1.357	0.692
	0.324	0.086	1.220	0.509
URINE	0.161	0.105	0.620	0.665
	0.080	0.055	0.069	0.056
	0.043	0.056		
BACTERIA	0.456	0.436		
	0.398	0.331		
	0.336	0.275		
	0.275	0.247		
	0.251	0.243		
PROTOZOA	0.240	0.229		
	0.220	0.210		
	0.209	0.201		
	0.190	0.189		
	0.174	0.165		

TABLE 53

ENRICHMENT (E) OF URINE, FAECES AND MILK AFTER ADMINISTRATION OF <sup>15</sup>N INTO THE RUMEN OF THE EWES MAINTAINED ON BASAL HAY AND CONCENTRATE SUPPLEMENTS

	Day	No. 72	No. 90
		A T O M % E X C E S S O F <sup>15</sup> N	
URINE	1	0.189	0.188
	2	0.071	0.084
	3	0.039	0.047
FAECES	1	0.158	0.065
	2	0.040	0.070
	3		0.078
MILK	1	0.032	0.047
	2	0.037	0.053
			0.043
			0.032
			0.030

MILK SECRETION IN LACTATING SHEEP

No. 72

D A Y

	1	2	3	4	5	6	7
Milk (ml)	330	330	150	220	240	200	150
gN	2.98	2.98	1.35	1.99	2.17	1.81	1.35
No. 90							
Milk (ml)	100	60	60	60	70	70	60
gN	0.88	0.84	0.74	0.86	0.71	0.98	0.61



TABLE 54

PER CENT N, OM AND CR IN DIGESTA OF THE WEST

AFRICAN DWARF SHEEP

Sheep No.	Feed	Omasum	Abomasum	Proximal Small Intestine	Distal Small Intestine	Rectum
% N	1.23	1.54	1.26	2.17	0.84	1.27
336 %OM	92.50	93.40	92.40	89.60	89.60	90.40
%CR	0.097	0.175	0.230	0.149	0.230	0.208
% N	0.76	1.89	1.19	1.82	1.12	1.96
314 % OM	93.50	90.30	92.40	88.60	90.00	83.80
% CR	0.072	0.175	0.145	0.145	0.215	0.218
% N	1.04	1.82	1.05	2.45	1.05	2.11
343 % OM	92.77	93.90	94.20	88.60	89.90	89.40
% CR	0.104	0.215	0.185	0.190	0.223	0.358
% N	1.32	2.38	1.61	1.40	1.26	1.83
399 % OM	93.04	95.30	88.60	88.00	88.00	90.80
% CR	0.086	0.160	0.160	0.177	0.145	0.254
% N	1.67	1.67	1.40	2.03	1.19	2.32
499 % OM	93.01	91.80	77.30	84.60	88.60	88.40
% CR	0.072	0.074	0.043	0.066	0.120	0.296
% N	1.98	3.78	2.66	2.31	1.47	2.38
510 % OM	93.32	90.59	87.30	86.00	87.50	86.60
% CR	0.072	9.137	0.160	0.152	0.200	0.370

TABLE 54

PER CENT N. OM AND CR IN DIGESTA OF THE WEST AFRICAN  
DWARD SHEEP (CONTINUED)

Sheep No.	Feed	Omasun	Abomasun	Proximal Small Intes- tine	Distal Small Intes- tine	Rectum
% N	1.23	1.61	1.82	3.01	1.54	1.47
320 % OM	92.50	92.30	92.50	89.70	89.90	92.60
% CR	0.097	0.043	0.043	0.045	0.300	0.150
% N	0.79	2.10	3.57	4.06	1.20	2.00
513 % OM	93.44	89.90	89.20	84.70	85.00	89.00
% CR	0.135	0.205	0.274	0.175	0.420	0.654
% N	1.01	2.03	2.66	3.64	1.00	1.86
518 % OM	92.82	93.60	93.00	87.90	87.70	89.00
% CR	0.156	0.240	0.274	0.145	0.310	0.546
% N	1.27	4.55	4.20	5.18	1.40	1.82
484 % OM	92.73	87.20	82.20	87.20	83.30	90.00
% CR	0.190	1.300	0.520	0.215	0.496	0.795
% N	1.40	1.16	4.62	5.60	1.00	2.33
358 % OM	92.69	90.80	90.00	85.00	84.90	87.60
% CR	0.190	0.370	0.320	0.240	0.440	0.926
% N	2.15	2.87	2.10	4.06	2.03	2.78
570 % OM	93.51	93.30	69.50	87.80	88.00	89.00
% CR	0.067	0.024	0.024	0.010	0.430	0.314