QUALITATIVE AND QUANTITATIVE ASPECTS OF AMINO ACID DIGESTION IN SHEEP

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ABSTRACT

A conventional digestion trial was conducted with three sheep fed three rations in a latin square design. Twenty-day digestion periods were divided in two to check for repeatibility of results. A sampling regime designed to measure volume of duodenal flow and obtain representative samples for chemical analysis was superimposed on the digestion trial. The rations had equal protein levels, but varied in amino acid and digestible energy content. Ration components were ground corn and legume-brome hay mixed in different proportions to give three different levels of digestible energy.

The highest energy ration gave significantly higher coefficients of digestibility of ether extract, nitrogen free extract, and gross energy than the other rations. Digestion of crude fibre was significantly highest on the high roughage ration, while ash and protein digestibilities were ration independent.

Hourly flow estimates were averaged within four equally spaced divisions of the day, and significant differences among these divisions was taken as evidence of diurnal variation in rate of fore-stomach emptying. The diurnal variation was monophasic, corresponding to feeding the rations once a day. Although data obtained on flow within periods appeared acceptable there was a significant variation in ten-day total flows among periods. Composite duodenal

samples made on an aliquot basis from within period flow data were used to estimate average composition of duodenal contents on the three rations.

Changes in percentage composition from feed to duodenal level showed protein and ash to give large increases, with ether extract and gross energy remaining fairly constant, and nitrogen free extracts declining. Wide ration differences in amino acid levels failed to cause significant ration effects on composition of duodenal protein. The evidence indicates that rumen fermentation removed ration differences in amino acid levels. The high energy ration resulted in a higher percentage of soluble carbohydrate in the duodenal digesta than the other two rations.

Estimates of dry matter passage through the duodenum based on assumptions on the amount and location of fibre digestion showed the high fibre rations to have significantly larger dry matter passage than the high corn ration. Calculations based on dry matter passage showed a net addition of crude protein and ash by the fore-stomach for all rations. Gross energy, nitrogen free extracts, and ether extract were more highly digestible in the rumen on the rations containing the most corn. Amounts of soluble carbohydrate entering the small intestine varied from an average of 62 gm per day on the high roughage ration to 109 gm per day on the high corn ration.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

INTRODUCTION

Digestion in the ruminant is characterized by fermentation followed by enzymatic digestion and then finally fermentation. Products of rumino-reticulum fermentation include gases which may be absorbed or lost by eructation, and other materials which may be absorbed from the rumen or passed on to the lower gut. The residual material leaving the fore-stomach contains partially degraded ration components and microbial material. After enzymic digestion in the small intestine the remaining constituents are subjected to fermentative attack by microorganisms of the large intestine. By partitioning digestion into fore-stomach and intestinal effects one can determine the contribution of each region to nutrient synthesis and absorption.

Importance of this partitioning can be demonstrated by considering the differences in nutrient utilization associated with the two areas. Fermentation of protein in the rumen gives partial hydrolysis of protein to amino acids which in turn may be deaminated. Ammonia from this source when present in excess is absorbed into the portal blood stream and converted to urea in the liver. Ammonia in the rumen may be synthesized into microbial protein. Both the quantity and quality of protein leaving the rumino-reticulum can differ from that in the ration. An independent method

for measuring the absolute amount of protein leaving the fore-stomach and its amino acid content are required before quantitative information can be obtained about intestinal digestion and absorption of protein in the ruminant. There is almost nothing known about the effect of ration protein on amino acid composition of digesta entering the duodenum.

Fermentative microorganisms in the rumen attack starches and more complex carbohydrates, giving volatile fatty acids, methane, and carbon dioxide as the main breakdown products. Short chain fatty acids are absorbed by the rumen epithelium and passed into the portal blood system. This fatty acid absorption accounts for a large proportion of energy absorbed from the ration. Intestinal hydrolysis of soluble carbohydrate to monosaccharides provide another potential source of energy for the ruminant. It is generally considered that with high roughage rations the amount of soluble carbohydrate escaping fermentation in the rumen is negligible. However rations containing high levels of digestible energy can pass through the rumen rapidly enough to escape complete starch degradation. Since the heat loss from fermentation accounts for an appreciable amount of ration energy, any factors which can increase the amount of monosaccharides absorbed from the small intestine will show increased efficiency of energy utilization. Since little information is available concerning the amount of soluble carbohydrate entering the duodenum, a measurement of this

would be helpful in the partitioning of carbohydrate digestion between the fore-stomach and lower digestive tract.

The present experiment was designed to estimate the effects of different rations on digestion occurring before and after the fore-stomach with special emphasis on amino acids. In addition the amount of crude fibre, ether extract, energy crude protein, nitrogen free extract, and soluble carbohydrate digestion occurring in the rumen was studied.

In order to conduct a study of this type one has to quantitatively measure the flow of digesta into the duodenum. This was done using fistulated sheep and a marker dilution technique which has been described by Phillips and Dyck (1964).

LITERATURE REVIEW

FLOW MEASUREMENT

In discussing the difficulties associated with attempts to measure the volume of digesta entering the lower alimentary tract of ruminants, Singleton (1961) stated that, "an ideal method of measurement would be applicable to the animal without producing any disturbance in its normal behaviour, so that recordings could be made over several successive days without the animal being moved from its usual location". Methods described to date have usually fallen far short of this ideal.

The anatomical locations at which flow measurements have been made are the omasal-abomasal junction, the region of the pylorus, and the first few inches of the duodenum. Oyaert and Bouckaert (1961) cannulated the omasal-abomasal orifice of sheep in a method which allowed them to block the orifice and collect all the material which would normally flow into the abomasum. A solution of polyethylene glycol was introduced into the rumen and the recovery at the omasal-abomasal level provided an estimate of flow independent of the volume collected. Failure to return the collected material to the abomasum gave an increase in water consumption in the experimental animals, and the dry matter content of the digesta was found to

decrease with time on collection. These observations, combined with greater than 100% marker recovery, indicated that the collection technique was increasing flow above normal levels. Total collection values were corrected for 100% marker recovery to give an average hourly omasal outflow of 462 ml with a feed intake of one kg of dry matter.

This method of flow estimation has been more extensively used in the region of the pylorus and the first part of the duodenum. This region is more accessible for experimental surgery than is the omasal-abomasal orifice.

Flow data obtained at the duodenal level does not give an accurate estimate of the fore-stomach effect due to the volume of secretions added by the abomasum. However little or no absorption occurs from the abomasum so quantities of nutrients estimated at the duodenum will be very similar to the quantities passing through the omasal-abomasal orifice. Therefore duodenal flow values showed the amounts of materials entering the small intestine, and thus available for digestion and absorption.

Phillipson (1952) studied duodenal flow using two distinct experimental preparations. One method involved exteriorization of flow between a cannula in the pyloric part of the abomasum and another in the duodenum, but the sheep did not survive. The second preparation consisted of

three simple cannulae, one immediately caudal to the pylorus, and two more aboral to the first. In the preparation involving three cannulae a balloon was inserted in the second cannula and inflated. This blocked the duodenum completely while digesta was collected from the first cannula, measured, and returned via the third one. In these experiments two factors in the experimental technique were noted which altered flow values:

(1) Neglecting to reintroduce the collected material into the duodenum gave an abnormally high flow, (2) the presence of the inflated balloon used to block the duodenum decreased flows. It was shown that removal of the balloon resulted in an immediate sustained increase in flow. Maximum average flows for a 40 kg sheep consuming 1200 gm of feed per day were 800 ml per hour based on no return of collected samples to the duodenum. Average minimum values of 350 ml per hour were obtained with a balloon inflated in the lumen of the duodenum. It was suggested that true values for this sheep on this ration would lie at some intermediate value.

Hogan and Phillipson (1960) used sheep with duodenal flow permanently exteriorized according to the method of Markowitz (1949) in order to estimate duodenal flow under normal conditions of digestion. The duodenal flow was completely collected, measured, sampled, and then returned to

the small intestine as soon as possible after collection. Mean flow values of 360 ml per hour were obtained for sheep eating 1000 gm of ration per day. The possible existence of a diurnal variation in flow rate was also investigated, and results from twice daily feeding of the ration failed to demonstrate the existence of any marked diurnal trends.

Harris and Phillipson (1962) used similar techniques in the estimation of duodenal flow, but they replaced the amount of collected material retained for proximate analysis with digesta obtained from a second sheep which was not on observation at the time. They fed chromium sesquioxide as an inert marker and measured recovery at the duodenal level as an independent estimate of flow. Digestibility of the ration was not affected by measuring flow, but complete collection of duodenal contents over twelve-hour periods did not give complete marker recovery. Physiological disturbance caused by duodenal collection appeared to slow down the normal rate of abomasal emptying, with high compensatory flows apparently occurring between collection periods. Flows were corrected for complete duodenal recovery of marker fed in the ration, and the average duodenal flow for all sheep when receiving 750 gm of hay per day was 416 ml per hour, with a digestibility of

55% for organic matter. There was a diurnal variation in flow rate which depended on time after feeding and the amount of time spent on rumination.

Singleton (1961) obtained average flow rates of 464 to 493 ml per hour for sheep fed 700 gm of hay per day. Surgical preparation for these experiments involved the use of re-entrant duodenal cannulae similar to those of Phillipson's later experiments. Flow volume was measured by passing the tube connecting the cannulae through a powerful magnetic field and recording the EMF produced in electrodes placed in contact with the digesta and at right angles to the magnetic lines of force. This method is advantageous from the viewpoint of observing without interfering, but the animals were required to stand and allowed little freedom of movement. Therefore observation periods were limited to three hours. Such short observation periods are likely to give abnormal flow data according to the results of Phillipson (1962).

These flow experiments indicate the necessity for observations which can be made continuously over several days with the minimum of inconvenience to the experimental animals. Such a method was used by Phillips and Dyck (1964) in conjunction with digestibility studies. A soluble inert marker, polyethylene glycol was infused into the abomasum of sheep, and duodenal digesta was sampled at hourly inter-

vals. Samples were analysed for the marker and for proximate constituents. Volume of flow from the abomasum was calculated from the degree of marker dilution. The presence of the person collecting the samples should have had no effect on observed values since the dilution of the marker would be a continuous process influenced very little by momentary changes. The volume of polyethylene glycol solution entering the abomasum per hour was equal to that of the sample being removed from the duodenum. Average flows in four sheep varied from 671 ml per hour on a high roughage ration to 265 ml on a ration containing a high proportion of readily fermentable carbohydrate. Food intake varied both within and between rations. Calculation of flow corrected for intakes of organic matter gave flow rates of 0.90, and 0.57 ml per hour per gram of organic matter consumed per day for high and low roughage rations.

NUTRIENT DISAPPEARANCE

A study of nutrient disappearance along the gastrointestinal tract of the ruminant gives valuable information on sites of digestion and absorptive abilities of the various organs. Such a study can be used to predict the efficiency of utilization of energy and nitrogen in a ration. Furthermore estimates of the quantity and composition of the dry matter leaving the fore-stomach should make it possible

to test the hypothesis that the abomasum and intestines of ruminants function in a similar manner to the gastro-intestinal tract of monogastric animals.

Measurements of dry matter absorption from the digestive tract are the initial information required for estimating total nutrient utilization. Hogan (1957) used sheep with permanently exteriorized duodenal flow to estimate dry matter disappearance at the pylorus and at the ileo-caecal junction. Then from a knowledge of feed and feces values he was able to establish the amounts disappearing in the fore-stomach, in the small intestine, and in the large intestine. Seventy percent of the digestible dry matter was absorbed before the duodenum, 11% in the small intestine, and the remainder in the caecum and large intestine. A similar pattern was observed by Balch (1957). He used the lignin ratio technique and showed that approximately 65% of the digestible dry matter was lost in the reticulo-rumen, and the remainder was lost in the intestines when an all hay ration was fed.

Hogan and Phillipson (1960) found that 70% of the digestible dry matter in a sheep ration disappeared before the duodenum, with 10% being lost in the small intestine, and the remainder in the caecum and large intestine. Singleton (1961) showed fairly low proportions of digestible dry

matter being lost from the fore-stomach. In one sheep he actually observed a net increase in dry matter passing through the duodenum relative to that consumed.

Energy

The main substances being absorbed from the rumino-reticulum and the omasum are the steam volatile fatty acids (VFA). Their absorption can be demonstrated by arterio-venous differences in blood levels (Phillipson, 1947) and by measuring relative amounts of VFA in rumen contents and abomasal contents (Badawy et al., 1958a). Microbial fermentation of dietary carbohydrate is the main source of VFA production in the rumen (Lindsay, 1959). VFA production in and absorption from the rumen is the most important source of energy for the metabolism of the ruminant. Balch (1958) calculated the heat of combusion of VFA produced from fermentation of organic matter in the rumen. Rumen samples from cows on a coarse hay and concentrate diet showed VFA production in in vitro fermentation accounted for 49 - 63% of the digestible energy in the ration. Chopping the hay increased the relative proportion of propionate and, in this case VFA production totaled 71 - 75% of the energy absorbed from the ration. Blaxter (1961) has shown a marked difference in efficiency of energy utilization by ruminants of the various end-

products of microbial fermentation and glucose. Blaxter determined "calorimetric efficiencies of energy utilization for maintainance" of acetate, propionate, butyrate, and lactate based on glucose as 100. None of the individual VFA's or any combination thereof gave values as high as glucose for efficiency of energy utilization. This experimental observation draws some theoretical support from the classical energy derivation cycles of Embden-Myerhoff and Krebs (Fruton and Simmonds, 1958).

Balch (1958) has demonstrated that the major portion of useful energy in most rations comes from the VFA produced by carbohydrate fermentation. Increase in efficiency of energy use on high concentrate rations have been attributed to changes in the molar ratios of VFA's towards higher levels of propionate at the expense of acetate (Lindsay, 1959). This basic fact is not under dispute, but Blaxter et al. (1956) propose the existence of a situation where high feeding rates will speed food passage through the fore-stomach, and prevent complete fermentation of dietary carbohydrates such as starch. Such carbohydrates would then be subjected to enzymic digestion and absorption in the intestines. The absorbed sugars would be available as an energy source of relatively high efficiency.

Estimates of the amounts of starch escaping rumen

fermentation and entering the duodenum have been set as low as 7.8 gm per day by Weller and Gray (1954) when sheep were receiving 148 gm starch in the ration. The use made of starch in the intestine was studied by Larsen et al (1956). Significant increases in blood sugar levels were obtained when glucose and maltose were injected into the duodenum and jejunum of calves. The administration of ground corn or starch at the omasal level gave negligible increases in blood sugars. These results indicate a lack of amylolytic activity in the intestines, or possibly a specificity of intestinal amylases for substrates not used in this experiment.

Taylor (1962) investigated the volume and amylolytic activity of pancreatic secretion in sheep. He found that the sheep pancreas secreted only about one-seventh as much of both fluid and enzymes as the dog pancreas. Since the relationship between concentration of amylase and enzyme activity was linear, the amylolytic activity in sheep would appear to be one seventh of that in the dog. Such a level of amylolytic activity should result in considerable hydrolysis of starch in the sheep's intestine.

Protein Quantity

The nature of the ration can influence the proportion

of dietary nitrogen appearing in the duodenal digesta. The main ration characteristics which have an effect are the amount of nitrogen in the ration, digestible energy level of the ration, and the solubility of ration protein. Gray et al. (1958) have shown that by altering the level of nitrogen in the ration the utilization of nitrogen in the rumen was affected. Sheep were fed mixtures of wheat straw, wheat hay, and lucerne hay with nitrogen contents varying from 0.7% to 2.9%. Amount of nitrogen leaving the abomasum tended to be constant despite the variations in nitrogen content among rations. The 2.9% level showed a loss of nitrogen at the abomasal level, presumably due to hydrolysis of protein and loss of ammonia from amino acids by deamination and absorption by the ruminal epithelium. The intermediate levels showed almost no change in amount of nitrogen from feed to duodenum. On wheat straw an increase in the amount of nitrogen from feed to duodenum suggested nitrogen addition in the rumen and a net synthesis of protein. A source of nitrogen for this synthesis could have been urea from the sheep's saliva. McDougall (1948) estimated the nitrogen level in mixed sheep saliva at 9 - 36 mg per 100 ml, giving a maximum of two grams per day from this source. Movement of nitrogenous substances from the blood into the rumen could also have accounted for

some of the increase in protein. Lewis et al.(1957) found blood levels of ammonia to be so low that diffusion into the rumen would be negligible from this source. However Houpt (1959) measured an ammonia appearance of about 1.5 gm per day in the isolated rumino-reticulum of sheep and goats. Blood urea is considered the source of this ammonia. Active transport of blood urea into the rumen has been demonstrated by Gaertner (1961), indicating the amount of nitrogen added from this source was independent of blood urea concentration. These factors seem to tend toward the production of a constant nitrogen level in the duodenal digesta regardless of ration differences.

A second point to be considered is the effect of energy level of the diet on nitrogen utilization. Phillipson et al. (1959) considered the rumen concentration of ammonia to be dependent on the dietary protein source and on the availability of a readily fermentable energy source. Synthesis of protein requires a supply of nitrogen either as ammonia or amino acid, and an available energy source. Without a continuous energy supply a majority of ammonia released by fermentation will be absorbed into the blood instead of being formed into protein.

Physical properties of the dietary protein are a third

factor influencing its utilization in the ruminant. McDonald (1948) found the source of dietary protein to be a major factor in determining the concentration of ammonia in the rumen. Blackburn and Hobson (1960) fed sheep diets differing only in the solubility of casein, the protein source. They found a positive correlation between solubility and proteolytic activity in the rumen. Soluble protein was rapidly broken down to mostly non-ammonia non-protein nitrogen, while the less soluble protein gave a steady but slower breakdown. Nitrogen retention by the sheep, and levels of bacterial nitrogen in the rumen were higher for the more soluble protein.

Chalmers and Synge (1954) compared herring meal protein supplements with casein in nitrogen balance trials on sheep. They found the herring meal to give a higher nitrogen retention than casein. Peak concentrations of ammonia in the rumen were larger, and appeared at a later time after feeding on casein than on herring meal. Heat treatment of casein decreased its solubility, and gave improved nitrogen utilization which was associated with a more uniform release of ammonia with time after feeding. They concluded that a uniform rate of ammonia release from dietary nitrogen gave the greatest utilization.

Conclusions reached by Chalmers and Synge are not in agreement with those of Blackburn and Hobson. These differences might be explained by the level and source of energy present in the basal rations of the different experiments.

Protein Quality

The quality of protein available for digestion in the duodenum compared with the quality of food protein has received little attention. There is the indirect evidence that various ration protein and non-protein nitrogen sources support similar growth, indicating that they are used for the synthesis of a uniform type of protein in the rumen. Weller et al. (1958) studied the quantitative changes of plant nitrogen to microbial and soluble nitrogen in the rumen of sheep. They hoped to obtain estimates of improvement in biological value between food and duodenal protein by obtaining estimates of the relative values of the two proteins, and the degree of conversion occurring. A physical separation technique was used and corrections for contamination were made using diaminopimelic acid as an indicator of bacterial protein remaining in the food fraction. For a ration of wheaten hay it was estimated that 63 - 82% of the total nitrogen

in rumen contents was present as microbial protein, 11 - 27% was still in the plant form, and 5 - 10% was soluble.

McDonald (1954) fed sheep a semi-purified diet containing zein protein as the nitrogen source. Using the absence of lysine in zein as a marker, and assuming that all nitrogen left the rumen as either plant or microbial protein, he calculated that 40 - 50% of zein was converted to microbial protein.

McNaught et al. (1950, 1954) fed rumen bacterial protein to rats and established a biological value of 81 - 88%. Such high biological values for rumen bacterial protein, together with the high proportion of conversion of dietary protein to bacterial protein, should result in significant improvement in quality of dietary protein. However Weller (1957) investigated the effect of nitrogen source in the diet upon the quality of synthesized bacterial protein. He fed several different forms of hay, but was unable to detect any ration effect on amino acid composition of rumen protein. There was a remarkable similarity between amino acid composition of plant leaf protein and rumen bacterial protein. This suggested a biological value of 60 - 70% for the bacterial protein, a range in which the proteins of several common feeds are included.

The quality of protein reaching the duodenum could depend upon the rate of food passage through the fore-stomach. If the concentrate in a mixed ration remains in the cranial end of the fore-stomach it may be passed on rapidly without much protein conversion occurring. Therefore factors affecting rate of passage could have an effect on protein quality.

Abomasal and Duodenal Protein

In assessing the rumen effect on protein quality by analysis of digesta collected from the duodenum it is important to consider dilution of microbial and ration protein by enzymic protein of endogenous origin. Nasset (1962) analysed jejunal contents of dogs fed rations of varying amino acid composition and found little similarity between feed and jejunal amino acid levels. This situation was postulated to arise from digestive secretions, and from secretion of free amino acids into the gut lumen to provide molar ratios favourable to absorption. In ruminants the above factors could be combining with the rumen effect to mask ration differences in amino acid composition at the duodenal level.

EXPERIMENTAL PROCEDURE

The experiment consisted basically of digestion trials using three sheep and three rations in a latin square design. Concurrent with the digestion trials estimates were made of the volume of digesta leaving the abomasum. Chemical analyses of samples of duodenal digesta together with flow estimates allowed calculations of the net digestion occurring in the fore-stomach and in the intestines.

DESIGN

Each period in the 3 x 3 latin square was of 34 days duration, consisting of a 14-day adjustment to the ration followed by two 10-day collection periods (Figure 1). The two 10-day digestibility trials were considered as replicates of the 3 x 3 latin square, and were used to check the repeatability of observations. The sheep were maintained in metabolism crates for three days before, and throughout the replicate 10-day collection periods. The sheep were fed once daily at 9 a.m. and consumed the ration within about one hour. Water was offered *ad libitum*.

RATIONS

The three experimental rations consisted of mixtures

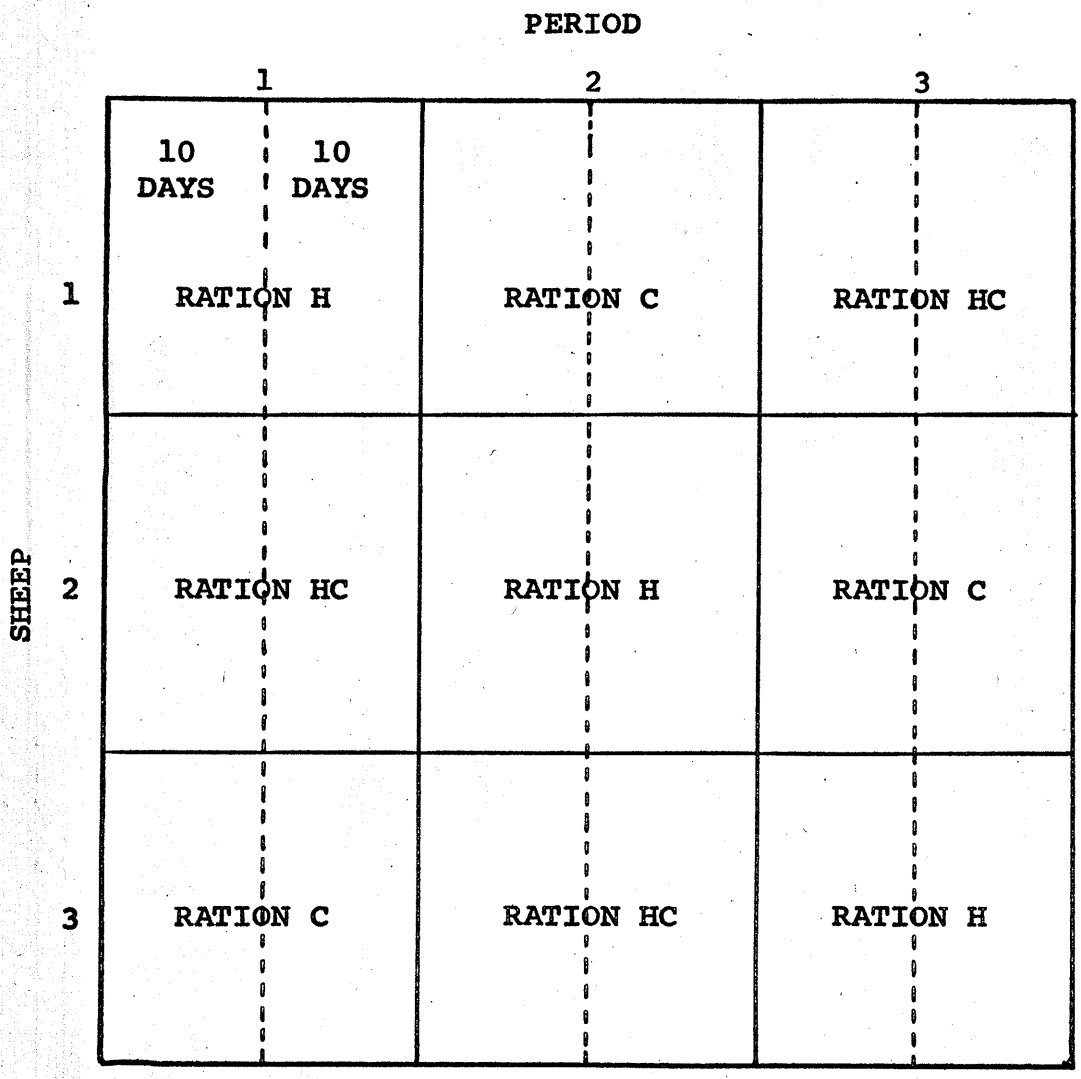

FIGURE 1

DIGESTION TRIAL LATIN SQUARE DESIGN

of coarsely ground corn and a composite alfalfa-clover-brome grass hay. The constituent hays and corn were analysed for crude protein. The chopped brome grass and legume hays were then mixed in suitable proportions to give the same crude protein content as the corn, thus giving final corn-hay rations of similar crude protein content. The three rations were designated H, HC, and C. Ration H consisted of one part corn to nine parts hay, ration HC of equal proportions of corn and hay, and ration C of nine parts corn and one part hay. Amounts of the ration sufficient for the whole experiment were weighed out in daily allotments of 1200 gm and stored in sealed polyethelene bags until required. Ten gm of cobalt-iodized salt were added to the ration daily just before feeding. The rations thus differed in content of readily digestible carbohydrate and also in the major protein source, i.e., plant leaf and corn protein. Both these factors were expected to affect the quantity and composition of the duodenal digesta. Chemical composition of the ration is shown in Table I and the amino acid composition of the ration protein in Table II.

ANIMALS

The experiment was conducted on three Suffolk wether

TABLE I. Chemical Composition of Rations

Nutrients (% of dry matter)	Ration C	Ration HC	Ration H
Crude protein	11.1	11.0	11.5
Ether extract	4.73	3.51	2.73
Crude fibre	5.10	16.05	28.60
Ash	3.8	5.9	9.2
N.F.E.	75.3	63.5	48.0
Gross energy (cal/gm)	4427	4413	4343
Organic matter	96.2	94.1	90.8
Soluble carbohydrate	56.2	34.0	22.5

TABLE II. Amino Acid Composition of Ration Protein (percent of protein)

Amino acid	Ration C	Ration HC	Ration H
Aspartic acid	8.51	10.02	10.14
Threonine	3.33	2.82	4.78
Serine*	3.42	3.36	2.78
Glutamic acid	32.7	31.3	33.7
Proline	8.74	5.09	8.52
Glycine	4.77	5.55	6.00
Alanine	7.21	7.46	7.30
Valine	2.25	2.00	3.39
Methionine	2.25	1.82	1.65
Isoleucine	5.86	5.91	6.09
Leucine	12.25	11.72	11.82
Phenylalanine + Tryptophane	1.26	1.73	2.96
Lysine	1.80	3.27	3.57
Histidine	1.26	1.82	2.09
Arginine	2.25	3.18	4.78
Total	97.9	97.1	109.6

* Uncorrected for losses on hydrolysis.

sheep ranging from three to four years of age. Initial weights of the experimental animals were 74, 78 and 85 kgm. Surgical preparation involved the insertion of permanent abomasal cannulae in the pyloric region of the stomach, and duodenal cannulae located two to three inches aboral to the pylorus. Experimental surgery on the animals had been completed for a previous experiment (Phillips and Dyck, 1964) which was terminated a year previous to commencing the present trial. All animals appeared healthy and normal despite the presence of the cannulae.

FLOW

Estimates of the volume of digesta flowing from the abomasum were made using the inert marker dilution principle according to the method of Phillips and Dyck (1964). Polyethylene glycol 4000 (PEG) was dissolved to give a concentration of 25 mg per ml in distilled water. The PEG solution was pumped into the abomasum at a constant rate, 24 hours a day, while collections were being made. Periodic sampling of duodenal contents and analysis for PEG were used to estimate the flow rate. From the amount of marker infused, and its final concentration in duodenal contents, the flow volume was calculated from the formula:

$$\text{Duodenal flow (ml)} = \frac{\text{amount of PEG infused (mg)}}{\text{concentration in duodenum (mg per ml)}}$$

Approximately 750 mg of PEG in 30 ml of water were pumped into the abomasum per hour. No attempts were made to adjust abomasal outflow for this higher than normal water input. Pumps used for infusion were two constant flow rate micropumps (Buchler Instruments Inc., Fort Lee, N.J.), operating on a reciprocating piston principle to give positive fluid displacement. The pumps were calibrated to deliver a volume of approximately 30 ml per hour, and reservoirs supplying the pumps were litre graduated cylinders. Records were kept of the amount of PEG solution pumped from the reservoirs to enable calculation of exact hourly infusion volumes. Infusion was carried out through 1/8 inch O.D. - 1/16 inch I.D. Tygon tubing which was connected to plastic inlets on the abomasal cannulae.

Although the flow experiment was designed primarily for estimation of the total volume of digesta leaving the abomasum over several days the variation within days could not be ignored. Previous methods of detecting within day variation include continuous observations based on total collection, and marker dilutions read every hour. Phillips and Dyck (1964) observed a monophasic diurnal varia-

tion in flow rates when feeding once a day and making flow observations for each hour of a 5-day digestion trial. The present experiment was designed to detect and allow for diurnal flow variations with the use of a less rigorous sampling technique. Statistical analysis of the data from the experiment of Phillips and Dyck (1964) showed that a division of the day into four 6-hour blocks such that the time of feeding lay in the fifth hour of one block, and randomly selecting a single value from each block indicated that most of the diurnal variation was due to variation among blocks rather than within blocks. On the basis of these calculations days were divided into four 6-hour blocks; from 5-10 a.m., 11 a.m.-4 p.m., etc., with the time of feeding at 9 a.m. Samples were taken at an hour randomly chosen from the six in each block. The period of collection was extended from 5 to 20 days. The overall change in design involved a reduction of samples from 120 to 80 per animal per ration, and was expected to give reasonably reliable flow estimates. Furthermore the longer sampling period was selected to test the reliability of 10-day estimates of flow, 10 days being the accepted desirable length of digestion trials.

SAMPLING PROCEDURE

Duodenal digesta samples were taken by removing a

gauze plug which extended through the entire length of the duodenal cannula and prevented accumulation of solid food residues in the cannula between collections. Approximately 30 ml of digesta were taken for each sample, five ml of which were used for PEG analysis with the remainder being frozen for storage.

Feces and urine collections were made once daily just before feeding. Feces weights were recorded and total feces collected were stored at 10°F in plastic bags. Urine volumes were recorded, and samples taken and stored at 10°F for nitrogen analysis.

CHEMICAL ANALYSIS

Composite samples of duodenal digesta were made by pooling individual samples using amounts proportional to the flow volume measured. Composite samples were frozen in dry ice and dried in a lyophilizer for 36 hours at a pressure of less than 50 microns of mercury. They were then ground and stored in airtight containers for analysis.

In preparation for analysis the feces were thawed and mixed thoroughly in a Hobart mixer at 50°F. The moisture loss on mixing was recorded for correcting dry matters and 500 gm samples of the homogenate were saved

for analysis.

Analyses for dry matter, ether extract, fibre, protein, and ash were conducted according to standard procedure (A.O.A.C., 1955). Energy determinations were made using a Parr adiabatic oxygen bomb calorimeter. Soluble carbohydrates in feed and duodenal digesta were separated from other fractions by the method of Weller and Gray (1954), and quantitative measurements made by the use of anthrone reagent.

Analyses for PEG were carried out according to the method of Hyden (1955). Amino acid analyses were made according to the fraction collector method of Stein and Moore (1958), except the eluting buffers were pumped through the column at a constant rate of 30 ml per hour as in the automatic analyser technique. Micropumps (Buchler Instruments Inc.,) were used to obtain constant flow rates.

STATISTICAL ANALYSIS

Flow data and digestibilities as well as amino acid composition of protein were tested by analysis of variance using a replicated latin square method (Cochran et al.1941). Duncan's new multiple range test (Steele and Torrie, 1960) was used to compare means in the investigation of diurnal flow variations.

RESULTS AND DISCUSSION

TOTAL DIGESTIBILITY

Data presented in this section are expressed as ration means for the sake of simplicity. This form of presentation is justified by the generally small contributions to variation made by the replicated digestion trials and individual sheep (Appendix Tables V - XI).

Organic Matter, Dry Matter, and Gross Energy

Within ration digestibilities for organic matter, dry matter, and grass energy were very similar. Mean organic matter digestibilities (Table III) were 62% for ration H, 74% for ration HC, and 83% for ration C, and the differences among these means were significant ($P \leq .01$). Dry matter digestibilities were slightly less, averaging 61%, 73% and 82% for the respective rations (Table III). Digestibility of energy on the three rations followed the same pattern (Table III) with respective ration averages of 58, 71 and 80%. Ration means for all three of these components differed significantly ($P \leq .01$). This ration trend in digestibility may be predicted from the N.F.E. content (48% for ration H as compared with 75% for ration C) which indicates large amounts of readily digestible carbohydrate supplied by the corn.

TABLE III. Feed to Feces Digestibility Coefficients (percentage)

Nutrients	Ration C	Ration HC	Ration H
Dry matter	81.6	72.6	60.5
Crude protein	68	65	63
Crude fibre	32	52	58
Ash	51	51	52
N.F.E.	89	81	64
Ether extract	79	71	47
Gross energy	80	71	58
Organic matter	83	74	62

Ash

Digestibility coefficients for ash were 51, 51 and 52 for rations C, HC, and H, respectively (Table III). Ration H in its complete form (10 gm of salt added per day) contained more than twice as much ash as ration C. Ash appeared to make up a fairly constant proportion of the fecal dry matter, being close to 10% for all rations. Similarity in digestion coefficients among rations could be entirely coincidental. Ration levels of ash decreased as dry matter digestibility increased, thus resulting in similar digestibility of ash for all rations.

Ether Extract

Ether extract content of the three rations ranged from 2.7% of ration H to 4.7% of ration C. Average digestibilities for ether extract were 47% for ration H, 71% for ration HC and 79% for ration C (Table III). These ration means were significantly different ($P \leq .01$). The apparently different digestibilities probably resulted from low ration levels combined with fairly high metabolic fecal excretion of ether extract.

Crude Fibre

Digestibility of crude fibre varied from 32% on ration C to 52 and 58% on rations HC and H, respectively.

This gave statistical significance for the ration effect on digestibility ($P \leq .01$). Depressions in fibre digestibility may result from increased energy levels in the high corn ration (Belasco, 1956). However, this may not have been the only factor involved since fibre levels were different among rations, and the source of the fibre and therefore its composition could have varied with rations. The relationship between fibre digestibility and fibre content of the ration was not linear, the digestibility tending to level off at high fibre level in the ration. This levelling could have been due to the presence of non-digestible fractions in the fibre such as lignin and lignin encrusted cellulose.

Crude Protein

Average crude protein digestibility was 63% for ration H, 65% for ration HC and 68% for ration C (Table III) with no significant difference among ration means. Ration levels were all approximately 11% of the dry matter (Table I), but fecal protein concentration was progressively higher as the fecal dry matter output declined from ration A to ration C. This increasing protein content just seemed to compensate for the declining dry matter output, resulting in almost equal total excretion of nitrogen and similar

coefficients of digestibility. This trend was the exact opposite of the situation occurring with ash where the rations contained varying percentages, but the fecal percentages were quite similar. This is an ample demonstration of differences in biological utilization of different nutrients.

The significance of a constant fecal excretion of nitrogen despite significant differences in dry matter excretion is difficult to explain (Hutchinson, 1958). Protein excretion in feces should consist of undigested food and microbial protein plus metabolic fecal protein originating in the body. Metabolic contribution to fecal protein could be constant among rations and could account for a considerable portion of the constant fecal excretion. Maynard and Loosli (1956) say the size of the metabolic fraction depends principally on the amount of dry matter consumed, its digestibility, and on body size. In this experiment differences in digestibility of dry matter would be the only factor militating against constant metabolic protein levels in feces, and excretion of constant amounts of metabolic protein among rations would tend to increase protein percentage where fecal dry matter output decreases. If the assumptions made so far are valid then similar amounts of diet and microbial protein

are being excreted on all rations since total excretions are the same. This means that any ration effect on quantitative rumen synthesis of protein must be accompanied by a compensatory intestinal disappearance. Alternatively there could be equal rumen synthesis of protein on all rations with protein leaving the abomasum having a similar amino acid composition and being absorbed in similar amounts for the three rations.

Amino Acids

Amino acid levels in the feces expressed as a percentage of fecal protein were not directly influenced by rations (Appendix Table I). This similarity in composition of fecal protein among rations must be due to rumen synthesis and metabolic protein having a large diluting effect on the original ration protein. This conclusion is based on the assumption that there is no ration effect on the composition of protein in the abomasal digesta (see below), and that the composition of metabolic fecal protein does not vary with rations.

Since there was no ration effect on composition of fecal protein, averages were made of amino acid levels in all fecal samples. Digestibility coefficients of amino acids calculated from individual observations were not significantly different among rations due to some variation

within rations. However digestibilities calculated from average fecal composition appeared to show a ration effect on utilization. Amino acids showing lower coefficients of digestibility on ration C than ration H include arginine, histidine and lysine. Methionine was low in the hay protein, so appeared to be more highly digestible in ration C.

Nitrogen Balance

Positive nitrogen balance was found for all sheep on all rations (Table IIIa). Average nitrogen retentions for 20-day periods were 60.8, 81.0, and 112.1 gm for rations H, HC, and C, respectively. These levels were not significantly different due to the limited number of observations. Table IIIa shows nitrogen losses in both feces and urine to be higher for ration H than ration C, but most of the difference is attributable to different urine excretions. Since the amount of ration provided was minimal for maintenance the more positive balance on ration C could be attributed to protein being stored rather than oxidized to meet body energy requirements.

FLOW

Diurnal Effect

Preliminary statistical analyses were carried out for

TABLE IIIa. Nitrogen Balance.
All Figures are gm of Nitrogen
Involved in 20-day Feeding Trials.

Ration	Sheep	Intake	Excretion		Balance
			Urine	Feces	
H	1	396	185	143	+ 68
	2		210	148	+ 38
	3		162	154	+ 80
HC	1	384	159	136	+ 89
	2		181	129	+ 74
	3		166	132	+ 86
C	1	383	116	126	+141
	2		142	117	+124
	3		189	121	+ 73

each sheep on each ration to gauge the relative contribution of within and between days to the total variation. Analysis of variance showed that the time of collection within the day (block effect) gave a highly significant contribution to variation in six of the nine trials ($P \leq .01$), and to reach significance at the 5% level in eight of the nine.

Duncan's new multiple range test was used to test for differences among the means for the four different times of day. A significant F value is not required for application of this test. In all nine trials the time period from 5 - 10 a.m. which included the time of feeding (block four), had significantly higher flows than block one (11 a.m.-4 p.m.). This significance was present even with sheep three on ration H where analysis of variance did not detect a significant block effect (Table IV). Means for blocks two and three lay intermediate between four and one in all cases. Variation among daily flow totals was significant in seven of the nine trials.

With a clearly established diurnal variation for individual sheep and rations, data were combined to give average flows for the four divisions of the day for each sheep on each ration. These data were analysed as four replicated 3 x 3 latin squares with time of day as repli-

TABLE IV. Mean Hourly Flows for the Four Blocks.

	Block 1	2	3	4
Ration H				
Sheep 1.	430	529	542	588
Sheep 2.	688	719	865	1086
Sheep 3.	517	539	598	736
Ration HC				
Sheep 1.	453	469	498	637
Sheep 2.	484	489	531	588
Sheep 3.	491	595	635	765
Ration C				
Sheep 1.	535	601	711	767
Sheep 2.	458	497	522	637
Sheep 3.	341	368	421	507
Average	492	532	590	701

All means not underscored by the same line are significantly different ($P \leq .05$)

cations. There were significant differences among means for blocks, rations, and periods ($P \leq .01$). The significant contribution of block means to the total variation indicates the presence of the expected diurnal variation in flow volume. Since the interaction term was very small Duncan's new multiple range test was used to test for differences due to time of collection, when averaged over the entire experiment. Ranking of these means can be accomplished merely by arranging them in block sequence, giving 492, 532, 590 and 701 ml per hour for blocks one, two, three, and four, respectively. Each of these means was significantly different from the other three ($P \leq .01$). This gives a general picture of lowest flows in the blocks from two to seven hours after feeding. Flow gradually increased through the day and night to reach maximum levels during the block including the next feeding time. Figure 2 shows a histogram of the means, and clearly indicates the monophasic diurnal flow pattern similar to that detected by Phillips and Dyck (1964). The widely differing nature of the rations had no effect on the diurnal flow pattern. Feeding the ration twice a day (Harris and Phillipson, 1962) resulted in a diphasic diurnal flow pattern with the peaks occurring immediately before or coinciding with the daily feeding times.

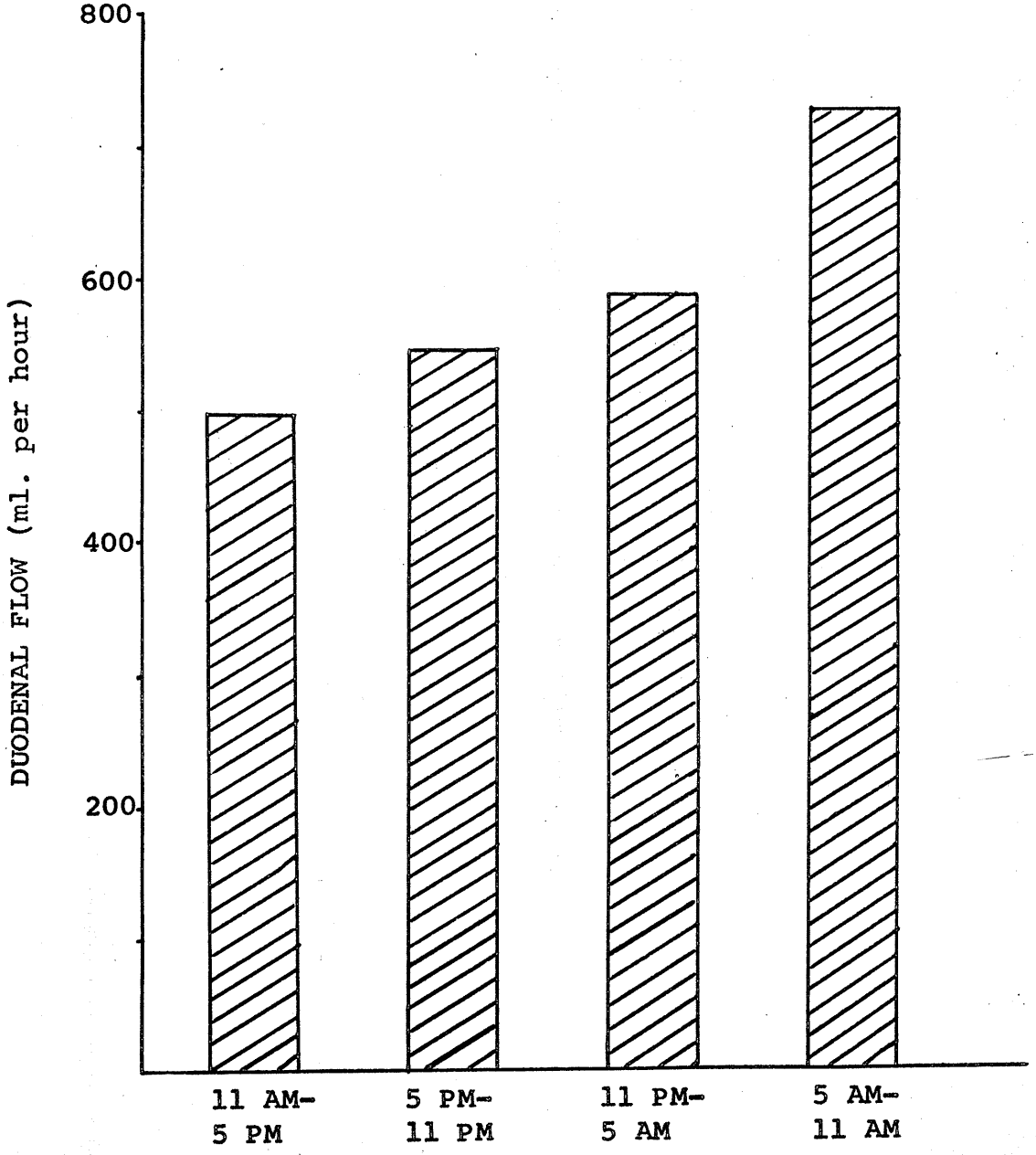

FIGURE 2

AVERAGED DIURNAL DUODENAL DIGESTA FLOWS

Increased flow during the six hour block including feeding suggests some relationship between prehension and increased rates of rumen emptying. This implied relationship did not seem to hold true since the time required to collect 30 ml of digesta was greatest when the animals were eating. The period of greatest flow therefore must have occurred just before eating, and psychic control is suggested since the increase occurs twice a day when rations are fed twice daily, and only once when fed once a day. The apparent reduction in flow rate with the onset of feeding suggests direct nervous control of rumen motility and emptying. The practical importance of this diurnal variation to workers attempting to measure flow is the implication that short term observations on flow may not be reliable for estimating flow over longer periods of time. Discontinuous short term observations may be held suspect also, since nervous control of rumen emptying may be strong enough to depress flow during observations, and to give compensatory increases after the removal of external interference (Harris and Phillipson, 1962). Depressing the rate of rumen emptying may affect the composition of the digesta. The composition of dry matter in the digesta leaving the abomasum in times of normal flow may thus be difficult to estimate from total collection data.

Total Flow Volume

Total flow values for ten day digestion trials were obtained by summing the four daily observations over the ten days and multiplying by six. These values for ten day duodenal flow are shown in Table V. Statistical analysis was carried out to estimate the effects of animals, rations, replicated ten day trials and periods, on total flow volume. Values for mean squares of the variables shown in Table V indicate a calculated F value approaching significance at the five % level of probability for rations. The trend which almost reached significant levels was towards greater flows on the high roughage ration (H) where disappearance of dry matter in the fore-stomach could not be expected to be as large as on ration C. Unfortunately the mean square for replicated digestion trials also approached significance, and the period effect was significant. The most likely explanation for the period effect lies in the chemical methods used in measuring PEG concentration. The reagents were made up in quantities sufficient to analyse all the samples from one period. Different reagent batches gave different base lines when plotting concentration of PEG versus optical density. Due to this base line variation a new standard curve was made up for each new reagent

TABLE V. Flow Volumes and Estimated Dry Matter Passage.

Ration	Sheep	10 Day Totals		
		Flow ml	Dry Matter %	Dry matter gm
H	1	130356	4.5	5766
		120252	4.5	5503
	2	218766	5.0	11048
		184152	6.4	11668
	3	154416	5.4	8339
		132264	4.3	5687
HC	1	130326	6.4	8258
		116388	5.2	5992
	2	130320	5.4	7108
		120810	5.0	6041
	3	149802	4.8	7119
		148458	5.0	7497
C	1	181560	6.1	10743
		131988	6.5	8558
	2	134508	6.6	8966
		119202	7.2	9012
	3	98682	4.6	4494
		97602	4.4	4252

batch, but plotting a daily standard curve would seem desireable for greatest accuracy.

The sampling design may have been at fault for taking too few samples to give data representative of the average hourly flow over 6-hour blocks. With only 10 samples per block the results could be biased estimates of flow. With a larger sample size this type of error should disappear if the hours for sampling are chosen at random from within the block.

It is possible that the observed values were fairly accurate estimates of flow, but that flow varied much more than is generally assumed. The only environmental factor which could be expected to influence flow rate would be temperature variation. Changes in mean temperature could have some effect on water consumption, and consequently affect duodenal flow. It might have been expected that increased duodenal flow resulting from increased water intake would contain less dry matter than normal. However the results from dry matter analyses did not bear this out.

Dry Matter Flows

Composite samples based on aliquots of one-fiftieth of the individual measurements of flow volume were analyzed for dry matter content. The dry matter content of the com-

posites varied from 4.3 to 7.2% with ration averages of 5.00, 5.03 and 5.09% for H, HC and C, respectively. Dry matter flows (Table V) calculated by multiplying volume of flow by dry matter percentage show just as much unexplainable variation as the total flow volumes. Thus it becomes apparent that there was no compensatory reduction in dry matter content for periods with high flow estimates. These results throw serious doubt on the validity of the total flow measurements and any results derived from them. The consistency of ration averages for dry matter content indicate flow measurements as the most likely source of error.

There is a remote possibility that there was a reduction in dry matter percentage with increased flow volume, but the sampling method used was not sensitive enough to detect these changes. Transferring duodenal contents containing relatively large undigested corn particles by wide tip pipette could have resulted in errors in dry matter transfer, but this seems unlikely. It could even be postulated that the observed dry matter flows were correct and the wide variation in dry matter flow was due to some physiological mechanism which regulates the amount of dry matter absorbed from the rumen. Existence of this type of mechanism seems unlikely since it

violates the "steady state" concept of rumen metabolism.

Validity of Composites Based on Flow

The composite samples were based on within period flow estimations. Flow estimates within periods would be independent of any systematic among period variations dependent on chemical reagents. Therefore the composite samples of digesta were considered as being representative of average digesta composition on any ration.

Workers using slaughter techniques (Boyne et al., 1956) are dependent upon results obtained from small numbers of animals sampled only once. Such an experiment must be repeated at intervals to measure the effect of time after feeding on digesta composition.

Results based on total collection from, or measurement of flow through, re-entrant cannulae have the disadvantage of all results based on discontinuous short term observations. Composition of the flow over three, or even over twelve hours may not be representative of the average quantity or composition for a particular ration. Even if results obtained on this experiment were based on no more than pooled samples of duodenal flow then the fact that they were taken at four randomly assigned hours per day over a 10-day digestion period should make them reasonably representative of the average composition of duodenal

contents for that ration.

COMPOSITION OF DUODENAL DIGESTA

Organic Matter

Organic matter content of duodenal digesta as shown on Table VII was 82.6, 85.0, and 86.5% of the dry matter for rations H, HC and C. This ration trend was similar to that appearing in the feeds.

Ash

Ash percentage of the duodenal dry matter was 17.4, 15.0, and 13.5 (Table VII) as compared with ration levels of 9.2, 5.9 and 3.8% for rations H, HC and C. There was a pronounced increase in ash from feed to duodenal contents on all rations. These results agree with those of Badawy *et al.* (1958a) who found increases in ash from about eight percent in the rumino-reticulum to 12% of dry matter in the proximal small intestine. If there is a significant ration effect on dry matter flow then there must be some relationship between dry matter passage and secretion of ash by the abomasum. Otherwise the ration trend would not be so closely duplicated by levels in the digesta. This concept is in agreement with work done by Ash (1961) who established a positive correlation between

TABLE VI. Mean Squares From Analysis of Variance of Flow Data.

Source	d.f.	Mean Square
Replicates	1	38,491,538
Periods	4	61,844,987
Animals	4	10,084,029
Rations	2	40,976,570
Reps. x Rations	2	2,634,476
Error	4	7,285,320

TABLE VII. Chemical Composition of Duodenal Digesta (Ration Averages)

Nutrients (% of dry matter)	Ration C	Ration HC	Ration H
Crude protein	24.9	23.3	20.1
Ether extract	8.1	5.8	4.4
Crude fibre	9.3	12.7	20.8
Ash	13.5	15.0	17.4
N.F.E.	44.2	37.8	37.3
Gross energy (cal/gm)	4425	4394	4223
Organic matter	86.5	85.0	82.6
Soluble carbohydrate	21.5	12.7	7.2

rate of abomasal emptying and acid secretion.

Ether Extract

Observed ether extract content of duodenal dry matter was 4.73, 5.80 and 8.13% (Table VII) while corresponding ration levels of 2.73, 3.51 and 4.73% were obtained for H, HC, and C, respectively. These comparisons indicate very low or negative fore-stomach disappearance of ration ether extract. This agrees with Blaxter (1962) who found little rumen absorption of the longer chain fats in either acid or combined form.

Crude Fibre

The proportion of crude fibre decreased from 28.6 and 16.1% of the feed to 20.8 and 12.7% of the duodenal dry matter (Table VII) for rations H and HC respectively. For ration C the trend was reversed and fibre represented a larger proportion of duodenal dry matter (9.25%) than feed (5.1%). Thus a ration effect on fibre digestion in the rumen was apparent even without quantitative flow data. The high level of digestible energy in ration C was probably responsible for the low fibre digestibility (Maynard and Loosli, 1956).

Nitrogen Free Extract

Average N.F.E. content of duodenal dry matter was

37% for ration H, 38% for HC and 44% for ration C(Table VII). If dry matter flow were equal between rations there would clearly be a ration effect on amount of carbohydrate available for intestinal digestion. Interpretations of ration effect on N.F.E. content at the duodenal level are limited by large within ration variation.

Crude Protein

Crude protein averaged 20.1% of duodenal dry matter for ration H, 23.3% for ration HC and 24.9% for ration C. There was a fairly large variation in protein level within rations which prevents statistical significance among ration means of protein concentration. There was considerable variability among sheep on any given ration, indicating that the nature of the ration may not exert a very precise effect on nitrogen metabolism of rumen microorganisms. It could also be possible that the three sheep had different dry matter flows on the same ration, and thus had similar amounts of protein leaving the abomasum on the same ration, and similar rumen nitrogen metabolism.

Amino Acids

Statistical analyses failed to detect a significant ration effect on amino acid composition of duodenal digesta.

Average essential amino acid content of the duodenal protein appears in Table VIII.

These findings indicate the rumen effect is one of removing ration variations in amino acid levels, and presenting a protein of fairly constant amino acid composition for enzymatic digestion in the small intestine. Table VIII shows the essential amino acid composition of whole egg protein in comparison to the protein of the three rations, duodenal protein, and averaged fecal protein. Values for egg are taken from Oser (1951) and used to calculate biological values of the different proteins by the chemical score method (Block and Mitchell, 1946) and by Oser's essential amino acid index. Values for tryptophane and cystine were taken from Morrison (1958) since hydrolysis of the samples interfered with the detection of these amino acids. Phenylalanine was measured on the short column, and its values may not be too reliable.

Chemical score involves expressing the most limiting amino acid as a percentage of its level in egg protein. This gave ration C a chemical score of 21 or 26 depending on phenylalanine or lysine being limiting. Ration H was lowest in methionine, having 43% of the level in whole egg protein. Duodenal protein appears to be lower in quality than hay protein with chemical scores of 27 (phenyl-

TABLE VIII. Amino Acid Compositions
(% of Protein)

Amino Acid	Whole Egg	Ration C	Ration HC	Ration H	Average Duodenal	Average Fecal
Lysine	7.0	1.8	3.3	3.6	5.0	4.5
Isolbucine	7.7	5.9	5.9	6.1	6.4	5.9
Valine	7.2	2.3	2.0	3.4	3.4	2.8
Arginine	6.6	2.3	3.2	4.8	2.4	2.4
Methionine	4.0	2.3	1.8	1.7	1.9	4.7
Threonine	4.3	3.3	2.8	4.8	5.6	4.5
Leucine	9.2	12.3	11.7	11.8	9.8	7.0
Phenylalanine	6.3	1.3	1.7	3.0	1.7	4.0
Histidine	2.4	1.3	1.8	2.1	1.5	1.8
Tryptophan	1.5	0.7	0.8	0.9	-	-
Cystine	2.4	0.9	1.4	1.8	-	-
Chemical Score	100	21	27	43	27	36
Oser's Essential Amino Acid Index	100	47	54	69	61	68

alanine) or 36 with arginine limiting. Rumen synthesis of amino acid is clearly shown in ration C where the level of lysine went from 1.8% of feed protein to 5.1% of protein in duodenal digesta.

Essential amino acid index gives ration biological values of 47.4, 53.8 and 68.7% for rations C, HC, and H respectively. Duodenal protein lies between ration HC and ration H at 60.8. On this basis the rumen effect appears to have reduced the biological value of the hay, but increased that of the corn protein.

Soluble Carbohydrate

Ration levels of soluble carbohydrate were 22.5, 34.0 and 56.2% for rations H, HC and C while duodenal dry matter averaged 7.2, 12.7 and 21.5% for the respective rations. Appendix Table IV shows the levels for each individual digestion trial and points out within ration variation in soluble carbohydrate content. Despite this variation there appears to have been a ration effect on the amount of soluble carbohydrate available for intestinal digestion.

ESTIMATED DRY MATTER PASSAGE

In view of the failure of the PEG method to provide

reliable values on average flow of digesta into the duodenum, attempts were made to estimate the quantities of dry matter being passed based on the passage of acid insoluble ash, and on crude fibre.

Acid Insoluble Ash

A naturally occurring ration component which should be inert to digestion and act as a digestibility marker is acid insoluble ash. The use of acid insoluble ash as a reference substance could give an independent estimate of dry matter passage through the duodenum. Boyne et al. (1956) and Badawy et al. (1958b) used this method with varying degrees of success. The disadvantage in applying this method in the present experiment arises from large ration differences in insoluble ash which varied from 3% in ration H to less than one percent in ration C. Duodenal dry matter passage calculated from insoluble ash content clearly shows a ration effect. Flows were lowest on ration H and highest on ration C (Table IX). Further calculations using these estimates indicated a net synthesis of fibre in the rumen for every sheep on ration C, clearly an improbable event. These results and those obtained by Badawy et al. (1958b) indicate that the chemical method may not be suitable for this experiment.

TABLE IX. Dry Matter Passage from the Abomasum.

Based on the use of acid insoluble ash as an inert digestibility marker.

Ration	Sheep	Ten Days Totals (gm)
H	1	4492
		4348
	2	4749
		6767
	3	5170
		4851
HC	1	5518
		5765
	2	5692
		5765
	3	5312
		5140
C	1	6930
		9580
	2	7081
		5474
	3	6580
		6264

Partition of Fibre Digestion

The amount of fibre digestion which occurs in the fore-stomach provides a basis for the following calculations. Preliminary work involved setting upper and lower flow limits based on absolute amounts of fibre in the feed and feces, and the percentage in duodenal dry matter. Limits are based on the assumption that no fibre synthesis occurs in any portion of the gastro-intestinal tract. The upper flow limit assumes all fibre digestion to occur caudal to the duodenum, then upper flow limit (gm. of dry matter)

$$= \frac{\text{gm. fibre in ration}}{\text{percent fibre in duodenal dry matter}} \times 100$$

Similarly the lower flow limit assumes all fibre digestion occurred in the rumen and fibre in feces replaces fibre in ration in the equation.

With rations H and HC there was a large amount of fibre digestion, and a correspondingly large range between upper and lower limits. With ration C the total feed and total fecal fibre were not too different and the flow limits were narrow. To obtain flow estimates for rations H and HC some assumptions must be made about the proportion of the digestible fibre disappearing in the rumen

versus the large intestine. Weller and Gray (1952) found that approximately 70% of the digestible fibre was lost in the rumen compared to 30% in the large intestine on an all hay ration. Balch (1957) found a similar partitioning of fibre digestion on roughage rations. Adding concentrates at half the ration or more depressed total fibre digestion, and disappearance was equally divided between the rumen and the large intestine.

Table X shows dry matter passage through the duodenum estimated from the assumption that partition of fibre digestion between rumen and large intestine was in the ratio of 7:3 for ration H and 1:1 for rations HC and C. It must be realized that these flow values are only as valid as the assumptions used to derive them, but under the circumstances they are the best available, and are used to estimate the net effects in the fore-stomach.

ESTIMATED RUMEN EFFECT ON CHEMICAL CONSTITUENTS

Ash

Digestibility values from Table XI show a net increase in ash from the feed to duodenal level with all rations. This increase in ash averaged 55 grams per day for ration H and 31 gm per day for ration C. This indicates

TABLE X. Dry Matter Passage from the Abomasum.

Based on partition of fibre digestion.

Ration	Sheep	Upper Limit gm	Estimate gm	Lower Limit gm
H	1	16,846	9767	6732
		16,074	9202	6258
	2	13,796	8272	5905
		13,864	8345	5980
	3	14,016	8571	6237
		14,629	8883	6421
HC	1	11,712	8520	5327
		12,588	9242	5896
	2	10,074	7495	4915
		12,588	7013	4406
	3	11,863	8970	6077
		10,428	7928	5427
C	1	6,987	5594	5003
		6,554	5508	4463
	2	4,573	3933	3293
		4,682	3759	2835
	3	6,220	5331	4442
		8,246	6836	5426

TABLE XI. Disappearance of Proximate Constituents Along the Digestive Tract.

	Ration		
	H	HC	C
Ash			
Stomachs[1]	-56	-91	-77
Intestine[1]	69	74	72
Intestine[2]	108	142	129
Total	52	51	51
Ether Extract			
Stomachs[1]	-42	-24	17
Intestine[1]	63	77	74
Intestine[2]	89	96	62
Total	47	71	79
N.F.E.			
Stomachs[1]	36	55	72
Intestine[1]	45	58	59
Intestine[2]	28	26	17
Total	64	81	89
Gross Energy			
Stomachs[1]	20	23	51
Intestine[1]	48	61	59
Intestine[2]	38	46	28
Total	58	71	80
Crude Protein			
Stomachs[1]	-44	-59	-13
Intestine[1]	74	78	72
Intestine[2]	108	125	72
Total	63	65	68

[1] As a percent of that ingested, negative values indicating synthesis.

[2] As a percent of that entering the intestine.

a possible relationship between mineral secretion in digestive juices, and the amount of dry matter passing through the abomasum. The percentage disappearance of ash entering the intestines was similar on all three rations. Since the amount entering the intestine varied with rations the total amount absorbed was ration dependent.

Ether Extract

Partitioning ether extract digestion shows a significant ($P \leq .01$) ration effect on fore-stomach digestibility (Table XI). There was a net synthesis of ether extract for rations H and HC and a net disappearance with ration C. No ration trend is apparent with digestion of fat entering the duodenum, so different feed to feces digestibilities were dependent upon the rumen effect.

Nitrogen Free Extract

Table XI shows an increased fore-stomach digestibility of N.F.E. from ration H to ration C. Disappearance of N.F.E. as a percent of that entering the intestine was again highest for the high roughage ration. Also the actual quantitative removal from the intestines was greatest for ration H. The explanation for this observation is that more N.F.E. enters the intestines from ration H than from the other two rations.

Gross Energy

The high level of digestible energy in ration C gives higher coefficients of disappearance (Table XI) from feed to duodenum and duodenum to feces than either of the other two rations. Again the total amount digested in the intestines was greater on ration H than on ration C. The reasons are probably the same as those given for N.F.E.

Crude Protein

All three rations showed a net gain of protein at the duodenal level (Table XI) with the increase being slightly greater for the roughage rations. Intestinal digestion coefficients were similar on all rations resulting in a higher apparent total digestibility with ration C than with H or HC. Ration HC appears to give the largest absolute rumen synthesis and intestinal absorption of protein of the three. Ration H supplied more of the limiting amino acids for absorption at the duodenal level than either HC or C even though the total amount of protein absorption appeared to be larger for ration HC.

Soluble Carbohydrate

Fore-stomach digestibilities of soluble carbohydrate

(Appendix Table IV) averaged 74.4% for ration H, 71.4% for HC, and 82% for ration C. Since the amount in the ration was greatest with ration C, there was more soluble carbohydrate entering the intestine with ration C than H or HC. This ration effect was not significant.

SUMMARY

A digestion trial was carried out to measure utilization of proximate constituents and amino acids on three different rations. The rations were 90% legume-brome hay and 10% ground corn, equal parts of each, and 10% hay with 90% corn. Attempts were made to measure duodenal flow volume, and to obtain representative samples from the three rations to determine the composition of this flow.

Experimental results provided a basis for the following conclusions:

1. Gross energy, nitrogen free extract, and ether extract digestibilities varied significantly among rations, and were highest on the high corn ration. Crude fibre digestibility was significantly highest on the hay ration. No ration effect was detectable on crude protein or ash digestibilities.
2. The regime used for sampling duodenal contents to measure flow detected a monophasic diurnal variation in flow. Highest flows occurred before and during eating.
3. No significant ration or sheep effects on total duodenal flow were detected, but the effect of digestion periods was significant. It is sug-

gested that the period effect resulted from inadequate controls in the chemical analysis for PEG.

4. The percentage of protein and ash in dry matter increased from feed to duodenum on all rations, N.F.E. percentage declined while ether extract and gross energy showed little change. Crude fibre percentage declined on the high roughage rations, but increased on the corn ration.

5. Differences in amino acid composition of protein in the ration were not reflected in the duodenal digesta. Synthesis of amino acids in the rumen and possible addition of endogenous protein to the digesta appeared to mask ration differences.

6. There was a significantly higher percentage of soluble carbohydrate in duodenal dry matter from the high corn ration than from either of the other two rations.

7. Duodenal dry matter flows estimated with the use of acid insoluble ash as an inert digestion marker appeared to be unreliable for the conditions of the experiment.

8. Calculations of duodenal dry matter passage by assumptions on the partitioning of fibre diges-

tion gave a highly significant ration effect on the quantity of dry matter passage. With similar dry matter intakes the quantity of passage was higher for rations with more crude fibre.

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APPENDIX TABLE I. Amino Acids in Ration H
(% of Protein)

	Feed	Duodenum	Feces
Methionine	1.65	1.38 ± 0.07	2.18
Isoleucine	6.09	6.38 ± 0.51	5.46
Leucine	11.82	9.71 ± 3.68	7.02
Phenylalanine	2.96	1.96 ± 0.15	4.13
Lysine	3.57	5.14 ± 0.17	4.49
Histidine	2.09	1.53 ± 0.30	1.90
Arginine	4.78	2.29 ± 0.92	2.24
Valine	3.39	3.28 ± 0.11	2.76
Serine	2.78	2.72 ± 0.08	2.30
Threonine	4.78	5.15 ± 0.72	3.95

APPENDIX TABLE II. Amino Acids in Ration HC
(% of Protein)

	Feed	Duodenum	Feces
Methionine	1.82	1.85 ± 0.30	2.32
Isoleucine	6.09	6.38 ± 0.13	6.27
Leucine	11.72	9.88 ± 0.39	7.68
Phenylalanine	1.73	1.46 ± 0.15	3.84
Lysine	3.27	4.88 ± 0.13	4.56
Histidine	1.82	1.56 ± 0.23	2.28
Arginine	3.18	2.81 ± 0.30	2.39
Valine	2.00	3.31 ± 0.08	3.03
Serine	3.36	3.03 ± 0.19	2.35
Threonine	2.82	6.15 ± 1.37	4.76

APPENDIX TABLE III. Amino Acids in Ration C.
(% of Protein)

	Feed	Duodenum	Feces
Methionine	2.25	2.35 ± 0.50	3.34
Isoleucine	5.86	6.36 ± 0.73	5.95
Leucine	12.25	9.65 ± 4.57	6.21
Phenylalanine	1.26	1.75 ± 0.69	3.98
Lysine	1.80	5.06 ± 0.37	4.41
Histidine	1.26	1.13 ± 0.28	1.31
Arginine	2.25	2.11 ± 0.42	2.41
Valine	2.25	3.53 ± 0.12	2.81
Serine	3.42	2.94 ± 0.07	2.29
Threonine	3.33	5.55 ± 0.65	4.76

APPENDIX TABLE IV. Soluble Carbohydrate

Ration	Sheep	Passage From The Abomasum (gm per 10 days)	Fore-Stomach Digestibility (%)
H	1	488	79.2
		341	85.9
	2	827	65.8
		1127	55.4
	3	720	70.2
		222	90.8
HC	1	1755	52.7
		1571	57.6
	2	585	84.2
		786	78.8
	3	886	76.1
		777	79.0
C	1	1708	71.8
		1372	77.4
	2	983	83.8
		985	83.7
	3	618	89.8
		882	85.4

APPENDIX TABLE V. Apparent Digestibility Coefficients for Crude Protein

Ration	Sheep	Fore-Stomach	Total
H	1	-77	63.0
		-65	64.5
	2	-31	62.3
		-11	62.5
	3	-30	58.6
		-53	63.5
HC	1	-56	63.3
		-75	65.6
	2	-43	65.7
		-33	67.0
	3	-94	68.5
		-57	63.0
C	1	-15	65.9
		-12	68.2
	2	36	70.3
		42	68.3
	3	-28	65.6
		-102	71.1

Negative values represent synthesis.

APPENDIX TABLE VI. Digestibility Coefficients for Fibre.

Ration	Sheep	Fore-Stomach	Total
H	1	42.0	60.0
		42.7	61.1
	2	40.0	57.2
		39.8	56.9
	3	38.8	55.5
		39.3	56.1
HC	1	27.3	54.5
		26.6	53.2
	2	25.6	51.2
		27.1	54.2
	3	24.4	48.8
		24.0	47.9
C	1	14.2	28.4
		16.0	31.9
	2	14.0	28.0
		19.7	39.5
	3	14.3	28.6
		17.1	34.2

APPENDIX TABLE VII. Apparent Digestibility Coefficients for Ether Extract.

Ration	Sheep	Fore-Stomach	Total	
H	1	-59.5	54.0	46.0
		-74.0	52.8	47.2
	2	0.7	50.9	49.1
		-24.5	54.0	46.0
	3	-39.1	53.5	46.5
		-55.8	51.5	48.5
HC	1	-22.5	30.4	69.6
		-49.3	30.6	69.4
	2	8.9	33.8	66.2
		-12.3	26.5	73.5
	3	-34.7	23.9	76.1
		-36.0	28.2	71.8
C	1	26.0	14.1	85.9
		26.2	22.8	77.2
	2	34.6	28.1	71.9
		31.1	28.0	72.0
	3	14.8	20.1	79.9
		-32.5	13.9	86.2

Negative values represent synthesis.

APPENDIX TABLE VIII. Apparent Digestibility Coefficients for Ash.

Ration	Sheep	Fore-Stomach	Total	
H	1	-86.4	50.1	49.9
		-72.9	47.3	52.7
	2	-49.4	48.0	52.0
		-12.1	47.7	52.3
	3	-57.5	46.9	55.1
		-57.0	46.5	53.5
HC	1	-94.7	48.2	51.8
		-102.6	48.8	51.2
	2	-77.1	53.2	46.8
		-66.8	49.4	50.6
	3	-106.5	45.0	55.0
		-96.1	49.9	50.1
C	1	-86.1	46.3	53.7
		-63.1	50.3	49.7
	2	-16.4	49.7	50.3
		- 9.3	46.3	53.7
	3	-105.9	50.1	49.9
		-182.3	45.1	54.9

Negative values represent a net addition.

APPENDIX TABLE IX. Digestibility Coefficients for Nitrogen Free Extract.

Ration	Sheep	Fore-Stomach	Total
H	1	32.7	64.1
		38.5	64.9
	2	41.2	63.8
		26.1	62.1
	3	39.9	63.9
		39.8	65.7
HC	1	47.1	81.4
		55.8	80.8
	2	56.8	81.8
		61.8	81.7
	3	53.0	80.1
		57.6	81.2
C	1	62.9	85.9
		67.2	87.4
	2	76.7	89.8
		77.3	89.7
	3	74.6	89.1
		73.7	89.3

APPENDIX TABLE X. Digestibility Coefficients for Gross Energy.

Ration	Sheep	Fore-Stomach	Total	
H	1	12.4	41.7	58.3
		17.5	39.8	60.2
	2	24.9	41.7	58.3
		23.0	42.7	57.3
	3	23.5	43.1	56.9
		19.4	41.4	58.6
HC	1	23.1	28.8	71.2
		17.2	29.5	70.5
	2	26.0	29.6	70.4
		36.6	27.4	72.6
	3	20.4	29.7	70.3
		28.4	30.1	69.9
C	1	46.7	21.6	78.4
		50.8	20.8	79.2
	2	62.6	20.0	80.0
		64.7	19.8	80.2
	3	49.7	19.4	80.6
		35.0	18.8	81.2

APPENDIX TABLE XI. Dry Matter Excretion
(10 Day Totals)

Ration	Sheep	Intake gm	Feces	
			gm Dry Matter	Digestibility
H	1	10,757	4198	61.0
			4071	62.2
	2		4278	60.2
			4290	60.1
	3		4377	59.3
			4182	61.1
HC	1	10,902	2949	73.0
			2991	72.6
	2		3000	72.5
			2886	73.5
	3		3048	72.0
			3085	71.7
C	1	10,769	2205	79.5
			2101	80.5
	2		1919	82.2
			1874	82.6
	3		1950	81.9
			1827	83.0