A COMPARISON OF VOLUNTARY FOOD INTAKE AND DIGESTIBILITY IN SWAMP BUFFALOES AND AYRSHIRE CATTLE

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ABSTRACT

The voluntary food intake and digestibility in Swamp buffaloes and Ayrshire cattle were compared. Four animals of each species were used and offered a medium quality diet, AA6, or a lower quality diet, Viton 10, at an ambient temperature of 21°C or 31°C. A double cross-over design was used so that every animal would be fed each diet at both temperatures. Water intake, respiration rate and serum thyroid hormone concentration were determined to indicate the degree of heat stress to which an animal was being subjected.

Voluntary food intake of the AA6 diet was significantly greater by cattle than by the buffaloes. There was no significant difference between buffaloes and cattle in the intake of the Viton 10 diet. The buffaloes consumed significantly more of the Viton 10 than the AA6 diet, whereas in the cattle there was no significant difference between the intake of the AA6 and Viton 10 diets. Cattle consumed significantly less at the higher temperature than at the lower one, however, temperature had no significant effect upon food intake of the buffaloes.

There was no significant difference between buffaloes and cattle in the Neutral detergent fibre or dry matter digestibility of either diet. Dry matter digestibility in both buffaloes and cattle was greater on the AA6 diet than on the Viton 10 diet, whereas Neutral Detergent fibre digestibility was greater for the Viton 10 diet than for the AA6 diet. Temperature did not affect dry matter

or Neutral detergent fibre digestibility significantly in either species of animal or for either diet.

1. INTRODUCTION

In many developing countries the domesticated water buffalo (*Bubal~~i~~us bubal~~u~~is*) is an important multi-purpose animal. Although there are few recognised breeds, two general types can be identified. The River buffalo and the Swamp buffalo. These two types predominate in different areas of the world. The River buffalo is found from India and Pakistan through Southwest Asia to Southeast Europe, whereas Swamp buffalo are common from the Phillipines west to India. The two types also differ in their agricultural roles. River buffalo are frequently kept for milk production as well as draught power, whereas Swamp buffalo are used primarily as draught animals.

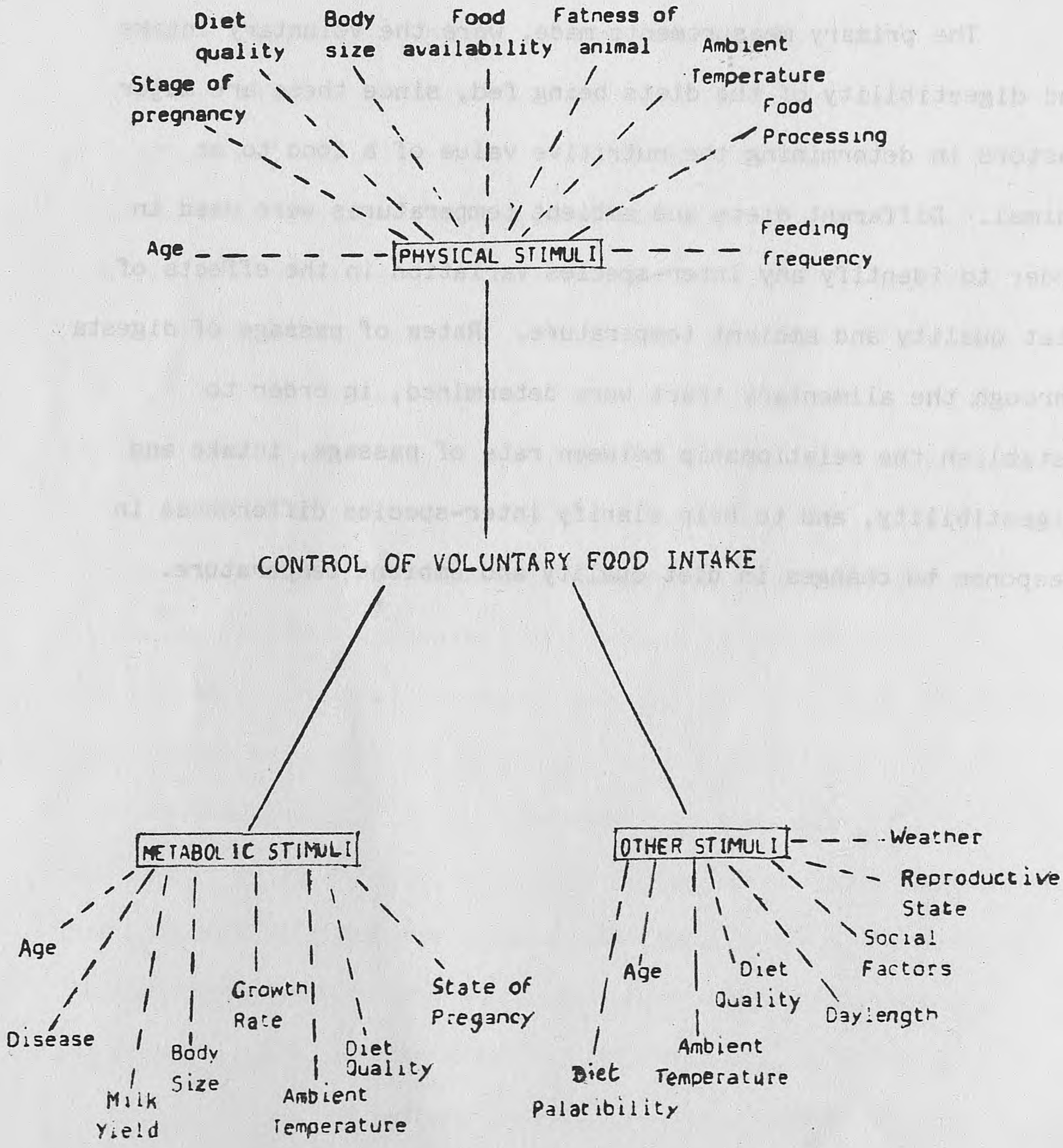
The human populations of many countries where buffaloes are kept, are beginning to exert an increasing pressure on land, the area under cultivated fodder crops and pastures is small, and in some cases decreasing. Consequently buffaloes are usually fed poor quality roughages and crop by-products. This situation is often aggravated by the occurrence of a long dry season when food resources are scarce. When the animals are used for draught, this becomes especially important, since the main energy requirements for work occur at the onset of the rainy season, which is also the time when the level of nutrition is at its worst.

Because of the importance of River buffaloes as milk animals there is somewhat more information about their nutrition than is available for Swamp buffaloes. The object of the work reported here was to obtain some fundamental nutritional data for

Swamp buffaloes, and to compare directly, these animals with Ayrshire cattle (*Bos taurus*). As such, it was an expansion of previous experimental work conducted at this Institute.

The primary measurements made, were the voluntary intake and digestibility of the diets being fed, since these are major factors in determining the nutritive value of a food to an animal. Different diets and ambient temperatures were used in order to identify any inter-species variation in the effects of diet quality and ambient temperature. Rates of passage of digesta through the alimentary tract were determined, in order to establish the relationship between rate of passage, intake and digestibility, and to help clarify inter-species differences in response to changes in diet quality and ambient temperature.

Fig. 2.1 Diagram to illustrate the relationship between factors, stimuli and the control of VFI.



Words in small print represent factors.

2. LITERATURE REVIEW

2.1 Voluntary food intake

Voluntary food intake (VFI) sets the limit on the intake of nutrients. As such it is a fundamental aspect of nutrition and it is influenced by a wide range of factors. It is likely that these factors affect the generation of stimuli in an animal, which in turn affect the control of food intake. This inter-relationship is demonstrated in Fig. 2.1. For the purposes of this report factors are considered to be any aspect of an animals environment, its diet or its own physical or physiological condition that affects VFI. Stimuli are considered to be the nervous or hormonal responses of an animal sent to the brain, where they act as a stimulus for the control of food intake, as a direct or indirect result of these factors. Recent reviews by Baile and Forbes (1974); Bines (1979); Van Soest (1982) and Baber (1982) give descriptions of many of the factors that affect VFI and the possible nature of some of the stimuli.

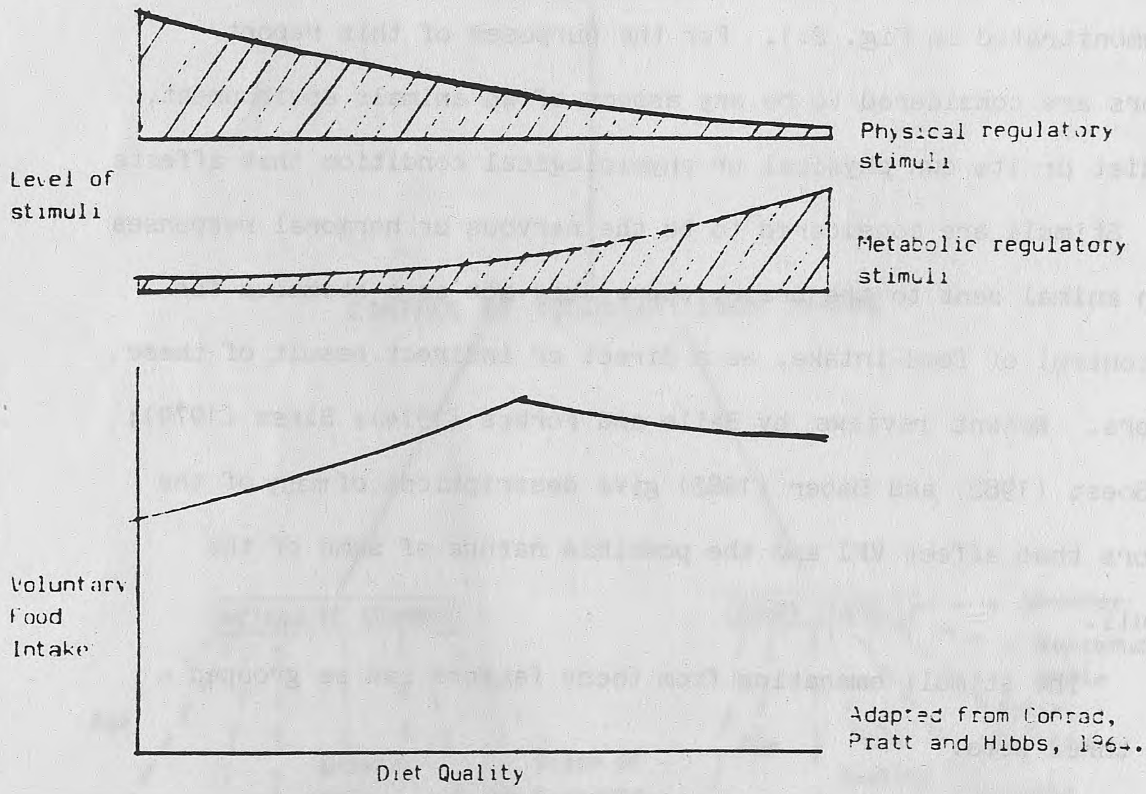
The stimuli emanating from these factors can be grouped into three sets:

Those emanating from the factors affecting metabolic regulatory mechanisms of the animal which are due to the need to regulate energy intake;

those emanating from physical regulation of food intake due to the need to regulate rumen and lower tract fill and

those emanating from other facets of nutrition, such as palatability or the need for temperature homeostasis.

Fig. 2.2 Diagram to represent suggested changing importance of stimuli with variation in diet quality.



Adapted from Conrad, Pratt and Hibbs, 1964.

As diet quality increases, there is a gradual reduction in the level of physical regulatory stimuli. Conversely there is a gradual increase in metabolic regulatory stimuli.

While the actual mechanism for the control of VFI is not known, it is likely that these stimuli and the factors which cause them, have a cumulative effect.

Factors may directly or indirectly affect the production of stimuli, for instance the effect of palatability, which can be considered to be the overall sensory perception of a food, will be direct, whilst that of body size will be indirect and via an effect upon rumen size or upon energy requirements for maintenance. Each factor may affect more than one set of stimuli, and indeed some factors such as diet quality, will affect all three sets of stimuli. As diet quality decreases, metabolic stimuli will also decrease tending toward an increase in VFI. Conversely, physical regulatory stimuli will increase as diet quality decreases, due to a decrease in the rate of passage of food from the rumen. Thirdly, the palatability of the diet is likely to be affected by changes in the quality of the diet. Therefore as diet quality alters, there is likely to be a gradual change in the relative importance of the three sets of stimuli. It will be the sum total of the changes in each set of stimuli that will determine the actual effect of diet quality upon VFI. This is illustrated in Fig. 2.2. In Swamp buffaloes, because their normal diet is of low quality, it is likely that physical fill regulatory stimuli will play the major role in controlling food intake.

Cattle and buffaloes have been shown to have different rumen microbial populations (Fujita, Imai and Ogimoto, 1979; Panjarathinam and Laxminarayana, 1974; Pant and Roy, 1970) and different rumen biochemical reactions (Mehra, Chetal, Singh and Saxena, 1978; Nangia, Aggarwal and Singh, 1972; Sharma and Mudgal, (1975a).This would suggest that cattle and buffaloes will differ in their ability to digest the fibre fraction of a diet and consequently that the effect of diet quality on physical regulatory stimuli will be different. Furthermore, Van Soest (1982) suggests that the palatability of foods will vary between different groups and species of animals.

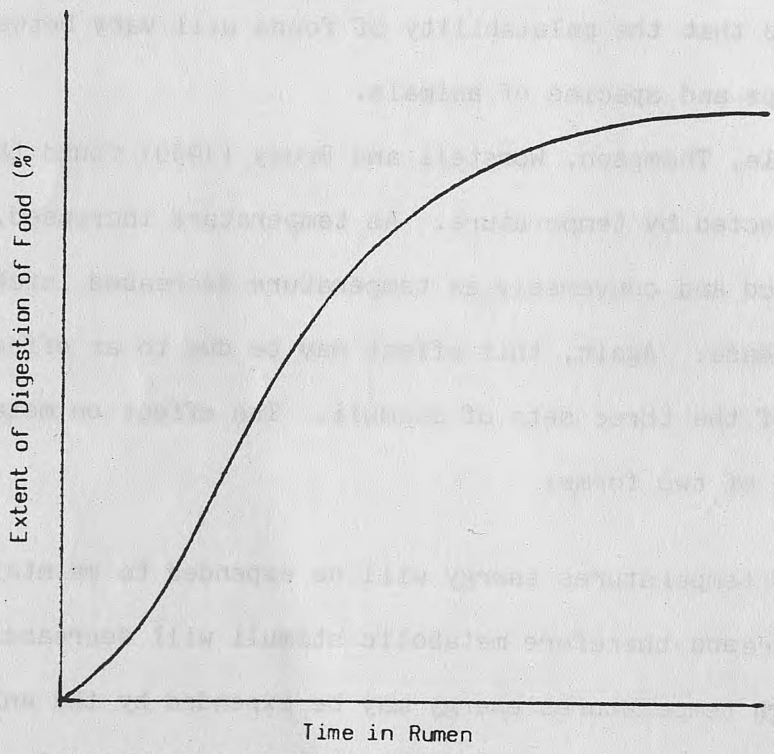
Ragsdale, Thompson, Worstell and Brody (1950) found that intake was affected by temperature. As temperature increased, intake decreased and conversely as temperature decreased intake tended to increase. Again, this effect may be due to an effect upon any one of the three sets of stimuli. The effect on metabolic stimuli may be of two forms:

at low temperatures energy will be expended to maintain body temperature and therefore metabolic stimuli will decrease;

at high temperatures energy may be expended by the animal whilst trying to keep cool, due to an increase in respiration rate, and again metabolic stimuli would decrease.

Warren, Martz, Asay, Hilderbrand,Payne and Vogt (1974) found that ambient temperature affects the retention time of food in the digestive tract and therefore it will also affect physical regulatory stimuli. Similarly, ambient temperature will also affect temperature homeostasis and therefore, the third set of stimuli will be affected.

Fig. 2.3 Typical extent of digestion-with-time curve for the rumen.



(Adapted from Van Soest, 1982).

Hamid, Richards and Sykes (1977) and Tilakaratne, Ranawana, Srikandakumar and Rajaratne (1980) have shown that cattle and buffaloes differ in their ability to withstand heat stress as shown by changes in their respiration rate, pulse rate and rectal temperatures. Therefore, it is unlikely that the effect of ambient temperature on VFI will be the same in both cattle and buffaloes. Indeed, Ragsdale *et al.* (1950) found that the effect of ambient temperature upon VFI varied with different types of cattle.

2.2 Digestibility

Along with VFI, digestibility is a major factor determining the nutritive value of a diet. Cell contents are almost totally digestible and therefore, the digestibility of a diet will be closely related to the proportion of cell wall in the diet and its digestibility. This in turn, is dependent upon the degree of lignification of the cell wall (Van Soest, 1982). The digestibility of the cell contents of a diet is unlikely to vary greatly between species. As different species of animals have different rumen microbial populations, the same does not necessarily apply to the cell wall fraction of a diet. This may cause between species differences in the dry matter digestibility of a diet.

The extent of digestion of food material within the rumen can be described by a cumulative curve as shown in Fig. 2.3. Since the extent of digestion increases with time spent in the rumen, and since ambient temperature is known to affect the rumen retention time, it is likely that ambient temperature will affect digestibility.

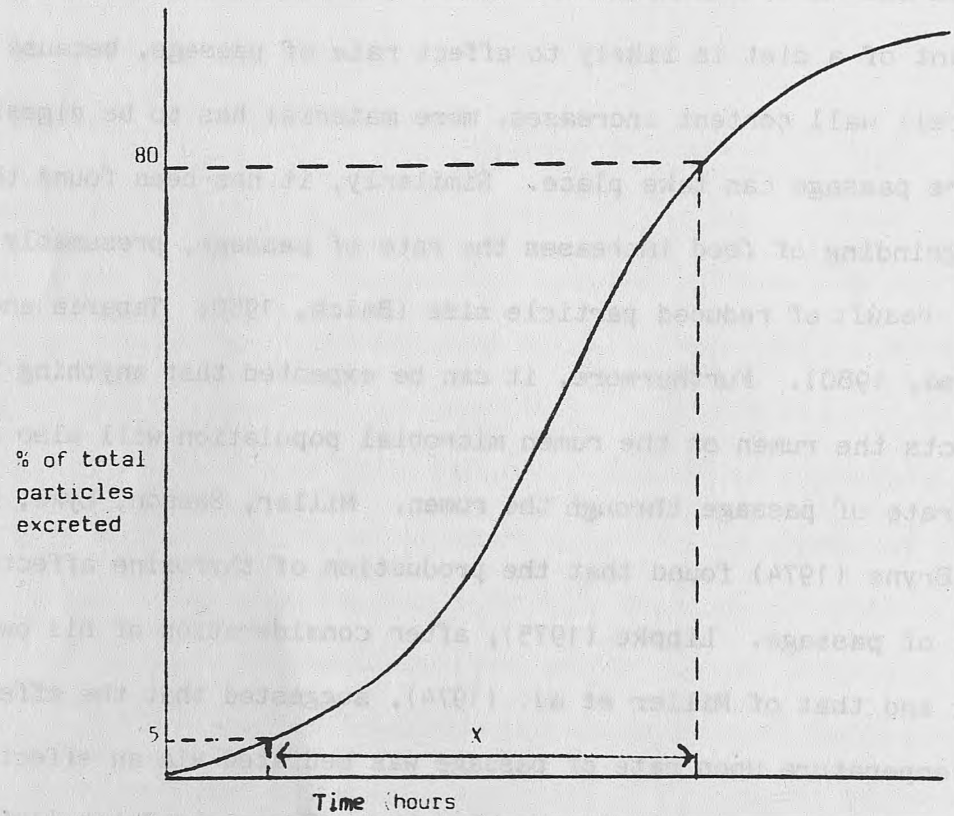
Mertens (1977) found that the actual form of the curve varied with the diet being fed, and so any effects of temperature upon digestibility are also likely to vary with the diet. Furthermore, as cattle and buffaloes respond differently to heat stress, the effect of temperature upon digestibility may also vary with the species of animal being considered.

2.3 Rate of passage of digesta

Rate of passage of digesta, refers to the flow of digesta through the gastrointestinal (GI) tract. It is directly related to retention time, and it can be measured over the whole of the GI tract, or for a specific part, such as the rumen. Ingested food will normally only leave the rumen if it is digested and absorbed into the body, or through passage on to the omasum and the rest of the GI tract. As such, digestion and passage can be considered to be competing fates for food particles (Mertens and Ely, 1982). The relative rates of digestion and passage through the rumen will determine the extent of digestion within the rumen and hence will be a major determinant of diet digestibility (Van Soest, 1982). Similarly, the combined effects of rate of digestion and rate of passage will determine how rapidly food leaves the rumen and how rapidly rumen fill is alleviated. The rate of passage will therefore, also affect VFI. In this manner, the rate of passage will have a profound effect upon both intake and digestibility. This effect is likely to be in opposite directions, namely, that as rate of passage increases, digestibility will decrease and intake increase.

Poppi, Norton, Minson and Hendrickson (1980) found that as the size of the particles in the rumen increased, there is an increasing resistance to their flow out of the rumen. From this, it would appear that the rate of passage of digesta from the rumen, will be largely determined by the rate of breakdown of food by rumination and microbes to a size suitable for transfer to the omasum and on to the abomasum. In this manner, the cell wall content of a diet is likely to affect rate of passage, because as the cell wall content increases, more material has to be digested before passage can take place. Similarly, it has been found that the grinding of food increases the rate of passage, presumably as a result of reduced particle size (Balch, 1950; Taparia and Sharma, 1980). Furthermore, it can be expected that anything that affects the rumen or the rumen microbial population will also affect the rate of passage through the rumen. Miller, Sanson, Lyke, Moss and Bryne (1974) found that the production of thyroxine affected rate of passage. Lippke (1975), after consideration of his own work and that of Miller *et al.* (1974), suggested that the effect of temperature upon rate of passage was mediated via an effect of thyroxine upon rumen motility. Campling, Freer and Balch (1962) found that urea supplementation of a straw based diet increased rate of passage, as Mehrez, Orskov and McDonald (1977) showed a close relationship between rumen ammonia concentration and fermentation rate, the effect noticed by Campling *et al.* (1962) was presumably due to an effect upon rumen fermentation. Finally, the differences in the rumen microbial population between cattle and buffaloes, are likely to affect the relative rates of passage of food for these animals, which may also lead to differences in the

Fig. 2.4 An example to demonstrate the method used by Balch (1950) to calculate rumen retention time



Adapted from Balch (1950)
The time difference marked X is the calculated rumen retention time.

relationship between intake and digestibility.

2.4 Measurement of rate of passage

There are various ways in which rate of passage can be measured. These can be separated into two classes. The first involves the measurement or estimation of the level of an indigestible, recoverable reference substance in the rumen. This value is then divided by the intake of this substance to obtain a calculated turnover time for material in the rumen (Van Soest, 1982). Alternatively, a dose of marker can be administered, followed by a series of collections over a period of several days. Dosage can be orally or via a fistula and collection from fistula sites or from faecal samples. Balch (1950) originated this method, using food dyes as markers. He calculated rumen retention time as the time interval between 5% and 80% of the stained particles appearing in the faeces. This is demonstrated in Fig. 1.4. Since Balch (1950), Castle (1956); Blaxter, McGraham and Wainman (1956), and Grovum and Williams (1973) have modified the method of determining rumen retention time and rate of passage. A complex mathematical analysis is now performed to determine the rate of passage of material through the rumen and the rate of turnover of food in the lower tract. If rate of passage is considered to be a continuous and steady process and if it is expressed as a proportion of contents passed per unit time, then the reciprocal of this value is the retention time.

Again, since the early work of Balch, a variety of markers have been used, such as plastic particles, chromium oxide, EDTA and polyethylene glycol. Frequently, these markers have been used singly, but there is now a tendency to use two or more markers, so that different fractions of the digesta can be identified separately. Ideally, the markers are 100% recoverable and they should flow with the digesta or the portion of the digesta being examined. Previous markers did not achieve these objectives and so the use of Carbon^{14} labelled forages or heavy metals such as Chromium (Cr) mordanted to plant fibre has been suggested (Van Soest, 1982).

3. MATERIALS AND METHODS

3.1 Background Information

The aim of the experiment, was to compare the effect of diet quality and ambient temperature on the nutrition of Swamp buffaloes and Ayrshire cattle. VFI and Dry Matter (DM) digestibility are major determinants of a diets nutritive value. As such, they were determined. Since rate of passage is related to both intake and digestibility and as it may account for interspecies differences it was examined. Van Soest (1982), suggests that Neutral detergent fibre (NDF) content gives the best indication of the cell wall content of a diet and so the NDF content of the diets being fed were measured. Differences in rate of degradation of food in the rumen and overall cell wall digestibility may be major factors in determining DM digestibility and VFI and so these were examined. Respiration rates, blood thyroid hormone levels and water intake were determined to provide an indication of the degree of heat stress experienced by an animal. Energy intake was determined in order to gain information on the response of animals to heat stress.

3.2 General Experimental Design

The experiment was conducted at the Centre for Tropical Veterinary Medicine (CTVM), Edinburgh, Scotland. Four adult Swamp buffaloes and four adult Ayrshire cattle, none of which were pregnant or lactating were used. The animals had all been bred in Britain and had been kept at the CTVM for at least 18

TABLE 3.1 Animals used and their allocation to Climate Chambers

Animal	Species	Approximate age (years)	Sex	Climate Chamber
E	Buffalo	7	Steer	7
F	Ayrshire	4	Steer	7
G	Ayrshire	4	Steer	8
H	Buffalo	4½	Cow	8
J	Ayrshire	5	Cow	9
K	Buffalo	4½	Cow	9
L	Buffalo	6	Bull	10
M	Ayrshire	5	Cow	10

TABLE 3.2 Experimental Design

Climate Chamber		7	8	9	10
Animal		E F	G H	J K	L M
Period	Dates				
Acclimatisation	10/5-17/5	High Temperature		Low Temperature	
1	17/5-7/6	AA6	Viton 10	Viton 10	AA6
2	8/6-28/6	Viton 10	AA6	AA6	Viton 10
Acclimatisation	29/6-5/7	Low Temperature		High Temperature	
3	5/7-25/7	Viton 10	AA6	AA6	Viton 10
4	26/7-15/8	AA6	Viton 10	Viton 10	AA6

months. During the course of the experiment, the animals were housed in pairs in climate chambers. In the allocation of animals to positions in climate chambers, each climate chamber was considered to be a unit and would contain one animal from each species. The animals were then allocated at random to their position within the chamber. The animals and their allocation to climate chambers are described in Table 3.1. The animals were chained up, and during collection periods they were separated by the use of two wooden pillars. Each animal had individual food and water troughs. Each chamber was fitted with max.-min. and wet and dry thermometers. Lighting was by electric light and a constant day length of 12 hours from 07.00-19.00 hours was used. Each climate chamber was cleaned and each animal washed once every morning, when faeces were being collected this occurred immediately following the first faecal collection of the day (see Section 3.6). During this time the door of the climate chamber was open whilst at all other times the door was kept closed.

The experiment was divided into four periods each of three weeks duration. Two diets and two temperatures were used with each animal being fed each diet at each temperature. The imposition of treatments to each chamber was arranged according to a balanced design as shown in Table 3.2

3.3 Feeding Regime

Two diets, each provided *ad libitum*, were used. One was AA6 a medium quality complete diet (see Appendix 1 for composition) and the other was Viton 10 which is commercially prepared sodium hydroxide (NaOH) treated straw. Both diets were supplied as pellets. Animals consuming AA6 were given 300g/day of molassine meal to facilitate acceptance of the markers for the rate of passage studies (see Section 3.7.1). Animals consuming Viton 10 received 120g/day of a proprietary mineral and vitamin premix (Beef Cow 300) and a supplement containing a mixture of urea and sodium sulphate in molassine meal, to provide adequate nitrogen (N) and sulphur (S) for maximum rumen microbial activity. The calculation of the amount and composition of supplement to be fed with the Viton 10 diet is given in Appendix 2.

3.3.1 Calculation of Food Intake

Consideration was given to the points raised by Blaxter, Wainman and Wilson (1961) about the difficulties encountered in the determination of food intake, before the following procedure was adopted. For the first thirteen days of any period, the animals were allowed to adapt to the diet being fed. At 09.00 and 15.30 hours food refusals were removed and fresh food was offered with the appropriate supplementation. Usually 8kg of food were offered in the morning and 12kg in the afternoon. However, this depended upon how much food had been left over the previous 2-3 days. If at any time an animal was without food it was offered more. During the final eight days of a period, henceforth called the collection period, the weight of food offered was recorded and similarly the amount of food refused was recorded. When food refusals had been weighed, a sample was taken for a DM determination. Food intake was calculated by considering food offered, supplements fed and food refused, an example of a calculation of food intake is given in Appendix 3.

3.4 Temperature Regime

The climate chambers were set at either a low temperature (low) of 20°C or a high temperature (high) of 33°C. Two chambers were set at each temperature with a cross-over occurring between periods two and three as shown in Table 3.2. Prior to the first and third periods, the animals were allowed a week in which to adapt to the change in temperature. This period has been called the acclimatisation period, and the first one began three days after the animals entered the climate chambers.

3.4.1 Measurement of Climate Chamber Environmental Conditions

During the collection periods, readings were taken from the two sets of thermometers at 10.00, 12.00 and 16.00 hours. Consideration was given to the work of Raff (1980), before the following method was decided upon for expressing environmental conditions. The mean dry bulb reading from the wet and dry bulb thermometer, was calculated for each climate chamber during each period. This value was taken as the best description of

TABLE 3.3. <u>Faecal Collection Times</u>

Day	Times of Collection							
1	08.00	10.00	12.00	14.00	16.00	18.00	20.00	23.00
2	08.00	10.00	12.00	14.00	16.00	18.00		22.00
3	08.00		12.00		16.00			22.00
4	08.00		12.00		16.00			22.00
5	08.00		12.00		16.00			22.00
6	08.00				16.00			
7	08.00				16.00			
8	08.00							

the environmental conditions within the climate chamber. The Relative Humidity (RH) in each climate chamber as well as the maximum and minimum temperature over a collection period were also recorded.

3.5 Collecting Feed Samples

On the last day of the first three periods, samples of Viton 10, AA6, molassine meal, Beef Cow 300 and the urea: sodium sulphate:molassine meal mixture, henceforth called the mixture, were taken for DM determination. Following drying at 100 °C, the samples were ground through a hammer mill with a 1mm screen and stored in airtight plastic jars for subsequent laboratory analysis. For the last collection period, samples of Viton 10 and AA6 were taken every day as it was felt that sampling on one day only may not provide a sufficiently representative sample. These samples were dried for DM determination and then subsampled by quartering to obtain a sample for grinding. The three supplements were sampled on the last day as for the first three periods.

3.6 Collecting Faecal Samples

At 23.00 hours on the day preceding a collection period, all faeces were scraped from the climate chamber and discarded. Faeces were then collected at various intervals during the collection period as shown in Table 3.3. At each collection the faeces from each animal were scraped from the floor and shovelled into pre-weighed clean dustbins. Care was taken to ensure the correct appropriation of faeces to each animal in a climate chamber. However, this was not always easy. The dustbins were weighed, their contents thoroughly mixed and a sample of approximately 1.5 to 1.8kg taken. From this sample a 2% sub-sample of the total faecal output for that collection was taken placed in a labelled bag and frozen. The remaining faecal sample was then weighed, dried for a DM determination and ground as for the food sample. These ground up faecal samples were then used in the rate of passage studies (see Section 3.7). If sufficient space was not available for immediate drying then the faecal sample was frozen and dried at a later stage. Total faecal output for a collection period was calculated by the summation of DM weight for each faecal collection except the collection at 08.00 hours on Day 1 of a period. At the end of each collection period, all the 2% sub-samples were thawed and pooled separately for each animal. A sample of the pooled sub-samples, henceforth called the pooled faecal sample was taken, dried and ground as for the feed sample. These pooled faecal samples were used to determine the NDF and ash content of the faeces (see Section 3.13).

3.7 Determination of Rate of Passage

After consideration of the work of Uden Colucci and Van Soest (1980) two markers were used. Chromium (Cr) mordanted to hay fibre was used as a solid phase marker and Cobalt ethylene diamine tetra-acetic acid (Co-EDTA) as a liquid phase marker.

Markers, were prepared by methods devised by Mathers (unpublished) and used previously by Shandomo (1982). The procedures followed are described in Appendices 4a and 4b

3.7.1 Feeding of Markers

On the afternoon immediately preceeding a collection period namely the 30th May, 20th June, 17th July and 7th August, food refusals were removed as usual , however, the animals were not fed. At 23.00 hours the markers were given. For animals on the Viton 10 diet, 125g of Cr mordanted fibre were mixed with 300g of the molassine meal:urea:sodium sulphate mixture, this was then mixed with 2kg of Viton 10. 100ml of Co-EDTA were then mixed with the food before it was fed. A similar procedure was adopted for animals on the AA6 diet, except that 300g of molassine meal were used instead of the mixture, and 2kg of AA6 instead of Viton 10. When most of the marker and the feed had been consumed, the animals were given more of their respective diets. The 120g of Beef Cow 300 required to supplement the Viton 10 was fed with this extra food.

3.7.2 Marker Analysis Trial

The wet acidic digestion method of Milner (1965, reported by Owen *et al*. 1967 as a personal communication) was to be used as the basis for analysing the Chromium content of a faecal sample. However, as it was intended to estimate Chromium by atomic absorption spectroscopy and not by colorimetry as the dichromate ion, a trial experiment was undertaken to determine whether the

TABLE 3.4 Marker Analysis Trial, Treatments Used and Distribution of Treatments

Tube Number	Digestion Time	Diluent	Heating Block	Position in Heating Block
1	0 minutes	Water	1	6
2	15 minutes	Water	1	2
3	30 minutes	Water	1	4
4	45 minutes	Water	1	5
5	60 minutes	Water	1	1
6	120 minutes	Water	1	3
7	0 minutes	Sulphuric acid	2	9
8	15 minutes	Sulphuric acid	2	12
9	30 minutes	Sulphuric acid	2	7
10	45 minutes	Sulphuric acid	2	8
11	60 minutes	Sulphuric acid	2	11
12	120 minutes	Sulphuric acid	2	10

method could be adapted to increase the rate and ease of sample preparation. The trial experiment consisted of taking a faecal sample and following the method of Milner (1965), except that varying digestion times were used instead of relying on colour changes, and the digest was diluted in either 1.1M sulphuric acid or in distilled water. The design used is shown in Table 3.4. Two pre-heated, heating racks were used, timing commenced as soon as the contents of the tubes boiled, which took less than one minute, therefore the tubes for 0 digestion time were removed from the heating rack almost immediately. Each heating rack held six tubes and the treatments were allocated according to a randomised block design with sulphuric acid and distilled water being the blocks.

3.7.3 Marker Analysis

After consideration of the result of the above experiment (see Section 4.15) Milner's (1965) method was modified and the following procedure adopted. Two sub-samples of ground faeces of approximately 5g were weighed and placed in large boiling tubes, 20ml of concentrated Nitric acid were added to each tube, which was then left overnight in a fume cupboard. The tubes were then heated in a heating rack until the evolution of brown fumes ceased. When the tubes had cooled, 15ml of digestion mixture, the preparation of which is described in Appendix 5, were added, before further heating for 30 minutes, during which time the contents were mixed twice by shaking the tube. The tubes were again allowed to cool, prior to the digest being transferred to 100ml volumetric flasks using distilled water to rinse out the tubes and to make the volumetric flasks up to 100ml. The contents of the volumetric flasks were allowed to settle overnight before the supernatant was decanted off into Universal bottles. The supernatant was then analysed for Chromium and Cobalt using an atomic absorbtion SP90 spectrophotometer. Standard solutions were made and used to obtain standard curves for Cobalt and Chromium concentrations, from which the concentration of each marker in the supernatant, and hence, the concentration of each marker in the faecal sample could be determined by the procedure outlined in Appendices 6a, 6b and 6c.

3.7.4 Mathematical Analysis of Faecal Marker Concentration

The results obtained above, were analysed by the method of Grovum and Williams (1973). A computer programme designed by Dr. J.S. Blake of Cambridge University and modified by Mathers (1983) was used to determined TT, the minimum time required for a particle of feed to pass from the mouth to the faeces, k_1 the rate constant for passage through the rumen and k_2 the rate constant for passage through the hind gut.

3.8 Measurement of Water Intake

Each animal had an individual water trough which was attached to a water meter, readings were taken at 09.30 and 16.00 hours on each day of a collection period. From these readings, water intake was calculated as shown below:

$$\frac{\text{Water Intake (litres)}}{\text{DM Food Intake (kg)}} = \text{Water Intake (l /kg food)}$$

3.9 Measurement of Animal Weight

On the day preceding the first experimental period, and on the last day of each collection period, the animals were removed from the climate chambers and weighed. Metabolic body weight was calculated as shown below:

$$\text{Body Weight}^{0.75} = \text{Metabolic Body Weight}$$

3.10 Measurement of Thyroid Hormone and Serum Urea Levels

On the last day of each collection period, blood samples were taken from the jugular vein of each animal, into non heparinised vacutainer tubes. After the blood had clotted the tubes were centrifuged and the serum removed and deep frozen until analysed. Serum T_3 (triodothyronine) and T_4 (thyroxine) levels were determined using Amerlex T_3 R1A and Amerlex T_4 R1A kits following the procedure recommended by the makers of these kits (Amersham International Ltd.). Serum urea levels were determined at the Veterinary Field Station using a Gilford Diagnostics urea determination kit. For Period 1 only, heparinised tubes were used for the blood collection and subsequent analysis was performed on plasma instead of serum.

3.11 Measurement of the Effect of Heat Stress

Respiration rates were recorded at 12.00 and 16.00 hours on each day of the collection periods. These measurements were made by observing the movement of the flank of the animals. The position of animals, lying or standing was recorded at 10.00, 12.00 and 16.00 hours. Ideally, pulse rate and rectal temperature would also have been recorded. However, previous experience with these animals had shown that attempting these measurements caused too much stress to the animals.

3.12 Determination of Cr Mordant Stability and the Degradability of the Diets in the Rumen

Six rumen fistulated sheep of the Animal Nutrition department at Edinburgh University were used to determine the stability of the Cr mordant used in the rate of passage studies, and the rumen degradability of the two diets being fed. After consideration of the reports by Mehrez and Ørskov (1977) and Ørskov, Hovell and Mould (1980) the following approach was adopted. Samples of AA6 and Viton 10 were ground through a hammer mill with a 3mm screen. The Cr mordant was used as prepared. Thirty-six washed, dried and weighed polyester Dacron bags measuring 9.5 x 140cm, 12 for Cr mordant and 12 for each of the diets, were taken and a weighed sample of approximately 5g placed into each bag. After sealing the bags at the neck by tying, the 12 samples of Cr mordant were separated into two groups. Five bags from each group were then tied individually with nylon string onto 2 separate plastic coated cords. The same procedure was followed for AA6 and Viton 10. At 09.30 hours on the first day of this trial the cords were inserted

TABLE 3.5 Allocation of Sample Groups to Rumen Fistulated Sheep

Sheep Number	1	2	3	4	5	6
Sample Group	Viton 10I	Mordant I	AA6I	AA6II	Viton 10II	Mordant II

TABLE 3.6 Time of Removal of Dacron Bags From Rumen Fistulated Sheep

Day	Time	Hours spent in rumen (Hrs.)
1	09.30	0
1	12.30	3
1	15.15	5 hrs. 45 mins.
1	21.30	12
2	09.30	24
3	09.30	48

into the six rumen fistulated sheep as shown in Table 3.5. At the same time, the remaining bag from each group was placed into the rumen contents of the appropriate animal, and then immediately removed. This bag represented time 0, the other bags were removed from each animal at the specified times over the next two days as shown in Table 3.6. As soon as a bag was removed from the rumen, it was rinsed under running tap water until the draining fluid was clear, the neck of the bag was then untied and rinsing repeated. The bag was then dried in a forced air oven at 65°C for two days, and then weighed.

The extent of disappearance of the feed samples was calculated. The relationship between extent of disappearance of food DM from the bag and time in the rumen could be described by single exponentials which were fitted (Mathers, pesonal communication) by a standard computer package (Maximum Likelihood Program developed by the Rothamstead Experimental Station). This relationship could be combined with information on the rate of passage of digesta through the rumen to determine the degradability of the feed in the rumen according to the method of McDonald (1981). The stability of the Cr-mordant was determined by comparing the degree of disappearance of the sample with time spent in the rumen.

3.13 Laboratory Analyses

The neutral detergent fibre (NDF) content, the acid detergent fibre (ADF) and the lignin content of the ground AA6, Viton 10 and molassine meal samples and the NDF content of the pooled faecal samples from each period were determined by the method of Goering and Van Soest (1970). The mixture could not be ground and so the above analyses were not carried out. The ash content of all the food and supplement samples and the pooled faecal samples was determined by ashing a weighed sample overnight at 500°C and then reweighing.

Finally, a DM determination was made on the ground faecal samples used for marker analysis, the ground pooled faecal samples, the ground food and supplement samples, the Cr mordant and the ground AA6 and Viton 10 samples that were used in the rumen fistulated animals. This determination was made, so that all values could be expressed on a DM basis. Except for the DM determination of the faecal samples used for marker analysis, the above analyses were all made in replicate.

The degree of lignification of the cell wall of the diets was calculated as shown below:

$$\%\ \text{lignification} = \frac{\%\ \text{Lignin}}{\%\ \text{NDF}}$$

The ash content of a sample was used to determine the Organic Matter (OM) content of a sample using the formula given below:

$$\text{OM} = 100 - \text{Ash}\ \%$$

3.14 Calculation of Digestibility

The apparent dry matter and NDF digestibility of a diet were calculated using the following formula. All weights are on a DM basis.

$$\text{Apparent DM Digestibility (\%)} = 100 \times \frac{\text{Total Food Intake} - \text{Total Faecal Output}}{\text{Total Food Intake}}$$

$$\text{Apparent NDF Digestibility (\%)} = 100 \times \frac{\text{NDF Intake} - \text{NDF Output}}{\text{NDF Intake}}$$

An example of an apparent NDF digestibility calculation is given in Appendix 7. As the mixture could not be analysed for NDF content, the assumption was made, that urea and sodium sulphate had a zero NDF content and so the NDF content of the mixture was entirely due to its molassine meal content.

3.15 Calculation of Energy Intake

Energy intake was calculated after the following assumptions had been made (ARC, 1980).

1. Gross Energy of digested organic matter is 19 MJ/kg.
2. Conversion factor of Digestible Energy (DE) intake to Metabolisable Energy (ME) intake is 0.82.

An example of the calculation of energy intake is given in Appendix 8.

3.16 Statistical Analysis

The results obtained were examined by Analysis of Variance as illustrated in Appendix 13. The initial analysis considered all the results together whilst in a further analysis, the data from buffaloes and cattle were examined separately. The F ratio for species differences was calculated as shown below:

$$\text{Species F ratio} = \frac{\text{Species Mean Square}}{\text{Animal within species Mean Square}}$$

All other F ratios were calculated using the Error Mean Square as the denominator.

Animals within species by diet interaction, animals within species by temperature interaction and animals within species by diet and temperature interaction were all considered to be estimates of the same value and were pooled in the residual error term. Correlation coefficients were calculated between the various measurements which had been made.

3.17 Statement of Authors Involvement in the Experiment

At this juncture, the author would like to comment that he was not personally involved with the collection of data or of food faecal and blood samples during the first two periods. However, he was involved in the collection of data and samples during the last two periods. He was also involved in the analysis of the blood samples from all four periods and in the analysis for Cr and Co of the faecal samples from period 1 which were used in the rate of passage studies. The author was responsible for the collation of the data obtained during all four periods, for the DM, OM, NDF and Lignin determinations on the feed and faecal samples from all four periods and for the experiments with the rumen fistulated sheep.

Table 4.1 Mean Climate Chamber Temperature for Each Treatment

	Low Treatment	High Treatment
Mean dry bulb temperature	21.55°C	31.19°C

Each value is the mean of the dry bulb temperatures over all the collection periods. It is calculated from the individual values given in Appendix 9.

Table 4.2 Animal Weights at the end of Each Collection Period (kg)

Animal	Initial Weight	Period 1	Period 2	Period 3	Period 4
E	740	755	794	793	795
F	600	587	623	632	629
G	510	514	509	544	597
H	605	603	592	535	602
J	565	578	590	581	633
K	590	585	615	544	631
L	690	729	738	780	758
M	420	443	450	438	459

4. RESULTS

4.1 General

Throughout the experiment the animals remained in overall good health, except that animal M developed a slight oedema during period 4, which did not require treatment.

4.2 Temperature

Climate chamber temperatures were not maintained constantly at the desired levels, and a smaller difference between the high and low temperature treatments occurred during the early part of the day than was intended. This was partly because while the hot chambers were being cleaned out, the temperature within the climatic chamber decreased and partly because when the external air temperature was high, it was difficult to maintain the lower temperature. Following cleaning, the temperature of the hot climate chambers increased rapidly and a subjective assessment of the duration of the lower temperature was about 30 minutes. The mean dry bulb temperature, measured between 10.00 and 16.00 hours for the climate chambers at the high and low temperature treatments, are given in Table 4.1. Individual dry bulb temperature values, max-min values and relative humidity values for each climate chamber during each period are given in Appendix 9.

4.3 Weight of Animals

The animal weights at each of the five weighings are presented in Table 4.2. Although consideration was given to

Table 4.3 Summary of the Laboratory Analysis of Feed Samples

	AA6	Viton 10	Molassine Meal	Mixture	Beef Cow 300
NDF content (%)	42.97	71.30	25.56		
Lignification (%)	12.95	13.73			
Organic Matter (%)	90.00	88.67	88.85	85.21	9.41

The values given are means of the estimates obtained for the feed sample from each collection period. All values except those for Lignification (%) are as a % of DM. Lignification (%) calculated as shown in Section 3·13 calculating the theoretical weight of an animal midway through a collection period, the weight of an animal at the end of the collection period was used in all subsequent calculations. This was because it was felt that the accuracy of the weighings, and the fact that no account could be taken of changes in the weight of gut contents, did not justify a more sophisticated treatment of the data. For the same reasons, a statistical analysis of the weight change of an animal over each experimental period was not carried out, although the weight changes are presented in Appendix 10.

4.4 Feed Analysis

The results of the laboratory analysis of the feed samples, are presented in detail in Appendix 11 and are summarised in Table 4.3. Differences between the periods were relatively small in comparison with differences between feeds and are presumably associated with the sampling procedure. Although the NDF content of the Viton 10 was considerably greater than that of the AA6, the percentage lignification of the two diets was not significantly different.

4.5 Voluntary Food Intake

The total food DM intake for each animal during each collection period is given in Appendix 12. The mean daily food consumption, expressed as gDM/kg liveweight day (g/kg lwt day) and gDM/kg Metabolic lwt day (g/kg lwt$^{.75}$ day) was compared for each of the eight treatment combinations. Individual animal

Table 4.4 Mean Voluntary Food Intake for Each Treatment Combination (gDM/kglwtday)

	AA6		Viton 10		
	Low	High	Low	High	
Buffaloes	16.54	16.47	21.27	19.29	
Cattle	30.17	23.58	26.11	25.28	SE mean = 1.512

Each value is the mean of four individual animal values.

Table 4.5 Mean Voluntary Food Intake for Each Treatment Combination (gDM/kglwt$^{.75}$day)

	AA6		Viton 10		
	Low	High	Low	High	
Buffaloes	84.30	83.35	108.38	99.10	
Cattle	145.13	113.13	126.58	121.75	SE mean = 6.969

Each value is the mean of four individual animal values.

values and the statistical analyses of the results are given in Appendices 13 and 14. Summaries based on the mean value for each treatment combination are given in Tables 4.4 and 4.5. The analysis of the combined results for Buffaloes and Cattle of VFI expressed on a liveweight basis, showed that the intake of the cattle was significantly greater than the intake of the buffaloes ($p < 0.01$). Intake was not affected significantly by the diet, however there was a significant species x diet interaction ($p < 0.05$). Finally, intake at the low temperature was significantly greater than intake at the high temperature ($p < 0.05$). Analysing the results of the buffaloes and cattle separately, showed that buffaloes consumed significantly more of the Viton 10 than of the AA6 diet ($p < 0.05$), whereas the intake of cattle was not affected significantly by diet. Conversely, the intake of cattle was significantly greater at the low temperature than at the high temperature ($p < 0.05$), whereas the intake of the buffaloes was not affected significantly by temperature. Analysing the results of the two diets separately, showed that for the AA6 diet, the intake of the cattle was significantly greater than that of the buffaloes ($p < 0.05$). However, the intake of the Viton 10 diet was not significantly affected by the species of animal.

The results of the analysis of intake on a metabolic liveweight basis were similar to those for intake on a liveweight basis except that, when the diets were analysed separately there was no species effect for either diet. However, the same trend could be identified in that for the AA6 diet, cattle tended to

Table 4.6 Mean NDF Content of the Faeces for Each Treatment Combination (%)

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	63.51	61.90	63.35	59.92
Cattle	64.73	62.51	60.44	61.44

SE mean = 1.288

Each value is the mean of the four individual animal values given in Appendix 16.

Table 4.7 Mean DM Digestibility for Each Treatment Combination (%)

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	66.23	71.65	59.75	64.13
Cattle	68.70	66.55	54.18	56.73

SE mean = 2.378

Each value is the mean of four individual animal values.

Table 4.8 Mean NDF Digestibility for Each Treamtent Combination (%)

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	49.81	58.84	62.20	67.78
Cattle	52.57	51.62	59.00	60.62

SE mean = 3.207

Each value is the mean of four individual animal values.

have a greater intake than buffaloes. The F ratio value for the species effect on the AA6 diet was 9.3 , which is not significant at the 0.05% level because for 1 and 3 degrees of freedom an F ratio value of 10.1 or over is required, although it is significant at the 0.10% level.

4.6 Faecal Analysis

The total faecal DM output for each animal during each period is given in Appendix 15. The mean NDF content of the faeces from each treatment is shown in Table 4.6, whilst individual estimates for each animal and the statistical analysis of this data may be found in Appendix 16. There was no significant difference between the mean NDF content of the faeces from any treatment combination. The results of the laboratory analysis of the faeces for OM are given in Appendix 17.

4.7 Apparent Digestibilities

The mean apparent digestibilities of the DM and the NDF contents of the diets for each treatment combination, are presented in Table 4.7 and 4.8. The apparent DM and NDF digestibility coefficients for individual animals and their statistical analyses are shown in Appendixes 18 and 19. Neither species of animal nor temperature had a significant effect upon DM or NDF digestibility. However, in the buffaloes there was a trend towards a greater apparent digestibility at the higher temperature. Diet affected significantly both DM and NDF digestibilites ($p < 0.001$), but in different manners. For DM digestibility, AA6

Table 4.9 Mean Water Intake for Each Treatment Combination (l/kg food DM)

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	4.46*	4.83*	5.88*	6.38*
Cattle	4.34**	4.86***	4.78**	5.09***

* Each value is the mean of four individual animal values
SE mean = 0.397

** Each value is the mean of three individual animal values
SE mean = 0.459

*** Each value is the mean of two individual animal values
SE mean = 0.562

had a significantly higher digestibility than Viton 10, whereas for NDF digestibility Viton 10 had a significantly higher digestibility than AA6. Furthermore, for DM digestibility, the absolute difference between the AA6 and Viton 10 diets was greater in cattle than in buffaloes (12.17 versus 7.00; SE difference = 2.378)

4.8 Water Intake

Three results for water intake were lost due to malfunctioning equipment. Two other results for animal M may be unreliable as they were very low in comparison with other results, and were obtained from a water meter which needed to be repaired at a later stage of the study. As all the missing and questionable results were for cattle, no attempt was made to estimate the missing values. Furthermore, the values from animal M were ignored in the subsequent analysis. The mean water intake for each treatment, excluding values for animal M, are presented in Table 4.9. Individual water intakes for each animal and their statistical analysis are given in Appendix 20. Diet had a significant effect upon water intake ($p < 0.01$) but neither temperature nor species of animal affected water intake significantly.

4.9 Estimated Metabolisable Energy Intake

The ME intake of individual animals during each treatment combination were calculated from DE intake and expressed as MJME/kglwt$^{.75}$day. The values obtained are presented in Appendix 21

Table 4.10 Mean Estimated Metabolisable Energy Intake ($MJ\ ME/kglwt^{.75}day$)

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	0.815	0.847	0.901	0.889
Cattle	1.437	1.106	0.963	0.963

SE Mean 0.0964

Each value is the mean of four individual animal estimates which are calculated as shown in Appendix 8.

Table 4.11 Mean Respiration Rates for Each Treatment Combination (counts/minute)

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	16.53	32	17.95	21.9
Cattle	39.98	84.93	30.08	70.67

SE mean = 2.909

Each value is the mean of four individual animal values given in Appendix 22.

along with their statistical analysis. The mean estimated ME intake for each treatment combination is shown in Table 4.10. The cattle had a significantly higher energy intake than the buffaloes ($p < 0.01$), and there was a significant diet species interaction. Cattle had a significantly higher energy intake on the AA6 diet than on the Viton 10 diet ($p < 0.05$). Whereas, for buffaloes, diet had no significant effect. Finally, temperature did not affect energy intake significantly.

4.10 Respiration Rates

Respiration rates, were used to indicate the degree of heat stress to which an animal was being subjected. Mean respiration rates are shown in Table 4.11 with individual values and their statistical analysis in Appendix 22. Cattle had a significantly higher respiration rate than buffaloes ($p < 0.001$). Similarly, respiration rates at the higher temperature were significantly greater than respiration rates at the lower temperature ($p < 0.01$). There was also a significant temperature-species interaction ($p < 0.001$) which was probably due to the fact that the increase due to temperature was greater in the cattle than in the buffaloes (42.77 versus 9.71; SE difference = 2.909). Analysing the combined buffalo and cattle data, showed a significant diet effect ($p < 0.001$). However, the separate analysis of this data showed that the respiration rate of buffaloes was not affected significantly by diet, whereas cattle had a significantly higher respiration rate on the AA6 diet than on the Viton

Table 4.12 Mean T_3 Levels (ng/ml) for Each Treatment Combination

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	1.31	1.11	1.23	1.14
Cattle	1.69	1.08	1.51	1.27

SE mean = 0.108

Each value is the mean of the values from four individual animals, these individual values are given in Appendix 23.

Table 4.13 Mean T_4 Levels (ng/ml) for Each Treatment Combination

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	57.0	54.5	60.8	50.8
Cattle	63.3	70.8	55.3	69.0

SE mean = 4.40

Each value is the mean of four individual animal values, these individual values are given in Appendix 24.

Table 4.14 Mean Serum Urea Levels (mmol/litre) for Each Treatment Combination

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	6.47	8.50	5.81	7.22
Cattle	6.98	6.59	5.29	4.48

SE mean = 1.157

Each value is the mean of four individual animal values, these individual values are given in Appendix 23.

10 diet ($p < 0.001$).

4.11 Concentration of Thyroid Hormones in the Blood

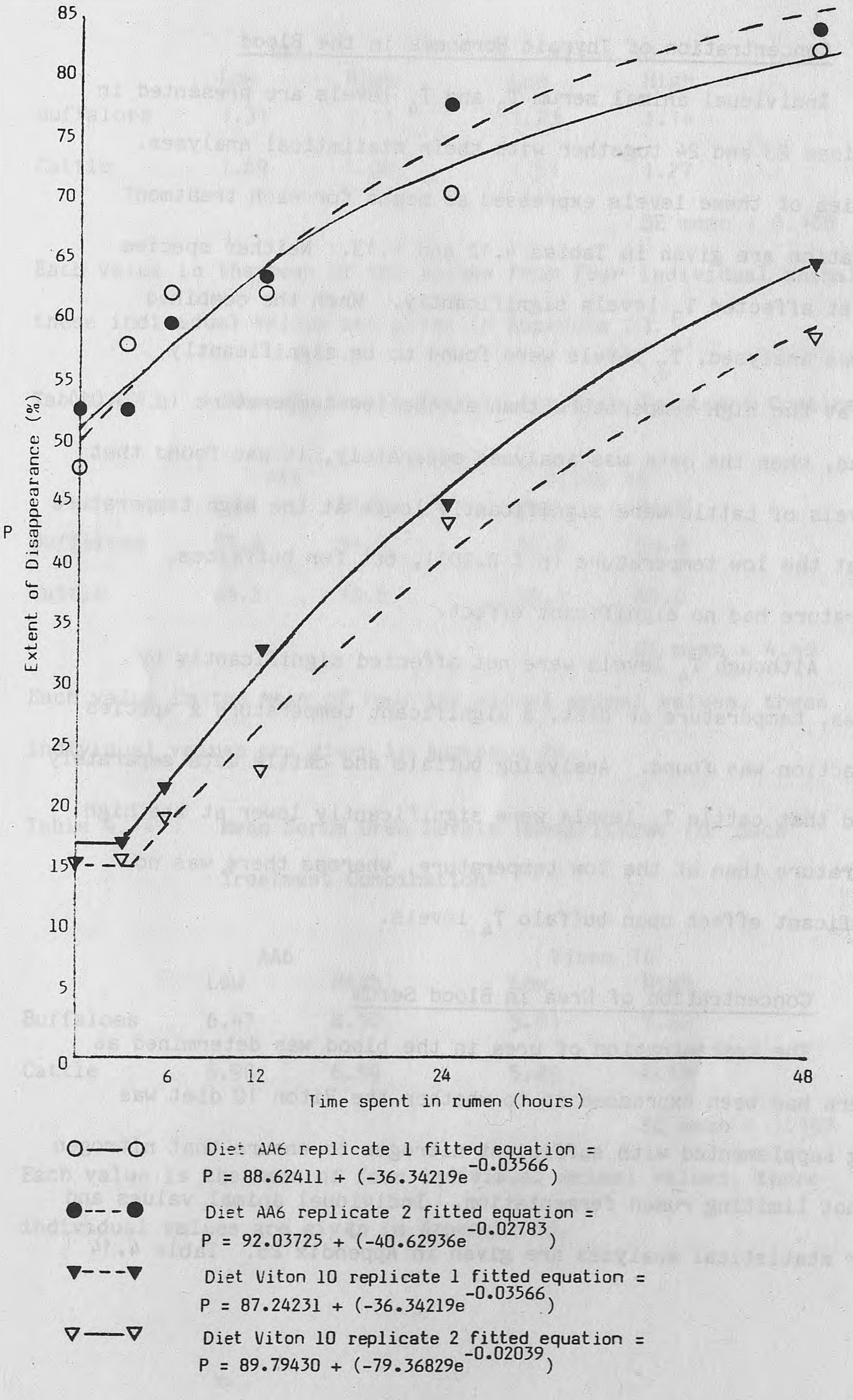
Individual animal serum T_3 and T_4 levels are presented in Appendices 23 and 24 together with their statistical analyses. Summaries of these levels expressed as means for each treatment combination are given in Tables 4.12 and 4.13. Neither species nor diet affected T_3 levels significantly. When the combined data was analysed, T_3 levels were found to be significantly lower at the high temperature than at the low temperature ($p < 0.01$). Whereas, when the data was analysed separately, it was found that T_3 levels of cattle were significantly lower at the high temperature than at the low temperature ($p < 0.001$), but for buffaloes, temperature had no significant effect.

Although T_4 levels were not affected significantly by species, temperature or diet, a significant temperature x species interaction was found. Analysing buffalo and cattle data separately showed that cattle T_4 levels were significantly lower at the high temperature than at the low temperature, whereas there was no significant effect upon buffalo T_4 levels.

4.12 Concentration of Urea in Blood Serum

The concentration of urea in the blood was determined as concern had been expressed as to whether the Viton 10 diet was being supplemented with sufficient nitrogen to ensure that nitrogen was not limiting rumen fermentation. Individual animal values and their statistical analyses are given in Appendix 25. Table 4.14

Fig. 4.1 Extent of Disappearance-with-time curves for AA6 and Viton 10



○——○ Diet AA6 replicate 1 fitted equation =
$P = 88.62411 + (-36.34219e^{-0.03566})$

●---● Diet AA6 replicate 2 fitted equation =
$P = 92.03725 + (-40.62936e^{-0.02783})$

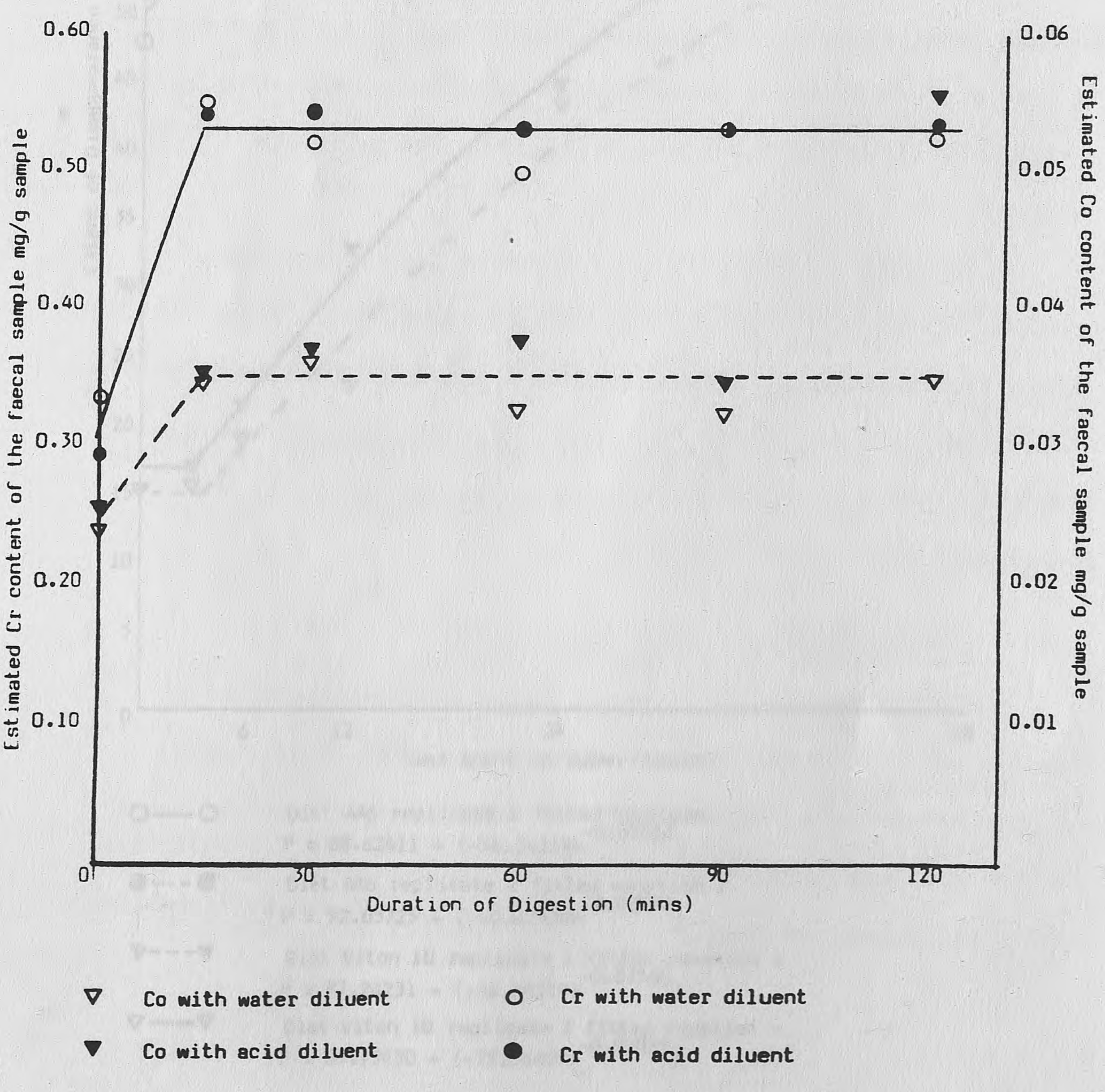
▼---▼ Diet Viton 10 replicate 1 fitted equation =
$P = 87.24231 + (-36.34219e^{-0.03566})$

▽——▽ Diet Viton 10 replicate 2 fitted equation =
$P = 89.79430 + (-79.36829e^{-0.02039})$

Table 4.15 Extent of Disappearance (%) of Chromium Mordant after incubation for Various Times in the Rumen of Sheep.

Time in Rumen	Sheep Number 2	Sheep Number 6
0 hours	-1.65	-1.75
3 hours	-5.75	-2.83
5.75 hours	-2.32	-2.77
12 hours	-3.31	-3.68
24 hours	-1.33	-3.24
48 hours	-2.83	-3.65

Fig. 4.2 Results of the Marker analysis trial, estimated Co and Cr concentrations in a faecal sample.



gives mean values for each treatment combination. Diet did not affect serum urea levels significantly. Similarly, temperature and species had no significant effect upon serum urea levels.

4.13 Disappearance of Feed Sample Within the Rumen

The values obtained from this experiment, are given in Appendix 26. The relationship between extent of disappearance of the feed sample and time in the rumen was determined using a computer. Different models were used to describe this relationship for the AA6 and the Viton 10 diets. The graphs obtained and the exponential relationships describing the graphs are given in Fig. 4.1. The AA6 diet disappeared more rapidly from the dacron bags than the Viton 10 diet.

4.14 Chromium Mordant Stability

The results of the Cr mordant stability trial are demonstrated in Table 4.15. These results show clearly that the Cr mordant was not degraded during the 48 hours it was present in the rumen. Indeed the weight of Cr mordant recovered actually increased slightly.

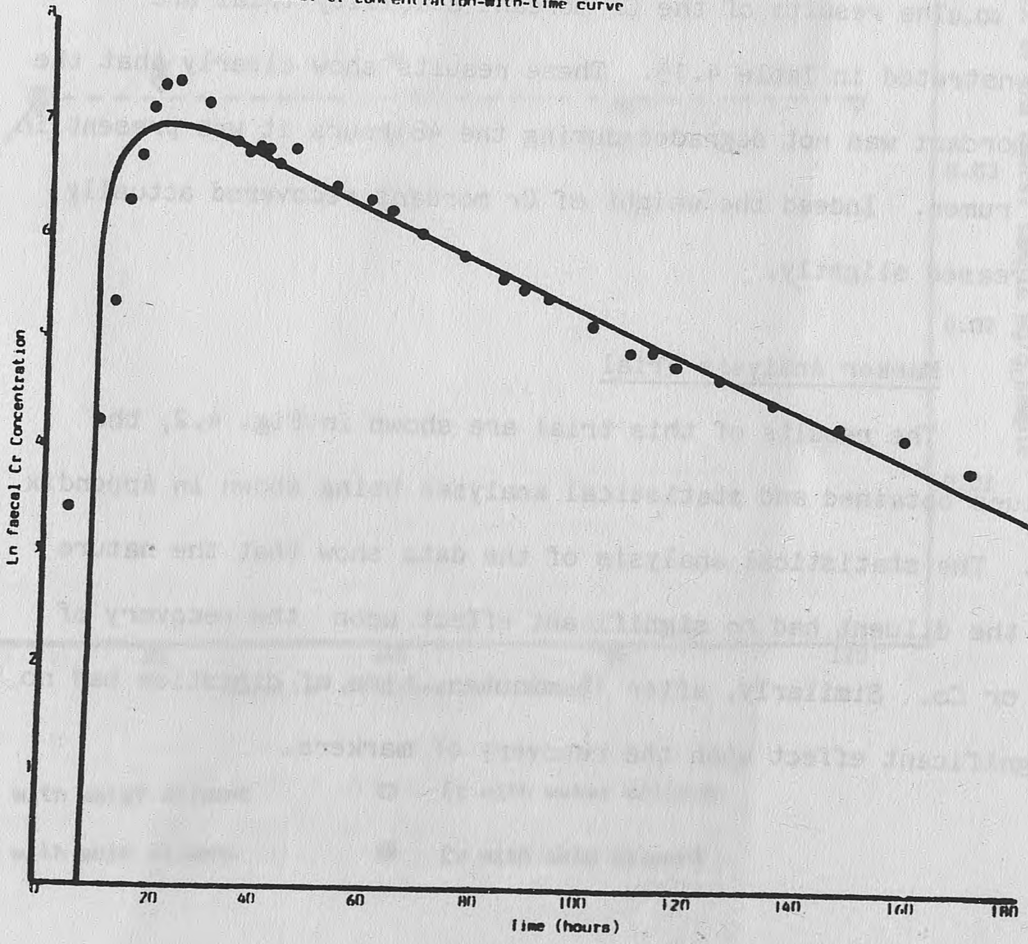
4.15 Marker Analysis Trial

The results of this trial are shown in Fig. 4.2, the values obtained and statistical analyses being shown in Appendix 27. The statistical analysis of the data show that the nature of the diluent had no significant effect upon the recovery of Cr or Co. Similarly, after 15 minutes, time of digestion had no significant effect upon the recovery of markers.

Table 4.16 Rate constants (k_1 and k_2), Time of first appearance (TT) and the mean retention time (MRT) for Cr mordant marker.

Species	Temperature	Diet	Animal	k_1 (%/h)	$^1/k_1$ (hrs)	k_2 (%/h)	$^1/k_2$ (hrs)	TT (h)	MRT (h)
Buffaloes	High	AA6	E	2.5	40.0	-	-	-	-
		Viton 10	H	2.3	43.5	10.3	9.7	9.76	63.0
	Low	Viton 10	K	2.4	41.7	9.9	10.1	6.72	58.7
		AA6	L	2.8	35.7	14.3	7.0	5.68	48.9
Cattle	High	AA6	F	2.4	41.7	17	5.9	8.04	55.6
		Viton 10	G	2.9	34.5	9.2	10.9	8.61	54.3
	Low	Viton 10	J	2.6	38.5	7.1	14.1	8.58	61.7
		AA6	M	2.6	38.5	9.5	10.5	3.89	52.3

Fig. 4.3 Faecal Cr Concentration-with-time curve



4.16 Rate of Passage of Digesta

The dried faecal samples from one complete collection period were digested, and the concentrations of Cr and Co in the faeces were estimated. Unfortunately, the computer programme used to analyse the faecal marker concentrations-with-time curves did not function properly and so the data were analysed manually. In the short time available, only the faecal Cr concentration-with-time data were analysed. Of the two markers, Cr was considered to be more important since it was used to study the rate of passage of solid particles through the GI tract. A typical set of faecal Cr concentration-with-time data is given in Appendix 28 and these data together with the fitted curve are shown in Fig. 4.3. The values for the two rate constants k_1 and k_2 and their reciprocals, for TT calculated time of first appearance of marker in the faeces and the mean retention time (MRT) for each of the eight animals are given in Table 4.16. Animal E produced relatively few faecal samples during the first two days of the collection period which prevented estimation of K_2. As a consequence, estimation of TT and MRT for this animal was not possible. The two rate constants k_1 and k_2 refer to the proportion of a pool of matter that leaves the pool each hour. Grovum and Williams (1973) suggest that k_1 refers to the rumen and so the reciprocal of k_1 refers to the mean retention time of matter in the rumen. In the present experiment this was of the order of 40 hours. K_2 has been assumed to represent a pool of digesta in the large intestine and so the reciprocal of k_2 refers to the retention time of matter in the large intestine.

Table 4.17 Extent of Digestion of the Diet in the Rumen (%)

Diet	Species	Extent of digestion in the rumen(%)	Proportion of total digestion occuring in the rumen(%)
AA6	Buffaloes	74.20	99.3
	Cattle	74.74	105.90
Viton 10	Buffaloes	44.54	69.18
	Cattle	41.53	70.15

Table 4.18 Correlation Coefficients

		r	Significance
Intake (g/kg) vs DM dig.	combined	-0.0635	NS
	Buffaloes	-0.1077	NS
	Cattle	0.322	NS
Intake (g/kg) vs NDF dig.	combined	0.1177	NS
	Buffaloes	0.6175	($P < 0.01$)
	Cattle	0.282	NS
Intake (g/kg) vs T_4	Combined	0.2985	NS
Intake (g/kg) vs T_3	Combined	0.383	($P < 0.05$)
Energy intake vs Resp. rate	Combined	0.3106	NS
T_3 vs NDF dig.	Combined	-0.0536	NS
T_3 vs DM dig.	Combined	0.0043	NS

This latter value is quite variable and ranges between 5.9 hours and 14.1 hours. TT is the theoretical time of first appearance of marker in the faeces and thus it represents the minimum time required for a food particle to pass from the mouth to the anus. MRT is made up of three components, $^1/k_1$ and $^1/k_2$ and TT and it refers to the mean time a particle spends in the animals GI tract which for this experiment ranged from 48.9 to 63.0 hours. As the faecal samples from only one collection period were digested and analysed no meaningful statistical analysis could be carried out on the data. However, a visual assessment of the data, showed no obvious trends. The data for each diet, combined over both temperatures, was used to make an estimation of the extent of digestion of the two feeds in the rumen of buffaloes and cattle. An example of the calculation involved is shown in Appendix 29 and the results obtained are given in Table 4.17. As only two values were used in the estimation of the extent of digestion of the diet in the rumen no inter-species comparisons can be made. However, the AA6 diet would appear to be digested in the rumen to a much greater extent than the Viton 10 diet.

4.17 Correlation Coefficient

Correlation coefficients were calculated between the various measurements that had been made. These coefficients were generally low and non significant even when the data for buffaloes and cattle was separated (Table 4.18).

Table 5.1 Proportion of Cell Wall in Some Subtropical and Tropical Forages

Forage Name	Cell Wall %
Cenchrus	66
Cynoden dactylon plectostachyus	76
Digitaria decumbens	68
Panicum maximum	68
Pennisetum purpureum	66

(Adapted from Van Soest, 1973)

5. DISCUSSION

5.1 General

The aim of the experiment, was to obtain fundamental data on the nutrition of Swamp buffaloes and to compare them directly with cattle. Two diets of different quality and two ambient temperatures were used to determine the effect of diet quality and ambient temperature upon various facets of nutrition that were to be studied. The two diets had very different cell wall contents. The AA6 was a medium quality diet with a cell wall content of about 40%, whereas the Viton 10 diet had a cell wall content of just over 70%. The latter is comparable to the values obtained by Van Soest (1973) for many tropical and subtropical grasses, see Table 5.1. The Viton 10 diet was supplemented with amounts of urea and sodium sulphate to ensure that neither nitrogen nor sulphur was limiting rumen fermentation. As the blood urea concentrations were not affected significantly by diet (Table 4.14) it is possible to assume that sufficient supplementary nitrogen had been provided. Although the mean temperatures of the two temperature treatments did not differ by as much as was intended, they may still be regarded as representing a high and a low temperature treatment. This is supported by the heat stress symptoms observed with the higher temperature (see Section 5.5).

Table 5.2 Some Comparative Values for the VFI of Swamp Buffaloes and Cattle

	INTAKE					
	(g/kg $lwt^{.75}$ day)		(g/kg lwt day)			
Diet	Buffaloes	Cattle	Buffaloes	Cattle	Significant Difference	Reference
Various			18—21	22—26	Yes	Grant *et al.* (1974)
Guinea Grass	93	95			No	Johnson *et al.* (1968)
Sorghum Hay	65.0	88.1			No	Moran *et al.* (1979)
Various	54 — 94	54 — 99			No	Moran *et al.* (1983)
Pangola Grass	65	59			Yes	Williams and Dudzinski (1982)
AA6 and Viton 10	83 — 108	113—145	16—21	24—30	Yes	Present Experiment

Table 5.3 Some Comparative Values for the VFI of River Buffaloes and Cattle

	INTAKE					
	(g/kg $lwt^{.75}$ day)		(g/kg lwt day)			
Diet	Buffaloes	Cattle	Buffaloes	Cattle	Significant Difference	Reference
Hay/Straw/ Concentrate			22.1	28.2	Yes	Bhatia *et al.* (1979)*
Various			22	25	Yes	Bhatia *et al.* (1980)*
Wheatbhoosa and Groundnut Cake	76	87	20	22	Yes	Ponappa and Nooruddin (1971)
Various	63 — 67	54 — 60			Yes	Sharma and Mudgal (1975 b)
Apluda aristata	72	64			Yes	Sharma and Rajora (1977)

* Bhatia, Pradhan and Singh, 1979

**Bhatia, Pradhan and Singh, 1980

5.2 Voluntary Food Intake

There are conflicting reports of the relative VFI of Swamp Buffaloes and cattle. Moran, Satoto and Dawson (1983) found no significant difference between the intakes of Swamp Buffalo and zebu cattle. Likewise, Moran, Norton and Nolan (1979) found no significant difference between the intakes of Shorthorn cattle, Brahman crosses and Swamp Buffaloes, and Johnson and Hardison, Ordoveza and Castillo (1968) found no significant difference between Holsteins and Swamp Buffaloes. In contrast Williams and Dudzinski (1982) found that Swamp Buffaloes had a significantly greater intake than shorthorn cross Brahmans. In the present trial, it was found that the Swamp Buffaloes consumed significantly less than Ayrshire cattle which is in agreement with the findings of Grant, Van Soest, McDowell and Perez (1974) who were working with Swamp Buffaloes and zebu cattle (see Table 5.2). The results from the present trial cannot be directly compared with those from the other trials as different diets were being fed. However, it should be noted that the VFI of animals in the present trial tended to be greater than those recorded in the other trials.

Direct comparisons between River Buffaloes and cattle show a similar picture to that for comparisons betwen Swamp Buffaloes and cattle, in that some trials show that buffaloes have a higher VFI than cattle, whilst others show that cattle have a higher VFI than River Buffaloes (see Table 5.3).

The discrepancies between the results from the trials comparing Swamp or River Buffaloes with cattle can be explained by one of three factors. The first is that if the animals being compared did not have the same nutritional histories, then this might affect their relative intakes as it may have affected their rumen development. Unfortunately, many of the trials discussed above, did not record the past nutritional history of the animals on trial. It is interesting to note that Moran *et al.* (1979) used animals of the same nutritional history and found no significant differences between the intakes of cattle and Swamp Buffaloes. In the present study, the animals had all been kept at the CTVM for at least 18 months prior to the beginning of the experiment and during this time, had all been fed on the same diets.

The second possible cause of a discrepancy is that the quality of the diet is likely to affect the relative intakes of buffaloes and cattle. In the present trial there was a significant species difference on the AA6 diet but not on the poorer quality Viton 10 diet. Similarly, the diets used by Grant *et al.* (1974) who found significantly higher intakes for cattle than for buffaloes, had higher DM digestibilities than the DM digestibilities of the diets used by Moran *et al.* (1983) or the OM digestibility of the diet used by Moran *et al.* (1979) who found no significant difference between the intake of cattle and Swamp Buffaloes. This would suggest that as the quality of the diet decreases, the relative intake of buffaloes in relation to cattle improves. This may be due to some of the differences in rumen physiology that have been discussed previously, or may be due to differences in the relative rates of passage between cattle and buffaloes which will be discussed later. Unfortunately, Williams and Dudzinski (1983) who found a higher intake in Swamp buffaloes than in cattle, did not record the quality of the diet being fed. However in that trial, the animals were freely grazing pangola grass pasture during the monsoonal dry season, as such the quality of the diet was likely to be low. Thirdly whether buffaloes or cattle have the higher intake will also be affected by the type of cattle being used. Bhatia *et al.* (1979) who found that cattle had a higher intake than River buffalo used *Bos taurus* cross *Bos indicus* cattle, whereas Sharma and Mudgal (1975 b) who found the opposite relationship used pure *Bos indicus* cattle. Furthermore, Frisch and Vercoe (1977) found that VFI was significantly greater in *Bos taurus* cattle than in *Bos taurus x Bos indicus* cattle.

The intake of the buffaloes was affected significantly by the diet with the buffaloes eating more of the poorer quality diet, whereas the intake of the cattle was not affected significantly by the diet. This difference, can be accounted for by consideration of ME intake. The buffaloes had a significantly lower estimated ME intake than the cattle. Furthermore, the estimated ME intake of the buffaloes was not affected significantly by the diet whilst that of cattle was. This would suggest that the relative importance of the different stimuli controlling intake are different for cattle and buffaloes. It would appear that the energy intake ceiling for buffaloes was lower than that

Table 5.4 Metabolisable Energy Requirements and Supply (MJ ME/day)

BUFFALOES

Animal	Requirement*	SUPPLY AA6 Low	AA6 High	Viton 10 Low	Viton 10 High
E	78.14	133.3	152.1	155.1	162.3
H	63.22	65.5	83.3	113.7	124.0
K	63.86	81.3	99.4	99.2	97.4
L	65.88	156.5	110.0	112.1	101.4

CATTLE

Animal	Requirement*	SUPPLY AA6 Low	AA6 High	Viton 10 Low	Viton 10 High
F	64.22	143.3	160.1	98.4	136.2
G	58.67	182.5	125.9	111.1	126.2
J	62.81	146.3	101.9	118.0	84.0
M	48.29	172.2	102.6	112.7	88.9

* Energy requirement is calculated using the formula shown below:-

ME requirement (MJ ME/day) = $8.3 + 0.091\,W$ (MAFF, 1975)

W = Animal weight. The mean weight of an animal calculated from the weights given in Table 4.2 was used.

for cattle, and that for buffaloes, stimuli from the control of energy intake, are the main stimuli in the control of VFI. Therefore as the digestible energy content of the AA6 diet is higher than that of Viton 10 (see Section 5.3), the intake of AA6 is lower. If for cattle, energy intake regulation is not as important as gut fill then the greater rate of disappearance from the rumen of the AA6 diet compared with the Viton 10 diet (see Fig. 4.1), means that physical fill stimuli are less on the AA6 diet than on the Viton 10 diet and so intake can potentially be higher.

Why the buffaloes should be regulating their ME intake at a lower level than cattle is a question of obvious importance. There are various possible reasons for this difference, but first it should be noted that all the animals had an energy intake far in excess of their maintenance requirements (Table 5.4). Presumably therefore, energy was going into liveweightgain, which is supported by the fact that all the animals except Animal H gained weight during the experiment.

The first possible reason, is that the buffaloes may have a lower ceiling for liveweight gain or more generally, a lower potential for production. However, it should be noted, that as all the animals were over 4 years of age any liveweight gain is likely to be due to an increase in the condition of an animal rather than growth. The second possible reason, is that the buffaloes and cattle may have entered the climate chambers at different body condition levels and therefore would have different potentials for liveweight gain. As no measurement was taken of the condition score of the animals when they entered the climate chambers, it is not possible to ascertain whether this was important. The third possible reason, is that the buffaloes may have a lower energy requirement for maintenance than cattle, which could also be associated with a lower potential for liveweight gain. Indeed, Frisch and Vercoe (1977) found differences in the fasting metabolic rate of different breeds of cattle and that fasting metabolic rate was related to liveweight gain.

Temperature did not affect the intake of buffaloes significantly, whereas it did affect the intake of cattle significantly. However, this latter effect was largely due to the temperature differences on the AA6 diet, as the intake of the cattle on the Viton 10 diet at the high temperature was only slightly less than the intake at the low temperature (25.28 vs 26.11 g DM/kg lwt day SE 1.51). This can be compared with the work of Ragsdale *et al*. (1950) who found that temperature affected intake significantly and Warren *et al*. (1974) who found no significant temperature effect. Both these groups of workers were using cattle.

The above effect can be explained in a variety of ways. Firstly, it could be said that the high temperature used did not put the animals under sufficiently severe heat stress, especially as the climate chamber temperature did decrease once each day. However, the respiration rate of both cattle and buffaloes were affected significantly by temperature and so it would appear that the animals were under some degree of heat stress. Secondly, it is possible that the species of animals differed in their susceptibility to heat stress, (see Section 5.5) and so differences in the effect of temperature upon intake are only to be expected. Thirdly, the different effects of temperature upon the intakes of cattle and buffaloes can be explained by differences in the importance of the stimuli controlling VFI. For the buffaloes, it has been mentioned that stimuli from the control of energy intake would appear to be very important in the control of VFI. This control of energy intake, may reduce the need to decrease energy intake to maintain temperature homeostatis, hence the lack of effect of temperature upon the intake of buffaloes.

For the cattle, physical fill regulatory stimuli were likely to be more important in the control of VFI. On the poorer quality Viton 10 diet, energy intake was lower than on the AA6 diet, and so the need to decrease energy intake to maintain temperature homeostasis may again not have arisen. In contrast, on the AA6 diet, potential energy intake was higher and so the need to regulate energy intake to maintain temperature homeostasis arose. This would explain why for the cattle, there was a large difference between the intake of AA6 at the high and low temperature and also why the combined intakes of the AA6 diet over both temperatures, was not greater than the combined intake of the Viton 10 diet over both temperatures.

5.3 Apparent Digestibilities

Buffaloes, have often gained the reputation of being better

Table 5.5 Some Comparative Values for Apparent Digestibility (%) in Swamp Buffaloes and Cattle.

APPARENT DIGESTIBILITIES

Diet	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes	Cattle
	OM		DM		NDF		CF	
Various[1]			56-57	54-57	52-59	50-56		
Guinea Grass[2]			48-64	45-60			60-70	51-66
Sorghum Hay[3]	54.5	54						
Various[4]			37-53	38-53				
AA6 and Viton 10[5]			60-72	54-69	50-68	52-61		

1 = Grant et al. (1974)

2 = Johnson et al. (1968)

3 = Moran et al. (1979

4 = Moran et al. (1983)

5 = Present Experiment

able to digest a diet and particularly the fibre fraction of a diet than cattle, however, scientific evidence does not uniformly support this view point (Ickhponani, Gill, Makkar and Ranjan, 1977). Grant *et al.* (1976); Moran *et al.* (1979) and Moran *et al.* (1983) all found no significant difference between the apparent digestibility of various fractions of the diet in Swamp Buffaloes and cattle. This supports the data from this trial which showed that neither DM nor NDF digestibility were affected significantly by species of animal. However Johnson *et al.* (1968) did find that Swamp Buffaloes had a significantly higher apparent DM digestibility than cattle but no significant difference in crude fibre (CF) digestibility was found (see Table 5.5).

In River Buffaloes, similar contrasts have been found, Sharma and Mudgal (1975 b); Sharma and Rajora (1977) and Bhatia *et al.* (1979) have all found that DM and crude fibre digestibility were higher in buffaloes than in cattle. Conversely, Bhatia *et al.* (1980) found no significant difference in the DM or CF digestibility of various good quality diets between buffaloes and cattle.

Again, the difference in the results obtained from different trials are likely to be partly due to differences in the past nutritional history of the animals on trial and partly to differences in the quality of the diet being fed. Indeed, in the present experiment, there was a greater difference in DM digestibility between buffaloes and cattle on the low quality diet than there was on the high quality diet (6.49 vs. 1.32; SE difference = 2.378). In both cases, the DM digestibility was higher in the buffaloes.

Diet significantly affected the apparent digestibility of both DM and NDF but in opposite ways. For DM digestibility, the AA6 diet was more digestible, which is to be expected as it has a much lower cell wall content. However, for NDF digestibility, Viton 10 had the higher digestibility. There are various factors that might have caused this difference. If the lignification of the cell wall had been different between the two diets, then a difference in cell wall digestibility might have been expected, but both diets had similar contents of lignification of the cell wall fraction (Table 4.3). Secondly, the rumen fermentation conditions for cellulolytic bacteria may have been more favourable on the Viton 10 diet than on the AA6 diet. This could have been brought about in one of three ways. The Viton 10 diet was supplemented with urea and so a readily available source of N was present, however, it should be noted that, the principal protein sources in AA6 are groundnut meal and barley. Mathers, Horton and Miller (1977) found that the protein in groundnut meal was rapidly and extensively degraded in the rumen, and Mathers and Miller (1981) found the same for barley. The AA6 diet contained a relatively high proportion of barley, which being readily fermentable, may have caused a lowering in rumen pH, thereby making the rumen conditions for cellulolytic bacteria less favourable. Indeed, Conrad, Pratt and Hibb (1966) found that the addition of concentrates to a diet may increase overall

DM digestibility, but it decreases cellulose digestibility. Van Soest (1982) commented that the concentration and relative proportions of the various classes of rumen organisms vary according to the composition of the diet. Therefore, as Viton 10 contains a high proportion of cell wall it is likely to favour the development of a rumen population that digests the cell wall.

Thirdly, the NDF digestibility of the Viton 10 diet may have been higher than that of the AA6 diet, because of the effect of the NaOH treatment of the diet. Mehrez, El-Shinnawy, Abou-Raya and El Ayek (1981) found that NaOH treatment of rice straw and maize stalks significantly increased their NDF digestibility. This is thought to be because the major effect of the NaOH treatment of straw is to breakdown links between lignin, hemicellulose and cellulose within the cell wall, thereby making hemicellulose and cellulose more available for digestion by microbial enzymes. Fourthly, NDF does not measure the microbial cell wall content and as such NDF is almost a true NDF digestibility, which may account for the higher NDF digestibility than DM digestibility on the Viton 10 diet.

Temperature did not significantly affect DM or NDF digestibility, however in the buffaloes there was a trend towards a higher digestibility at the higher temperature. A similar trend was found by Warren *et al.* (1974) and Lippke (1975) who were both working with cattle. The effect of temperature upon digestibility will be discussed further in Section 5.4.

Both the diets being fed, would appear to be of a reasonable quality, the DM digestibility of the Viton 10 diet ranged from 54 to 65% and that of AA6 from 67 to 72%. Furthermore, the VFI of the Swamp Buffaloes on the present trial tended to be higher than the VFI of Swamp Buffaloes from other trials. Because of this, the applicability of these results to the areas where Swamp Buffaloes are kept, can be questioned. Differences in diet quality would appear to be partly responsible for many of the discrepancies between the various reported values for the DM digestibility and VFI for cattle and buffaloes. Therefore it is interesting to contemplate whether any differences in the relative VFI and DM digestibility of buffaloes and cattle would have been seen if a poorer quality diet had been fed. In the present experiment, consideration was given to using untreated straw as a poor quality diet. However, problems would have been encountered in the feeding of untreated straw in the climate chambers and so the decision to use the commercially prepared Viton 10 diet was made.

5.4 Rate of Passage

The results of the Cr-mordant stability trial (see Section 4.14) would suggest that the Cr-mordant was a suitable marker substance in that it was not degraded in the rumen. Mathers and Aitchison (1981) found that food residues in dacron bags can become contaminated with microbial matter during incubation within the rumen, this is the likely cause of the slight increase in the DM, recorded during incubation of Cr-mordant in the rumen.

The modified wet acid digestion method for preparation of faecal samples for Cr and Co analysis would appear to be suitable. It is interesting to note that the original method of Hill and Anderson (1958) which was modified by Czarnocki *et al.* (1961) and then by Milner (1965) used water as the diluent. The analysis of the marker concentration-with-time curve could have been made using the concentration of the marker in the faeces or the amount of marker excreted per unit time. Both these values were recorded in Appendix 28. However, it was decided that the concentration-with-time curve should be analysed.

Blaxter *et al.* (1956) found a positive correlation between food intake and rate of passage and a negative correlation between digestibility and rate of passage. Similarly Poppi, Minson and Ternough (1980) and Hendrickson, Poppi and Minson (1981) found that the stems of grasses and legumes were retained longer in the rumen than leaves and that the VFI of stems was lower. However, there was not a significant different between the digestibility of the stems and leaves. Kennedy (1982) found that the retention time of pasture hay which had a higher cell wall content that alfalfa hay was longer, but that the digestibility of the pasture hay was lower than that of alfalfa hay. Similarly, Blaxter *et al.* (1961) found that as the quality of food increased, so did rate of passage and digestibility. Therefore, simple relationships cannot be drawn between rate of passage of food residues, voluntary intake of food and its digestibility. However, a knowledge of the rate of passage of food residues may help to explain differences in intake and digestibility for different diets, species of animals and temperatures. For a given diet, increased intake is likely to provoke increased rate of passage of food residues through the gut and overall digestibility may decrease (Grovum and Williams, 1977). However, for different diets the relationship will be less clear and digestibility may be positively correlated to rate of passage. Interpretation of the species difference in VFI and digestibility from the present experiment, may be eased when the faecal Cr and Co analyses have been completed.

Kumar and Raghaven (1974) and Rana and Langar (1980) found that the rate of passage of digesta was slower in buffaloes than in cattle, the former workers also found that buffaloes had higher DM and CF digestibilities than cattle. Whereas Moran *et al.* (1983) found the opposite trend, with cattle having a slower rate of passage than buffaloes. Ludrai, Rai and Pandey (1981) and Shandomo (1982) found no significant difference between the mean retention time of food in cattle and buffaloes. Therefore as for digestibility and VFI, species differences in rates of passage of digesta in cattle and buffaloes would appear to be unclear, and are likely to depend upon the diet being fed and the past nutritional history of the animal.

The work of Poppi *et al.* (1980); Hendrickson *et al.* (1981) and Kennedy (1982) showed that as the cell wall content of a diet increases its retention time in the rumen will also increase. Therefore one may predict that the Viton 10 diet is likely to have a longer retention time than the AA6 diet which would also help to account for the higher NDF digestibility of the Viton 10 diet. Furthermore, if differences are shown between cattle and buffaloes in the response of rate of passage to changes in diet quality then this would help to explain why diet quality does not have the same effect upon DM digestibility and VFI in cattle and buffaloes.

Warren *et al.* (1974) found that mean retention time was increased as temperature increased from 18 to 32°C and that this was associated with increases in the digestibility of DM and NDF. As discussed previously, the increase in the retention time in the GI tract is believed to be due to the effects of heat stress upon blood thyroxine levels. In the present experiment, the buffaloes tended to have higher DM and NDF digestibilities at the higher temperature than at the low temperature . This would be expected, if rate of passage decreased, at the high temperature, however, thyroxine levels in the buffaloes were not significantly affected by temperature. Furthermore, there was no trend in the cattle to higher DM and NDF digestibilities at the higher temperature and cattle appeared to be more heat stressed than the buffaloes (see Section 5.5). Which makes explanation of the increase in digestibility of the DM and NDF fractions of the diet in the buffaloes at the high temperature difficult until the information on retention times is complete.

Using the rate of passage data, estimations were made of the extent of digestion of the two diets in the rumen. No firm conclusions can be drawn as rate constants from only one period could be used. However, the data would indicate that the extent of digestion of the AA6 diet in the rumen is greater than that of the Viton 10 diet. It would also suggest that the proportion of total digestion occurring in the rumen was greater for the AA6 diet than for the Viton 10 diet. These values are closer to true digestibilities than apparent digestibilities which accounts for the fact that the estimated digestion of the AA6 diet in the rumen of the cattle was greater than the calculated apparent DM digestibility. The extent of disappearance-with-time curves (Fig. 4.1) showed that 50% of the AA6 diet disappeared from the dacron bags almost immediately. This figure is very high and would imply that a high proportion of the AA6 diet is instantly soluble.

5.5 Heat Stress

Water intakes, respiration rates, and blood thyroid hormone levels were measured so as to assess the extent of heat stress which the animals encountered. Water intakes, expressed per unit of DM consumed were not affected by temperature which is surprising as Warren *et al.* (1974) and many others (ARC, 1980) have found that water intake increased as temperature increased. Similarly, species of animal did not affect water intake. However, the water intake on the Viton 10 diet was significantly higher than on the AA6 diet which is presumably because of the high Na content of the Viton 10 diet. Respiration rates were very significantly affected by temperature and the increase in respir-

Table 5.6 Mean Estimated Heat Output for Each Treatment Combination* (MJ/square metre body surface day)

	AA6		Viton 10	
	Low	High	Low	High
Buffaloes	13.79	14.01	15.26	14.88
Cattle	20.33	16.64	15.93	15.80

*Individual animal estimates are given in Appendix 30 and a sample calculation of estimated heat output is given in Appendix 31.

ation rate was greater for the cattle than the buffaloes which would suggest that cattle were suffering greater heat stress than the buffaloes. This is contrary to the findings of Hamid *et al.* (1977) but is in agreement with Tilakaratne *et al.* (1980). For the cattle, respiration rate tended to be higher on the AA6 diet than on the Viton 10 diet. Differences in T_3 and T_4 levels would further support the suggestion that cattle were more heat stressed than buffaloes since exposure to the higher temperature depressed serum T_3 and T_4 concentrations significantly in the cattle with no significant effect in the buffaloes. Many of these differences can be explained by differences in the heat output of animals (see Table 5.6). The heat output of the cattle tended to be greater than the heat output of the buffaloes, furthermore, the heat output of the cattle on the AA6 diet appeared to be higher than the heat output on the Viton 10 diet. Interestingly, the heat output of cattle on the AA6 diet at the high temperature was much lower than at the low temperature which would further support the suggestion that for cattle on the AA6 diet, intake is decreased at the high temperature in order to help maintain temperature homeostasis.

5.6 Conclusion

The present experiment suggests that urea supplemented sodium hydroxide treated straw is suitable for at least maintenance in adult Swamp buffaloes. The most important stimuli in the control of voluntary food intake in buffaloes and cattle are likely to be different. Furthermore, the relative voluntary food intake and dry matter digestibility between Swamp buffaloes and cattle would appear to be determined to a large extent by the quality of the diet being fed. Further research is required using a poorer quality diet such as untreated straw, to simulate the type of diet that might naturally be fed to Swamp buffaloes.

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Appendix 1 Composition of AA6

Ingredient	Percentage
Chopped Barley Stray	30
Extracted groundnut meal	7
Barley	22.25
Wheatfeed	23.25
Molasses	10
Urea	1
Tallow	1.25
Salt	1.5
Di Calcium Phosphate	1.5
Sodium carbonate	2
Lime	0.25
Vitamins A D_3 and E are added	

Source: Wainman, Smith and Dewey, 1975.

AA6 is a compounded diet produced by the University of Edinburgh and intended for use in animal experiments.

Appendix 2 Calculation of Supplements needed for Viton 10 Diet

a) Calculation of amount of urea supplement required

If Dry Matter intake = 10kg/day and Viton 10 contains 9 MJ ME/kg DM

Then microbial requirements for (N) = 1.25 x 9 x 10 gN*

= 112.5 gN/day

Viton 10 contains 38g protein/kg

If assume 40% of this is available in the rumen then

$$\text{supply of N from urea needed} = 112.5 - \frac{38 \times 10 \times 0.40}{6.25}$$

$$= 112.5 - 24.32$$

$$= 88.18 \text{ gN}$$

The urea source used contains 46% N and the efficiency of capture of Ammonia (NH_3) from urea is 0.8

$$\text{then need } \frac{88.18}{(0.46 \times 0.8)} = 239.62\text{g Urea for each 10kg of Viton 10}$$

b) Calculation of amount of Sodium sulphate required

Walli and Mudgal (1982) suggest that a N:S ratio of 10:1 was required for optimal cellulose digestibility in buffaloes and cattle.

then need 112.5 x 0.1 = 11.25gS

therefore as we are using Na_2SO_4 and molecular weight of

Na = 30, S = 32, O = 16

$$\text{need } \frac{11.25}{(32/124)} = 44.3\text{g } Na_2SO_4 \text{ for each 10kg of Viton 10}$$

c) If we wish to feed 300g of a molassine meal:urea:sodium sulphate mixture with 4kg of Viton 10, then we need

$$\frac{239.62}{10} \times 4 = 94.48\text{g urea}$$

$$\text{and } \frac{44.3}{10} \times 4 = 17.72\text{g } Na_2SO_4$$

therefore each 300g lot of mixture should contain 94.48g urea

17.72g $NaSO_4$

187.8g Molassine meal

These figures were rounded off and the following amounts were used in 300g of mixture:-

95g Urea

18g Na_2SO_4

187g Molassine meal

d) Amount of Beef Cow 300 to be fed

The suppliers of the Beef Cow 300 vitamin and mineral premix suggested that 4oz/day or approximately 120g/day was a suitable level of supplementation.

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* ARC (1980)

Appendix 3 Sample Calculation of Food Intake

The example given is that of Animal E in Period 4 the diet being fed was AA6.

AA6 DM = 83.6%

Molassine meal DM = 72.3%

Day	Time	AA6 offered kg	DM AA6 offered kg	Molassine meal offered kg	DM Molassine meal offered kg	Total DM offered kg	Food Refused kg	DM % of food refused	DM refused	DM consumed
1	am	10	8.36			8.36	1.06	68.61	0.73	7.63
	pm	16	13.38	0.3	0.22	13.60	3.74	81.07	3.03	10.57
2	am	8	6.69			6.69	2.87	78.91	2.27	4.42
	pm	16	13.38	0.3	0.22	13.60	4.78	78.63	3.76	9.84
3	am	8	6.69			6.69	3.76	81.88	3.08	3.61
	pm	12	10.04	0.3	0.22	10.26	4.7	80.40	3.78	6.48
4	am	8	6.69			6.69	0.3	75.26	0.23	6.46
	pm	16	13.38	0.3	0.22	13.60	6.62	83.24	5.51	8.09
5	am	8	6.69			6.69	1.3	83.82	1.09	5.60
	pm	16	13.38	0.3	0.22	13.60	8.88	84.11	7.47	6.13
6	am	8	6.69			6.69	3.96	85.22	3.37	3.32
	pm	12	10.04	0.3	0.22	10.26	4.36	81.28	3.54	6.72
7	am	8	6.69			6.69	3.01	85.55	2.58	4.11
	pm	12	10.04	0.3	0.22	10.26	0.16	71.05	0.11	10.15
								Total food intake =		93.13

Appendix 4a Preparation of Cr mordanted fibre

The following procedure (Mathers, unpublished) was used:

1. Fibre preparation: 150g milled hay (ground to pass 1mm screen) were suspended in approximately 2 litres of 3% sodium lauryl sulphate and the mixture boiled gently for about 3 hours. The suspension was then filtered through a nylon stocking and washed very thoroughly with boiling water until the filtrate was clear. After a final wash with acetone, the fibre was spread on a tray and dried overnight at 65°C.
2. Mordant preparation: The dried hay fibre was weighed and then suspended in a solution of sodium dichromate containing an amount of Chromium (Cr) equivalent to 14% of the fibre weight e.g. if weight of fibre = 100g then sodium dichromate required = $100 \times 0.14 \times \frac{298}{100} = 41.72$g. This quantity of sodium dichromate was dissolved in sufficient water (about 1 litre) to cover and thoroughly wet the hay fibre. This suspension was made in a glass beaker. After thoroughly mixing the hay fibre with the sodium dichromate solution, the beaker was covered tightly with aluminium foil and baked in an oven at 100°C for 24 hours.

The fibre was then filtered through a nylon stocking and washed thoroughly with tap water, before resuspending in tap water. Ascorbic acid equivalent to 0.5 times the weight of the fibre, 50g in this example, was then added to the suspension, mixed thoroughly by stirring and the suspension was then left to stand for 1 hour. Finally, the fibre was washed thoroughly, again through a nylon stocking, until the filtrate was free of soluble green matter, before it was spread on a tray and dried at 65°C.

Appendix 4b Preparation of Co-EDTA solution

The following procedure (Mathers, unpublished) was used.

75g of CoII acetate were dissolved in 300ml of distilled water, 60ml of Hydrogen Peroxide (H_2O_2) were added slowly, and the solution mixed well. 100g of Disodium EDTA were dissolved in approximately 500ml of distilled water. This required heating and mechanical stirring on a magnetic stirrer and hot plate for several minutes. When both solutions were cool, they were both transferred to a 1 litre beaker containing a few glass beads. This beaker was then covered with aluminium foil and the mixture boiled gently for one hour, after which time its pH was raised to pH7 by the addition of NaOH. The mixture was allowed to cool before filtering under vacuum through Whatman 541 filter paper. Finally, the filtrate was transferred to a 1 litre volumetric flask and made up to 1 litre using distilled water.

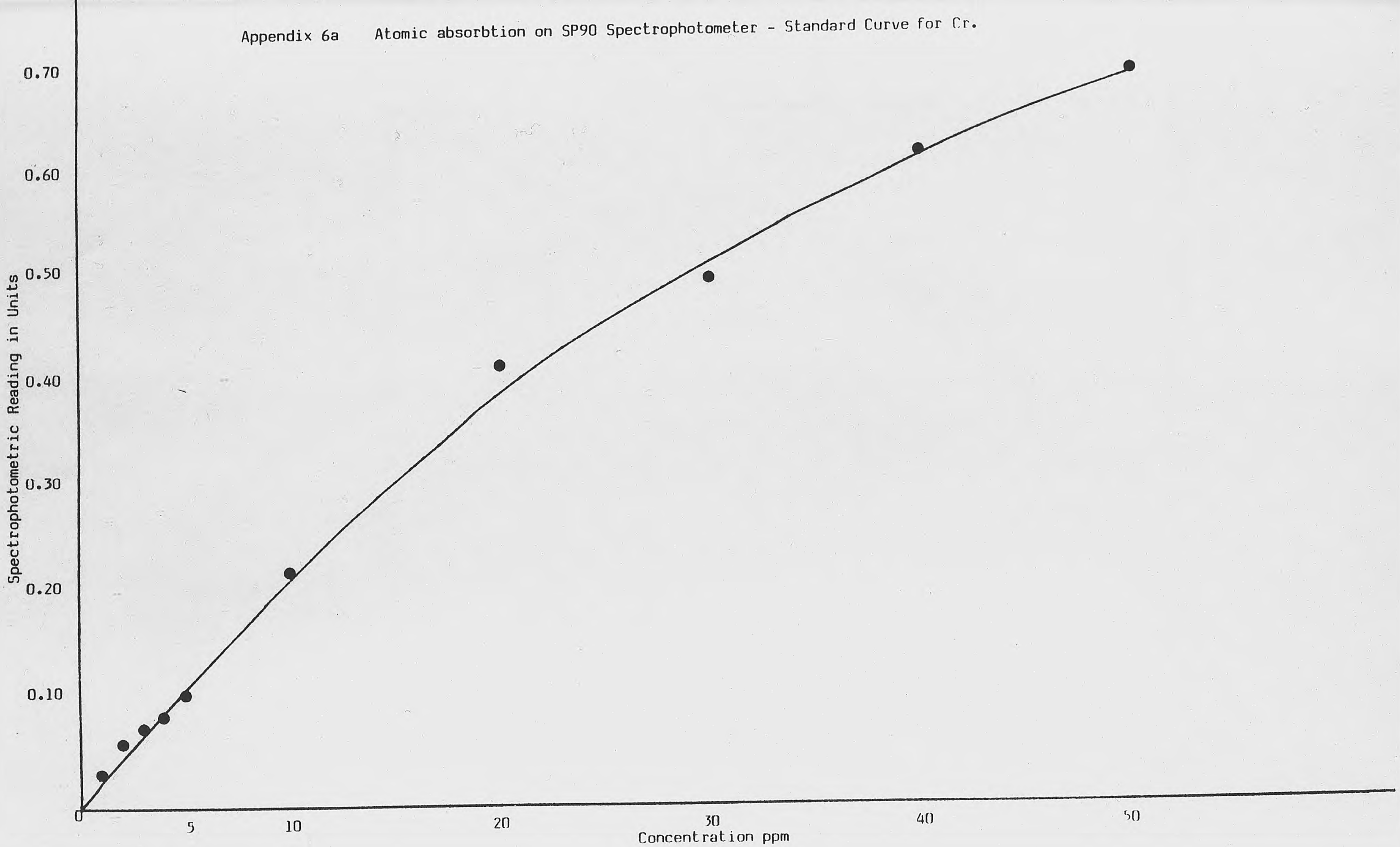
Appendix 5

Preparation of the digestion mixture

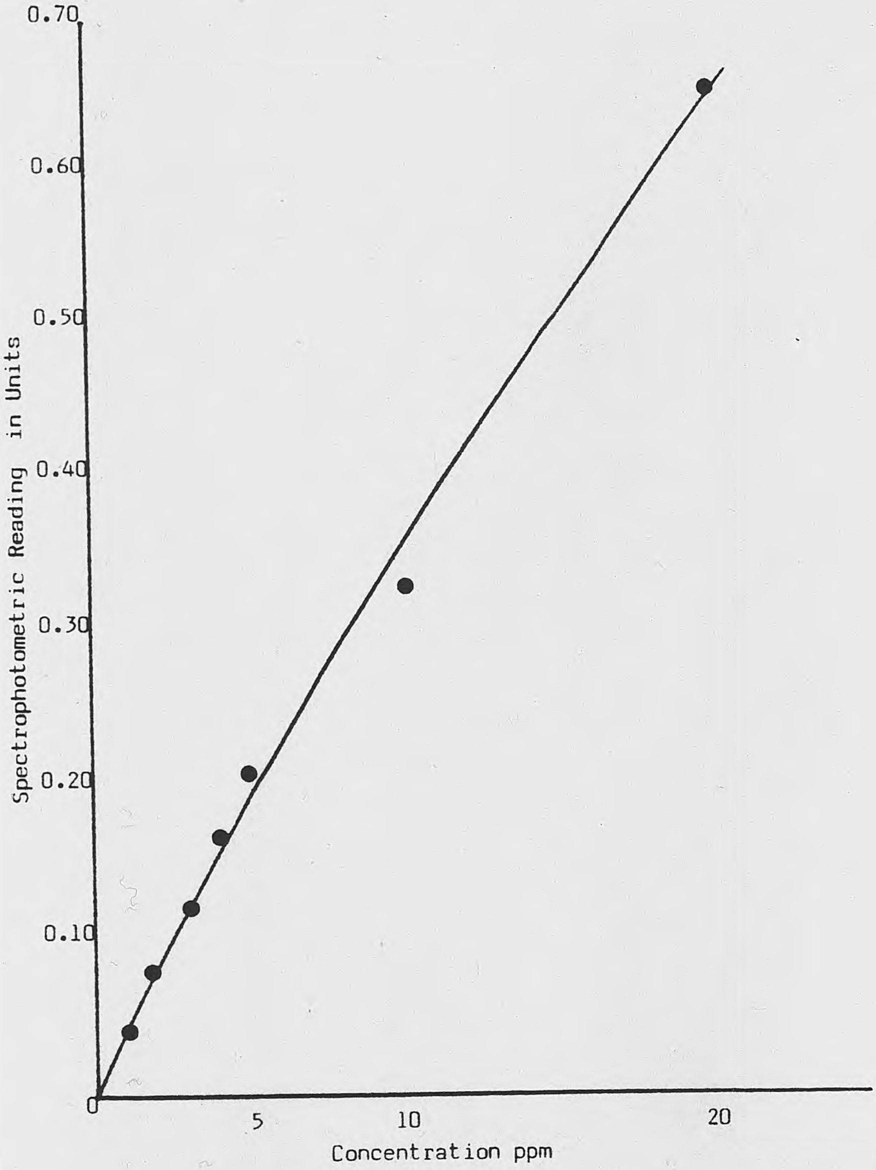
The digestion mixture used by Milner (1965) which was the same as the digestion mixture used by Czarnocki, Sibbald and Evans (1961) was used. It was prepared as shown below.

> 20g of sodium molybolate were dissolved in 300ml of distilled water. To this solution 300ml of concentrated sulphuric acid was added slowly with careful stirring and colling in ice-water. When chilled, 400ml of 70% perchloric acid was added followed by a final stirring.

Appendix 6a Atomic absorbtion on SP90 Spectrophotometer - Standard Curve for Cr.



Appendix 6b Atomic absorbtion on SP90 spectrophotometer - Standard Curve for Co.



Appendix 6c Calculation of Marker Concentrations from SP90 Spectrophotometer Determinations

From the standard curves in Appendix 6a and 6b, the concentration of marker in the supernatant is determined by interpolation. Let this value be Xppm.

Therefore marker concentration in faecal sample =

$$\frac{X \times 100^{*}}{\text{DM wt of faecal sample (g)}} \quad \mu g/g$$

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* This value is from the volume of distilled water in which the digested faecal sample was diluted.

Appendix 7 Example Calculation of NDF Digestibility

The example given is for animal E, period 3, when the diet Viton 10 was being fed.

A.

Food Component	Food DM offered kg/week	Calculated Intake* (kg DM/week)	NDF Content of food %	NDF Intake kg/week	Total NDF Intake kg/week
Viton 10	141.70	115.66	71.30	82.47	83.74
Mixture	9.34	7.62	16.70**	1.27	
Beef Cow 300	0.80	0.65	0***		

* Total food intake DM was 123.94kg/week and it was assumed that the proportions of food components in food refusals were the same as those in the food offered.

** Assuming that all NDF in mixture was present in molassine meal. Molassine meal which contains 26.55% NDF provided 62.9% of the mixture.

*** NDF content of mineral premix (Beef Cow 300) was assumed to be zero.

B. NDF Output

Faecal DM output = 45.62kg - see Appendix 15.

NDF content of faeces = 63.01% - see Appendix 16.

Therefore NDF output = 45.62 x 0.6301 = 28.75kg.

C. NDF Digestibility = $100 \times \frac{(83.74 - 28.75)}{83.74} = 65.67\%$

Appendix 8 Example Calculation of Energy Intake

The example used is for animal E, period 3 when the diet being fed was Viton 10.

A OM Intake

Food Component	Food DM Consumed kg/week*	OM as % DM	OM Intake kg/week	Total OM Intake
Viton 10	115.66	88.67	102.56	109.11
Mixture	7.62	85.21	6.49	
Beef Cow 300	0.65	9.41	0.06	

* See Appendix 7.

B OM Output

Faecal DM output = 45.62kg/week - see Appendix 15

OM content of faeces = 86.41% - see Appendix 16

Therefore OM output = 39.42kg/week

C Digestible OM intake = 109.11 - 39.42 = 69.69kg/week.

D DE intake

Assuming energy content of digested OM = 19.0 MJ/kg (ARC, 1980)

Then DE intake = 19.0 x 69.69 = 1324.11 MJ/week.

E ME Intake

Assuming conversion factor of DE to ME is 0.82 (ARC, 1980)

then ME intake = 1324.11 x 0.82 = 1085.77 MJ/week

Therefore ME intake/day = 1085.77 ÷ 7 = 155.11 MJ ME/day

Live weight of animal E = 793kg (See Table 42)

Therefore Live Weight$^{.75}$ = 149

Therefore ME intake/lwt$^{.75}$day = 1.041 MJ ME/kg lwt$^{.75}$ day

Appendix 9 Climate Chamber Temperatures and Relative Humidities

			Period 1	Period 2	Period 3	Period 4
Room 7	Temp (°C)	Max	34.0	32.5	24.0	23.5
		Min	18.5	17.0	20.0	20.0
		Mean	30.7	30.6	22.2	22.9
		Relative Humidity(%)	71.6	67.8	85.7	87.2
Room 8	Temp (°C)	Max	34.0	34.5	24.5	25.0
		Min	18.0	19.0	20.0	20.0
		Mean	30.0	30.6	22.8	22.5
		Relative Humidity(%)	76.6	74.5	91.4	89.4
Room 9	Temp (°C)	Max	24.0	22.0	35.0	33.5
		Min	12.0	16.5	24.0	25.0
		Mean	19.6	20.9	32.3	32.0
		Relative Humidity(%)	91.4	91.3	93.5	71.3
Room 10	Temp (°C)	Max	24.0	24.0	35.0	34.0
		Min	17.0	19.5	24.0	25.0
		Mean	20.9	20.6	31.8	31.5
		Relative Humidity(%)	95.8	94.1	96.1	75.7

The max-min values are the maximum and minimum temperatures recorded in a climate chamber during each collection period. The mean temperature and the relative humidity values are the mean of readings from each day of the collection period.

Appendix 10 Weight Change of Animals Over the Experimental Period (kg/day)

BUFFALOES	AA6		Viton 10	
	Low Temp.	High Temp.	Low Temp.	High Temp.
E	+0.095	+0.682	-0.037	+1.857
H	-2.111	-0.524	+3.190	-0.091
K	+1.429	-2.630	-0.227	+4.143
L	+1.773	-1.048	+0.429	+1.555

AYRSHIRES	AA6		Viton 10	
	Low Temp.	High Temp.	Low Temp.	High Temp.
F	-0.143	-0.591	+0.333	+1.714
G	+1.296	-0.238	+2.524	+0.182
J	+0.571	-0.333	+0.591	+2.476
M	+1.045	+1.000	+0.333	-0.444

Each value is calculated as shown below.

$$\text{Weight change (kg/day)} = \frac{\text{kg weight change in a period}}{\text{number of days in the period}}$$

Appendix 11 Laboratory Analysis of Feed Samples

		Period 1	Period 2	Period 3	Period 4	Mean
AA6	NDF Content %	44.58	41.96	43.01	42.31	42.97
	Lignin %	6.05	5.31	6.28	4.65	
	% Lignification	13.57	12.65	14.60	10.99	12.95
	Organic Matter %	90.00	89.59	91.25	91.15	90.00
Viton 10	NDF %	67.79	69.26	74.83	73.30	71.3
	Lignin %	9.93	9.53	10.18	9.47	
	% Lignification	14.65	13.76	13.60	12.92	13.73
	Organic Matter %	88.56	88.15	88.71	89.27	88.67
Molassine-Meal	NDF %		22.85	30.30	26.52	26.56
	OM %		88.81	88.96	88.77	88.85
Mixture	OM %		86.29	84.93	84.41	85.21
Beef Cow 300	OM %		9.23	9.78	9.21	9.41

Each value is the mean of two or more replicates except for the values for NDF content of molassine meal which are single values.

All data except Lignification %, are as %DM

Appendix 12 Food DM Intake (kg/week)

	BUFFALOES			
	AA6		Viton 10	
	Low	High	Low	High
Animal E	93.13	93.65	123.94	114.66
Animal H	50.95	55.55	90.77	86.44
Animal K	62.06	73.58	88.72	83.89
Animal L	109.17	81.88	100.91	93.22
	CATTLE			
Animal F	108.02	107.73	104.21	112.64
Animal G	124.39	93.05	107.48	108.91
Animal J	104.42	74.53	96.14	80.28
Animal M	118.41	76.02	98.91	82.43

Appendix 13 Intake of DM by Individual Animals (g/kg lwt day)

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	16.73	17.72	22.32	20.63
Animal H	13.60	13.41	21.54	20.48
Animal K	14.42	19.32	21.67	18.99
Animal L	21.39	15.43	19.53	17.07
Mean	16.54	16.47	21.27	19.29

CATTLE

Animal F	24.53	26.22	23.56	25.83
Animal G	32.67	26.12	25.72	30.27
Animal J	25.28	18.33	23.76	18.12
Animal M	28.18	23.66	31.40	26.89
Mean	30.17	23.58	26.11	25.28

Statistical Analysis of Food Intake g/kg lwt day

a) Combined ANOVA table

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Species (S)	1	498.414	498.414	15.64**
Animals within species	6	191.186	31.864	3.49 **
Diet (D)	1	13.481	13.481	1.47 NS
Temperature (T)	1	44.675	44.675	4.88*
D X T Interaction	1	7.382	7.382	<1
D X S Interaction	1	49.129	49.129	5.37*
S X T Interaction	1	14.459	14.459	1.58 NS
S X D X T Internaction	1	29.318	29.318	3.20 NS
Residual	18	164.578	9.143	
TOTAL	31	1012.618		

SE mean = $\sqrt{\frac{9.143}{4}} = 1.512$ NS = Not Significant * p <0.05 ** p < 0.01

*** p < 0.001

b) Separate ANOVA table

BUFFALOES

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Temperature	1	4.15	4.15	<1 NS
Diet	1	57.04	57.04	8.50*
Animals	3	9.00	3.00	<1 NS
D X T Interaction	1	3.64	3.64	<1 NS
Residual	9	60.42	6.71	
TOTAL	15	134.25		

CATTLE

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Temperature	1	54.98	54.98	4.75*
Diet	1	5.57	5.57	<1
Animals	3	182.14	60.73	5.24*
D X T Interaction	1	33.06	33.06	2.86 NS
Residual	9	104.15	11.57	
TOTAL	15	379.96		

c) Separate ANOVA tables for diets

AA6

Source	d.f.	Sum of Square	Mean Square	F ratio
Species (S)	1	430.25	430.25	10.1*
Animals within species	3	127.90	42.63	3.99*
Temperature (T)	1	44.19	44.19	4.14 NS
S X T Interaction	1	42.48	42.48	3.98 NS
Residual	9	96.05	10.68	
TOTAL	15	740.87		

VITON 10

Source	d.f.	Sum of Square	Mean Square	F ratio
Species (S)	1	117.29	117.29	3.77 NS
Animals within species	3	93.37	31.12	7.29**
Temperature (T)	1	7.87	7.86	1.84 NS
S X T Interaction	1	1.30	1.30	<1 NS
Residual	9	38.44	4.27	
TOTAL	15	258.27		

Appendix 14 Intake of DM by Individual Animals (g/kg lwt$^{.75}$ day)

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	88.8	92.9	118.5	109.5
Animal H	65.4	66.2	106.7	101.5
Animal K	71.8	93.3	106.5	95.2
Animal L	111.2	81.0	101.8	90.2
Mean	84.30	83.35	108.38	99.10

CATTLE

Animal F	122.9	129.0	118.1	129.0
Animal G	157.8	124.0	127.1	144.1
Animal J	124.6	90.0	116.5	90.9
Animal M	175.2	109.5	144.6	123.0
Mean	145.13	113.13	128.58	121.75

Statistical Analysis of Food Intake (g/kg lwt$^{.75}$ day

a) Combined ANOVA table

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Species (S)	1	8639.551	8639.551	14.76**
Animals within species	6	3512.829	585.471	3.01**
Diet (D)	1	447.005	447.005	2.30 NS
Temperature (T)	1	1106.851	1106.851	5.70*
D X T Interaction	1	177.661	177.661	<1 NS
D X S Interaction	1	12347.540	1237.540	6.37*
S X T Interaction	1	353.779	353.779	1.82 NS
D X T X S Interaction	1	630.110	630.110	3.24 NS
REsidual Error	18	3497.201	194.289	
TOTAL	31	19602.535		

$$\text{SE mean} = \sqrt{\frac{194.289}{4}} = 6.969$$

b) Separate ANOVA table

BUFFALOES

Source	d.f.	Sum of Square	Mean Square	F ratio
Temeprature (T)	1	104.551	104.551	<1 NS
Diet (d)	1	1586.031	1586.031	9.78*
Animals	3	648.737	316.246	<1 NS
D X T Interaction	1	69.306	69.306	<1 NS
Residual	9	1458.830	162.093	
TOTAL	15	3867.464		

CATTLE

Source	d.f.	Sum of Square	Mean Square	F ratio
Temperature (T)	1	1356.081	1356.081	5.9*
Diet (D)	1	98.506	98.506	<1 NS
Animals	3	2864.092	954.693	4.22 *
D X T Interaction	1	738.481	738.481	3.26 NS
Residual	9	2038.361	226.484	
TOTAL	15	7095.519		

c) Separate ANOVA tables for diets

AA6

Source	d.f.	Sum of Square	Mean Square	F ratio
Species (S)	1	8208.36	8208.36	9.3 NS
Animals within species	3	2653.03	884.34	4.0*
Temperature (T)	1	1085.70	1085.70	4.9 NS
S X T Interaction	1	964.10	964.10	4.4 NS
Residual	9	1992.71	221.41	
TOTAL	15	14903.91		

VITON 10

Source	d.f.	Sum of Square	Mean Square	F ratio
Species (S)	1	1668.72	1668.72	3.17 NS
Animals within species	3	1632.89	544.30	6.7*
Temperature (T)	1	198.81	198.81	2.45 NS
S X T Interaction	1	19.80	19.80	<1 NS
Residual	9	731.40	81.27	
TOTAL	15	4251.62		

Appendix 15 Faecal DM Output (kg/week)

	BUFFALOES			
	AA6		Viton 10	
Animal	Low	High	Low	High
E	28.72	19.15	45.62	32.49
H	19.64	14.81	34.22	23.88
K	22.07	22.93	40.52	35.59
L	32.96	28.71	43.20	42.16
	CATTLE			
F	38.80	30.13	55.05	44.22
G	35.61	32.24	52.85	46.44
J	32.67	26.02	37.46	36.40
M	34.78	26.48	41.83	37.88

Appendix 16 NDF Content of the Faeces (%)*

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	63.81	59.58	63.01	58.83
Animal H	60.96	63.97	60.69	54.97
Animal K	67.59	59.62	63.41	60.94
Animal L	61.69	64.43	66.27	64.92
Mean	63.51	61.90	63.35	59.92

CATTLE

	AA6		Viton 10	
	Low	High	Low	High
Animal F	65.55	61.99	60.44	61.43
Animal G	61.35	63.51	60.87	59.24
Animal J	68.82	61.63	60.15	60.36
Animal M	63.21	62.91	60.29	64.71
Mean	64.73	62.51	60.44	61.44

* Each value is the mean of two replicate determinations.

Statistical Analysis of NDF Content of Faeces

ANOVA Table

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Species (S)	1	0.098	0.098	<1 NS
Animals within species	6	46.203	7.701	1.16 NS
Diet (D)	1	28.300	28.300	4.26 NS
Temperature T	1	19.650	19.650	2.96 NS
D X S	1	5.170	5.170	<1 NS
D X T	1	0.968	0.968	<1 NS
S X T	1	7.277	7.277	1.09 NS
T X D X S	1	12.704	12.704	1.9 NS
Error	18	119.397	6.633	
Total	31	239.768		

$$\text{SE mean} = \sqrt{\frac{\text{EMS}}{4}} = 1.288$$

Appendix 17 Organic Matter Content of the Faeces (%)*

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	83.24	83.30	86.41	86.02
Animal H	83.55	84.85	84.06	84.96
Animal K	87.43	85.33	86.19	84.53
Animal L	84.76	84.52	88.79	86.42

CATTLE

	AA6		Viton 10	
	Low	High	Low	High
Animal F	84.63	83.00	86.23	85.77
Animal G	84.05	84.30	84.57	84.25
Animal J	86.48	81.77	84.25	84.14
Animal M	83.93	84.28	86.63	86.00

* Each value is the mean of two replicate determinations.

Appendix 18 Apparent DM Digestibility (%) for Individual Animals.

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	69.2	79.6	63.2	71.7
Animal H	61.5	73.3	62.3	72.4
Animal K	64.4	68.8	56.3	57.6
Animal L	69.8	64.9	57.2	54.8
Mean	66.23	71.65	59.75	64.13

CATTLE

	AA6		Viton 10	
	Low	High	Low	High
Animal F	64.1	70.6	47.2	60.7
Animal G	71.4	65.4	50.8	57.4
Animal J	68.7	65.0	61.0	54.7
Animal M	70.6	65.2	57.7	54.1
Mean	68.70	66.55	54.18	56.73

Statistical Analysis of DM digestibility (%)

Combined ANOVA Table

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Species (s)	1	121.68	121.68	2.89 NS
Animals within species	6	252.395	42.066	1.86 NS
Diet (D)	1	735.361	735.361	32.52***
Temperature T	1	52.020	52.020	2.30 NS
D X T Interaction	1	6.667	6.662	<1 NS
D X S Interaction	1	53.562	52.562	2.37 NS
S X T Interaction	1	44.180	44.180	1.95 NS
D X T X S Interaction	1	16.531	16.531	<1 NS
Residual Error	18	407.095	22.611	
TOTAL	31	659.4		

$$\text{SE mean} = \sqrt{\frac{(22.611)}{4}} = 2.3775$$

Separate ANOVA Table

BUFFALOES

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Diets (D)	1	196.00	196.00	8.27*
Temperature (T)	1	96.04	96.04	4.05 NS
Animals	3	245.75	81.92	3.45 NS
D X T Interaction	1	1.10	1.10	<1 NS
Residual Error	9	213.35	23.71	
TOTAL	15	752.24		

CATTLE

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Diets (D)	1	592.92	592.92	27.55**
Temperature (T)	1	0.16	0.16	<1 NS
Animals	3	6.64	2.21	<1 NS
D X T Interaction	1	22.09	22.09	1.02 NS
Residual Error	9	193.66	21.51	
TOTAL	15	815.48		

Appendix 19 Individual Animal Apparent NDF Digestibility (%)

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	53.99	71.53	65.67	75.35
Animal H	45.02	60.09	66.13	77.53
Animal K	43.75	56.56	58.99	61.74
Animal L	56.49	47.17	58.00	56.51
Mean	49.81	58.84	62.20	67.78

CATTLE

	AA6		Viton 10	
	Low	High	Low	High
Animal F	45.00	59.49	52.72	64.34
Animal G	58.96	48.55	55.71	62.67
Animal J	49.68	49.67	65.35	59.49
Animal M	56.63	48.77	62.22	55.96
Mean	52.57	51.62	59.00	60.62

Statistical Analysis of NDF Digestibility %

Combined ANOVA Table

Source	d.f	Sum of Square SS	Mean Square	F ratio
Species (S)	1	109.927	109.927	1.63 NS
Animals within species	6	404.860	67.477	1.64 NS
Diet (D)	1	675.557	675.557	16.42 ***
Temperature (T)	1	116.701	116.701	2.83 NS
D X S Interaction	1	17.420	17.420	<1 NS
D X T Interaction	1	0.385	0.385	<1 NS
T X S Interaction	1	97.197	97.197	2.36 NS
D X T X S Interaction	1	133.016	133.016	3.23 NS
Residual Error	18	740.427	41.135	
TOTAL	31	2180.488		

$$\text{SE mean} = \sqrt{\frac{41.135}{4}} = 3.207$$

Separate ANOVA Tables

BUFFALOES

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Diets (D)	1	454.97	454.97	9.88*
Temperature (T)	1	213.45	213.45	4.63 NS
Animals	3	382.45	127.48	2.77 NS
D X T Interaction	1	11.83	11.83	<1 NS
Residual Error	9	414.59	46.07	
TOTAL	15	1477.29		

CATTLE

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Diet (D)	1	238.01	238.01	6.19*
Temperature (T)	1	0.45	0.45	<1 NS
Animals	3	2.41	0.81	<1 NS
D X T Interaction	1	6.57	6.57	<1 NS
Residual Error	9	345.84	38.43	
TOTAL	15	593.27		

Appendix 20 Individual Animal Water Intakes (l/kg food DM)

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	4.72	5.45	5.88	7.91
Animal H	4.48	4.11	4.60	6.50
Animal K	5.30	5.11	6.28	5.39
Animal L	3.35	4.63	6.74	5.72
Mean	4.46	4.83	5.88	6.38

CATTLE

	AA6		Viton 10	
	Low	High	Low	High
Animal F	4.93	-	5.01	-
Animal G	4.13	4.65	4.24	5.28
Animal J	3.96	5.07	5.10	4.90
Animal M	1.93	5.26	1.76	-
Mean	3.74	4.99	4.03	5.09
	(4.34)	(4.86)	(4.78)	

Figures in brackets are values calculated ignoring the values for M.

Statistical Analysis

Combined ANOVA Tables

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Species (s)	1	2.669	2.669	5.505 NS
Animals within species	6	2.909	0.485	<1 NS
Temperature (T)	1	1.518	1.518	2.41 NS
Diets	1	7.176	7.176	11.37**
Residual Errors	12	7.570	0.631	
TOTAL	25	23.486		

Appendix 21 Individual Animal Energy Intakes (MJME/ kg lwt$^{.75}$ day)

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	0.889	1.056	1.041	1.082
Animal H	0.590	0.694	0.932	1.016
Animal K	0.661	0.880	0.834	0.773
Animal L	1.118	0.759	0.795	0.685
Mean	0.815	0.847	0.901	0.889

CATTLE

	AA6		Viton 10	
	Low	High	Low	High
Animal F	1.137	1.345	0.781	1.090
Animal G	1.615	1.177	0.919	1.169
Animl J	1.219	0.863	1.000	0.666
Animal M	1.775	1.036	1.15	0.926
Mean	1.437	1.106	0.963	0.963

Statistical Analysis of Energy Intake (MJ ME/kgLWt$^{.75}$/day)

Combined ANOVA Table

Source	d.f	Sum of Square SS	Mean Square	F ratio
Species (S)	1	0.5159	0.5159	8.80**
Animals within species	6	0.3516	0.0586	1.58 NS
Temperature (T)	1	0.1195	0.1195	3.21 NS
Diet (D)	1	0.0480	0.0480	1.29 NS
S X D Interaction	1	0.2769	0.2769	7.94*
S X T Interaction	1	0.0620	0.0620	1.67 NS
T X D Interaction	1	0.0412	0.0412	1.11 NS
T X D X S Interaction	1	0.0706	0.0706	1.90 NS
Residual Error	18	0.6698	0.0372	
TOTAL	31	2.1555		

$$\text{SE mean} = \sqrt{\frac{0.0372}{4}} = 0.0964$$

Separate ANOVA Tables

BUFFALOES

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Diet (D)	1	0.0163	0.0163	<1 NS
Temperature (T)	1	0.0005	0.0005	<1 NS
Animals	3	0.1323	0.0441	1.52 NS
D X T	1	0.0019	0.0019	<1 NS
Residual Error	9	0.2600	0.0289	
TOTAL	15	0.4110		

CATTLE

Source	d.f.	Sum of Square SS	Mean Square	F ratio
Diet (D)	1	0.3801	0.3801	7.60 *
Temperature (T)	1	0.1096	0.1096	2.19 NS
Animals	3	0.2192	0.073	1.46 NS
D X T	1	0.1098	0.1098	2.19 NS
Residual Error	9	0.4499	0.0500	
TOTAL	15	1.2286		

Appendix 22 Individual Animal Respiration Rate (counts/minute)

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	13.6	26.1	19.5	24.1
Animal H	12.7	23.3	13.6	23.6
Animal K	18.6	27.6	20.8	17.4
Animal L	21.2	51.0	17.9	22.5
Mean	16.53	32.00	17.95	21.9

CATTLE

	AA6		Viton 10	
	Low	High	Low	High
Animal F	28.9	75.9	26.4	64.4
Animal G	39.9	80.3	27.6	62.8
Animal J	32.6	83.6	29.4	63.3
Animal M	58.5	99.9	36.9	92.2
Mean	39.98	84.93	30.08	70.67

Each value is the mean of all recordings over a collection period.

Statistical Analysis of Respiration Rate (counts/minute)

Combined ANOVA Table

Source	d.f	Sum of Square	Mean Square	F ratio
Species (S)	1	9422.213	9422.213	37.106***
Animals within species	6	1523.544	253.924	7.501***
Diet (D)	1	538.740	538.740	15.916***
Temperature (T)	1	5509.875	5509.875	162.772***
D X T Interaction	1	126.006	126.006	3.722 NS
D X S Interaction	1	119.738	119.738	3.537 NS
T X S Interaction	1	2196.258	2186.258	64.586***
D X T X S Interaction	1	25.742	25.742	<1 NS
Residual	18	609.303	33.850	
TOTAL	31	20061.420		

$$\text{SE mean} = \sqrt{\frac{33.850}{4}} = 2.909$$

Separate ANOVA Tables

BUFFALOES

Source	d.f	Sum of Square	Mean Sqaure	F ratio
Diet (D)	1	75.256	75.256	1.124
Temperature (T)	1	377.330	377.330	5.635*
Animals	3	214.672	71.558	1.069
D X T Interaction	1	132.826	132.826	1.984
Residual Error	9	602.700	66.967	
TOTAL	15	1402.784		

CATTLE

Source	d.f.	Sum of Square	Mean Square	F ratio
Diet (D)	1	583.222	583.222	23.227***
Temperature (T)	1	7318.802	7318.802	291.447***
Animals	3	1308.867	436.289	17.374***
D X T Interaction	1	18.923	18.923	<1
Residual	9	225.983	25.109	
TOTAL	15	9455.797		

Appendix 23 Individual Animal Serum T_3 Concentration (ng/ml)

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	1.83	1.5	1.25	1.20
Animal H	1.25	0.90	0.90	1.25
Animal K	1.10	0.85	1.22	0.90
Animal L	1.05	1.20	1.55	1.20
Mean	1.31	1.11	1.23	1.14

CATTLE

Animal F	2.05	1.28	1.55	1.78
Animal G	1.73	1.15	1.40	1.13
Animal J	1.60	0.80	1.35	0.95
Animal M	1.38	1.10	1.75	1.20
Mean	1.69	1.08	1.51	1.27

Each value is the mean to two replicates.

Statistical Analysis of Serum T_3 levels (ng/ml)

Combined ANOVA Tables

Source	d.f.	Sum of Square	Mean Square	F ratio
Species (S)	1	0.291	0.291	1.85 NS
Animals within species	6	0.943	0.157	3.34**
Temperature (T)	1	0.653	0.653	13.88**
Diet (D)	1	0.001	0.001	<1 NS
T X S Interaction	1	0.161	0.161	3.42 NS
T X D Interaction	1	0.107	0.107	2.27 NS
S X D Interaction	1	0.002	0.002	<1 NS
T X D X S	1	0.033	0.033	<1 NS
Residual	18	0.846	0.047	
TOTAL	31			

$$\text{SE mean} = \sqrt{\frac{0.047}{4}} = 0.108$$

Separate ANOVA Tables

BUFFALO

Source	d.f.	Sum of Square	Mean Square	F ratio
Diet (D)	1	0.002	0.002	<1
Temperature (T)	1	0.082	0.082	1.43 NS
Animal	3	0.445	0.148	2.66 NS
D X T Interaction	1	0.010	0.010	<1 NS
Residual	9	0.501	0.056	
TOTAL	15	1.042		

CATTLE

Source	d.f.	Sum of square	Mean Square	F ratio
Diet (D)	1	0.000	0.000	<1 NS
Temperature (T)	1	0.731	0.731	19.07 **
Animal	3	0.498	0.166	4.33*
D X T Interaction	1	0.130	0.130	3.38 NS
Residual	9	0.345	0.038	
TOTAL	15			

Appendix 24 Individual Animal Serum T_4 Concentration (μg/100ml)

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	6.6	7.4	5.7	6.7
Animal H	5.1	4.8	5.4	5.0
Animal K	4.7	2.6	5.4	3.4
Animal L	6.4	7.0	7.8	5.2
Mean	5.70	5.45	6.08	5.08

CATTLE

	AA6		Viton 10	
	Low	High	Low	High
Animal F	6.7	5.2	6.0	6.4
Animal G	7.8	8.7	6.3	8.2
Animal J	5.2	6.1	4.3	5.6
Animal M	5.6	8.3	5.5	7.4
Mean	6.33	7.08	5.53	6.90

Each value is the mean of two replicates.

Statistical Analysis of Serum T_4 levels (μg/100ml)

Combined ANOVA Tables

Source	d.f.	Sum of Square	Mean Square	F ratio
Species (S)	1	6.212	6.212	1.16 NS
Animals within species	6	31.877	5.310	6.84 ***
Temperature (T)	1	0.382	0.382	<1 NS
Diet (D)	1	0.475	0.475	<1 NS
D X T Interaction	1	0.008	0.008	<1 NS
D X S Interaction	1	0.475	0.475	<1 NS
T X S Interaction	1	5.695	5.695	7.34 *
T X D X S Interaction	1	0.945	0.945	1.22 NS
Residual	18	13.950	0.776	
TOTAL	31	60.022		

$$\text{SE mean} = \sqrt{\frac{0.755}{4}} = 0.440$$

Separate ANOVA Tables

BUFFALOES

Source	d.f.	Sum of Square	Mean Square	F ratio
Diet	1	0.00	0.00	0.00 NS
Temperature (T)	1	1.563	1.563	1.78 NS
Animals	3	19.015	6.330	7.23**
T X D Interaction	1	0.563	0.563	<1 NS
Residual	9	7.89	0.877	
TOTAL	15	29.03		

CATTLE

Source	d.f.	Sum of Square	Mean Square	F ratio
Diet	1	0.950	0.950	1.41 NS
Temperature (T)	1	4.575	4.575	6.79*
Animals	3	12.861	4.287	6.36 *
D X T Interaction	1	0.330	0.330	<1 NS
Residual	9	6.061	0.673	
TOTAL	15	24.779		

Appendix 25 Individual Animal Serum Urea Levels (mmol/l)

BUFFALOES

	AA6		Viton 10	
	Low	High	Low	High
Animal E	6.74	8.91	6.78	6.89
Animal H	7.78	8.86	8.19	5.08
Animal K	3.60	10.75	2.92	12.38
Animal L	7.75	5.46	5.36	4.56
Mean	6.47	8.50	5.81	7.22

CATTLE

Animal F	4.93	7.42	4.93	3.79
Animal G	8.32	7.43	7.96	3.49
Animal J	7.57	7.72	3.89	7.29
Animal M	7.09	3.80	4.38	3.33
Mean	6.98	6.59	5.29	4.48

Statistical Analysis of Serum Urea Levels

Combined ANOVA Tables

Source	d.f.	Sum of Square	Mean Square	F ratio
Species (S)	1	10.893	10.893	3.10 NS
Animals within species	6	21.036	3.506	<1 NS
Temperature (T)	1	2.514	2.514	<1 NS
Diet (D)	1	16.420	16.420	3.06 NS
D X T Interaction	1	0.540	0.540	<1 NS
D X S Interaction	1	1.772	1.772	<1 NS
S X T Interaction	1	10.776	10.776	2.01 NS
D X S X T Interaction	1	0.017	0.017	<1 NS
Residual	18	96.314	5.350	
TOTAL	31	160.268		

Appendix 26 Extent of Disappearance of Food Samples from Dacron Bags (%).

Time (hrs)	Diet AA6 Replicate		Viton 10 Replicate	
	1	2	1	2
0	48.65	53.41	16.07	16.02
3	58.61	53.03	17.53	16.32
5.75	62.84	60.41	22.11	19.76
12	62.85	64.23	33.55	23.62
24	71.71	78.10	45.56	44.03
48	82.79	84.66	65.46	59.36

Appendix 27 Values Obtained in the Marker Analysis Trial

Estimated Chromium Content of the Faecal Sample (µg/g sample)

Time	Water Diluent	Acid Diluent
0	338	299
15 mins	551	544
30 mins	521	545
60 mins	501	532
90 mins	533	532
120 mins	525	535

Analysis* of Chromium Content of Faecal Sample

Source	d.f.	S.S.	M.S.	F ratio
Time	4	971.4	242.85	1.87 NS
Diluent	1	324.9	324.9	2.51 NS
Residual	4	518.6	129.65	
Total	9	1814.9		

Estimated Cobalt Content of the Faecal Sample (µg/g sample)

Time	Water Diluent	Acid Diluent
0	24.4	25.9
15 mins	34.8	35.6
30 mins	36.3	37.4
60 mins	32.9	38.0
90 mins	32.7	34.9
120 mins	35.3	55.9

* Analysis is performed ignoring Time 0

Analysis of Cobalt Content of Faecal Sample

Source	d.f.	S.S.	M.S.	F ratio
Time	4	178.286	44.572	1.28 NS
Diluent	1	88.804	88.804	2.54 NS
Residual	4	139.726	34.932	
Total	9	406.816		

Appendix 28 A Typical Set of Cr Concentration - with time data

The data set reported is that of Animal F.

Sample No.	Elapsed Time (hrs)	μgCr/g Faecal DM		Mean	Faecal Dry Weight (kg)	mgCr	mgCr/hr
1	9	31.1	33.3	32.2	1.522	49.01	5.45
2	11	69.7	75.5	72.6	0.251	15.22	9.11
3	13	221.7	226.5	225.1	0.544	121.91	60.96
4	15	584.0	571.0	577.5	0.321	185.38	92.69
5	17	529.5	553.6	541.6	0.441	371.15	185.57
6	19	1354.9		1354.9	0.510	691.00	345.50
7	21	1626.4	1698.7	1662.6	0.423	703.28	351.64
8	24	1729.1	1757.7	1743.4	0.546	951.90	317.30
9	33	1350.2	1407.2	1292.7	2.242	3124.68	347.13
10	35	1010.6	994.1	1002.4	0.290	290.7	145.35
11	37	921.6	918.5	920.1	0.179	164.7	82.35
12	39	955.1	947.5	951.3	0.801	761.99	381.00
13	41	936.6	972.9	953.3	0.360	343.19	171.59
14	43	874.3	793.9	834.1	0.488	407.04	203.52
15	47	958.7	952.6	955.7	0.926	884.98	221.24
16	57	681.8	658.7	670.3	1.918	1285.64	128.56
17	61	594.9		594.9	0.773	459.86	114.96
18	65	542.5	553.3	547.9	0.637	349.01	87.25
19	71	432.6	458.7	445.7	1.103	491.61	81.93
20	81	369.9	365.5	367.7	1.769	650.46	65.05
21	85	293.8	290.8	292.3	0.661	193.21	48.30
22	89	259.6	280.0	269.8	0.637	171.86	42.97
23	95	235.2	260.3	247.8	1.044	258.7	43.12
24	105	185.1	192.8	189.0	1.054	199.21	19.92
25	109	141.3	158.6	150.0	0.711	1154.65	28.91
26	113	156.2	153.3	154.8	0.679	105.11	26.28
27	117	131.4	141.1	136.3	1.747	200.91	50.23
28	129	110.4	129.3	119.9	1.184	141.96	11.83
29	137	95.6	97.1	96.4	1.400	134.96	16.87
30	153	71.9	80.1	76.0	2.311	175.64	10.98
31	161	73.4	66.5	80.0	2.074	145.18	18.15
32	177	55.5	48.9	52.2	2.378	124.13	7.76

Appendix 29 Example Calculation of the Extent of Digestion of a Feed Sample in the rumen.

The example chosen is that for the Viton 10 diet in the buffaloes. The formula involved are obtained from McDonald (1981).

1. $P = a + \frac{b^1c}{C+R} \exp (- (c + k) to)$

2. The extent of disappearance of food in the rumen against time curves given in Fig. 4.1, are described by the following exponential relationship.

$$P = A + (B \times e^{-c})$$

The values obtained were $P = 89.7943 + (-79.36829 \times e^{-0.02039})$

and $P = 87.24231 + (-74.96805 \times e^{-0.02553})$

3. $a = A + B = 89.7943 - 79.36829 = 10.42601$

$87.24231 - 74.96805 = 12.27426$

Therefore mean $a = 11.350135$.

4. $-B = b^1$ therefore mean $b^1 = (79.36829 + 74.96805) \div 2 = 77.16817$

5. mean $c = (0.02553 + 0.02039) \div 2 = 0.02296$

6. k from rate of passage experiments (Table 4.16) = 0.023 for high temperature and 0.024 for low temperature.

7. to = 3 hours (see Fig. 4.1).

8. Therefore $P = a + \frac{b'c}{C+R} \exp (-(c + k) to)$

$$\text{becomes } 11.350135 + \frac{77.16817 \times 0.02296}{0.02296 + 0.0235} e^{(-(0.02296 + 0.0235)3)}$$

$$= 11.350135 + 33.174081 = 44.52\%$$

9. DM digestibility from Appendix 17 = 72.4 + 56.3

Therefore mean DM digestibility = 64.35%

Therefore proportion of total food digested that is digested

$$\text{in the rumen} = \frac{44.52}{64.35} \times 100 = 69.18\%$$

Appendix 30 Individual Animal Estimated Heat Output

(MJ/square metre body surface day)

BUFFALOES

	AA6		Viton 10	
Animal	Low	High	Low	High
E	14.94	16.10	17.18	17.22
H	10.94	12.12	15.35	15.82
K	11.95	14.31	14.32	13.59
L	17.32	13.49	14.18	12.90
Mean	13.79	14.01	15.26	14.88
CATTLE				
F	17.68	19.12	14.02	17.49
G	22.05	17.53	15.67	18.25
J	18.18	14.15	16.16	12.30
M	23.40	15.76	17.87	15.15
Mean	20.33	16.64	15.93	15.80

Appendix 31 Sample Calculation of Heat Output of Animals

The example chosen is that of Animal E, period 3.

1. Metabolisable Energy (ME) intake = 155.1 MJ ME/day (see Appendix 8).

2. ME requirements for maintenance = 8.3 + 0.091 W (MAFF, 1975)

   where W = body mass in kg.

   Therefore ME required for maintenance

   = 8.3 + 0.091 (793) = 80.5 MJ/d

3. ME available for production = 155.1 - 80.5 = 74.6 MJ/day

4. Efficiency of metabolisable energy use for liveweight gain

   = 0.0435 M/D

   where $M/D = \frac{155.1 \text{ MJ ME/day}}{17.71 \text{ kg DM/day*}} = 8.75$

   Therefore efficiency of ME use = 0.38

5. Heat Output = Metabolisable Energy required for maintenance + Metabolisable Energy not used in Liveweight gain

   = 80.5 + (1 - 0.38) x 74.6 = 126.7 MJ/day

6. Heat Output/Surface Area

   body surface area = $0.09\ W^{0.66}$ (ARC, 1980)

   therefore body surface area = 7.37 square metres

   Therefore estimated Heat Output = $\frac{126.7}{7.37}$ = 17.18 MJ/square metre body surface day.

*obtained from Appendix 12