"THE RELATIONSHIP BETWEEN THE CRUDE FIBRE CONTENT OF PASTURE GRASS AND THE VOLATILE FATTY ACIDS OF THE RUMEN CONTENT OF WHITE FULANI (ZEBU) CATTLE"

T H E S I S

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by

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CHAPTER 1

1.1 General Introduction

Pastures are different from natural grasslands, in that they are generally used for livestock production. In developed countries and in many governments and university farms in the tropics pastures are usually enclosed by fences, and are rotationally grazed. Some pastures are included in crop rotation in arable farming, while others are used indefinitely for grazing and they form permanent pastures. Rotation pastures and permanent pastures are found in Europe, America and Africa and other parts of the world.

1.2 Importance of Pasture in Nigeria

The vegetation of Nigeria was mapped out by Keay (1953). The Savannah occupies a total area of 309,000 square miles in Nigeria, about 83% of Nigeria's total land area (Oyenuga, 1957). The Savannah in Nigeria is divided into four zones, namely Sahel, Sudan, Northern guinea and Southern guinea. There are some small areas in the South where grass has invaded farm clearings. The grasslands in Nigeria are located in the Savannah. The grasses found in the Savannah are

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Aristida stipoides, *Schoenofeldia gracilis*, *Cenchrus biflorus*, *Cenchrus prieurii*, *Ctenium elegans*, *Eragrostis tremule*, *Pennisetum pedicellatum*, *Andropogon psendapricus*, *Andropogon gayanus*, *Loudetia*, *Hyparrhenia rufa*, *Hyparrhenia cyanescens*, *Hyparrhenia subplumosa*, *Hyparrhenia dissoluta*, *Cymbopogin giganteus*, *Imperata cylindrica*, *Brachiaria spp*, *Thelopogon elegans*, *Digitaria gayana*, *Pennisetum polystachyon*, *Setaria sphacelata*, *Setaria pallidifusca*, *Pennisetum purpureum*, *Andropogon tectorum* and *Panicum maximum*.

This natural grassland is located in Northern Parts of Nigeria. The Savannah forms the chief source of food for white Fulani (Zebu) cattle in the North. In Nigeria both in the North and South, there are no parcels of land set aside mainly for grazing. The grasses fed to livestock have not been seeded or fertilized, to improve production and quality of the herbage for animal feed. Improved pastures are only found in government owned ranches and Dairy Farms.

There is a very good opportunity to improve pastures in Nigeria. Pastures are fundamental to increased food production and to the conservation of soil and water.

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1.3 Importance of pasture on livestock feeding

The *Graminae* is the most important of all plant families from an economic point of view because of its important products. For instance cereals such as wheat, maize, rice, barley, oats, millet, guinea corn as well as others of minor importance belong to this family. They form the stapple foods of man in all parts of the world. Cereal seeds, apart from providing food for man are also used for live stock and their foliage is also used as live stock feed. Numerous species of grasses form the chief source of food of domestic and farm animals. They are grown as fodders or pasture crops for various animals in different parts of the world.

The grass family is a very large one containing over 300-400 genera and over 4,000 - 5,000 species. Its members are widely distributed all over the world. Some grow in all parts of the world where flowering plants also exist, and they may thrive well from sea level to places with perpetual snow. They however, do well in the open where there is plenty of air and light. Only a few species of grasses are of agricultural importance.

Grasses form important natural vegetation in some places

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around the world, and may form large expanse of vegetation in some parts such as the Prairie of America, Pampas of Argentina, and Savannah of Nigeria. There are many grasses and legumes in Nigeria that are being used and can be used as improved pastures for livestock feeding. The good and promising grasses for livestock feeding are Gamba, Giant star, Bahama, Kyasuwa, Elephant, Guinea, Digitaria, Cenchrus, and Brachiaria. The good and promising legumes for livestock feeding are Centrosema, Stylosanthes, and Alyce clover. Some of these grasses and legumes have been used successfully in agricultural stations and farms throughout the Country, both for dairy, beef and horse production.

Even though there are few planted pastures in Nigeria, the results obtained from government farms have proved that the pastures can perform as excellently as those in other parts of the world. Nigeria's livestock depend on natural pastures and most of them do not get enough nutrients from these pastures, particularly during the non-growing or dry season when they feed on sub-maintenance rations and so many of them become lean and emaciated.

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1.4 Variation in quality of Pasture

The quality of a pasture is usually judged by its carrying capacity of grazing cattle, the performance of the cattle in terms of milk, beef and growth and calving percentages of the animals. To be able to produce a good yield per acre and good palatable fodder for animals pasture quality depends on such factors as good crop husbandry, fertile soil, and climate.

In the tropics extensive grazing is the chief form of land use for livestock production. The rainfall in these areas are low and variable and lead to poor pasture yield. Usually there are two growing seasons in the year, the period of good rainfall and abundant growth alternating with the period of low to no rainfall and poor growth.

1.5 Some promising Nigerian grasses and legumes

Guinea grass. *Panicum maximum* (Jacq)

The genius *Panicum* is a large and important one in Africa. It contains a considerable number of species which grow in varying habitats. Rattray (1960) observed some species growing in very wet and very dry places, while some grow on land from sea level to places of high altitudes. Oyenuga (1960) and Miller (1963) observed guinea grass to

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be a part of natural grassland in Nigeria. Davies and Skidmore (1966) reported that it is the best known tropical grass cultivated experimentally or on a large scale throughout the tropics. Oyenuga (1957; 1960) in his investigations found guinea grass to be a tufted perennial grass with heights ranging between 3ft and 10ft. It is heavy yielding, it has high nutritive value and is resistant to drought. It is palatable to livestock and is selectively grazed by livestock when included in pastures. Guinea grass reaches its maximum development under moist, warm conditions, where the soils are fertile. It grows together with Pennisetum purpureum and Panicum turgidum in sub-desert areas, and Panicum repens in marshy areas (Rattray 1960) and Andropogon tectorum and Imperata cyclindrica in Nigeria, (Oyenuga 1960). Davies and Skidmore (1966) recognised two main groups of guinea grass throughout the tropics; The larger and the smaller types. The larger type is very good fodder grass that is propagated vegetatively on a commercial basis, and it requires wide spacing. The smaller type is grown from seeds. Seed formation in guinea grass is poor since the greater percentage of the seeds produced are not viable. Guinea grass is an apomict, (Davies and Skidmore, 1966) and it can be established as a permanent

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pasture where the dry season is not too severe. Guinea grass has been introduced to South America, Central America, Asia and Australia from its home in West Africa.

Elephant grass. Pennisetum purpureum (Schum).

It is a tall growing fodder grass indigenous to Nigeria. It produces broad leaves and is higher yielding in the wetter South than in the drier Northern Nigeria. Oyenuga (1957) and Davies and Skidmore (1966) reported it to be a deep rooting and drought resistant grass. Elephant grass has been used in East and South Africa to prevent soil erosion. Usually it is restricted to the margins of lakes and rivers and other permanently wet areas and in zones with annual rainfall ranging between 45 inches and 60 inches. Rattray (1960) reported it to be a characteristic grass of the moist climate of the coastal zone. Elephant grass grows in some woodland or savannah of varying density, which has been derived from tropical evergreen forest of the high rainfall belt that extends from the Sudan and Uganda in East Africa, through the Belgian Congo (Zaire), the Central African Republic, the Republic of the Congo, the Republic of Gabon, the Republic of Chad, to Sierra Leone in West Africa. It spreads as far south as Angola,

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Southern Rhodesia and Nysaland. It is an apomict that crosses easily, and it is propagated vegetatively.

Giant Star Grass.

Cynodon plectostachyus (Pilger) Giant star grass originated from East Africa where many varieties of it exist. It is a perennial grass with good stolons which root readily and rapidly on the ground to form a dense mat. It produces vertical branches which rarely grow above 4ft (Olubajo 1969). It grows well in the tropics and it tolerates heavy grazing, and wet and dry climates as well as a wide range of tropical soils. Ogor (1961); and Davies and Skidmore (1966) found it to combine well with legumes like Centrosema. Giant star is a deep rooting tropical grass that needs high soil fertility to persist as a sole crop. It is one of the tropical grasses that respond well to nitrogen and phosphorus fetilizers.

Centrosema pubescens (Bentham)

It is a deep rooting herbaceous perennial legume widely used in grass and legume mixtures, in parts of tropical Africa including Nigeria. It was introduced from South America (Miller and Rains, 1963). It will climb at times and when thus supported it produces more seeds. The germination of its seeds is poor even after

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scarification. It is better suited to high rainfall areas and it persists in the dry season. It becomes woody after one year, so it is better grown as a companion legume. It combines well with Cynodon plectostachyus (Miller and Rains 1963); Oyenuga (1957); and Chloris gayana, Melinis minutiflora and Panicum maximum. It covers the soil well, since it has a very rapid growth, and can eliminate completely all weeds when it is fully established. It is palatable to grazing cattle either as sole plant (Whyte, Moir and Cooper 1953), or as a part of grass/legume (Oyenuga,1957). It fixes nitrogen in the soil and therefore increases the nitrogen content of the grass. It is one of the self pollinating legumes in Africa (Davies and Skidmore,1966).

Stylosanthes gracilis (H.B.K)

The common name for this legume is stylo. It is also known as Brazilian lucerne because it originated from tropical South America. It is one of the most important pasture legumes used throughout Nigeria. Adegbola (1965) showed that it can be grown successfully as pure stands or in a mixture with grasses such as Andropogon gayanus, different species of Cynodon, and Melinis minutiflora.

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Horrell (1964) also reported stylo as one of the best legumes that improved the yield of unfertilized pastures at Serere. Miller and Rains (1963), and Adegbola (1964) showed that is does well without innoculation, and that cattle will graze it selectively during the dry season when the grasses are less palatable in Northern Nigeria. Careful grazing encourages it to spread, but it rarely survives the long dry season in the extreme north. It is superior to Centrosema pubescens and Desmodium in terms of nutritive value in Northern Nigeria (Miller and Rains, 1963). Hedrick (1961) reported that stylo can be used as livestock feed in the dry season in Southern Nigeria. Adegbola (1964; 1965) showed that it can be used for silage when grown with some grasses, and when grown as pure stands it can be cut and fed as hay during the dry season in the South. This was also found to be in other parts of the world (Vivian, 1959). Grinding Stylo into a meal for livestock feeding during the dry season in the South was suggested by Nwosu (1960). It is a trifoliate, self pollinating plant that can be propagated by cuttings in the high rainfall areas of the South (Nwosu, 1961) and from seeds which need scarification to encourage good germination. The

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seeds are small and pigmented. The colour ranges from black to yellow, and the lighter coloured seeds germinate more readily (Cowdry and Verhoeven, 1961). Ode (1970), Foster (1961) and Gilchrist (1967) showed that depth of sowing is very important since deep sowing causes slow rate of germination. A depth of 1½ inches has been found to be the best at Moor Plantation in Western Nigeria, 2.5 cm in Northern Nigeria and Queensland. Fertilizers like phosphorus and sulfur (Horrell and Court, 1965; Gilchrist, 1967; Horrell and Newhouse, 1966; Dada, 1972) improved yield of stylo and pure stands and as stylo/grass mixtures.

1.6 Crude Fibre as an undefined component of Pasture Herbage

"Crude fibre" was a term evolved by the Weende Chemists about a Century ago. Crude fibre consists of that part of plant that cannot be digested by dilute alkali and dilute acids. It therefore contains a mixture of substances in varying proportions. The replacement of crude fibre determination by a detailed analysis of plants into distinct chemical groups like pectins, lignin, cellulose, hemicellulose, starch, sugars, crude protein and ash was advocated by Van Soest (1966, 1967), Richards and Reid (1953), Deinum and Van Soest (1969). This detailed

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analytical procedure is however very expensive.

It is generally believed that crude fibre is related to forage quality. This is true to some extent, but it is very unsatisfactory measure of forage quality because of its variable composition and digestibility. It is some times more digestible than the nitrogen free extract which is supposed to be the most digestible fraction of the forage.

Crude fibre composition varies appreciably between species and within species at different stages of growth. This was shown in the results of investigations carried out by Oyenuga (1957; 1959; 1960), Armstrong, Thomas and Cook (1950), Woodman, Evans and Norman (1934), and Arnold Kivimae (1966).

Generally crude fibre content increases with advance in stage of maturity of forages, but it may decline after reaching the peak, (Oyenuga, 1958; Ademosun, 1970; Ademosun and Baumgardt, 1967; Wilson and Carrick, 1966; and Kivimae, 1966). Oyenuga and Olubajo (1966) obtained crude fibre range of 31-38%, and Ademosun (1970) obtained a range of 29-34%, while Ademosun and Baumgardt (1967) obtained a wider range of 22-51%.

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The crude protein of most forages decreases with age and frequency of cutting while the crude fibre increases with age. This was shown by Armstrong, Thomas and Cook (1950); Woodman, Evans and Norman (1934); Tilley and Terry (1964); White, Johnston and Armstrong (1964); and Oyenuga (1958.) Smith (1969) obtained more crude protein in the first regrowth than in the second regrowth of Hyparrhenia veld of Northern Rhodesia.

The livestock in Nigeria and in most parts of Africa and the tropics depend on the natural grassland for their feed supply. The livestock industry is limited by the general malnutrition in the dry season caused by the seasonal decline in the nutritive value of the natural grassland. The nitrogen content of the pasture herbage and the structural constituents of which crude fibre is a major constituent, play very important role in determining the nutritive value of the grassland. Low crude protein content and high crude fibre content lead to lower feed intake and poor animal production, particularly in the dry season.

When the feed value was calculated with crude fibre as an index substance, reliable values were obtained until

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early flowering. The varying content of the slightly soluble carbohydrates and crude fibre during the vegetative period contributes to the difficulties of estimation of the feed value.

Most prediction equations for nutritive value are based on regressions of digestibility, voluntary intake, or nutritive value index with one or more chemical components. The use of crude fibre to estimate digestibility of protein or cellular contents is not valid.

Richards and Reid (1953) reported high negative correlations between the amounts of digestible dry matter and crude fibre. High negative correlations were also observed between the faecal contents of crude fibre, and the digestible dry matter content of the herbage used. Only small amounts of the dry matter of forages and faeces were unresolved by the analysis of lignin, hemicellulose, cellulose, pectin, starch, sugars, crude protein, ash and ether extract. These detailed analyses are expensive even though the constituents represent distinct chemical groups.

The replacement of crude fibre analysis with an analysis for lignin, and the measurement of total carbohydrates by difference would result in the grouping

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of nutrients groups having more meaning biologically than the presently employed proximate analysis.

1.7 Effect of Climate and soil on crude fibre content and pasture production in the Tropics

Tropical soils are formed on land surfaces that are, geologically speaking very old (Davies and Skidmore, 1966). The natural drainage of tropical soils is very poor. The soils are shallow because they have become compact below the surface, and plant roots penetrate with difficulty. If the parent rock is rich in iron, the compact layer forms laterite. They are very low in plant nutrients because they have been exposed to leaching for millions of years. The plant nutrients are almost all contained in the soil organic matter at the surface, and in the plant debries on the soil surface, and in the plants growing on the soil.

In Equatorial regions the transpiration of pastures can be as high as 90 inches at sea level, falling to 45 inches at 8,000 ft. above sea level, but in the tropics the annual rainfall is less and usually appreciably less than the amount needed for transpiration. Tropical pastures have the quality of being able to withstand drought for months and are rapid growing and rapid seeding. Tropical

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grasses are much deeper rooting than temperate grasses. Some will extend their roots as far down as 15 feet or more for example Giant Star Grass, and will exert a suction greater than 15 atmospheres for a depth of at least 12 feet. This is below wilting point. British pasture grasses rarely root below 4 feet and will not dry soil to wilting point.

The fainfall in the tropics is variable and there are two seasons, the rainy and dry seasons. There is good pasture growth during the rainy season. Animals have enough and even more than enough of good food to eat. The excess pasture herbage is stored as silage or hay. Animals gain weight rapidly. In the rainy season also most of the good top soil is washed away by floods and the soil becomes poor and this leads to poor pasture production as time goes on, particularly in hilly areas. Good management, contouring, ridging, and manuring or fertilization will then be needed to bring the land to good production, Pereira and Beckley (1953).

In the dry season most of the pastures have become straw and shrubs. They cannot supply enough good material to grazing animals, so the animals fed on the poor quality pasture herbage lose weight.

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Grown grasses and Natural grasslands respond very well to fertilizer treatments (Davies and Skidmore, 1966; Poultney, 1959; Smith, 1961, 1964); and to mixing with legumes (Horrell, 1964; Moore, 1962; Motta, 1953).

The pasture herbage contains higher crude fibre at the young stages of growth, and the crude fibre content may not be too high at maturity (Whyte, Moir and Cooper, 1959).

The protein, minerals and vitamin contents of tropical pastures are also low (Milford and Minson, 1965b). Deinum (1966) reported that the crude fibre content of herbage dry matter was decreased by light intensity and increased by temperature. In the fresh matter it was increased by both. The crude fibre consists of the cellulose of the parenchymatous cell walls and of part of the lignin and hemicellulose from the vascular bundles. Water shortage decreased crude fibre content of grasses.

Because of the larger concentrations of carbohydrates in the cells at higher light intensity, the amount of crude fibre in relation to the total dry matter yield is lower. The smaller concentration of carbohydrates in the cells at a higher temperature causes the crude fibre content to

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increase. Where conditions for growth are good, pasture yields in the tropics, and also the output in terms of animal production compare favourably with those of temperate countries. This was shown by the results of many workers (Smith, 1961; 1964; Moore, 1962; Pereira and Beckley, 1953; Pereira, 1959; Pratt and Knight, 1964; and Motta, 1953). The quality and yield of pasture in Nigeria and other parts of the tropics decrease with frequency of cutting (Oyenuga, 1959a; b; 1960a; b; Akinola, Chheda and Mackenzie, 1971; Ademosun, 1970; and Patterson, 1933.)

The carrying capacity of pastures depends on the quality of the pasture. During the rains the good and abundant growth supplies enough food materials for animals. The animals therefore gain weight. During the dry season when the pasture grasses have declines appreciably in quality and digestibility, the animals lose weight. Pasture grasses seeded with legumes produce good quality animals and the average daily live weight increase per animal will be good. Cultivated land divided into paddocks and rotationally grazed will give good increase in live weight of the animals. The pastures may be

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fertilized to produce better quality forage, or hay plus some supplements fed to increase live weight gains. Rotational grazing of fertilized pastures gave good live weight gains in Kenya (Pereira, 1959). Hay plus nitrogen supplements improved live weight gains of animals in Northern Rhodesia (Smith, 1961), while herbage yield was increased and improved in quality by fertilizers in Kenya (Poultney, 1959). Mixtures of pasture grasses with legumes increased pasture production and reduced the rate of decline in productivity, at Serere, East Africa (Horrell, 1964).

With good quality pastures, fewer number of acres will be needed per animal.

1.8 Milk fat content of the Zebu cattle

The Zebu cattle including the white Fulani cattle in Nigeria produce milk of high butterfat content. The milk of the Zebu cattle in Asia, Eastern and Southern Africa were analysed by various workers (Mason and Maule, 1960; Williamson and Payne, 1959); and the butterfat content found to range from 4-7%. Investigations carried out in Nigeria (Olaloku, 1968; Adeneye, Oyenuga, and Olaloku, 1970; and Olaloku, Egbuiwe and Oyenuga, 1971) showed the butter-

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fat content to range from 3-9%. On the average the White Fulani cattle produce milk of over 5% betterfat content. The White Fulani Cattle are better as beef than as dairy animals (Oke, 1961) because they do not produce enough milk to make dairy farming economical. Imported breeds of cattle, Holsteins, Friesians and Jerseys, from America and Europe are currently being introduced into government and some University farms to increase production and to produce crosses which will produce more milk.

There are many factors that affect the yield and composition of milk. Some of these factors are the age of the dam, the older dams produce more milk, the live weight of the cow and time of calving, its health, the number of calves, the climate and plane of nutrition, and breed. In Nigeria and tropical Countries in general, reports of investigations (Oke, 1961; Deinum, 1966; Williamson and Payne, 1959; Davies and Skidmore, 1966; Smith, 1961; 1964; Milford and Minson, 1965a, b; Minson, Whyte, Moir and Cooper, 1959; Paterson, 1933; Oyenuga, 1957; 1959; 1960; Payne and Hancock, 1957; Pereira and Beckley, 1953; Pereira, 1959; Motta, 1953; Poultney, 1959; Horrell, 1964; ,

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Birch, 1964; and Pratt and Knight, 1964; Enlow and Dutton, 1954); showed that the climate, plane of nutrition and breed are the major factors affecting the yield and composition of the Zebu Cattle.

Effect of Climate on Zebu Cattle, milk yield and milk composition

Temperature in the tropics, (Nigeria is one of the tropical Countries,) varies widely and is generally high. The ideal temperature according to Williamson and Payne (1959) should be between 50° and $80^{o}F$. Very high temperature causes rise in rectal temperature, and a decline in feed intake, in productive processes like growth, milk and beef production and change in milk composition.

The length of daytime for grazing is long in the tropics, but the ambient air temperature, particularly in the dry season, depresses appetite, reduces feed intake, and grazing time. The animals are subjected to strong solar radiation and they prefer to rest under shades provided by the few trees in the paddocks or open grassland. Cattle, both young and old, in the tropics and temperate areas graze during the day time (Godfrey, 1961).

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Most of the animals rest about noon when the temperature is highest, and grazing is broken by frequent rests more in the afternoons than in the mornings, particularly by the younger animals. Older animals graze continously than the younger ones. All these lead to slow growth, low utilization of pasture, low production in terms of beef and milk and long calving interval.

The animals grazing the savannah of Nigeria, and other tropical natural grasslands, except those in government stations, receive no supplements either in the dry or rainy seasons. Their performances are poor in terms of production of beef and milk. These natural grassland provide sub-optimal food particularly in the dry season. The animals do not grow as rapidly as expected and they show alternate gain and loss in weight. The milk production is very low and the fibrous pasture herbage they feed on lead to the production of milk of high butterfat content. Protein is the more important constituent of milk needed by man although butterfat and milk protein are both of commercial importance in dairy industry.

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1.9 Fibre content of tropical pasture harbage and volatile fatty acid production

Crude fibre and other structural constituents, except lignin, are degraded by the microflora in the reticulo-rumen of the ruminant into fatty acids, and gases; and with increasing age the pasture herbage becomes less palatable, more fibrous and less digestible due to increased crude fibre and structural constituents production. The structural carbohydrates of grasses represent a considerable portion of the plant dry matter. It ranges from about one third of the dry matter in the young plant to over half of the dry matter in the mature grass (Waite, Johnston, and Armstrong, 1964; Arnold Kivimae, 1966; Richards and Reid, 1953; Van Soest, 1966; Ademosun and Baumgardt, 1967).

Acetic acid forms the major part of the total acids produced in the rumen (Bills, Khatri, and Day, 1963; Storey and Millard, 1965; Jennings, 1957; Ademosun and Baumgardt, 1967.)

The amount, rate and proportion of the fatty acids depend on the type of feed offered the animals. In supplemented feeds the acetic acid production is just over half of the total acid produced in the rumen (Donefer,

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Lloyd and Crampton, 1963; Stanley, Morita and Ueyama, 1964; Raymond, 1961). For such feeds the acetic acid to propionic acid ratio narrows down to just over 2. In highly fibrous feeds more acetic acid is produced and the propionic and butyric acids contents fall. The acetic acid to propionic acid ratio increases and may be as high as 7 in highly fibrous and unsupplemented feeds (Armstrong, 1964). With increasing maturity of pasture, animals tend to increase their feed intake, and this increase in feed intake causes a reduction in digestibility, and increased faecal loss (Armstrong, 1964; Armstrong and Blaxter, 1964). The decrease in digestibility and faecal loss are more in poor quality feeds.

The fatty acids produced in the rumen from the structural carbohydrates and other feed components are absorbed and utilized for energy, maintenance, milk fat synthesis, and depot fat synthesis, (Craplet, 1963; Armstrong, 1964; Armstrong et al.,1958; 1957 a, b, c). Acetic and butyric acids are lipogenic, (Maynard and Loosli, 1962; Tyler, 1958; West, Todd and Mason, 1968; and Crampton, 1956), while propionic acid is glucogenic.

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When more acetic acid is produced then more fat will be deposited in the milk and body depots. This may be one of the reasons why the milk fat in the White Fulani (Zebu) cattle in Nigeria is higher than that of the Friesian. When more propionic acid is produced and the acetic acid to propionic acid ratio is small, more protein and less fat is deposited in the milk. The ruminant animal has a four-chambered stomach, namely rumen, reticulum, omasum and abomasum. These compartments develop from the embryonic stomach and not from the oesophagus. The rumen, reticulum and omasum are not glandular, and they represent the fore-stomach. Food eaten is macerated and well mixed in the rumen, while the rumen, reticulum and omasum subject the food to digestion by micro-organisms before passing it to the glandular abomasum and then through the intestinal tract. The rumen requires a large amount of water for its normal functioning. This explains in part, the enormous salivary secretion of ruminants, and indicates that ruminants should have access to water all the time.

Rumination embraces the mechanical factors of digestion whereby food in the stomach is regurgitated, remasticated,

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re-insalivated and re-swallowed. These four-phases with a small pause after re-swallowing, make up a cycle of rumination.

Objective of the thesis

The present work is carried out to investigate the relationship between the crude fibre content of four forages on the volatile fatty acid composition and milk fat of the White Fulani (Zebu) cattle.

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CHAPTER 2

LITERATURE REVIEW

2.1 Digestion in the Ruminant

McCarthy and Kesler (1956) in their investigations on the young ruminant found that at birth, and for a short time afterwards, the calf relies on glucose for its major energy needs. Later, the glucose level in the blood begins to fall while the concentration of volatile fatty acids in the blood increases. At this time rumen activities increase as well as the cellulose digestion of the feed. The rumen, reticulum, omasum, abomasum also undergo some development before the volatile fatty acids can be absorbed at the maximum rate. Becker, Marshall and Dix Arnold (1963), Godfrey (1961a), also found that the volume of the omasum increased 60 times between 10 and 150 days of age. Sutton, McGilliard and Jacobson (1963), Godfrey (1961b), Wardrop (1961), Benzie and Phillipson (1957) observed that the forestomach epithelia developed rapidly to adult form when roughage was given early to calves.

Kameoka and Morimoto (1954) in their investigations on the adult ruminant reported that all the digestible crude fibre of the feed disappeared from the forestomach of the

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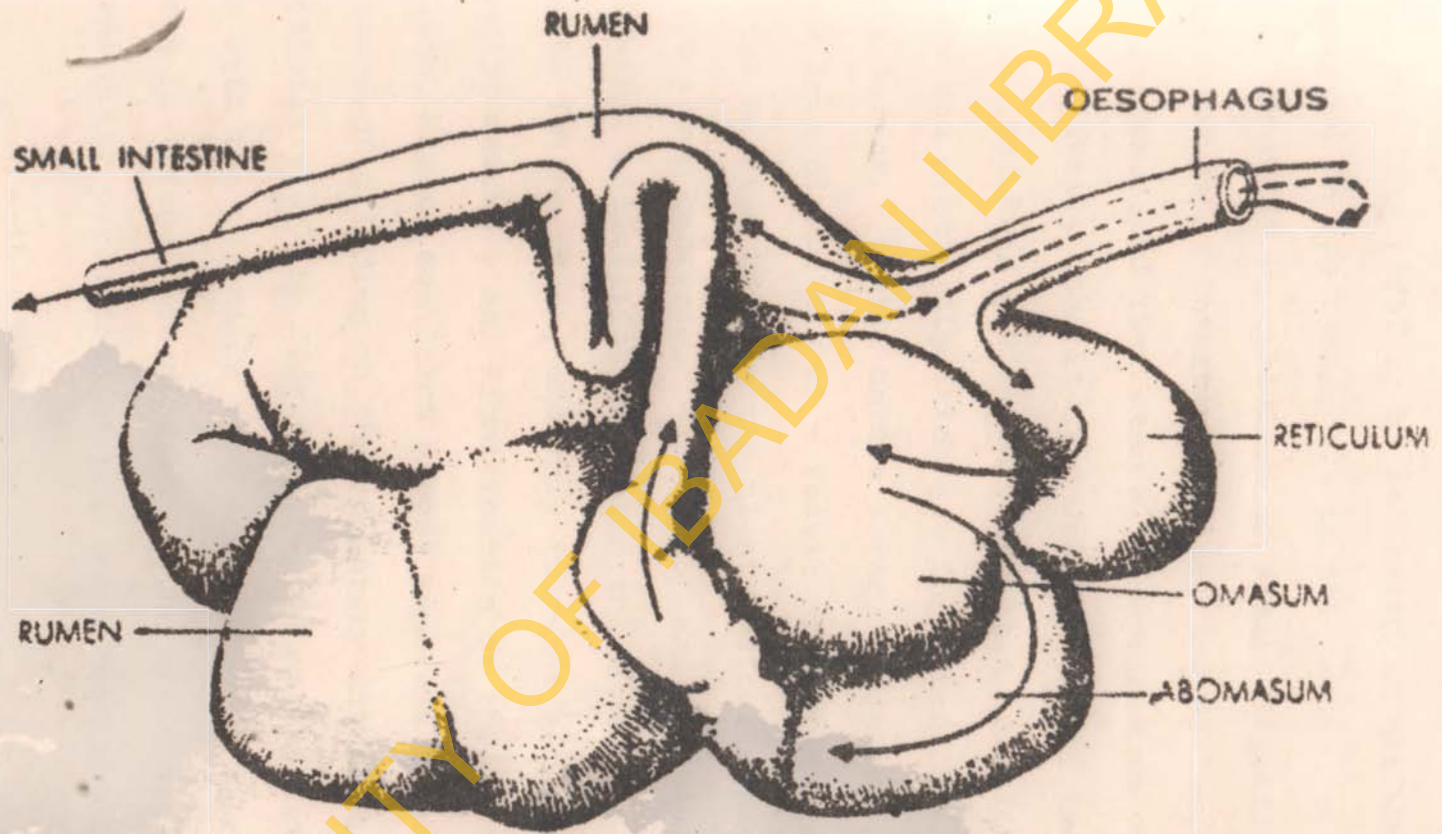

Schematic representation of the organs that comprise the stomach of the ruminant: rumen, reticulum, omasum, and abomasum. (After *Scientific American* (1958), **198**, 34.)

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goat. But Gray (1957 a; b) indicated in his work that 70% disappeared in the rumen, 17% in the caecum, and 13% in the colon. There is no cellulose or starch digestion in the abomasum or small intestines (Gray, and Weller, 1954 a; b). They also observed that starch is degraded in the omasum. Dehority (1961), Dehority and Johnson (1961; 1962) and Richards and Reid (1953) reported that cellulose, hemicellulose and pectin digestion are influenced by the maturity of the forage. In the mature forage these constituents become less digestible as a result of lignin forming a physical barrier between the plant hemicellulose or pectin and the rumen bacteria. "In vitro" and "in vivo" studies showed that ballmilling increased the amount of cellulose digested in the rumen, and this increase became larger with advancing maturity and lignification of the forage. Campling, Freer, and Balch (1963) reported that the influence of grinding on the voluntary intake and the digestibility of the diet will depend on the extent to which both the retention time and rate of breakdown of digesta in the gut are altered by the grinding. Pelleting increased the percentage of propionic acid and the total

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concentration of the rumen acids (Jorgensen and Schulty, 1963; Shaw, Ensor, Tellechea, and Lee, 1960; Bensadoun, Palladines, and Reid, 1962; Salsbury, Hoefer and Lisecke, 1961; Van Soest, 1959; Stanley and Morita, 1964; and Balch, 1958). Pelleting also increased the blood sugar levels (Jorgensen and Schulty, 1963; Shaw, Ensor, Tellechea and Lee, 1960); but decreased milk fat and percentage of acetic acid in the rumen liquor (Balch, Balch, Bartlett, Cox Rowland and Terner, 1954b; Hawkins, 1963; Bishop, Loosli, Trimberger and Turk, 1963; Van Soest, 1959). Beardsley (1964); Moore (1964); Bishop et al. (1963); reported that pelleting increased the rate of fermentation in the rumen, feed intake and feed efficiency which then result in increased milk yield and daily live weight gain. Pelleting also causes increase in the degree of unsaturation of body fat (Shaw et al., 1960) and a decrease in the dry matter and crude fibre digestibility (Moore 1964). When high roughage was fed, the molar percentage of acetic acid was greater than when low roughage was fed, (Bishop et al., 1963). But Balch et al. (1963) working with cows and feed low in hay but high in concentrate reported that even when the hay intake was only 4 lb. per day, the milk fat content did not

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fall because it was fed along with a concentrate cube having the physical property of roughage. A concentrate mixture with highly digestible carbohydrate like starch caused a fall in the fat content of milk. Starch in flaked maize is thought to affect the rumen flora. Even when hay was fed in the ground form, the fat content of the milk fell considerably. The greater depression in milk fat with flaked maize than with the others (in flaked maize the starch granules are raptured and the starch partly dextrinized by passage between rollers) is thought to be associated with the effect of the starch on the flora of the rumen. Generally, a diet low in hay and high in concentrate causes a fall in milk fat content, a high iodine value and lower softening point and also a lower Reichert Meissel value. Shaw et al. (1959) reported that concentrates with even lower levels of roughage produced milk with only slight decreases in the milk fat content. The physical property of roughage is not found in straw. Balch (1958) reported that relatively more propionic acid was produced from the diet containing finely ground hay than from the long-hay diet. The result of Gray et al. (1958) showed that only well digested material reached the

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omasum and abomasum from the rumen even in the period immediately after feeding. For this reason the extent of digestion in the rumen could not be determined from the changing coefficients in the rumen itself, but was indicated by the more constant values in the omasum and abomasum.

The extent of digestion of cellulose in the rumen of sheep fed wheaten hay and wheat straw was about 30%, on wheaten hay about 40%, and on luceme hay more than 50%. The extent of digestion of pentosans and solids in the rumen was very similar to that of cellulose. Badawy (1958) reported that the reticulo rumen sac 5 hours after feeding was found to contain 74% of the total dry matter of the whole digestive tract. Owing to dehydration, the dry matter percentages were considerably higher in the omasum and the colon than in the other parts of the tract, the omasum having the highest value. The highest values for the lower volatile fatty acids as milli equivalent per 100 ml contents or as a ratio to lignin corresponded to the main sites of microbial activity, mainly the reticulo rumen sac, caecum and colon. The mean total digestion coefficient of energy was 68%. Rogerson (1958) also reported that the rumen and reticulum together contained,

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on a dry matter basis approximately two thirds of the digesta found in the alimentary tract. There were indications that, as the amount of roughage in the diet decreased, the proportion in the omasum and small intestine decreased. Secretions into the small intestine resulted in greater quantities of digestible nutrients in the contents of this section than in those of the abomasum, and in some instances an even greater weight of wet digesta was found than the weight of food ingested. Increases in the quantity of nitrogenous material constituted the greatest change, although increases in fat and carbohydrate content, together with the higher protein value, resulted in substantially enhanced energy value.

Researches into the feeding frequency were carried out by Knox and Ward (1961); Putnan, Gutierrez, and Davis (1961). The former reported a significant increase in volatile fatty acid concentration when feeding frequency was increased from 2 to 8 times a day. Putnan et al. (1961) fed calves 2 and 10 times a day and also observed an increase in the average volatile fatty acid concentrations. The increases however were not significant. When the animals were fed 2 times a day there were two peaks one

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to two hours after feeding, which dropped to the lowest point the hour before feeding. On the other hand when they were fed 8 times a day there was a more constant concentration of volatile fatty acids during the twenty four hour period. Acetic acid was produced more than the other acids when the animals were fed 2 times, but propionic acid predominated when they were fed 8 times a day. Butyric acid also increased to a lesser extent when fed 8 times a day. The result of the work of Knox and Ward (1961) showed that feeding ruminants several times a day produced the same effect as feeding pellets. These similar effects are the significant increase in volatile fatty acid concentration and increased propionic and butyric acid production together with increased weight gain and increased efficiency with which digestible energy is converted to 4% fat corrected milk.

The result of the work of Palmquist, Smith and Ronning (1964) showed that when cows are fed roughage at 6 a.m. and 6 p.m. and Concentrates at 2 a.m. and 2 p.m. the milk fat percentage decreased significantly. The same thing happened when the roughage and concentrates

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were fed at 6 a.m. and 6 p.m. The decrease, however, was significantly less when the concentrates were fed separately from the hay than when they were fed at the same time. They postulated the theory that feeding hay pellets and grain at separate times provides a more even supply of rumen fermentation products for milk fat synthesis.

Volatile fatty acid production in the rumen is affected by the type of carbohydrate in the feed. Shaw et al. (1959) reported that cows fed rations made up primarily of cooked high starch feeds produced milk with low fat content. A ration composed primarily of bread effected a decrease of more than 30% in the fat content of milk and a low level of butyric acid in the milk fat but had little effect on the iodine number of the milk fat. There was a similar effect when feeds composed mainly of cooked rice, cooked potato meal, bread and molasses were fed. The decrease of milk fat was worsened by adding skim milk to the ration. The decrease of milk fat can be prevented by feeding low energy intake. This result supports the earlier work of Balch et al. (1955).

Johnson, Dehority, Conrad, and Davis (1962) observed a significant difference between the digestibilities of the

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dried and undried samples of mixed forages. The undried samples were about 7% more digestible than the dried ones. Bowden and Church (1962) reported that the digestibility of oven dry samples of Tall fescue differed from fresh frozen samples, oven dry samples being better digested. But these two samples produced essentially the same molar percentages and total amount of volatile fatty acids. Fisher and Elliot (1966) reported that the infusion of glucose resulted in reductions in fat yield and percent fat. Infusion of propionate also decreased milk fat, but to a lesser extent. Richards and Reid (1953); Ademosun (1970); Waite et al (1964); Armstrong (1964); Ademosun et al. (1967) in their investigations observed that pasture grasses became more unpalatable and less digestible as they matured. With increasing age the amounts of cell wall constituents (lignin, cellulose, hemicellulose) increased progressively and pectins, sugars, crude protein and ether extract decreased. Lignin was completely undigested, while sugars were almost completely digested in the herbage at the three stages of growth (vegetative, boot to early head, and full bloom). The grazing animals usually

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select leafier and more highly digestible portions of the plant, but hand-fed or stall-fed steers consumed the herbage cut and offered to them.

Saliva production also affects rumen digestion. Wilson (1964) in his work on digestion in the ruminant reported that section of parotid ducts and nerves decreased the intake of lucerne chaff diet of hay, but had no effect on the intake of straw or milled lucerne hay. Artificial saliva increased food intake to 15% below the initial intake. The passage of food and rate of cellulose digestion were also decreased, but the digestibility of dry matter was slightly increased. After section of the nerve and duct to the left parotid gland there was an increase of 64% in the secretion of the right parotid gland. After section of one or both ducts and nerves there was an increase in the secretion of residual saliva. McDougall (1948) showed the importance of saliva in rumination.

The type and number of micro-organisms in the fore-stomach and the type of end products affect the rate of digestion. Bowden and Church (1962) reported that the digestive capacities of the rumen liquor collected from the

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same steer on different days differed considerably. Montgomery, Schultz and Baumgardt (1963) reported that "*in vitro*" cellulose digestion showed that there is very little difference in the ability of the micro-organisms taken from the rumen of cows of varying hay intake, to digest cellulose. The micro-organisms need some fatty acids for efficient work. Wegner and Foster (1960) reported that some of the micro-organisms isolated from the rumen failed to grow on subculture in the basal medium unless it was enriched with rumen fluid or a mixture of fatty acids commonly found in the rumen. Gray and Pilgrim (1952) reported that the rate of production of volatile fatty acids by the micro flora was rapid at first; 60% of the total volatile fatty acids was formed in the first quarter of the fermentation period; and thereafter declined to a lower but fairly steady level. They also need some catalysts for efficient function: for example *Veillomella gazogenes* and species of the genus *Propioni-bacterium.*

Gray *et al.* (1962) found that plant nitrogen was rapidly attacked and the micro-organisms grew rapidly. Different diets gave rise to different proportion of protozoal-nitrogen and bacterial-nitrogen in microbial-

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nitrogen fraction. In their early experiment Gray *et al.* (1958) found that 63-82% of the total nitrogen was present as microbial-nitrogen, 11-27% as plant-nitrogen, and 5-10% as soluble nitrogen. Gray *et al.* (1958) calculated the quantity of nitrogen reaching the abomasum and duodenum to be equivalent to nearly 100% of the dietary-nitrogen for sheep fed wheaten hay (N=1.1%), but only about 65% for animals fed on a mixture of wheaten hay and lucerne hay (N=1.8%), and as little as 48% for those fed on lucerne hay alone (N=2.9%). An amount in excess of the dietary nitrogen was found to reach the abomasun in the sheep fed on a diet of wheaten hay and wheat straw, very low in nitrogen (0.7%). In a sheep fed on lucerne hay, the concentration of ammonia in the rumen fluid was probably never less than 20 mg. nitrogen per 100 milliliter, and for 8 hours after feeding it was much greater, reaching a maximum value of more than 50 mg. nitrogen per 100 milliliter. In the sheep fed on wheaten hay the concentration of ammonia remained for the greater part of the day at 5-6 mg. nitrogen per 100 milliliter rumen fluid, and only rose above this value to about 12 mg. per 100

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milliliter for 3-4 hours after feeding. The extra sources of nitrogen are the salivary-nitrogen and the gastric juice-nitrogen. Badawy, Campbell, Fell, Cuthbertson and Markie (1958) reported that relative to lignin, the ratios (total nitrogen and non-protein nitrogen) indicated an increase of nitrogen in the abomasum, but the increase was much greater in the proximal half of the small intestine than in the distal half. When the intestine was divided into four parts, protein nitrogen, and non-protein-nitrogen concentrations increased considerably in the first quarter of the small intestine and decreased progressively in the other quarters. Lignin ratios indicated that the non-protein-nitrogen contributed a greater proportion to the increase in the small intestine than did protein-nitrogen, though the latter also rose. This considerable rise in nitrogen concentration in the first quarter of the small intestine was not found in living sheep fitted with permanent cannulas, or with an esteriorized flow of the small intestine when the cannulas were located within the first few feet from the pylorus. A comparison of the histology of the mucosa of the small intestine of sheep shot in the frontal region with a humane killer and then bled, with

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specimens removed from sheep under pentobarbitone anaesthesia revealed that the mucosa remained relatively intact when removed under this anaesthesia, on the other hand there was a very marked shedding of the epithelium in the animals that had been short and bled. This difference might be expected to account for part of the observed rise in nitrogen.

The possibility of feeding cows protein-free feeds for milk production was carried out by Virtanen (1966). Purified carbohydrates were fed along with labelled urea and ammonium sulphate. He established the fact that synthesis of bacterial protein in the rumen of lactating cows fed purified carbohydrates, with urea and ammonium sulphate as the sole source of nitrogen, could be increased, through the feed adaptation, to a level adequate not only for maintenance of the cow, but also for a relatively high milk production. The composition of the milk was similar to that of normal milk rich in fat and protein. In their work, Salsbury *et al.* (1961) reported that a ration of cellulose, starch, and urea was found to be the simplest combination capable of maintaining the cellulose

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digesting ability of rumen ingesta for three days. Cellulose plus a purified soyabean protein was more effective than cellulose plus urea. When the ration consisted of only cellulose, starch, and urea, a source of amino acids was required to maintain cellulose digestion for more than three days. With this ration, no deficiency of valeric or isovaleric acid was demonstrated, and yeast extract was the most effective additive.

2.2 Absorption in the Ruminant

Kameoka and Morimoto (1959) reported that 61.7 - 85.4% organic matter was absorbed in the rumen, reticulum, and omasum of goats. The absorption of digestible protein in the forestomach ranges from 20 - 52.1%, while about 65.7 to 96.9% of the nitrogen free extract was absorbed from the forestomach. About 33 - 64% of water present in the reticular content was absorbed through the wall of the omasum (Gray, 1954 a; b).

Many investigators, particularly those in the temperate regions, have worked on the end products of digestion in the ruminants, and the absorption and metabolism of such products. Some of the investigations were carried out on intact animals, while other workers found the "in vitro" method more convenient.

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John, Barnett and Reid (1957), Stanley, Morita and Ueyama (1964) carried out investigations on different roughage levels. They found that acetic acid was the major acid produced, since it formed over half of the total acids produced in the rumen. John <u>et al.</u> (1957) noted that rumen liquors containing the same substrate produced identical V.F.A. and that the potency of rumen liquor varies throughout the season. Acetic acid was also the major acid produced in their work. Propionic acid became the major acid produced in their "<u>in vitro</u>" procedure as the season advanced. Butyric and valeric acids gave a curve that followed parellel course throughout the season. Their findings were supported by the result of Donefer, Lloyd, and Crampton (1963) who used sheep in their experiment. On a molar percentage basis, acetic was the major acid produced, while the production of propionic acid was next to that of acetic acid, and butyric acid had the lowest percentage.

Stewart, Stewart and Schultz (1958) reported that the volatile fatty acid concentrations increased after feeding in all cases. The average volatile fatty acid concentra-

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tions by weight in their experiment were 5.2% for valeric acid, 16.5% for butyric acid, 18.3% for propionic acid, and 60% for acetic acid. The relative proportions of the volatile fatty acids in the rumen remained constant. The total quantities of volatile fatty acid increased abruptly after feeding, but by 8 hours after feeding, the total quantities decreased. The peak in total production rate was in some cases reached within one hour after feeding. Their work also showed that there is no preferential absorption of the various volatile fatty acids from the normally functioning rumen, since absorption is proportional to concentration of volatile fatty acids.

In investigations of Donefer, Lloyd and Crampton (1963) acetic to propionic acids ratios of 2.4 to 2.6 were obtained. These were not significant. Stanley, Morita and Ueyama (1964) obtained acetic to propionic acid ratio that ranged between 2.1 and 2.74.

Raymond and Terry (1966) using an "in vitro" method reported that fibrous feeds of low digestibility gave an acetic to propionic acid ratio greater than 7:2. As increasing amounts of carbohydrates are given, the radio

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tends to narrow reaching almost unity on a mainly concentrate diet. The investigation of Brown, Stull and Scott (1962) showed that low rouphage diet caused a significant increase and the high rouphage diet decreased the higher fatty acids. The molar ratios of any of the volatile fatty acid in the rumen were not significantly altered on the high rouphage diet. Sutton (1968) infused monosaccharides and observed increases in the concentration of the volatile fatty acid and decrease in the pH. The rate of change in the molar proportions of the volatile fatty acids was greatest in the first four hours and decreased thereafter.

Naga and El-Shazly (1969) reported that the volatile fatty acid concentration in the buffalo fed concentrate plus straw was significantly higher than in the bulls.

Raymond, and Terry (1966) in very extensive tests observed a wide range of pH values "in vivo". The pH ranges from 5.5 - 7.0. They are therefore of the opinion that "in vitro" experiments should be carried out at pH between 5.5 and 8.0.

John, Barnett and Reid (1957) in their work on fresh grass reported acetic acid as the major acid produced,

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but as the season advanced, propionic acid was the major product. Butyric and valaric acids gave a curve that followed more or less parallel course throughout the season. The potency of rumen liquor varied throughout the season.

In animals fed roughages only 61.9 - 73.2% of digestible ether extract was absorbed from the forestomach, but lower values were obtained when concentrates plus hay was fed, (Kameoka and Morimoto, 1959). On retention times of feed, Combe and Kay (1965) observed that in the small and large intestines the retention times were inversely related to the dry matter intake of a particular feed and faecal output. Conrad et al. (1958) working with 4-7 month old calves measured the blood flow of the forestomach and reported that about 63 gm. of acetic acid and 25 gm. of propionic acid were absorbed for each pound of dry feed consumed during a 24 hour period. Johnston, Kesler and McCarthy (1961) also working with calves reported that 51% of the volatile fatty acid is absorbed in the reticulum, while 83% is absorbed in the omasum. Calves can absorb fatty acids rapidly if they are introduced to roughage very early in life (Sutton et al., 1953; Godfrey,

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1961 a; b; Sijpesteijn and Elsden, 1952) in their research with sheep noted that the conversion of cellulose to propionic acid proceeded in part via succinic acid. Succinate accumulated for a short time after feeding, suggesting that a large amount of succinate was produced during digestion. Added succinate is rapidly absorbed from the rumen, at the same time there was an increase in the concentration of propionic acid only. Some of the volatile fatty acids produced are converted to other acids.

Johnston et al., (1961) working with calves injected Na-butyrate-C^{14} into the omasum and found that the butyrate C^{14} was rapidly absorbed from the omasum to the blood of the omasal vein, but 50% of the recovered label in the omasal blood was in the form of lactic acid. This indicated on unknown pathway for the convertion of butyrate to lactate by omasal epithelium. Luick (1960) used cattle and observed that butyrate is converted to β phydroxy butyric acid by rumen epithelium. Gray et al. (1952) suggested that a considerable part of the higher fatty acids in the rumen fluid may be produced by

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secondary reactions from both acetic and propionic acids. This was supported by their observation that at the end of fermentation some of the carbon originally present in the carboxyl group of acetic acid was distributed among all the higher fatty acids, while part of the carboxyl carbon of the propionic acid appeared in valeric but not in butyric acid. This indicated synthesis of higher acids by condensation of the lower ones with two carbon compounds in equilibrum with acetic acid. Joyner _et al_. (1963) in the result of their studies observed the rapid absorption of labelled butyrate through the omasal wall and saw evidence of its convertion to another volatile fatty acid. The butyrate is found as β phydroxy butyric acid in the blood and is in close association with blood lactate.

Gray (1948) in his work on absorption of acids reported that between 10 and 14% of the acetic acid and about 47% of the propionic acid introduced was absorbed within 6 hours. The amounts absorbed were not altered by the inclusion of inorganic phosphate in the mixture. In an early work on the absorption of acids in the

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ruminant, Gray (1947) reported that when sodium salt of acetic, butyric, and propionic acids are fed and the animals ate lucerne there was a more rapid absorption of propionic acid than of the other acids. On the basis of relative rates of absorption of these acids at concentrations in which they were found to occur in the normal rumen during the fermentation of wheaten hay chaff, it was concluded that a much greater proportion of propionic acid is formed than is indicated by analysis of the acids found in the rumen (Gray, 1947; 1961). Montogomery et al. (1963) reported that there was a slight decrease in blood urea, suggesting that the acid absorbed into the blood stream was being neutralized by the urea, which would prevent a drop in the blood pH. Barcroft, McAnally and Philipson (1944) in a very early work on absorption of acids observed that the concentration of volatile fatty acids of the blood draining the rumen is considerably higher than that of peripheral blood in which little volatile fatty acid is present. Volatile fatty acids in significant amount is present in blood draining the omasum and the caecum, but is absent from the blood draining the

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abomasum, the small intestine, and the empty rumen, and water filled rumen. Badawy (1958 a; b) reported that the apparent absorption of the volatile fatty acids was of the order of 18 gm (expressed as acetic acid) in the omasum and 4 gm in the abomasum in 24 hours. Dehydration took place in the omasum. It appeared to occur to the greatest extent in the digesta between the laminae at the deepest point within the greater curvature, with a tendency to further dehydration towards the abomasal orifice. The concentration of nitrogen in terms of the markers used was less in the omasum than in the reticulum, indicating that absorption occured in the omasum. The concentration of volatile fatty acids, decreased progressively through the deeper zones of the omasum and from the reticulum to the abomasum, indicating absorption during the time spent in the omasum. It was calculated to be of the order of 77% of that entering the organ. Rogerson (1958) observed a large degree of 'apparent' absorption of both nutrients and water took place from the omasum. Absorption of nutrients was almost completed by the time the caecum was reached. Apart from a small amount of absorption from the

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caecum, most of the dehydration occured in the terminal colon, where a significant amount of soluble ash was also absorbed.

Volatile fatty acids absorption is affected by the rumen pH, while the pH is also affected by feed and volatile fatty acid concentrations. Emery and Brown (1961) fed sodium and potassium bicarbonate to cows and observed that the sodium bicarbonate increased the rumen pH. Sutton et al. (1963) in their investigation of the absorptive ability of the rumen observed that the absorption rate of volatile fatty acids decreased as their chain length decreased at acid pH values for calves fed starter or milk. At alkaline pH a decrease with decreasing chain length was indicated. Gray (1948) reported that at acid reactions, acetic and propionic acids are absorbed readily, but absorption did not occur to any significant extent at pH of 7.5. Acetic acid was not absorbed from the rumen when introduced as a slightly alkaline pH of 7.5 solution of sodium acetate. Rook et al. (1963) observed that acetic acid depressed the pH of the rumen liquor slightly. Armstrong and Blaxter (1957 a; b) reported that a mixture

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of propionic and butyric acids in the molar ratio of 3:2 infused into the sheep caused a more rapid absorption of butyric acid than propionic acid. Later the reverse occurred. This change was associated with a rise in the pH of the rumen. Barcroft et al. (1944) reported that when judged by the concentration found in the blood leaving the rumen, the rate of absorption of the sodium salts of acetic, propionic and butyric acids, shows that acetate is more rapidly absorbed, propionate less so, while butyrate is slowly absorbed. The quantity of volatile fatty acids calculated as acetic acid absorbed in an hour from the reticulum and rumen together is estimated to vary from 1 to 5 gm. Bensadoun et al. (1952) reported that there was essentially no glucose absorption from the gastro intestinal tract. Appreciable amount of formic acid (4.7 to 13.4 gm/day) were absorbed. The total quantities of volatile fatty acids absorbed per day ranged from 73 to 89 gm/day. Gray and Pilgrim (1951) observed that there was considerable increase in the concentration of acid in acid rumen after feeding. By the end of the day, the concentration had returned to its original value.

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Gray *et al.* (1952) reported that propionic acid increases and acetic or butyric acid decreases for some hours after feeding, after which these changes are reversed and the mixture gradually returns to its original composition. Waldern *et al.* (1963) reported that the greatest absorption of acids occurred between 3 and 9 hours after feeding, when the concentration of the volatile fatty acids in the rumen was maximal.

2.3 SYNTHESIS OF FAT IN THE RUMINANT

The pathways of conversion of volatile fatty acids to fat in the milk or depot fat were investigated by many workers. Popjack and Beakmans (1950; 1953) noted that in female rabbits cholesterol is synthesised in the liver, intestines and overies. The source of plasma cholesterol is the liver. Fatty acids are synthesised in the lungs and along the entire gastro intestinal tract. Acetate is used for the synthesis of fatty acids to a greater extent in the lungs. Folley and French (1949; 1950) also reported fatty acid synthesis from acetate in the lung of the goat. Phospholipin fatty acids and glyceride fatty acids in the lungs and intestine are not at equilibrum,

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the C^{14} being more in the former. In the liver the two types of fatty acids appear to come to equilibrum rapidly. There is the possibility that the fat precursors in the liver and intestines are not identical. Cholesterol is formed in the free form and esterified later. The two workers on further work with rabbits (1950) reported that non-lactating mammae of pregnant rabbits synthesise cholesterol and fatty acids. The foetus synthesized its own fatty acids and cholesterol, but also absorbs lipids from the maternal circulation. The mammae of lactating rabbits synthesise long and short chain fatty acids and cholesterol from acetate and carbohydrates. In 6 hours at least 30 - 70% of short-chain acids originate from acetate, or 25% from carbohydrates. Long chain fatty acids are however synthesised to a lesser extent from acetate and carbohydrate in the mammary gland. About 65-95% of the glycerol part of fat, derived from carbohydrate, was newly formed in the mammary gland in 6 hours. It was concluded that the glycerol part of fat is more rapidly metabolised by the animal than the fatty acid part. About 20% of the body's acetate used for acetylation of

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β-aminobenzoic acid was derived from glucose in fully fed lactating rabbits. Chemical degradation of octanoic acid from milk and mammary gland fat and the acetyl group of β-acetamidobenzoic acid showed that the conversion of glucose to fatty acids proceeds by the overall reactions thus, glucose ⟶ pyrurate ⟶ fatty acid. Continuing their research Popjak *et al.* (1952) concluded that glucose is the carbohydrate precursor of glycerol in the udder. This is supported by the work of Luick (1960). The acetate carbon entered glucose by way of the citric acid cycle followed by glycolytic process in reverse. The citric acid cycle provides a path for the entry of acetate carbon into pyruvate, and hence by the reversal of the glycolytic cycle, acetate carbon can find its way into glucose. It is likely that the breakdown of glucose to glycerol occurs within the udder itself (Luick, 1960). Glycerol could be formed from glucose at the triosephosphate stage of the glycolytic breakdown. Luick (1960) used labelled glucose and observed that 68% of milk fat glycerol is derived from plasma glucose. Their result showed that 2 pathways might be involved, one of which is

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the synthesis of glycerol from glucose in the udder and the other the synthesis of plasma lipid glycerol from glucose, presumably in the liver, and its subsequent absorption into the udder. There is no free glycerol in the blood of cattle so that glycerol as such cannot be considered an important precursor of milk fat glycerol. The trials indicated that milk fat is synthesised within the mammary gland from glycerol and free fatty acids. The bulk of the acetate produced in the rumen seems to pass unchanged through the liver into the general circulation, (Luick, 1960). This is supported by Joyner _et al._ (1963).

Propinate is thought to give most of its C to the synthesis of glucose. Butyrate is oxidised to β-hydroxy butyrate (BHBA) by the rumen epithelium and perhaps by the liver. Butyrate cannot give more than 13% of the C needed for the synthesis of short chain fatty acids. The mammary gland absorbs large quantities of triglycerides from the blood. Micro-organisms in the rumen produce transisomers of the dietary fatty acids, and large quantities of short chain fatty acids, especially acetate, propionate and butyrate synthesised from non lipid dietary precursors.

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Rook *et al.* (1963) observed that in growing heifers infusion of butyric acid increased blood BHBA, acetone and aceto acetic acid.

Cowie *et al.* (1951) used labelled acetic acid to perfuse the cow's udder and they observed that about 40% of the labelled C was found in the udder fat and some of the labelled C acetate was incorporated into fatty acid fractions of milk triglycerides. This experiment also showed that short chain acids are not formed directly by degradation of long chain acids. Some of the labelled C was absorbed into the cholesterol molecule. This showed that cholesterol is synthesised in the udder of the cow and the goat. Balman and Folley (1951) observed the effect of insulin on fat synthesis by lactating mammary gland slices of rats, mice, rabbits and sheep. They suggested that the stimulating effect of insulin on fat synthesis by mammary tissue may be due, partly, to the stimulation of the formation of glycerol from glucose. Mammary gland can synthesise and secrete glycerides, then the supply of glycerol might be a critical, rate-limiting factor in fat synthesis by this tissue. It is also possible that the

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insulin effect is related to the utilization of glucose as an energy source for fat synthesis.

Luick (1961) also infused labelled glycerol, acetate, glucose, propionate and butyrate into the udder of cows. The result indicated that glycerol is synthesised in the udder from glucose, but not from acetate, propionate or butyrate. The newly formed glycerol is incorporated into milk fat. This strongly implies that one pathway of milk fat synthesis involves its in situ synthesis in the mammary gland from pools of free fatty acids and glycerol. Folley and French (1949) working with rats, mice, rabbits, guinea pigs, lactating goats and cows observed the Respiratory quotient (R. Q) of the mammary slices in the presence of carbohydrates. In the presence of glucose the R.Q. of tissue from the mouse, rat, guinea pig and rabbit was well above unit, but that of the tissue of ruminants - cows, goats, was less then unity. This result did not support the theory that the lower fatty acids of the ruminant milk fat are synthesised from carbohydrates in the udder. The result with rat showed that under anaerobic conditions the rat mammary gland slices in the

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presence of glucose produce quite considerable amount of acid. It indicated that glycolysis is not the only process whereby the acid is produced. The mammary tissue of rat therefore exhibits a marked Pasteur effect. On further work with R.Q those workers reported that in the rat there exists some mechanism of fat synthesis in the mammary gland. The initiation of lactation is under endocrine control, so that there is likely to be a close relationship between the lactogenic hormone or hormone complex and changes in the mammary gland metabolism occurring after parturition. Folley and French (1950) observed the metabolism of acetate by mammary gland slices of sheep, goat, cow, rat and rabbit, and observed that ruminant mammary tissue in early or late lactation was almost inert to glucose. The tissues from lactating rumminants greatly utilise acetate, and glucose stimulates the use of acetate by mammary slices from rat, rabbit and sheep. Miller and Allen (1955) fed sodium acetate to lactating cows, and their result suggested that there is a minimum level of acetate required for production of milk of normal fat content, and that feeding of acetate up to this level

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will cause recovery from the diet-depressed milk fat. Additional amounts above this level will not cause further increases. Van Soest and Allen (1959) in their studies with cows observed that when ground and restricted roughage was fed to lactating cows and goats there was a decrease in arterial blood acetic acid. It appeared that the peripheral tissues as well as the mammary gland were utilizing less acetic acid under conditions of milk fat depression. The feeding of Na-acetate tended to increase low milk fat, and the feeding of Na-propionate tended to lower it further. This showed that acetic acid is not the only acid involved. In their investigation they observed that hydroxy butyric acid is a precursor of milk fat. The propionate is converted to succinate, then to oxaloacetate. This condenses with acetyl-CoA and is diverted from acetoacetate formation toward oxidation in the Kreb's cycle. This reaction leads to non synthesis of milk fat and causes a lowered utilization of acetate. Schmidt and Schultz (1958) fed Na-propionate to lactating cows and observed a non-significant fall in milk fat content. The propionate did not affect milk production. When the

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blood glucose and Ketone bodies are normal, propionate feeding had no appreciable effect. Terner (1951) carried out his investigation to ascertain the operation of the citric acid cycle in the udder. He used slices from rats, rabbits, goats and sheep and used pyruvate and glucose, and acids as substrates. Small amounts of citric acid accumulated when depleted mammary gland slices were incubated with fumarate and pyruvate. Fumarate reversed the inhibition of respiration caused by malonate. These observations suggest that the citric acid cycle is operative in the mammary gland. He also observed that pyruvate is oxidised more completely in the presence of dinitro phenol (DNP) than in its absence. Dinitro phenol appears to affect carbohydrate metabolism in two ways, one of which is by influencing the glycolytic breakdown of glucose to pyruvate, and the other by influencing the metabolic fate of pyruvate. Pyruvate is metabolished by mammary tissue by oxidation via the Kreb's cycle and by synthetic reactions. About 2 mol of pyruvate appear to be utilized in synthetic reaction for every mol of pyruvate oxidised.

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2.4 STRUCTURE AND COMPOSITION OF MILK FAT

Milk fat is not consistent in composition. Hensen and Shorland (1951; 1952) analysed butterfat and reported that based solely on the melting-point-evidence of branched chain fatty acids, presented by Cason (1948), one of the two C_{17} fatty acids isolated (M.P. 54.4°) appears to be the iso acid (15 methyl-hexadecanoic acid) while the other (M.P. 39.8°) appears to be the ante-iso-acid (14 methyl-hexadecanoic acid). These two C_{17} methyl branched chain fatty acids are isomeric with normal heptadecanoic acid. A C_{20} saturated acid fraction containing at least three and possibly four methyl groups was isolated from butter fat. This acid has a low solidifying point and very low melting point of -7°C.

Carolyn Boatman, Decoteau, and Hammond (1961) observed that the amount of trisaturated glyceride varied from 21.5 to 32.0% by weight. They found that there was no preferential selection or exclusion of any of the major saturated fatty acids from the trisaturated glycerides. The relation among the melting point of a fatty acid and the melting point and amount of trisaturated glyceride,

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which has been found to hold for many fats, does not hold for milk fat. The heat of fusion of the last triglyceride in the fat to melt changes from sample to sample.

Smith and Lowry (1962) separated the lipids in milk, and found the major phospholipids in milk to be

Phosphatidyl cholines (PC)

Phosphatidyl ethanolamines (PE)

and Sphingomyelins.

Patton _et al._ (1964) obtained the same classes of phospholipids, mainly cephalins, lecithin, and sphingomyelin, in similar proportions with much the same total fatty acid composition in milk, skim milk, cream, and butter milk.

Virtanen (1966) reported that when vegetable oil was fed, the unsaturated fatty acids which occur in lesser amounts especially C_{16} and C_{14} acids, were higher in the fat of the test milk than in the fat of the normal milk. The amount of branched chain fatty acids was increased in the fat of the test milk.

Laura Evans and Stuart Patton (1962) observed that the high density lipoproteins had a strong binding capacity for cholesterol and phospholipids and that low density lipo-

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proteins showed a great affinity for cholesterol palmitate and tripalmitin. Palmitin and linoleic acids were bound almost entirely by serum lipoproteins. Cholesterol exchanged between high and low density lypoproteins, but the rate of transfer is in the direction of the high density lipoproteins. Cholesteryl palmitate moved only from low to high density lipoproteins. Tripalmitin and phospholipid did not transfer in either direction. Palmitin and linoleic acids transfered from both lipoprotein groups to the serum proteins. Brown _et al._ (1962) in their analysis observed that cottonseed oil depressed the acids of milk fat from C_6 through C_{14}, but increased the C_{18} acids. Smiths and Jack (1954 a; b; c.) in their analysis of milk fat established the presence of conjugated and non-conjugated polyethanoid constitutents. The small values of conjugated trienoic constitutents were relatively constant and also the trace amounts of tetraenoic systems agree with the result of other workers. The non-conjugated dienoic acids fluctuated less and non-conjugated trienoic and tetraenoic acids were observed. Polyethenoid constituents were concentrated mainly in the

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C_{18}-C_{20} fraction. Relatively more of the conjugated components formed urea complexes. There is likely to be a non-conjugated pentaenoic constituent. The C_{18} to C_{12} monoethenoid fractions have treans isomers whose approximate concentration ranged from 14 - 27% of the monoethenoid methyl esters. The unsaturated bond of decenoic acid occurs between the 9th and 10th carbons. The conjugated double bonds are principally cis-trans. The fatty acids of milk fat with shorter carbon chains than 10 do not contain double bonds.

Zaletel, Bird, Cannon, Wise and Kempthorne Bartley (1951) observed that the lactation trends in the majority of animals appear to be characterized by the production of fats with a high iodine value at the peak of milk production followed by decline in the iodine number to the 4th or 5th month of lactation and later a slight increase in iodine value to the end of lactation. The changes in iodine value were associated largely with changes in estimated oleic acid content of the milk fat. The percentage of linoleic acid decreased slightly in a linear manner during the lactation period. Pasture grazing during

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periods in which the effect of lactations was at a minimum increased the iodine value by an average of five units. This level was maintained when the cows were on pasture, but when the pasture herbage was removed from the diet, the iodine value dropped rapidly to levels considered to be normal. Pasture feeding had little effect on the linoleic acid content of the milk fat. Hensen and Shorland (1952) noted a regular increase in the content of C_6 - C_{14} saturated acids, and total saturated acids, beginning in July up to November, and afterwards a slow decline to the end of the season. The unsaturated acids varied in the opposite direction to that of the C_6 - C_{14} constituents. The butyric acid content declined throughout the season. Palmitic acid showed little change, but when expressed as a percentage of the total saturated acids it showed a tendency to increase throughout the season. The variations in content of C_{18} unsaturated fatty acids reflect the influence of the changing plane of nutrition throughout the year.

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2.5 MEASUREMENT OF RUMINANT FEEDING STUFF

Basically chemical analysis shows that food is composed of the same elements which go to form the animal body, and its products. The amount and proportion of the elements in each food vary greatly. However, these elements are built together to form compounds which are similar in kind and can be classified. The dry matter of all foodstuffs can be divided into organic and in-organic groups.

The ruminant feeds are evaluated by their chemical compositions or by "*in vivo*" or "*in vitro*" digestibility methods.

Chemical analyses involve the estimation of water, ash, crude fibre, crude protein, organic matter, dry matter, ether extract and nitrogen-free-extract. The Weende Station investigators were the first to establish a system of analysis over a century ago. This is known as the "Proximate Analysis". Most of the nutrients analysed are more or less uniform, but the crude fibre part of plants consist of a mixture of variable cabohydrates. Despite its short-comings, these methods of

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analysis still form the basis of most analytical procedures in use today, although it is now being widely improved or modified (Van Soet 1966; 1967; Ademosun <u>et al.</u> 1967; Ademosun 1970).

Ruminants can digest about 50% of the crude fibre of most feeds. There are differences in the breakdown of crude fibre from different sources. The polysaccharides of mature plants are less digestible than those of young growing plants. The crude fibre of growing pasture grass, fresh or dried, is more digestible than that of hay. The difference is due to chemical and physical structure, and also to the presence of certain substances like lignin which as the plant matures get deposited in the cell wall. Intact fibre prevents the action of the digestive enzymes on other useful nutrients in the stems, leaves, seeds and every cell. Cell walls are ruptured by cellulose decomposing organisms as well as the movements of digestion. The amount of crude fibre present in the feeds of ruminants and the nature of the crude fibre determine the level of digestibility of the nutrients present in the feeds.

The end products of carbohydrate digestion in the

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rumen are volatile fatty acids (V.F.A's) and gases. Some of these acids are acetic, propionic, iso and n-butyric acids. Gases like ammonia, CO_2, CO and higher V.F.A's are also produced from microbial actions on proteins and nitrogenous compounds. Valeric and isovaleric acids and other higher V.F.A's and combustible gases like CH_4 are produced by this microbial fermentation of carbohydrates.

"In vitro" digestibility analysis offers a practicable method of examining the potential nutritive value of various feeds, and indicates those which may justify further animal evaluation. Among commercial varieties of cocksfoot, the early "stemmy" varieties were in general more digestible than the late "leafy" ones, while with any particular maturity type, the stemmy varieties tended to be more digestible than the leafy. This indicated that the generally held view that leafiness is associated with high nutritive value is not necessarily correct. Raymond, and Terry (1966) in their work on various animal feeds observed that in cocksfoot, immature stem and leaf sheath are at least as digestible as leaf. Only as the plant matures does the stem become less digestible than leaf. At

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maturity a leafy variety might be expected to be the more digestible. Alexander and Mary McGowan (1961) described a method for the "*in vitro*" determination of digestibility of herbage. In this procedure two stage digestion developed by Tilley and Terry was used. The rumen liquor stage ended after forty eight hours. The pH of the medium was adjusted to 1.2 and pepsin solution added to digest the remaining protein. The hyflo supercel filter aid used along with fibre glass filter paper quickened the filtration. Digestible dry matter content of the samples gave a coefficient of correlation of r = + 0.97 with the "*in vivo*" figures. Armstrong, Alexander, and Mary McGowan (1964) modified their former method by adding ammonium sulphate to the rumen liquor buffer mixture.

Barns (1965) in his own "*in vitro*" experiment calculated the energy intake, the relative intake, and the nutritive value index.

Fina, Teresa, and Bartley (1958) observed that the rumen liquor decomposed completely in less than 48 hours 500 mg. of cellulose. They reported that anaerobic conditions must prevail throughout the trials in order to

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obtain maximum cellulose digestion. Protozoal activity was sustained for twenty four hours, but after thirty eight hours this activity was greatly reduced. The rumen temperature and pH vary and they affect the rate of decomposition of the cellulose.

McDougall (1948) reported that saliva lubricates the feed and aids the process of mastication. In the ruminant it forms a fluid medium for transporting food back to the mouth for remastication and downwards through the stomach to the small intestine. It forms also a buffered medium in which the micro-organisms of the rumen can flourish. There are four salivary glands but the parotid produces quite a lot. The dry matter in gm. per 100 ml saliva was 1.28 while the ash content was 0.97. Sodium formed the largest part of the ash followed by phosphorus and chlorine, potassium and nitrogen are present in small amounts while calcium and magnesium are in traces. Carbon dioxide is also present in saliva. Saliva is slightly alkaline, its pH at room temperature ranges from 8.05 - 8.46, but this may decline to between 7.96 - 8.26 with rise in temperature. This is the reason why artificial

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saliva solution is added to the medium in "*in vitro*" digestibility studies.

Extensive grazing is the chief form of land use in the dry pastural tropics. These lands are usually sparsely populated, rainfall is variable and pasture yields are low. The patterns of growth of pastures have the main feature of laternate poor growth with good and abundant growth periods. Where conditions are good, pasture yields in the tropics and also the output in terms of animal production compare favourably with those of temperate countries. Live weight gain recorded with cattle fattened on Pangola grass pastures in Jamaica were as high as expected from temperate pastures.

The amount of herbage eaten by grazing animals is estimated from data from their faeces output together with estimates of the digestibility. Grazing animals choose the younger and presumably the more digestible components of the pasture. This shows that the pasture harvested mechanically is not likely to be of the same digestibility as that selected by the animal.

Direct and indirect methods have therefore been

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developed for predicting herbage digestibility. The direct method for determining herbage digestibility includes the daily measurement of feed intake and faces out put. This method is good for stall-fed animals, but leads to a lot of error when applied to grazing animals. Many investigators have therefore evolved some indirect methods for estimating the digestibility of herbage by grazing animals. Some of these workers are Forbes and Gerrigus (1948; 1950); Raymond (1951); Lancaster (1949); and Reid et al. (1952).

Markers have been used by nutritionist in trials involving omnivora and carnivora. A marker is substance consumed as a part of the meal in a digestibility trial and is also given as a part of the meal after the end of the experiment. Some of the makers that are in use are iron oxide, bone black and chromic oxide. Other substances used as index in digestibility trials are lignin, crude fibre and methoxyl and various dyes.

Marker substances are not suitable for use with herbivores, because the colouring substance from the first and second feedings are mixed either in the rumen or in the caecum. This causes no sharp division between feedings.

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In order to avoid quantitative collection of faeces and quantitative records of feed intake, index substances are used. Index substances are substances that may be consumed by, or administered to an animal, but are entirely inert in the digestive tract, and so are completely and regularly excreted uniformly mixed with the faeces.

Index methods:

Nitrogen as faecal index:

This method involves the estimation of nitrogen in the feed and faeces. Lancaster (1954), Raymond and co-workers (1948, 1954) Reid (1952) and Gallup and Briggs (1948) Kennedy, Carter, and Lancaster (1959) used this method. There have been differences in the digestibility predicted by different workers due to analytical methods, differences in animals and the types of herbage. Green-Halgh and Corbett (1960), GreenHalgh, Corbett and Mc Donald (1960), Minson (1958) and Raymond and co-workers (1956) also estimated digestibility of the forages used in their experiment from faecal nitrogen. Use of nitrogen as an index is prefered to chromogen because nitrogen is easier to determine. Lancaster (1949) showed that

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Metabolic faecal nitrogen is excreted in amounts directly proportional to the dry matter intake of the animal, its concentration in the faeces will be proportional to dry matter digestibility. GreenHalgh and Corbett (1960) and GreenHalgh *et al.* (1960b) observed that Spring and Summer trials showed the same differences as the digestibility and faecal nitrogen relationship. Faecal nitrogen differed in value for the first and aftermath growths.

Plant chromogen as Faecal Index:

The chromogen in the feed and faeces were analysed, and equations formulated to determine the digestibility of the feed. Plant chromogen as faecal index has been used by Raymond *et al.* (1954 a; b), Reid *et al.* (1952), Kane and Jacobson (1954), Kane *et al.* (1953), GreenHalgh and Corbett (1960a), GreenHalgh, Corbett and McDonald (1960). The handicap in chromogen experiments is that the equations were not more precise than nitrogen equations. In fact incomplete extractions leading to high recoveries and the extracts increasing in optical density cause a lot of problem.

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Chromic Oxide:

Chromic oxide appears to be the most generally satisfactory marker of dry matter yet tested. Difficulties in its use are probably due more to inadequate mixing with digesta than to its physical properties. It has been used successfully in digestibility trials by Corbett, GreenHalgh, Gwym and Walker (1958), Christian and Coup (1954), Coup and Lancaster (1952), Balch *et al.* (1957), Moore (1958), Olubajo (1969). It has been used on ruminants and non-ruminants.

Faecal chromic oxide concentrations are more variable in grazing than in hand fed animals. Corbett *et al.* (1958) observed that even when a marker is well mixed with the contents of the alimentary tract, variable excretion would occur if feed constituents of different digestibilities become stratified in passage through the gut or absorption varied at different times of the day. Variable marker excretion by ruminants arises usually from uneven clearance from the four-chambered stomach. Uneven excretion may also arise from the rapid passage of a portion of the dose before thorough mixing with digesta.

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Identification of faeces of grazing animals in digestibility trials:

The experimental animals used in digestibility trials are fed polystyrene particles of different colours. These ensure a rapid and good identification of faeces voided by each animal. In some cases the polystyrene is ground into powder or cut into cubes of variable sizes. Minson, Taylor, Alder, Raymond and Rudman (1960) and Corbett *et al.* (1960) also used coloured particles in their investigations.

Lignin and methoxyl groups have been used successfully by many investigators in digestibility studies (Ely, Kane, Jacobson and Moore, 1953; Richards and Reid, 1952; Anthony and Reid, 1958).

2.6 MEASUREMENT OF VOLATILE FATTY ACIDS

Fatty acids of compounds of plant and animal origin have been determined in the past by fractional distillation and by displacement of the lower homologues from substances like charcoal (Holman and Hagdahl, 1948; Clarke, 1928; 1943). James and Martin (1952) were pioneers in investigations involving gas - liquid chromatographic separation of volatile fatty acids. Since then chromotographic separation of short and long chain volatile fatty

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acids, and methyl esters of saturated and unsaturated acids, and other volatile compounds and gases in different feeds and animal products like silage, cheese, milk, fat, butter fat, tissue fluids, blood plasma and blood serum have been carried out by many investigators (Storry and Millard, 1965; Bills, Khatri and Day, 1963; Anderson and Hollenbach, 1965; Jennings, 1957; James and Martin, 1965; Deman, 1946; James and Web, 1957; Mobbitt, Gillian Mick-innon, 1963). The method of James and Martin has been modified by many workers (Storry and Millard, 1965; James, 1960; Bills, Khatri and Day, 1963; Anderson and Hollenbach, 1965). Other forms of chromatography like the thin layer, paper chromatography and displacement separation technique have also been evolved (Kuramoto, Jezenski and Holman, 1957). Chromatographic methods may be used to fractionate a natural product, isolate its components, characterize, and to a limited extent, classify the original mixture as well as its constituents components. Choosing a chromatographic technique to be employed for the separation of a compound depends on the amounts to be separated, and the physical state of the sample. No

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single chromatographic method at present is good enough for the complete separation of a complex mixture of compounds. Accurate temperature control of the column and often of the detector is necessary when highly reproducible results are required. Provided the temperature, quantity of stationary phase in the column, and the flow rate of gas are kept constant, a given acid always emerges at the same time. Peaks are recorded by the detector and the peak areas measured by triangulation, planimeter, by cutting out the peaks and weighing and by a recording integrator.

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CHAPTER 3

MATERIALS AND METHODS

3.1 HISTORY AND MANAGEMENT OF THE EXPERIMENTAL PLOTS

The experimental plots are located within the low-land rain forest zone with a mean annual rainfall of 48 inches. There are two main seasons in the year: one of which is the dry season which starts from November or late October to February, and the other is the rainy season which starts from March to October or November, with a short break in August.

The experimental plots are located on the Western part of the teaching and research farm. The plots are situated on the North Western side of the University of Ibadan Campus. Clearing of the site was started in March 1961, when a few acres were planted with Kyasua grass plus centrosema for silage. A few more acres were ploughed in 1965 to make a total of 12 acres. The 12 acres were divided into four randomized blocks of 3 acres each, and each was replicated 4 times. Each block contains the 4 treatments H, J, K and L. Each treatment is $\frac{3}{4}$ acre in size and each is sub-divided into $\frac{1}{4}$ acre size plots to faci-

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litate effective grazing. This makes a total of 12 sub-plots per treatment. Each plot was fenced round with barb wire and water troughs and salt licks provided in easily accessible spots.

After ploughing, tripple super phosphate was applied to the soil at the rate of 1 cwt per acre by tractor broad-caster. The two legumes were planted first, and the grass-es planted two weeks later. The grasses and legumes were planted in rows, and they alternated with one another within the rows. Cutting back was carried out at the end of every grazing experiment, by a hand slasher, to a height between 6" and 8" above the ground. At the onset of the rainy season, fertilizer application by hand is carried out yearly at the rate of 2 cwt of single super-phosphate plus 1 cwt of sulphate of ammonia per acre.

Experimental Design

The four established pastures used in this experi-ment, designated H, J, K and L are composed as follows:

Treatment H consists of *Cynodon plectostachyus*. Pilger
Centrosema pubescens Bentham
and *Stylosanthes gracilis* H.B.K.

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Plate 1

One of the fistulated cows on the field.

Treatment J	*Pennisetum purpureum* Schum
	Centrosema pubescens
	and *Stylosanthes gracilis*
Treatment K	*Pennisetum purpureum*
	Panicum maximum Jacq
	Centrosema pubescens
	and *Stylosanthes gracilis*
Treatment L	*Pennisetum purpureum*
	Panicum maximum
	Cynodon plectostachyus
	Centrosema pubescens
	and *Stylosanthes gracilis*

These grasses and legumes have been described in chapter 1.

3.2 The White Fulani (Zebu) cattle

The live-stock of West Africa were classified by Mason (1951) and later by Faulkner and Epstein (1957). The possession of hump was used to classify the live-stock. Mason and Maule (1960) in their further work on classification of livestock of Eastern and Southern

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Plate 2
Another fistulated cow on the field.

Africa also used the presence of hump as their basis of classification, while Joshi and Phillips (1953) used the presence of horn in their classification of cattle in India and Pakistan.

Tse-tse fly affects the belt of the African Continent that lies approximately between latitude 15°N and 15°S, covering some 4,500,000 square miles of territory. This fly causes trypanosomiasis in cattle, but the Zebu is fairly tolerant to this and other diseases like red-water-fever, foot and mouth and anaplasmosis disease. The Northern States of Nigeria are relatively free from tse-tse flies, while the Southern States are within the tse-tse fly belt.

The White Fulani cattle belongs to the Lyre-horned Zebu group also found in Senegal, and the Sudan. It is the most widely distributed and most promising cattle in Nigeria. This breed possesses a heat resisting mechanism that is of prime importance. It has a close glossy coat, thick skin with large folds at the dewlap which starts from the throat and goes as far as between the front legs. The dewlap and sheath give a much greater surface area per

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Plate 3

Fistulated steer grazing on the field.

unit of body weight than in European breeds. The White Fulani possesses an efficient sweating mechanism which helps it to survive the heat of the tropical climate.

3.3 Health of the Animals

All the animals were in good health at the beginning of the experiment. The veterinarians in the department of veterinary medicine were responsible for keeping the animals in good health. The animals were sprayed routinely with insecticide against tick, but one of the fistulated steers died at the end of the experiment on treatment J.

3.4 Plan of the Experiment

Eight White Fulani animals were used in these experiments. Six of them were cows while the remaining two were steers. The two steers and two of the six cows were fistulated. The remaining cows were lactating cows. All the animals had access to fresh water in troughs and salt licks daily. The animals weighed between 286 kg. and 376 kg. The cattle were divided into two groups of four animals. Each group consisted of two intact plus one fistulated cow and one fistulated steer. One group was fed treatment H

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Plate 4

Fistulated steers and cows in the field.

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at 4 weeks, while the other was fed treatment H at 12 weeks. All the fistulated animals were kept in stalls and fed the same forages as their group mates grazing in the field. The same procedure was adopted for treatments J, K and L.

3.5 <u>Feeding of Animals and Sample Collection</u>

During the experimental period, all the animals in the stall received 60 lb of fresh herbage daily. Representative samples of pasture herbage were cut and chopped into bits. 60 lb of the fresh herbage was divided into two equal parts, one part fed at 8.30 a.m. and the remaining part fed at 4.30 p.m. The residue for each day was collected, weighed, and 100 gm. (1 lb) representative samples taken for chemical analysis. 100 gm (1 lb) representative samples of the fresh herbage was also taken for chemical analysis. 50 gm. representative samples of fresh herbage and residue were also taken for Dry matter analysis.

Each animal in the stall and field was fed 20 gm. chromic oxide paper wrapped in duplicating paper. The marker was fed at 8.30 a.m. and 4.30 p.m., making a total of 40 gm. paper per day (14.2 gm. Cr_2O_3 per day). The

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Plate 5

Fistulated steer on the field.

paper containing the chromic oxide was lubricated with liquid paraffin and administered with a balling gun into the oesophagus of the animal. The administration of chromic oxide continued throughout the experimental period. For identification of faeces from each grazing animal, 20 gm. polystyrene particles encapsulated in gelatine capsules was given at 8.30 a.m. Administration started three days to faeces collection and ended two days to the end of the experiment.

Faeces Collection

The animals in the stall were not fitted with digestibility bags, but the faeces voided were collected and weighed as soon as they are voided. The faeces were then kept in polythene bags. The morning collections started from 9 a.m. and the evening collections started from 4 p.m.

The faeces of the animals grazing in the field were collected immediately they were voided. Faeces for each animal were compounded and weighed, and 100 gm. representative sample taken for chemical analysis. 100 gm. representative samples were also taken for Dry matter deter-

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mination. The faeces collection was for six days after a seven day preliminary period. Faeces samples not immediately analysed were kept in the deep freeze at -5°C to -8°C.

Drying of Samples and milling

100 gm. representative samples of the fresh feed offered to the animals were dried in a Gallenkamp air oven at 65°C to 70°C for 48 hrs. The dried samples were milled in an 8" Gallenkamp laboratory hammar mill to a fineness of 4 mm. diameter mesh. 100 gm. representative samples of the grass residues and faeces voided were also dried and milled in like manner. Sub-samples of the grass samples were taken and milled in a micro mill to a fineness of 1 mm. diameter. The milled samples were kept in well stoppered Killner jars until ready for analysis.

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CHAPTER 4

BOTANICAL ANALYSIS

4.1 INTRODUCTION

Grazing animals influence the botanical composition of pastures, their yield and chemical composition. Frequency of cutting pasture and climatological factors and topography of the land also affect botanical composition of pasture. When pasture is cut more frequently, the production is reduced and due to exposure of soil to rain and sunlight, the growth of other species of grasses or legumes may be encouraged (Akinola *et al.* 1971; Tuley, 1968; Chheda *et al.*, 1971; Smith, 1964; Patterson, 1933.) In grazing trials therefore it is necessary to carry out botanical analysis in order to know the changes in the pasture composition due to grazing and trampling, and the contribution of each component to the performance of the animal.

Methods of botanical analysis have been developed, but no one method is very accurate (Brown, 1954; Pasto, Allison and Washko, 1957; and Van Keuren and Ahlgren, 1957) Botanical analysis of pastures may be done by visual

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estimation of herbage as it is on the field, or by visual estimation of cut herbage samples in the laboratory or by hand separation of the cut herbage into the different species making it up. The last method is by far the best in that it is free from bias.

4.2 METHOD OF ANALYSIS

The botanical analysis of the pasture treatments was carried out in October to December 1969, and in December 1970 and January 1971. Eight samples were taken per plot during the experimental period. The samples were taken to the laboratory in cellophane bags and hand-separated into individual species. Each component was dried separately in tins in the oven at 100°C for 48 hours and the dry weight recorded. Each species was then calculated on dry matter basis.

4.3 RESULTS AND DISCUSSION

Table 4.1 shows the results of the botanical analyses for the last three months of 1969. The table showed that for treatment H, Giant star grass contributed about three quarters of the feed intake of the animals. Centrosema made up about 10 percent of the composition and weed

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growth made up just over 5 percent. STYLOSANTHES was not present at all in the grass samples. The absense of *Stylosanthes* throughout the experimental periods in 1969, 1970, and 1971 could be that it could not compete very well with the other species of grasses grown. The frequency of cutting and the trampling effect of the grazing animals may be another factor which led to its complete eradication. The seeds produced by *Stylosanthes* also need some scarification before they can germinate well but new scarified seeds were not planted every year, so that poor and slow rate of germination together with the depth to which they were buried did not favour their regrowth.

Gaint Star Grass population remained fairly constant for the three months. The *Centrosema* population decreased from October onwards. The drop in population from October to November was more than that from November to December. The weed population rose sharply from October to November, but rose only slightly from November to December.

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TABLE 4.1

MEAN DRY MATTER BOTANICAL COMPOSITION OF TREATMENT SWARDS

	TREATMENT H				TREATMENT J				TREATMENT K					TREATMENT L					
	G. Star	Centro-sema.	Stylosan-thes.	Weed	E grass	Cen tro-sema.	Stylo-santhes	Weed	E grass	G grass	Centro-sema.	Stylo-san-thes.	Weed	E grass	G Star grass	G grass	Centro-sema.	Stylo-san-thes.	Weed
1969																			
Oct.	72.88	24.35	0	2.78	78.25	20.08	0	1.67	47.29	40.98	10.97	0	0.94	42.43	21.22	24.80	10.41	0	1.14
Nov.	72.30	20.83	0	6.87	79.60	16.31	0	4.10	48.33	35.83	9.17	0	6.67	33.16	26.70	29.82	9.60	0	0.73
Dec.	72.08	20.10	0	7.82	78.81	16.84	0	4.35	46.52	37.60	8.68	0	8.20	32.75	27.52	30.25	8.00	0	1.48
Mean	72.42	21.77	0	5.82	78.89	17.74	0	3.37	47.38	38.14	9.55	0	4.94	36.11	25.15	28.29	9.34	0	1.12
1970																			
Dec.	71.97	21.20	0	6.83	77.56	17.00	0	5.44	47.86	38.24	9.20	0	4.70	33.12	27.50	28.88	8.50	0	2.00
1971																			
Jan.	70.00	22.50	0	7.50	75.60	18.20	0	6.20	45.95	40.00	9.00	0	5.05	33.20	24.15	30.00	10.00	0	2.65
Mean	70.99	21.85	0	7.17	76.58	17.60	0	5.82	46.90	39.12	9.10	0	4.88	33.16	25.83	29.44	9.25	0	2.33

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There seems to be a trend that as the population of the legume Centrosema decreases in the sward, the weed population increases.

In treatment J, Elephant Grass also contributed the major part of the animal intake. Centrosema made up just under 20% of the population while the weed population was under 4%. The range of the population percentage of the Elephant Grass was between 78.25% and 79.60% which is narrow. The range for Centrosema was from 16.31% to 20.08% which is wider than the one for grass. The range for the weed population during the three months was from 1.67% to 4.35%. The weed population increased from October to December. The increase was sharp from October to November. A similar trend observed in Treatment H was also observed in Treatment J. The weed population increased as the legume population decreased.

In Treatment K, Elephant Grass made up under half of the total sward population. Guinea Grass also made up an appreciable amount of the sward population. The two grasses made up 85.52% of the total sample. Both grasses contributed more to the animal feed than the single grass in

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treatment H or J. The centrosema population decreased appreciably in this sward, since it made up under 10% of the total population. It decreased progressively from October to December. The weed population also increased sharply from October to November, but the increase from November to December was not sharp. The weed population ranged from under 1% in October to over 8% in December.

In Treatment L, Elephant grass also contributed most to the animal feed than the remaining two grasses, although its contribution was less than in Treatment K. Guinea grass was more in the sward than Giant Star grass. The three grasses made up 89.55% of the total sward population. Centrosema accounted for just over 9% of the total population, while weed was just over 1%. Elephant grass population decreased sharply from October to November, and decreased just slightly from November to December. Giant Star and Guinea grasses populations on the other hand increased sharply from October to November, but thereafter increased slightly. The weed population showed very little change, while Centrosema population decreased steadily. An observation here is that the more grass in

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the sward the less weed is found, and the decrease in legume population is gradual.

In December 1970 and January 1971, botanical analyses of the swards showed similar trends. In Treatments H and J however, Giant Star Grass population was less, Centrosema population remained the same while the weed population increased. In Treatment K the two grasses together formed the largest component of the Sward than in 1969, but the increase was small, and the weed population also decreased slightly than the 1969 value. The three grasses in Treatment L were about 1% less than the 1969 value and the weed population increased by about the same value in 1970/1971.

The inability of Stylosanthes gracilis to remain permanently in pastures used mainly for grazing has been shown by the investigations of Okorie, Hill and McIlroy (1965), Adegbola (1965), Foster (1961), Nwosu (1961), and Miller et al. (1964).

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CHAPTER 5

MICROBIOLOGY

5.1 INTRODUCTION

The ruminants are noted for the large number of micro flora present in the rumen. The bacteria and protozoa are responsible for fermenting some part of the ruminant feed which are not normally digested by the digestive enzymes. However, the population growth and predominant species of the microbes, rate of fermentation, and type of end product of fermentation of the feed, are affected by many factors, some of which are, the pH of the medium, type of feed, enzymes, vitamins, essential minerals, amino acids, and fatty acids (Bryant and Doetsch, 1954b; Wegner and Foster, 1963; and El-Shazly, 1952a; Scott and Dehority, 1965; Bryant, Robinson and Chu, 1959; Salisbury <u>et al.</u>, 1961). The rumen bacteria function at pH between 5.5 and 7.0 anaerobically, although wider pH values (5.5-8.5) have been reported (Raymond and Terry, 1966). The optimum termperature for their function ranges from 38 - 40°C, and they also need some amount of fermentation products

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(Wegner and Foster, 1960). Naga, and El-Shazly (1969) collected rumen samples from water buffalo and Zebu cattle before feeding, on two successive days. In their microscopic examination they found the protozoa belonging to the genius *Entodinium* to be predominant under all conditions. There was a sharp drop in the total number of protozoa in the rumen liquor in water buffalo when sweet Sudan was fed. The relative concentration of *Entodinium* was also reduced in favour of *Isotricha* and *Eudiplodinium*. Almost similar number of ciliate protozoa were found in the bulls and buffaloes - fed on berseem, but when concentrate and straw were fed, the number in bulls were double those in buffaloes. Both bulls and buffaloes had similar genera of protozoa, but their relative concentrations and sizes were different. Large *Oligotricha* were abundant in buffaloes.

5.2 METHOD OF ANALYSIS

Rumen bacterial and protozoal populations have been counted by Culture counts (Kistner, Gouws and Gilchrist, 1962; and Claypool, Jacobson and Wiseman, 1961). In this procedure a special habitat similar to the rumen medium

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was used. Liquid or solid media may be used, certain precautions should be taken during dilution and counting: such precautions are to avoid oxygen and unfavourable pH and temperature. The other method is the Direct Counts, which takes into account the total number of bacteria present, the total dead and living bacteria, and the number of individual species present. It is the most reliable method. It is a quick and easy method also. Treatment H at 4 and 12 weeks was fed to three fistulated ruminants and rumen samples taken from them for bacterial and protozoa counts.

The Direct Counts method was adopted in this work. Since rumen bacteria are many, the rumen liquor was diluted, and the cells killed and stained to render them more visible. Good staining solutions have been developed by Hobson and Mann (1955; 1957), Moir (1951), and Hungate (1957). The actual counting for bacterial population was done with NEUBAUER COUNTING CHAMBER, although other counting chambers specially designed for bacterial counts have been developed (Ubrner, 1962).

Direct Counts for the protozoal population was done

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using a counting cell covered with cover slip. Counting procedures have been described by Warner (1962 a; b), Purser and Moir (1959), and Boyne Eodie and Raitt (1957). Microscopic examination was carried out to identify morphological types. Details of the Analytical procedures can be found in Appendix V.

5.3 RESULTS AND DISCUSSION

Table 5.1 showed the protozoa and bacterial population during the experimental period in 1969. The table showed that bacteria formed the greater component of rumen microflora. The protozoa were many also, but from the result they formed less than one million in cattle. The population varied a lot and many dead protozoa were visible. On two occasions no living protozoa could be found in the ruminal fluid of the stall-fed steer. There were more protozoa in the goat ruminal fluid when the goat was kept in the stall than when it was grazing in the field. The steer contained relatively more protozoa in the liquor than the cow. The liquor contained far less amount of protozoa before feeding in both steer and cow, while the bacterial population was more in the cow than the steer.

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TABLE 5.1

RESULT

Ruminal Protozoan and Bacterial Counts

Date	Feed	Stage of growth wks	Animal	In Stall	Protozoa per gm of content	Bacteria per gm of content
2/9/69	H	12	Steer 114	"	247,400	1,650,000,000
9/9/69	H	4	Goat	"	1,560,000	1,400,000,000
16/9/69	H	12	Steer 114	"	220,000	1,250,000,000
23/9/69	H	12	Steer 114	Grazing	90,000	1,700,000,000
23/9/69	H	4	Goat	"	78,000	1,200,000,000
30/9/69	H	12	Steer 114	Stall	Dead protozoa 84,000	3,000,000,000
3/10/69	H	12	Steer	"	Dead protozoa 66,000	3,200,000,000
6/10/69	H	12	Steer 114	"	120,000	6,500,000,000
14/10/69	H	12	Steer 114	"	118,000	12,250,000,000
22/10/69	H	4	Goat	"	3,080,000	6,500,000,000
4/11/69	H	4	Cow 49	"	340,000	7,750,000,000
11/11/69	H	4	Cow 49	"	180,000	10,200,000,000
18/11/69	H	12	Steer 114	"	72,000	7,500,000,000
18/12/69	H	4	Before feed Cow 49	"	4,000	10,200,000,000
18/12/69	H	12	Before feed Steer 114	"	Less than 2,000	3,550,000,000
18/12/69	H	4	Before feed Steer 198	"	Less than 2,000	4,550,000,000

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The number of bacteria present in the liquor taken from the steer when there were living protozoa did not vary much whether the steer was stall-fed or grazing. When all the visible protozoa were dead, the bacterial population increased rapidly to about three times the former population. The cow and steer contained more bacteria in their liquor when the protozoal population decreased, but in the goat, the greater the number of protozoa the greater was the bacterial population. The cows liquors contained more bacteria per gm of content than the Steer. The difference may be due to the fact that the Cows were fed the four week old forage.

Some of the bacteria seen under the microscope were rodshaped. They may belong to the genera Bacteroides and Clostridium. The members of these genera digest cellulose and sugars like glucose, cellobiose, maltose, sucrose, and starch.

Some roundish or cocci forms of bacteria were also identified. Some of them were in aggregation while others stood singly. They are likely to belong to the genus Ruminococcus. Ruminococcus species have been

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isolated by Sijpesteijn (1951); Hungate (1957); and Synge (1953). The cocci forms of bacteria are also cellulolytic.

The protozoa recognised were mostly ciliates which moved about the medium in fresh samples and a few flagellates. The flagellates were fewer in number, and the cilia in ciliates were all over or just in some parts of the body.

Both types of protozoa were easily visible from direct illumination. The ciliate protozoa recognised are probably members of the genera Isotricha, Entodinium, Eudiplodinium and Dasytricha. These have been identified by many investigators (Naga and El-Shazly, 1969; Clarke, 1964a; 1964b.) Most of them contained particles inside them, and these stained well with iodine solution. These are starch grains stored in their protoplasm. The protozoa also contained nuclei and contractile vacuoles.

The variation in protozoal and bacterial population, and the occurence of dead protozoa showed that the digestibility of a forage by "in vitro" procedure is affected to a large extent by the microflora. A check on the

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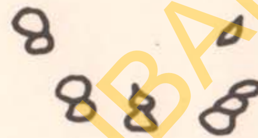
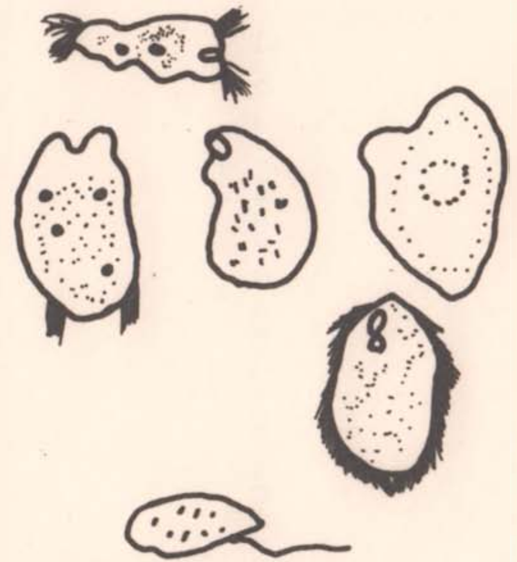
MICROBIOLOGY OF RUMEN LIQUOR.

ROD-LIKE BACTERIA



COCCI BACTERIA



RUMEN LIQUOR PROTOZOA

PLATE SHOWING THE TYPES OF BACTERIA AND PROTOZOA FOUND IN THE RUMEN LIQUOR OF WHITE FULANI CATTLE.

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microbiology also affords a measure of the health of the ruminant.

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CHAPTER 6

CHEMICAL COMPOSITION OF TREATMENTS H, J, K, AND L, AT 4 AND 12 WEEKS

6.1 INTRODUCTION

The nutritive value of a forage or pasture herbage may be estimated from its chemical composition. Chemical analyses show the amount of nutrients in the forages. Some of these nutrients are well defined (protein or nitrogen, minerals, glucose, starch, lignin, vitamins, cell-wall, N.F.E) while some are of mixed composition (crude fibre). These analyses show the extent at which a particular nutrient is present, the seasonal variation of each nutrient and whether a feed has toxic or inhibitory substances.

The Nutritive values of some tropical forages have been calculated from their chemical composition (Oyenuga, 1957; 1959a,1959b;1960a,1960b;Ademosun, 1970; Miller, 1963; Ademosun, et al., 1967; Miller et al., 1964; Patterson, 1933; Okorie et al., 1965) while those of temperate forages have also been carried out by many workers (Forbes et al., 1950; Ely et al., 1953; Richards et al.,

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1952; Anthony *et al.*, 1958.)

Chemical composition of forages has the disadvantage in that it does not show the availability of the nutrients to cattle.

The nutritive value of pasture forages can also be estimated from results of grazing trials (Smith *et al.*, 1955; Richards *et al.*, 1952; Topps, 1962; Olubajo, 1969; Olubajo et al., 1971; Raymond, 1954; Lancaster, 1954; GreenHalgh, 1960a, 1960b) This method is more reliable in that it shows the actual amount of a forage nutrient that is available to the animal.

6.2 ANALYTICAL METHODS

Sample collection and pretreatments for chemical analyses are described under chapter 3. The chemical analyses were carried out according to the official methods of analysis of the A.O.A.C. (1964). Determinations were carried out in duplicates and the results expressed on dry matter basis. All the reagents used were of the analytical grade.

Moisture: 2 gm duplicate samples of milled material were dried at 105°C in an electric oven to constant

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weight. The difference between the weights before and after drying gave the residual moisture content. Dry matter content was calculated from the results.

Total ash: The dried duplicate samples from the moisture determination were ignited at $600^{\circ}C$ in a muffle furnace for two hours or until the colour was whitish.

Organic matter: This was calculated as the difference between the weight of dry matter and the weight of total ash.

Ether extract: This was determined by using petroleum ether, (B.P. 40-$60^{\circ}C$) to extract the ether extract in the milled samples. Soxhlet Extraction apparatus was used, and each extraction lasted 6-7 hrs.

Crude fibre: The duplicate residues from the ether extraction were used. The fat free residues were boiled with 1.25% H_2SO_4 for 30 minutes followed by boiling with 1.25% NaOH for another 30 minutes. Each residue was filtered, washed, dried and weighed. The dried and weighed residues were ignited in a muffle furnace at $600^{\circ}C$ for 2 hrs, cooled and weighed. Crude fibre was calculated as the weight of dried material minus weight of ash.

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Crude protein was determined by the Kjeldahl method. Crude protein was calculated by multiplying total nitrogen content by the factor 6.25.

Nitrogen-free-extract (N.F.E). This represents the soluble carbohydrates in the feed. It was calculated as the difference between 100 and the sum of moisture, total ash, crude protein, ether extract and crude fibre.

6.3 RESULTS

The summary of the results is shown in table 6.1. Details of the results can be seen in Appendix VI. The mean monthly rainfall figures for the years 1967-1971 are shown in figure 6, while mean daily variations of treatment nutrients during the experimental periods are shown in figures 6.2-6.5.

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The results of the chemical composition of treatments, H, J, K and L cut at 4 and 12 weeks are shown in Table 6.1.

Crude Fibre

When all the forages were cut at four weeks, forage H had the highest crude fibre content, while forage J had the lowest crude fibre content. The difference between the highest and the lowest values being 2.84%. The difference between H and K was 1.65% and the difference between H and L was 2.80%. Forage K was 1.15% more than L, while forage L was 0.04% higher than forage J.

When all the forages were cut at twelve weeks forage H also had the highest crude fibre content, followed by J. Forage L had the lowest crude fibre content. The difference between forages H and J was 0.75%, between H and K was 2.76%, between H and L was 3.51%. The difference between forage J and K was 2.01%, between J and L was 2.76%, and between K and L was 0.75%.

Comparing the crude fibre content at the two stages of growth, forages, H, K and L contained higher levels at four weeks than at twelve weeks. Forage J was the only forage containing higher level of crude fibre at twelve

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TABLE 6.1

MEAN CHEMICAL COMPOSITION OF TREATMENTS H, J, K, AND L, CUT AT 4 AND 12 WEEKS

(% Dry Matter)

Feed Constituent	TREATMENTS				Time of Sampling (weeks)
	H	J	K	L	
Dry Matter	90.9	93.4	93.3	-	4
	89.8	92.6	94.7	-	12
Organic Matter	83.3	82.9	83.9	90.0	4
	82.3	81.3	82.9	7.5	12
Crude Protein	9.5	9.0	8.3	10.9	4
	9.8	8.6	7.2	9.9	12
Crude Fibre	35.3	32.5	33.7	32.5	4
	34.5	33.8	31.8	31.0	12
Ether Extract	1.1	1.1	1.1	1.0	4
	1.1	1.1	1.1	1.1	12
Ash	7.5	10.3	10.0	9.7	4
	7.4	11.4	12.1	11.6	12
N.F.E.	46.6	47.2	47.0	45.9	4
	47.2	45.1	47.8	46.5	12

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weeks than at four weeks. Forage H contained 0.81% more crude fibre at four weeks, while forage K contained 1.92% more crude fibre when four weeks, and forage L contained 1.52% more crude fibre when four weeks. Forage J contained 1.28% less crude fibre when four weeks.

However, analysis of variance for the crude fibre content of the four forages at the two stages of growth showed no significant differences between the forages and time of cutting ($P > 0.05$). All the forages except J contained higher crude fibre levels at four weeks than 12 weeks. One of the reasons for this may be due to leaf to stem ratio. At maturity many pastures plants produce a lot of leaves.

The treatments were cut at 4 and 12 weeks and a lot of chemical changes went on within the herbage during the 4 weeks interval. Pasture herbages increase in crude fibre content up to a point before the bvalue falls. This fall after a peak has been shown by Oyenuga 1958; 1960; and Ademosun 1970. The treatments except J must have reached the crude fibre peak production before 12 weeks. The crude fibre content at 12 weeks may be taken to be a

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decline as was shown by these workers. The lower crude fibre content at 12 weeks does not indicate better nutritive value or digestibility.

Another reason for the high crude fibre content at four weeks of growth may be because sampling was carried out while the forages were only a little height above ground. Therefore it was possible that a few old stumps have been sampled along with the young growing forages.

<u>Crude Protein</u>

Table 6.1 also showed that crude protein contents of all the forages except H were higher at 4 weeks than at 12 weeks. Considering all the forages at 4 weeks, forage L contained the highest level of crude protein, followed by H and then J and lastly K. Forage L exceeded forage K by 2.69%, it was also higher in crude protein than forages J and H by 1.90% and 1.40% respectively. H was slightly higher in crude protein than J by 0.56% and more than K by 1.29%. Forage K was only lower in crude protein than forage J by 0.73%.

At 12 weeks, forage L again contained the highest level of crude protein, followed by H and J and lastly K.

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Forage L exceeded K in crude protein by 2.67%, it was higher than J by 1.27% and H by as little as 0.06%. Forage H contained more crude protein (1.21%) than J and more than K by 2.61%. J contains more crude protein (1.40%) than K.

There were no significant differences between cutting intervals, but there were significant differences among treatments K and L, (Appendix VI).

Organic Matter

When the treatments were 4 and 12 weeks the organic matter content of all the treatments except L were above 80%, (Table 6.1.) Treatment K was consistently higher in organic matter than the rest at the two stages of growth. Treatment K was higher in organic matter than treatment L by as much as 3.88%, while it was higher than treatment J by only 0.94% and higher than treatment H by a still lower value of 0.54%. Treatment H was higher in organic matter than treatment J by only 0.40%, and higher than L by 3.34%. Treatment J was higher in organic matter than treatment L by 2.94%.

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At 12 weeks treatment K was higher in organic matter content than treatment L, and the amount this time was even higher, 5.44%, than at 4 weeks. Treatment K was higher than treatment J by 1.69% a value also higher than that of the early stage, while it was higher than treatment H by 0.69% a value also higher than that of the early state. Treatment H was higher in organic matter content than treatment J and L by just 1% and 4.75% respectively. Treatment J was higher than Treatment L in organic matter by 3.75%.

Analysis of variance showed significant differences among treatments at 5% level and between sampling periods at 5% level.

<u>Dry matter</u>

At 4 weeks the dry matter content of all the treatments except K was higher than at 12 weeks. The difference in each case was however not significant.

<u>Ash</u>

Ash content exhibited greater variation in treatments K and L at both stages of growth than in treatments H and J. Treatment H contained about the same ash content at both

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stages of growth, while the ash content increased with increase in maturity in treatments J, K, and L treatment K having the greatest increase with increase in maturity. The increases were not significant.

Ether Extract

This was the same for all the treatments at both stages of growth.

Nitrogen free extract (N.F.E).

The level of N.F.E. increased with maturity in all the treatments except treatment J. The increases were not significant, so also was the decrease in treatment J.

Figure 6.1 showed the monthly variations in rainfall (inches) for the years 1967-71. The total rainfall in 1967, 1968, 1969, 1970, 1971 was 32.87 ins, 79.4 ins, 41.7 ins, 55.3 ins, 48.2 ins, respectively. 1967 was the driest year since the amount of precipitation for each month was low. There was a small peak in June and the major peak was in September. The rainfall figure for September was only lower than that for 1969. January and December were the driest months, and the break in rainfall was always in August. The two rainfall peaks for every

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FIG. 6.1 MONTHLY VARIATIONS IN RAINFALL (INS) FOR THE YEARS 1967 AND 1968, 1969 1970 AND 1971.

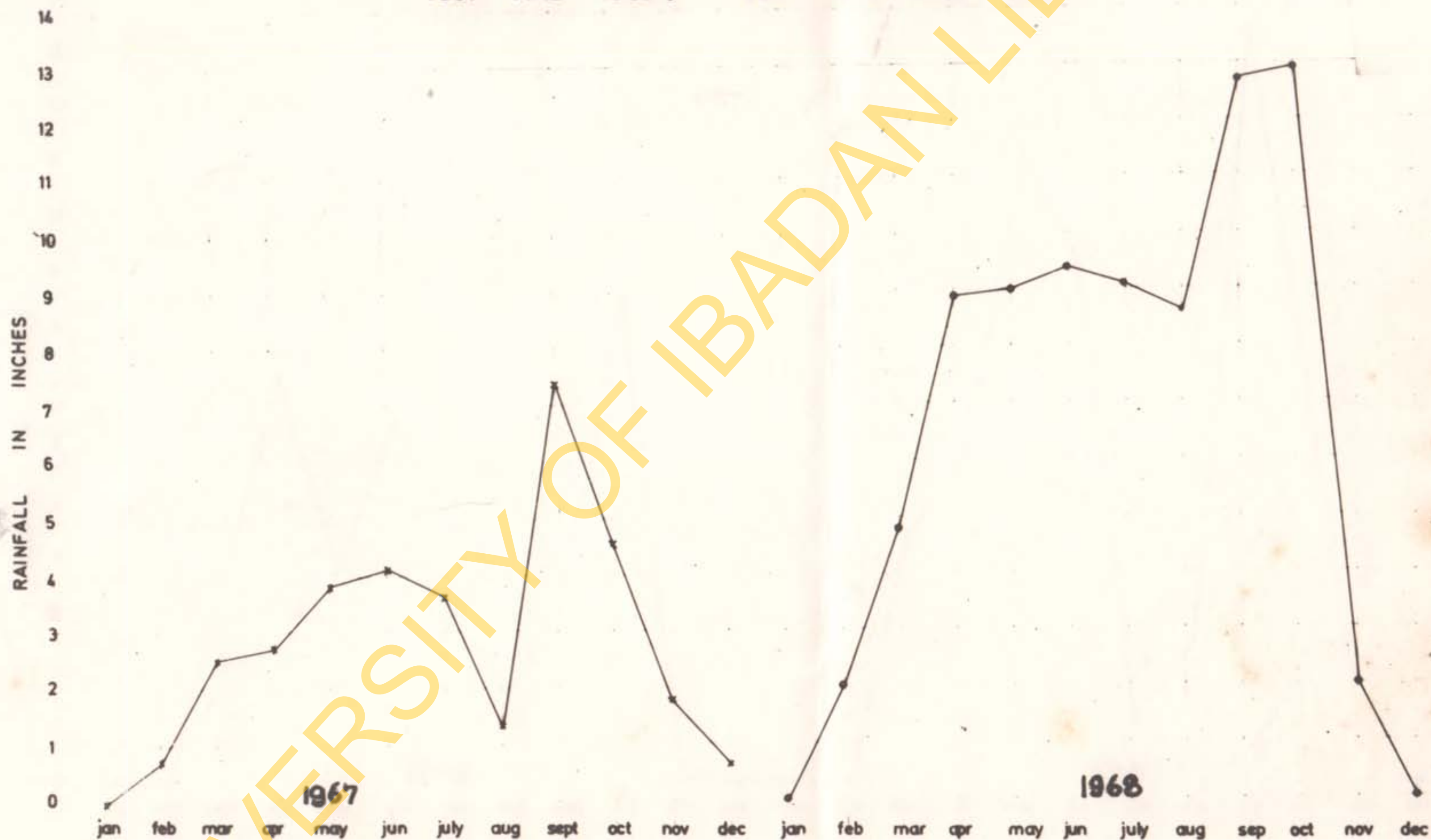

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FIG 6.1 MONTHLY VARIATIONS IN RAINFALL (INS) FOR THE YEARS 1969 AND 1970

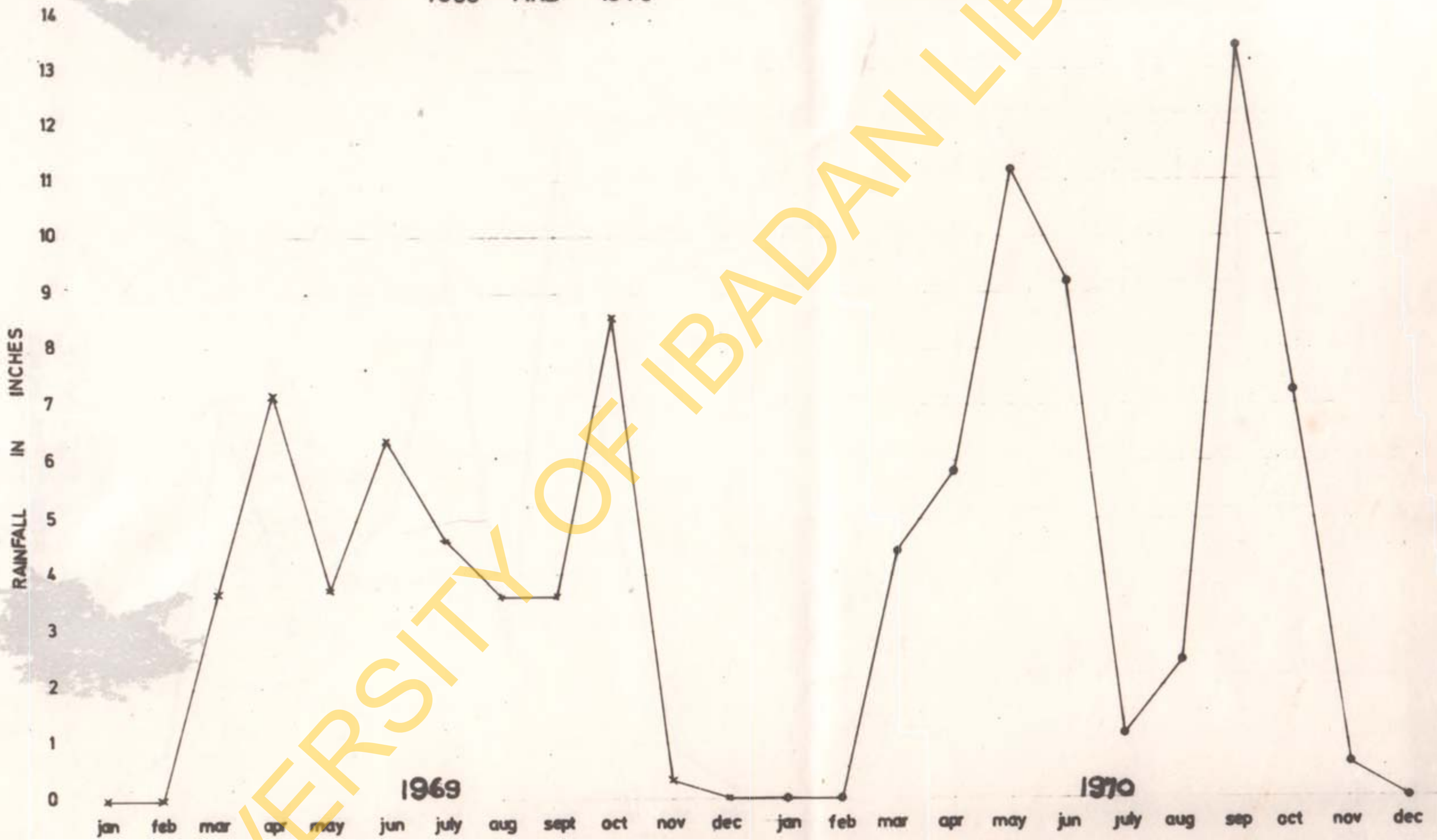

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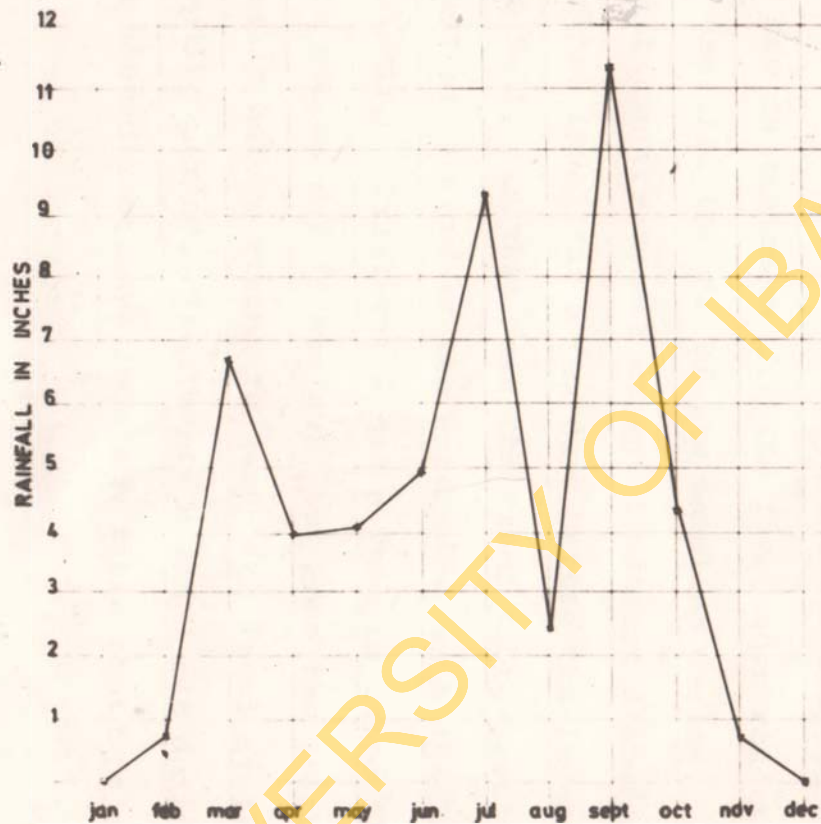
FIG. 6-1 MONTHLY VARIATIONS IN RAINFALL (INS) FOR THE YEAR 1971



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year were at June and September. There were however three rainfall peaks in 1969 and 1971.

Figure 6.2 showed the mean daily crude fibre variation of all treatments at 4 and 12 weeks for experimental period. The crude fibre content of all the treatments at 4 weeks except treatment J rose for a few days before falling. The fall was not continuous but broken by occasional rises during the experimental period. The levels at the end of the experiment were lower than those at the beginning of experiment. The crude fibre level however dropped after the first day in treatment J. The level then rose from the third day till the end of the sampling period. At 12 weeks all the treatments except treatment J increased in crude fibre level for a few days before the levels started to fall. In this case the crude fibre levels were still higher at the end of the experiment than the levels at the beginning. Treatment J had a fall in curde fibre content the second day it was cut, but rose the third day and fell again before rising to very high level at the end of the sampling period. Treatments H and J cut during the dry months showed increases in crude fibre contents of

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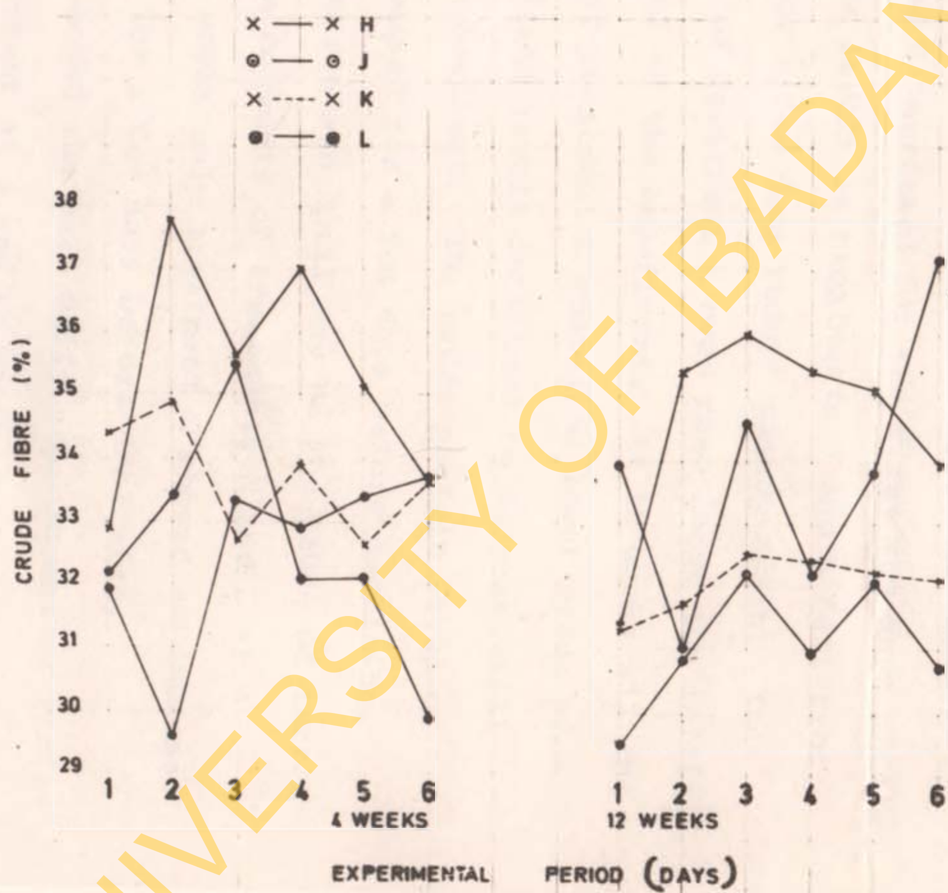
FIG. 6.2 MEAN DAILY CRUDE FIBRE VARIATION OF EACH TREATMENT AT 4 AND 12 WEEKS FOR EXPERIMENTAL PERIOD



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treatments K and L rose for a few days and dropped as the experiment progressed.

Figure 6.3 showed the mean daily crude protein variation of each treatment at 4 and 12 weeks during the experimental period. At 4 weeks, the crude protein content of treatments H and K increased for the first few days before falling, but the levels in treatments J and L fell from the first day for a few days before rising again. The protein content of treatment L even rose above the initial level at the end of the experiment. At 12 weeks all the treatments except treatment K contained lower crude protein levels, and the levels continued to decrease till the end of the experiment. The crude protein level of treatment K increased for a few days before declining. The rainfall in March and April may be the cause of increased crude protein contents of treatments K and L at 4 weeks, but at 12 weeks only treatment K showed an increase in crude protein for a few days before declining.

Figure 6.4 showed the mean daily organic matter variation of each treatment at 4 and 12 weeks during the sampling

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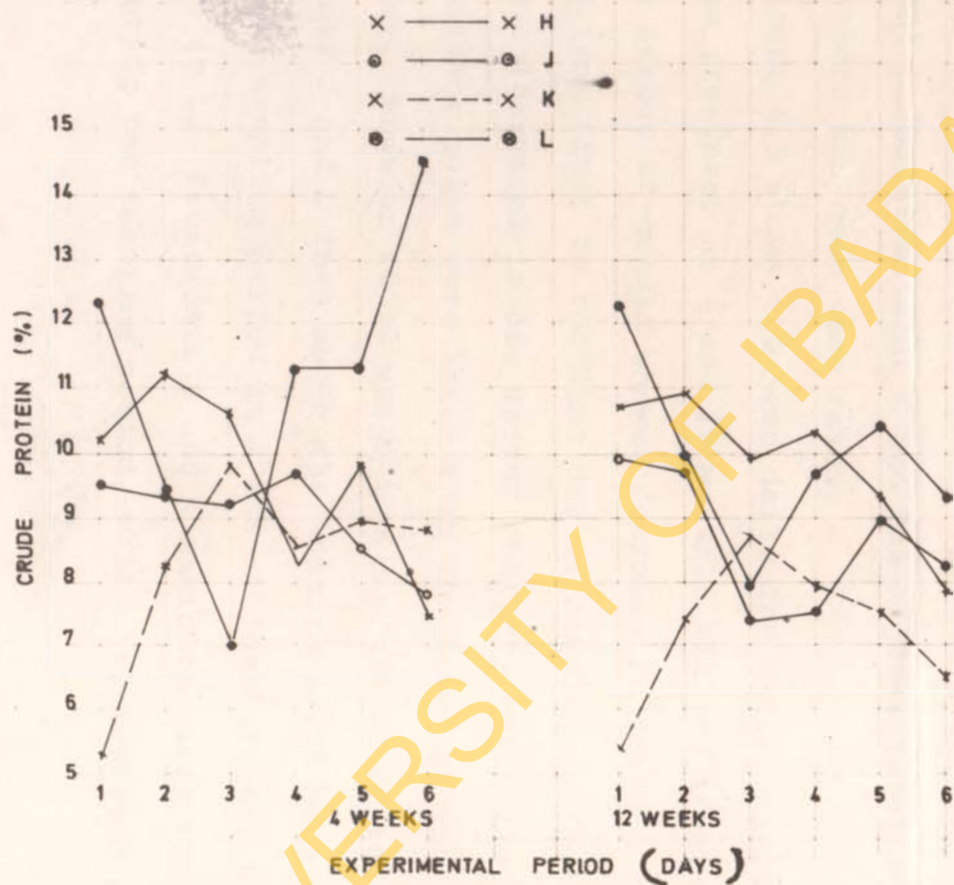
FIG. 6.3 MEAN DAILY CRUDE PROTEIN VARIATION OF EACH TREATMENT AT 4 AND 12 WEEKS FOR EXPERIMENTAL PERIOD



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period. At both stages of growth, treatment L contained the lowest level of organic matter and it exhibited the greatest variation during the experimental period. All the treatments showed a downward trend, with occasional increases, in organic matter level. At 12 weeks the organic matter level rose at the end of the experiment to the original level. At both stages of growth treatments H, J, and K contained more organic matter than treatment L, but treatment K had the highest level even though there had been some rains.

Figure 6.5 shows the mean daily dry matter variation for each treatment at 4 and 12 weeks. All the treatments at both stages of growth showed decrease in the dry matter content from first to the last day of sampling. There were some increases in dry matter content, but these inter-mittent peaks were lower than the level at the first day. However at 12 weeks the dry matter content of treatments J and L rose above the initial level before the end of the sampling period in J and at the last day of sampling in L. Treatments J and K exhibited less variations during the sampling period, while treatments H and

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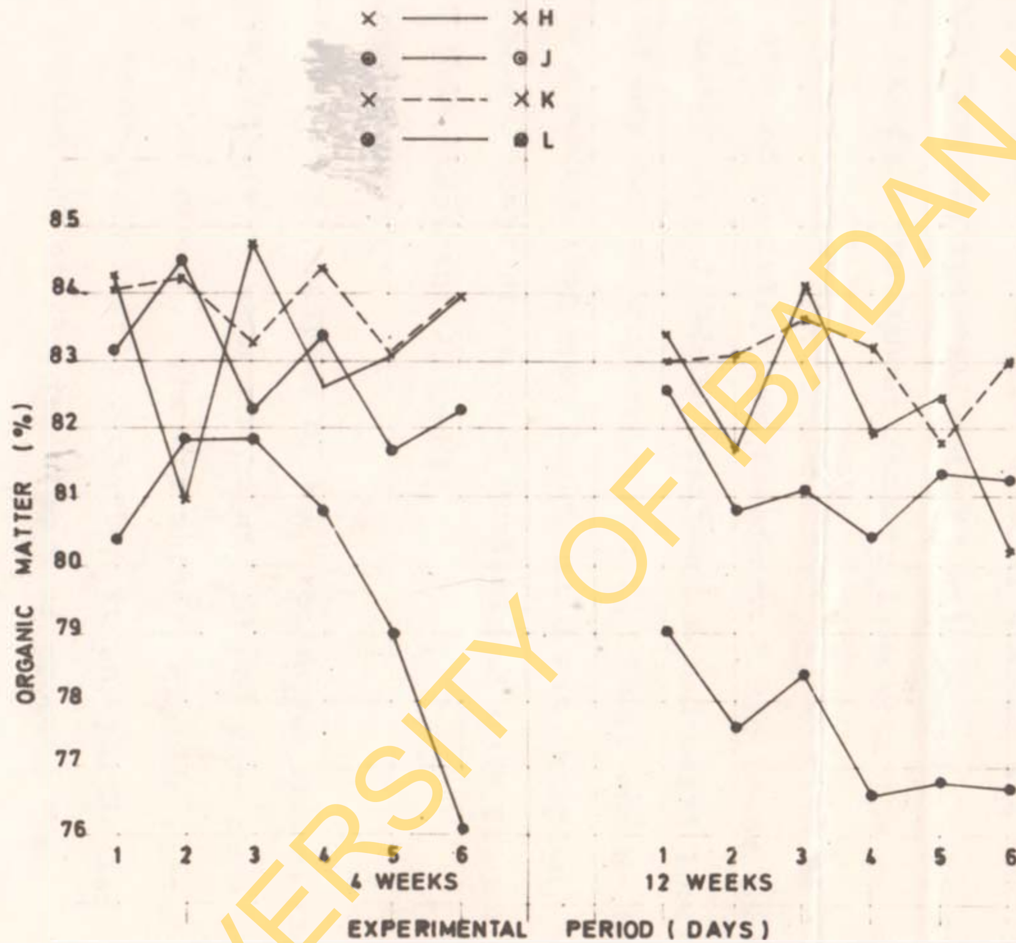
FIG. 6.4 MEAN DAILY ORGANIC MATTER VARIATION OF EACH TREATMENT AT 4 AND 12 WEEKS FOR EXPERIMENTAL PERIOD



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L exhibited greater variations at 4 weeks. Treatments L and H contained less dry matter than treatments J and K. At 12 weeks, treatments K and L showed less variation than treatments H and J. Treatments J and K contained higher levels of dry matter than H and L.

The dry matter content of J at 4 weeks was the highest and from the rainfall data it will be seen that there was no rainfall throughout January 1970 when the experiment was carried out. The dry matter level fell as the rains started to fall, thus treatment K had lower content of dry matter than J, while treatment L had still lower level of dry matter than treatment K. There was no rainfall in December 1966 when Treatment H was sampled, but it contained lower dry matter contents at both stages of growth than treatment J, and K.

6.4 DISCUSSION

The chemical analyses of the treatments showed that the constituents of each treatment did not vary much at both stages of growth. The dry matter, organic matter and N.F.E. showed very little variation at any particular growth period and at the two stages of growth. The

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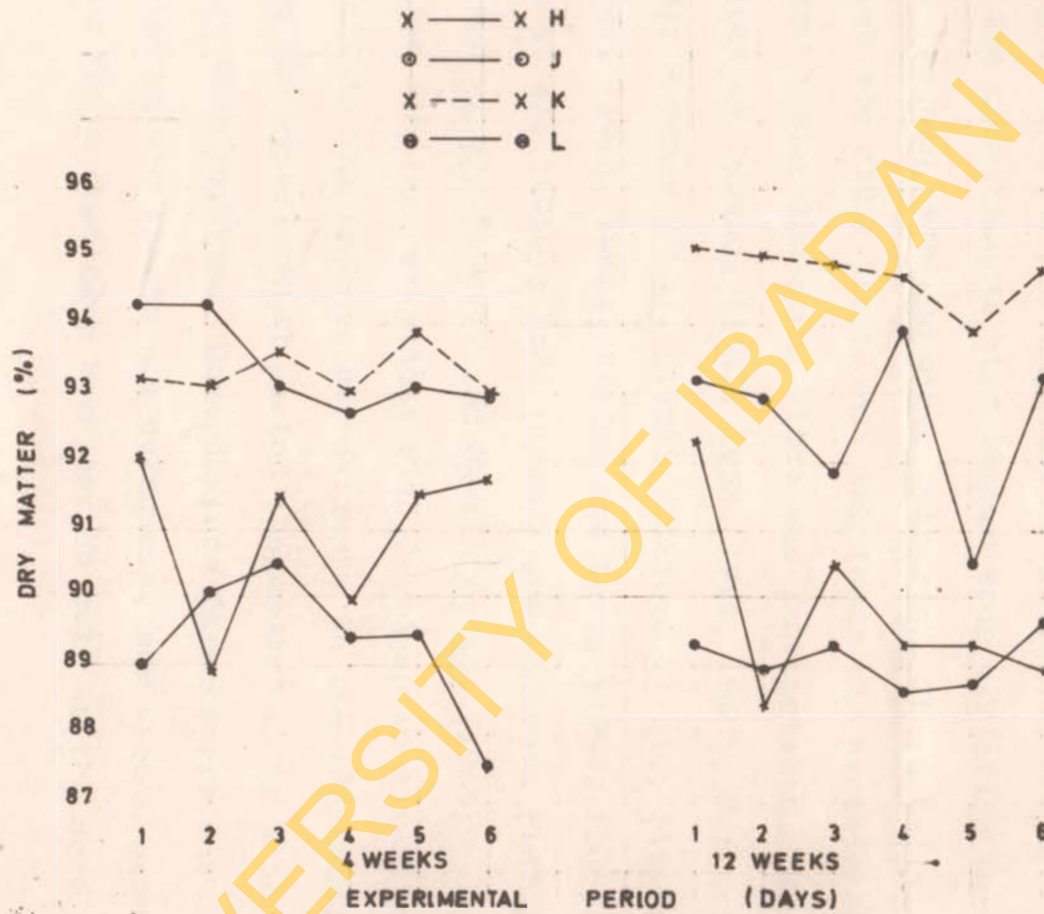
FIG. 6.5 MEAN DAILY DRY MATTER VARIATION OF EACH TREATMENT AT 4 AND 12 WEEKS EXPERIMENTAL PERIOD



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reason for the close chemical composition may be due in part, to the fact that the experiments were carried out during the late rains and dry season when there was not enough precipitation (table 6.1 and fig 6.1). There were many very hot and dry days, and the growth of pasture herbage was not maximal (figures 6.2-6.5).

The constituents that showed some variation were the crude protein, ash and ether extracts. The usual trend is that the older the plant, the lower the protein content, and the higher the crude fibre and ash contents. Investigations of Oyenuga (1957, 1958, 1959, 1960); Patterson (1933); Woodman et al. (1934); Milford et al., (1965a); VanSoest (1966, 1967); Tilley and Terry (1964, 1966); Whyte et al. (1959); Todd (1956); and Ademosun (1970); Ademosun et al. (1967); and Okorie et al. (1965) revealed similar variations with increase in maturity. The fall in the amounts of nutrients in the treatments during the experimental period (Appendix VI) is a normal process with forages. When pasture forages are cut and when the mature shoots are dying out, new shoots grow to replace those that have been cut as well as the dead shoots.

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The tender regrowths are succulent and contain more water than the mature herbage, so they contain less dry matter. The shock of frequent cutting, particularly when the former shock received due to cutting has not been overcome, leads to less forage production and a decline in the amounts of the various constituents.

These data show that the pasture forages under investigation contained adequate crude protein levels to meet the maintenance requirements of grazing cattle. This result is supported by an earlier work by Oyenuga (1957, 1960). Since the levels in all cases were higher than 7%, which many investigators including William, Davies and Skidmore (1966) in temperate regions regard to be the lowest limit, the forages may be said to have good nutritive value. Milford and Minson (1965a) observed that low crude protein content of tropical grasses lead to low productivity of tropical cattle. The crude protein content is however low for grass/legume mixtures. Higher values have been recorded by Oyenuga (1960), and Patterson (1933). The grasses contained higher crude protein levels at the young growing stages

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than at maturity. Pasture plants have passed the stage of active growth and are in the reproductive stage before they are twelve weeks. By this time also many of the leaves and stems are dead and dry. Minson and Milford (1965b) observed drastic falls in the crude protein levels of some tropical forages they investigated. There were cases where the falls in tropical pastures went drastically low and remained between 1% and 3%, particularly during the dry season. The high nitrogen content of these pastures during the dry season might be due to the presence of centrosema which featured in all the mixtures. This legume is known to be draught resistant. It could also explain the higher level of crude protein in mixture H at 12 weeks than at 4 weeks.

Comparing the organic matter contents at the two stages of growth, it is clear that forage K offered the highest organic matter (O.M.) at both periods, although the amount offered at the mature stage was slightly lower. All the other forages too offered lower amounts at maturity. The organic matter contents of the other forages were consistently lower at the late stage of

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growth than at the early stage of growth. Some tropical pasture forages offer lower amounts of organic matter at their late stages of growth because of high total ash content usually encountered at these stages of growth.

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CHAPTER 7

VOLATILE FATTY ACID PRODUCTION IN THE WHITE FULANI (ZEBU) CATTLE.

7.1 INTRODUCTION

The performance of the grazing animal depends on its health, nutritional status, climatological effects and breed. The proper function of the digestive system of the animal depends on the number and type of microflora in the fore-stomachs and the medium in which they operate. The efficiency of utilization of a feed can therefore be judged by the amount and variation of the volatile fatty acids in the rumen, their absorption and transport within the ruminant body and their utilization for energy, growth, and milk fat and depot fat synthesis.

7.2 COLLECTION OF SAMPLES

The animals used, treatments, and experimental design have been described in chapter 3. Rumen liquor and blood samples were collected from each of the fistulated animals during the last five days of each experiment.

About 300 ml rumen liquor were collected from each animal by suction through a perforated transparent rubber tubing inserted through the fistula into the ventral part

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Plate 6

Rumen liquor sampling in the stall.

of the rumen. The liquor poured directly into clean glass bottles. The bottles were well stoppered after collection. Sampling was started one hour before feeding, and continued hourly up to eight hours after feeding. The samples not immediately analysed in the laboratory were stored at $-5^{o}C$ until required for analysis.

Blood samples

About 30 ml blood samples from each of the fistulated animals were collected. Sampling was also started one hour before feeding and then hourly up to eight hours after feeding. The blood samples were drawn from the jugular vein. The well stoppered bottles containing the whole blood were kept in a cold room for twenty four hours to allow for a good separation of serum from the blood cells. The separated serum was centrifuged and decanted off and analysed in the laboratory. Samples not immediately analysed were stored below $-5^{o}C$ until needed. The sodium salt obtained by titrating the distillate against 0.01N NaOH was evaporated in a rotary evaporator to 2 ml, under vaccum. 3 ml 0.1N H_2SO_4 was added to release the acids. The clear liquid was poured into clean well stoppered bottle. The samples were kept at $0^{o}C$ until needed for chromatography. 3.0 μ.1 of each sample was injected into a Pye Chromatograph using hydrogen flame ionization detector. The operating

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condition was as for rumen liquor.

7.3 ANALYTICAL PROCEDURE

About 50 ml of the rumen samples were squeezed through fine cloth. 5 ml of the squeezed sample plus 5 ml 0.1N H_2SO_4 saturated with Mg SO_4 were distilled in a Markham Still. 350 ml distillate were collected and titrated against 0.01N NaOH to obtain the total **steam** volatile fatty acids.

For the estimation of the individual volatile fatty acids a Pye Chromatograph using hydrogen flame ionization detector was used. 25 ml of each of the squeezed liquor were poured into centrifuge tube and 5 ml of 25% Orthophosphoric acid in 5N H_2SO_4 were added to each liquor. It was left for at least 30 minutes and then centrifuged at 2,500 r.p.m. for 10 minutes. The supernatant was poured into a small bottle and well stoppered. 3 μ.l. of this was injected into a column packed with polyethylene glycol 20M (P.E.G. 20M) with phosphoric acid on celite 100-120 mesh. The working temperature was 125°C. Argon was the eluant gas and the attenuation was 1 x 500. Each peak was measured by triangulation and its area calculated in inches. The corrected peak area was used to calculate the milli equivalent percent of ech acid. The column had a recovery rate ranging from 97-106%.

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7.4 RESULTS

Ruminal volatile fatty acids (V.F.A.) production

Table 7.1 shows the results of treatments H, J, K and L at 4 and 12 weeks, fed to Zebu cattle and sampled one hour before feeding and hourly after feeding up to 2 hours. Analytical data for treatment H at 12 weeks are missing because most of the bottles used for storing the rumen liquor got broken and the samples were lost.

Ruminal pH

On the average, the pH of the rumen liquor produced by each treatment at the two stages of growth was over 7. There was no steady rise or fall in pH in all the forages. For treatment H at 4 weeks the pH fell very slightly to lower levels one and two hours after feeding. At 12 weeks of growth the pH level also fell one hour after feeding, but rose only very slightly 2 hours after feeding. Treatment J at 4 weeks had a lower pH level one hour after feeding, but rose again 2 hours after feeding. at 12 weeks the pH level fell at one and two hours after feeding. With treatment K the pH level rose one hour after feeding but dropped very little 2 hours after feeding. At 12 weeks the pH level rose one hour after feeding and continued to rise two hours after feeding. Treatment L

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at 4 weeks gave a rise in pH level one hour after feeding but dropped to lower level 2 hours after feeding. At 12 weeks the pH fell one hour after feeding but rose again two hours after feeding.

Total volatile fatty acids (V.F.A.)

The total volatile fatty acids (V.F.A's) produced in the rumen from the 4 week treatment H rose one hour after feeding, but dropped 2 hours after feeding. The high level also coincided with the higher pH level after feeding. At 12 weeks the peak for the total volatile fatty acids in the rumen occured one hour after feeding. For treatment J at 4 weeks the total V.F.A. rose one hour after feeding but dropped again two hours after feeding. The pH level dropped to lower level after feeding but rose again two hours after feeding. At 12 weeks the total V.F.A. rose to higher level after feeding and it dropped to lower level two hours after feeding. The highest total V.F.A. level coincided with the highest pH level in some cases.

Treatment K at 4 weeks produced more V.F.A. at one hour than at two hours. This higher V.F.A. content also coincided with the higher pH level. At 12 weeks treatment K gave a higher V.F.A. content one hour after feeding than at two hours after feeding, but the pH level was lower at this time.

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Treatment L produced more volatile fatty acids one hour after feeding when the treatment was 4 weeks. At this time also the pH was higher than two hours after feeding. At 12 weeks the V.F.A. produced was higher one hour after feeding than at two hours, but the pH was lower than at two hours after feeding.

It was observed that the higher total V.F.A. production before feeding than after feeding was not consistent.

Individual V.F.A.'s

The separation of the volatile fatty acids chromatographically showed that in all the treatments and at both stages of growth, acetic acid accounted for well over 70% of the total V.F.A. in the rumen. Propionic acid came next with just under 20% while Butyric acid formed the lowest amount, being under 10% in most cases except in treatment L at 12 weeks, when the mean was 11.6%.

With treatment H at 4 weeks there was not much variation in the acetic acid content before feeding and one and two hours after feeding. The peak production of acetic acid occured one hour after feeding. The propionic acid peak however, occured two hours after feeding. At 12 weeks the peak for acetic acid was also at one hour after feeding; propionic and butyric acids also showed their peak levels at two hours after feeding.

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Treatment J at 4 weeks produced the highest amount of acetic acid one hour after feeding and the highest propionic and butyric acids two hours after feeding. Similarly, at 12 weeks the peak production of propionic and butyric acids occured two hours after feeding.

Treatment K produced the highest amount of acetic acid two hours after feeding. The acetic acid level dropped after feeding when the treatment was 4 weeks, but increased only very slightly after feeding when it was 12 weeks.

Treatment L at 4 weeks produced acetic acid peak one hour after feeding. This coincided with the peaks for pH and total V.F.A. at this particular stage of growth. Propionic acid level was lowest one hour after feeding while butyric acid content was highest one hour after feeding. At 12 weeks the acetic acid peak was at 2 hours after feeding, the propionic acid peak was at 2 hours after feeding and the butyric acid peak was at one hour after feeding.

Just as the pH level and the total V.F.A. content of the rumen liquor, the prefeeding acetic, propionic and butyric acid levels did not show any clear relationship with the levels after feeding. In some cases the

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prefeeding levels were higher, and in other cases lower than the post feeding levels. There was a tendency however, for the various levels to come close to the prefeeding levels two hours after feeding.

Acetic acid to Propionic acid (A/P) ratio

The acetic to propionic acid ratio is of great importance in ruminal V.F.A. studies because it shows at a glance the type of feed being digested and absorbed, and the metabolic processes that may follow the absorption of the V.F.A. produced. All the treatments gave a ratio above 4 at the two stages of growth, except L at 12 weeks before feeding which gave 3.37. Treatment J gave the highest ratio of over 5 at both stages of growth. The ratio tends to be slightly higher at 4 weeks than at 12 weeks of growth.

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TABLE 7.1

Ruminal pH, total V.F.A., individual V.F.A., and acetic to propionic acid ratio (A/P) of treatments (H) at 4 and 12 weeks of growth fed to Zebu cattle.

Stage of growth of Forage (weeks)	Time of Sampling.	pH of liquor	Total V.F.A. Meq/ 100 ml rumen liquor	Individual V.F.A. Meq %			A/P
				Acetic Acid	Propionic Acid	Butyric Acid	
H 4	Before feeding	7.37	7.29	78.29	14.27	7.04	5.51
	Hours of Sampling. 1	7.30	7.33	78.72	15.24	6.04	5.17
	2	7.21	7.22	77.75	16.06	6.19	4.84
	Mean	7.29	7.28	78.39	15.19	6.42	5.16
12	Before feeding	7.13	7.42	72.43	20.44	7.13	3.54
	Hours of Sampling. 1	6.99	7.57	77.36	16.33	6.31	4.74
	2	7.03	7.09	75.00	17.69	7.31	4.24
	Mean	7.05	7.36	74.93	18.15	6.92	4.13

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TABLE 7.1

Ruminal V.F.A., pH, Acetic/Propionic ratio (A/P) of treatments (J) at different stages of growth fed to Zebu cattle.

Stage of growth of Forage (weeks)	Time of Sampl-ing.	pH of liquor	Total V.F.A. Meq/ 100 ml rumen liquor	Individual V.F.A. Meq %			A/P
				Acetic Acid	Propi-onic Acid	Buty-ric Acid	
J 4	Before feeding	7.05	14.82	77.97	14.20	7.83	5.49
	Hours after feeding						
	1	6.98	15.12	80.30	13.36	6.34	6.01
	2	7.10	14.48	79.29	14.19	6.52	5.59
	Mean	7.03	14.81	79.19	13.92	6.90	5.70
12	Before feeding	7.30	13.85	77.76	14.10	8.14	5.51
	Hours after feeding						
	1	7.25	14.63	79.94	12.86	7.20	6.22
	2	7.08	12.92	73.73	15.19	11.08	4.85
	Mean	7.21	13.80	77.14	14.05	8.81	5.53

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TABLE 7.1

Ruminal V.F.A., pH, Acetic/Propionic ratio (A/P) of treatment (K) at 4 and 12 weeks of growth fed to Zebu cattle.

State of growth of Forage (weeks)	Time of Sampl-ing.	pH of liquor	Total V.F.A. Meq/ 100 ml rumen liquor	Individual V.F.A. Meq %			A/P
				Acetic Acid	Propi-onic Acid	Buty-ric Acid	
K 4	Before feeding	7.61	8.06	79.59	17.86	8.55	6.71
	Hours after feeding 1	7.86	7.94	75.54	14.61	9.85	5.17
	2	7.78	7.27	79.73	14.03	6.24	5.68
	Mean	7.75	7.76	78.29	15.50	8.21	5.85
12	Before feeding	7.31	9.01	77.58	17.52	4.90	4.43
	Hours after feeding 1	7.60	8.11	78.60	16.82	4.58	4.67
	2	8.18	7.92	79.64	16.12	4.24	4.94
	Mean	7.70	8.35	78.61	16.82	4.57	4.68

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TABLE 7.1

Ruminal V.F.A., pH, Acetic/Propionic ratio (A/P) of treatment (L) at 4 and 12 weeks of growth fed to Zebu cattle.

Stage of growth of Forage (weeks)	Time of Sampling.	pH of liquor	Total V.F.A. Meq/ 100 ml rumen liquor	Individual V. F. A. Meq %			A/P
				Acetic Acid	Propionic Acid	Butyric Acid	
L 4	Before feeding	7.38	9.36	76.88	16.97	6.35	4.52
	Hours after feeding 1	7.50	7.87	76.98	13.44	7.58	5.88
	2	7.35	7.57	77.27	16.86	5.87	4.58
	Mean	7.41	8.27	77.64	15.76	6.60	4.99
12	Before feeding	8.15	10.89	63.81	18.91	17.28	3.37
	Hours after feeding 1	7.30	8.31	76.06	13.49	10.49	5.64
	2	8.10	8.12	78.69	14.24	7.07	5.53
	Mean	7.85	9.11	72.85	15.55	11.60	4.85

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Table 7.2 showed the results of pH, total V.F.A., the individual acids, and the acetic acid to propionic acid ratio of the rumen liquor when treatments H, J, K and L were fed to Zebu cattle at 4 and 12 weeks of growth. The sampling period was extended to 4 hours after feeding.

Treatment H at 4 weeks gave an almost constant pH level however, fell by a few units one hour after feeding from the prefeeding level and also at 4 hours after feeding. There were two small peaks, one at one hour after feeding and the other at 3 hours after feeding. The total V.F.A. rose after feeding reaching a peak two hours after feeding, The level thereafter fell to 7.02 meq.% at 3 hours after feeding but rose to 7.18 meq. % 4 hours after feeding.

At 12 weeks treatment H produced a pH peak at 4 hours after feeding. The pH level dropped from the prefeeding level but started to rise two hours after feeding. The total V.F.A. content also dropped after feeding, but started to rise 2 hours after feeding till it reached a peak 4 hours after feeding.

Treatment J at 4 weeks produced a fairly uniform pH level throught the sampling period. The highest level was at 3 hours after feeding. The level fell from the prefeeding level but rose again two hours after feeding. The

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total V.F.A. content of the rumen liquor dropped one hour after feeding and continued to drop up till 4 hours after feeding. The highest level was at one hour after feeding.

At 12 weeks the pH level also remained fairly steady throughout the sampling period. However, the pH rose by small units and the highest pH level was at 3 hours after feeding. The total V.F.A. content dropped from the pre-feeding level of 18.24 meq. % to 15.31 meq.% one hour after feeding. It rose by a small unit two hours after feeding but dropped again to the lowest level 3 hours after feeding. The level rose to a peak at 4 hours after feeding.

Treatment K at 4 weeks produced the highest pH level 4 hours after feeding. The pH level dropped from the pre-feeding level of 7.05 to 6.92 one hour after feeding. It rose 2 hours after feeding and remained at the level 3 hours after feeding, but rose to the peak 4 hours after feeding. The total V.F.A. content also dropped to lower level after feeding, but rose two hours after feeding, dropped again 3 hours after feeding and reached the peak 4 hours after feeding. At 12 weeks there was a slight drop one hour after feeding, it dropped again 2 hours after feeding but rose steadily till it reached a peak 4 hours after feeding. The total V.F.A. rose after feeding, but dropped to lower level 3 hours after feeding and latter

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rose to a peak 4 hours after feeding. The highest pH level and the total V.F.A. peak occured at the same time, at the two stages of growth.

Treatment L at 4 weeks produced rumen liquor pH that remained constant from one hour before feeding till 2 hours after feeding. The pH level then rose 3 hours after feeding till it reached its peak 4 hours after feeding. The total V.F.A. content rose after feeding till it reached a peak 4 hours after feeding. The pH peak and the total V.F.A. peak coincided.

At 12 weeks the pH remained constant till one hour after feeding but rose steadily till it reached a peak 4 hours after feeding. The total V.F.A. also rose after feeding but fell to lower level and rose again till it reached a peak 4 hours after feeding. The peaks for the pH and total V.F.A. also occured at the same time.

<u>Individual V.F.A.</u>

Acetic acid formed over 70% of the separated V.F.A.s in all the treatments and at the two stages of growth.

For treatment H at 4 weeks the acetic acid content dropped to lower level one hour after feeding and continued to drop but rose a bit 4 hours after feeding. The level was however still lower than the the level one hour after

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feeding. Propionic acid content also dropped to lower level one hour after feeding but rose to a peak 3 hours after feeding and dropped again 4 hours after feeding. Butyric acid content rose after feeding reaching a peak 2 hours after feeding but dropped thereafter till 4 hours after feeding. The content was still higher at 4 hours than at the prefeeding stage.

At 12 weeks the acetic acid content dropped to lower level one hour after feeding but rose to a peak 2 hours after feeding and it dropped again. The propionic acid content rose after feeding but dropped to lower level 2 hours after feeding. It rose from then onwards till it reached a peak 3 hours after feeding. Butyric acid also rose after feeding and reached a peak 4 hours after feeding.

Acetic acid was the major acid produced when treatment J was fed at 4 weeks of growth. Propionic acid was next to it in production while butyric acid had the lowest production. At 12 weeks of growth acetic acid also formed the greater part of the total volatile fatty acids produced in the rumen. Propionic acid came next while butyric acid came least in production.

Treatments K and L also produced more acetic acid than propionic and butyric acids. The individual V.F.A. production was similar at both stages of growth.

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A/P ratio

The acetic to propionic acid ratio did not fall below 3. Treatments H and J had the highest ratio at both stages of growth, while treatments K and L had lower ratios at both stages of growth.

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TABLE 7.2

Ruminal pH, Total V.F.A. and individual V.F.A., Acetic/Propionic (A/P) ratio of treatment (J) at 4 and 12 weeks of growth fed to Zebu cattle

Stage of growth of Forage (weeks)	Time of Sampling.	pH of liquor	Total V.F.A. meq/100 ml rumen liquor	Individual V. F.A. Molar %			A/P
				Acetic Acid	Propionic Acid	Butyric Acid	
J 4	Before feeding	7.27	11.90	77.56	17.16	5.28	4.52
	Hours after feeding 1	7.12	8.54	79.20	14.73	6.07	5.38
	2	7.23	8.24	80.97	12.97	6.06	6.24
	3	7.47	7.75	81.93	13.24	4.83	6.19
	4	7.22	7.45	79.44	14.65	5.91	5.42
	Mean	7.26	8.78	79.82	14.55	5.63	5.55
12	Before feeding	6.85	18.24	79.00	16.18	4.82	4.88
	Hours after feeding 1	6.93	15.31	78.61	14.02	7.37	5.61
	2	6.88	15.36	79.76	15.86	4.38	5.03
	3	6.98	13.02	80.39	15.34	4.27	5.24
	4	6.90	15.46	78.27	17.05	4.68	4.59
	Mean	6.91	15.48	79.21	15.69	5.10	5.07

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TABLE 7.2

Ruminal pH, Total V.F.A. and individual V.F.A., and Acetic/Propionic (A/P) acid ratio of treatment (H) at 4 and 12 weeks of growth fed to Zebu cattle

Stage of growth of Forage (weeks)	Time of Sampl-ing.	pH of liquor	Total V.F.A. meq/100 ml rumen liquor	Individual V.F.A. Molar %			A/P
				Acetic Acid	Propi-onic Acid	Buty-ric Acid	
H 4	Before feeding	7.46	6.09	82.51	14.24	3.25	5.79
	Hours after feeding 1	7.43	6.88	78.79	12.61	8.60	6.24
	2	7.42	8.65	76.96	13.84	9.20	5.56
	3	7.43	7.02	73.82	18.53	7.65	3.98
	4	7.35	7.18	75.98	17.02	7.00	4.46
	Mean	7.42	7.16	77.61	15.25	7.14	5.21
12	Before feeding	7.10	7.51	80.16	14.24	5.60	5.62
	Hours after feeding 1	6.80	6.80	77.34	15.13	7.53	5.11
	2	6.90	7.06	79.08	14.70	6.22	5.37
	3	7.05	7.22	75.75	16.84	7.41	4.49
	4	7.20	7.81	75.54	16.45	8.01	4.59
	Mean	7.01	7.28	77.57	15.47	6.95	5.04

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TABLE 7.2

Ruminal V.F.A., pH, Acetic/Propionic (A/P) ratio of treatment (K) at 4 and 12 weeks of growth fed to Zebu cattle

Stage of growth of Forage (weeks)	Time of Sampling.	pH of liquor	Total V.F.A. Meq/100 ml rumen liquor	Individual V.F.A. Molar %			A/P
				Acetic Acid	Propionic Acid	Butyric Acid	
K 4	Before feeding	7.05	7.82	68.20	16.69	15.11	4.09
	Hours after feeding 1	6.92	7.77	78.93	16.13	4.94	4.89
	2	7.00	9.00	77.70	16.62	5.68	4.68
	3	7.00	8.61	77.89	15.90	6.21	4.90
	4	7.25	9.41	76.40	17.64	5.96	4.33
	Mean	7.04	8.52	75.82	16.60	7.58	4.58
12	Before feeding	6.95	7.62	74.01	13.34	12.65	5.55
	Hours after feeding 1	6.90	7.72	78.34	11.61	10.05	6.75
	2	6.80	8.01	67.35	18.49	14.16	3.64
	3	7.20	7.92	77.09	17.02	5.89	4.53
	4	7.40	9.11	67.92	19.99	12.09	3.40
	Mean	7.05	8.08	72.94	16.09	10.97	4.77

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TABLE 7.2

Ruminal pH, total V.F.A., and individual V.F.A. Acetic/Propionic (A/P) ratio of treatment (L) at 4 and 12 weeks of growth fed to Zebu cattle

Stage of growth of Forage (weeks)	Time of Sampling.	pH of liquor	Total V.F.A. Meq/100 ml rumen liquor	Individual V.F.A. Molar %			A/P
				Acetic Acid	Propionic Acid	Butyric Acid	
L 4	Before feeding	6.73	7.78	76.25	16.81	6.94	4.54
	Hours after feeding 1	6.73	8.66	77.45	16.39	6.16	4.74
	2	6.73	9.16	76.85	16.86	6.29	4.56
	3	7.00	9.61	77.04	16.30	6.66	4.72
	4	7.13	10.20	75.21	16.77	8.02	4.48
	Mean	6.86	9.08	76.56	16.63	6.81	4.61
12	Before feeding	7.15	6.43	79.57	13.38	7.05	5.95
	Hours after feeding 1	7.15	8.61	78.60	16.01	5.39	4.91
	2	7.20	6.63	70.04	20.08	9.88	3.49
	3	7.35	7.72	71.21	18.25	10.54	3.90
	4	7.40	9.11	76.68	15.33	7.99	5.00
	Mean	7.25	7.70	75.22	16.61	8.17	4.65

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7.4 pH Values and total V.F.A.

In table 7.3, the pH for J at 4 weeks reached its maximum 4 hours after feeding, while the total V.F.A. reached its peak production at 1 hour after feeding. The greatest amount of acetic acid was produced 2 hours after feeding. The mean of the acetic acid to propionic acid was 5.42.

The pH of 7.57 and acetic to propionic acid ration of 5.42 are high. These are expected because the feed was not supplemented and it was fibrous.

At 12 weeks the pH reached its maximum at 6 hours after feeding, and the total V.F.A. reached its maximum production 1 hour after feeding. Acetic acid production reached its highest at 4 hours after feeding. The acetic acid to propionic acid ratio was not as high as at 4 weeks.

In treatment L the pH reached its maximum 1 hour after feeding, while the total V.F.A. reached its maximum production also 1 hour after feeding. The acetic acid reached its highest production 2 hours after feeding. The acetic to propionic acid ratio was 5.37.

When treatment L was 12 weeks the pH reached its highest at 1 hour and 6 hours after feeding. The total V.F.A. reached its peak at 1 hour after feeding, while the

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acetic acid production was at its highest 4 hours after feeding. The acetic to propionic acid ratio of 4.78 was lower than the value at 4 weeks.

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TABLE 7.3

Ruminal V.F.A., pH , Acetic/Propionic (A/P) ratio of treatment (J) at different stages of growth fed to Zebu Cattle.

Stage of growth of Forage (weeks)	Time of Sampl-ing.	pH of liquor	Total V.F.A. Meq/ 100 ml rumen liquor	Individual V. F. A. Molar %			Acetic Propi-onic Ratio
				Acetic Acid	Propi-onic Acid	Buty-ric Acid	
J 4	Before feeding	7.43	12.29	79.58	15.32	5.09	5.19
	Hours after feeding 1	7.10	13.51	77.32	16.13	6.55	4.79
	2	7.45	10.19	80.24	14.40	5.36	5.57
	4	7.83	10.64	79.55	14.33	6.12	5.55
	6	7.48	11.26	79.94	13.87	6.19	5.76
	8	8.13	10.33	80.04	14.08	5.88	5.68
	Mean	7.57	11.37	79.45	14.69	5.87	5.42
12	Before feeding	7.15	11.61	78.21	16.75	5.05	4.67
	Hours after feeding 1	6.83	13.70	75.10	18.85	6.05	3.98
	2	7.10	13.41	74.40	15.33	10.27	4.85
	4	7.38	12.62	77.89	16.53	5.59	4.71
	6	7.45	11.94	76.98	18.66	4.36	4.13
	8	7.43	10.96	75.64	19.50	4.87	3.85
	Mean	7.22	12.37	76.37	17.60	6.03	4.34

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TABLE 7.3

Ruminal pH total V.F.A., Individual V.F.A., Acetic/Propionic (A/P) ratio of treatment (L) at 4 and 12 weeks of growth fed to Zebu Cattle.

Stage of growth of Forage (weeks)	Time of Sampl-ing.	pH of liquor	Total V.F.A. Meq/100 ml rumen liquor	Individual V. F. A. Molar %			Acetic Propi-onic Ratio
				Acetic Acid	Propi-onic Acid	Buty-ric Acid	
L 4	Before feeding	7.10	7.92	83.19	14.84	1.97	5.61
	Hours after feeding 1	7.24	6.81	82.18	15.43	2.39	5.33
	2	7.16	6.57	83.82	13.79	2.39	6.07
	4	7.15	5.77	83.01	14.54	2.41	5.69
	6	7.08	5.67	81.43	14.08	4.49	5.78
	8	7.29	5.54	73.39	19.56	7.05	3.75
	Mean	7.17	6.38	81.17	15.38	3.45	5.37
12	Before feeding	7.52	8.17	70.91	14.74	14.35	4.81
	Hours after feeding 1	7.60	6.83	75.48	16.70	7.82	4.51
	2	7.58	6.43	75.39	16.91	7.70	4.45
	4	7.57	6.24	76.66	15.41	7.93	4.97
	6	7.60	5.25	76.42	15.31	8.27	4.99
	8	7.80	5.54	77.43	15.66	6.91	4.94
	Mean	7.61	6.41	75.38	15.79	8.83	4.78

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Tables 7.4, 7.5 and 7.6 showed the pH, total V.F.A. in rumen and blood, individual V.F.A. and acetic to propionic acid ratio of treatments H, J, K and L fed at 4, 8 and 12 weeks of growth. The sampling was carried out one hour before feeding and hourly after feeding up to 8 hours after feeding.

Table 7.4 and figures 7.11, 7.12, 7.13, 7.14 and 7.15 showed the pH, total V.F.A. in rumen liquor and blood serum and the individual V,F.A. in the rumen liquor, when H was 4 weeks and 8 weeks respectively. At 4 weeks, treatment H had a pH range between 6.93 and 7.42. The pH fell from the prefeeding level to a lower level one hour after feeding. The highest pH level was at 2 hours after feeding. This was also illustrated in figure 7.11. The total V.F.A. dropped from the prefeeding level of 7.07 meq. percent to 4.69 meq. percent. The peak V.F.A. production was at 4 hours after feeding. This is illustrated in figure 7.12. The acetic acid peak was at 8 hours after feeding, although there was a minor peak 5 hours after feeding. Propionic acid peak was at 4 hours after feeding, and this acid varied between 16.54 molar percent to 22.74 molar percent. Butyric acid varied from 0.78 molar percent 2 hours after feeding to 4.32 molar percent 3 hours after feeding. Acetic to propionic acid ratio was just over 4.

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGE (H) AT DIFFERENT STAGES OF GROWTH TO ZEBU CATTLE ON RUMINAL PH

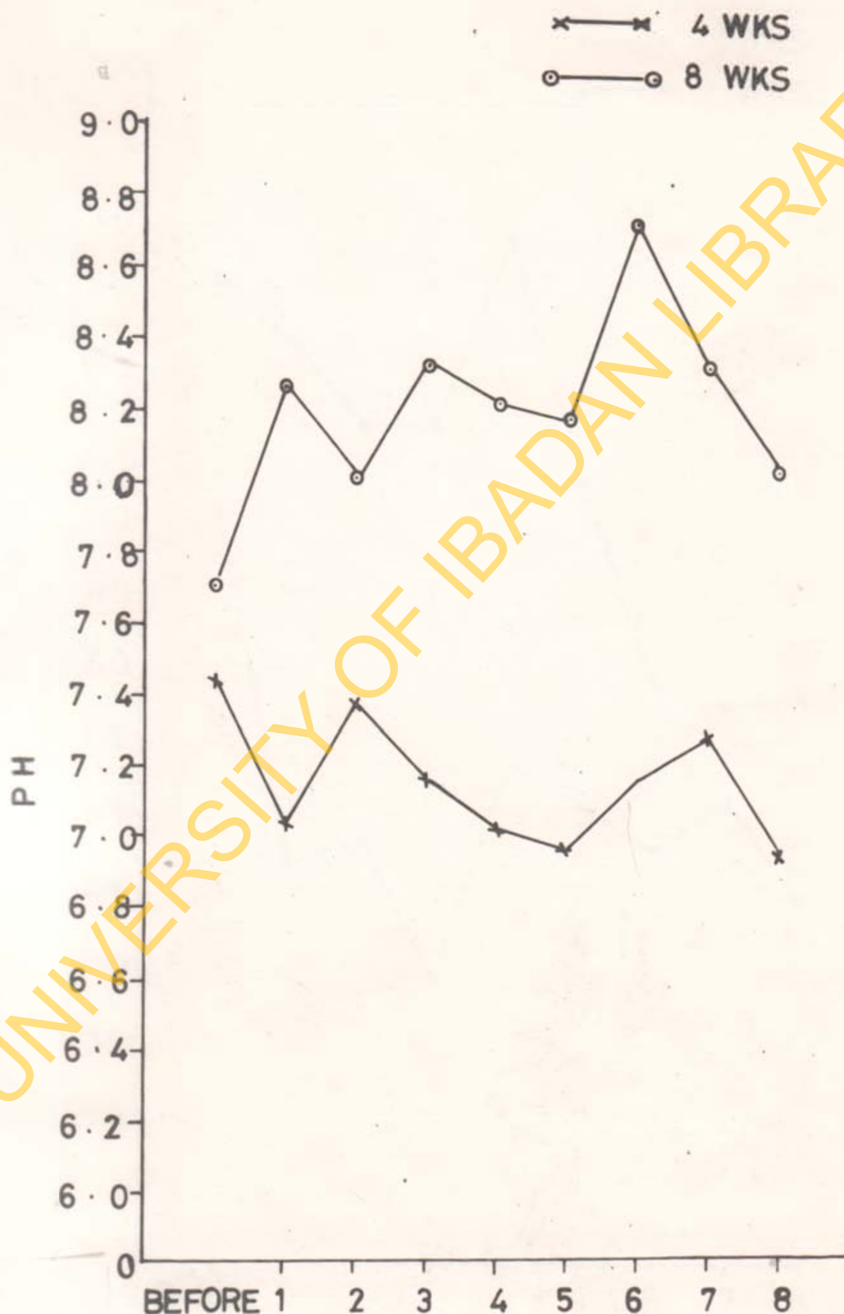

FIG.7.11 TIME OF SAMPLING (hrs) AFTER FEEDING

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (H) ON THE TOTAL VOLATILE FATTY ACID PRODUCTION BY ZEBU CATTLE.

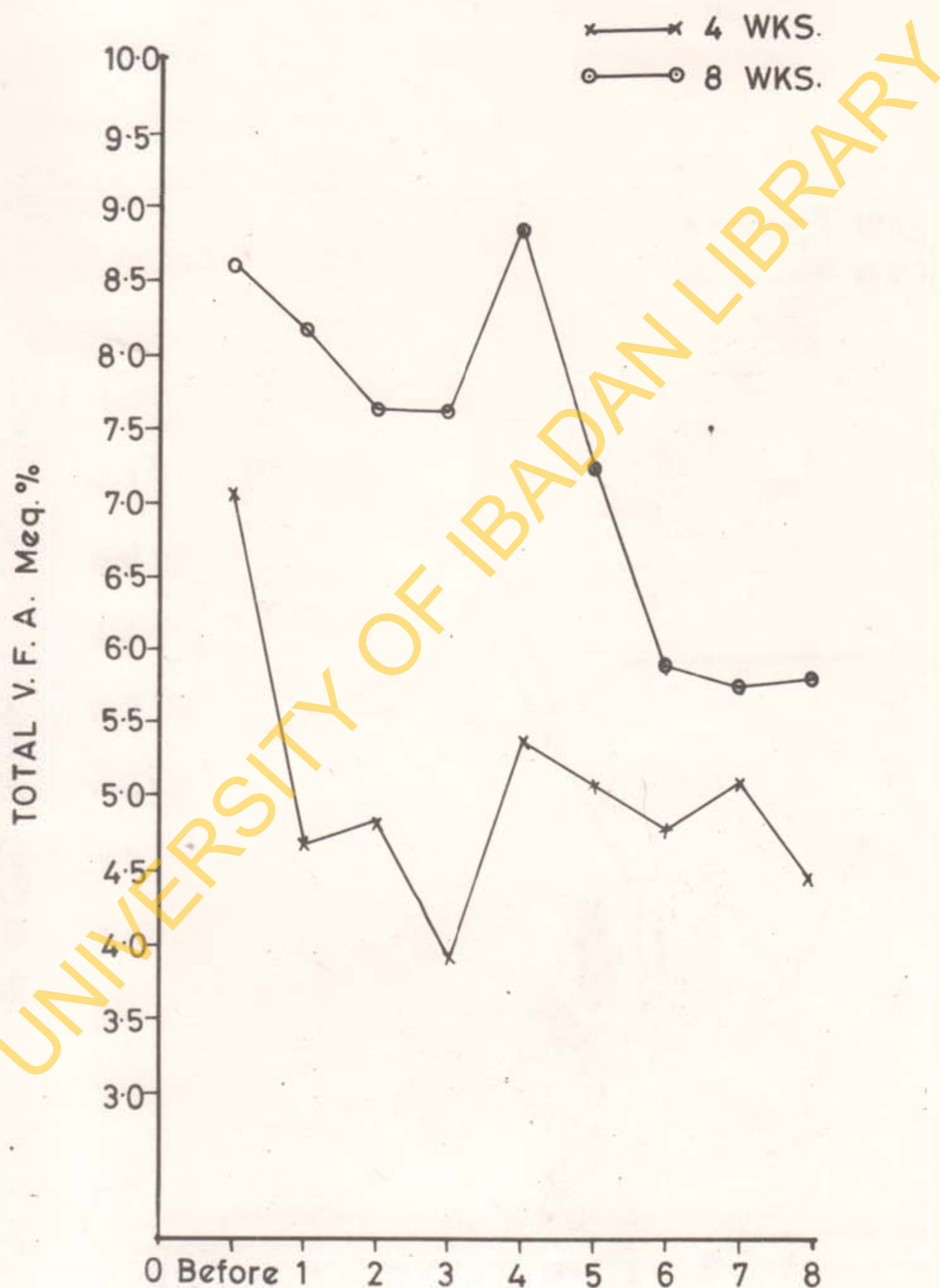

FIG. 7.12 TIME OF SAMPLING (hrs.) AFTER FEEDING.

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (H) ON THE TOTAL BLOOD VOLATILE FATTY ACID PRODUCTION (Meq. %) BY ZEBU CATTLE.

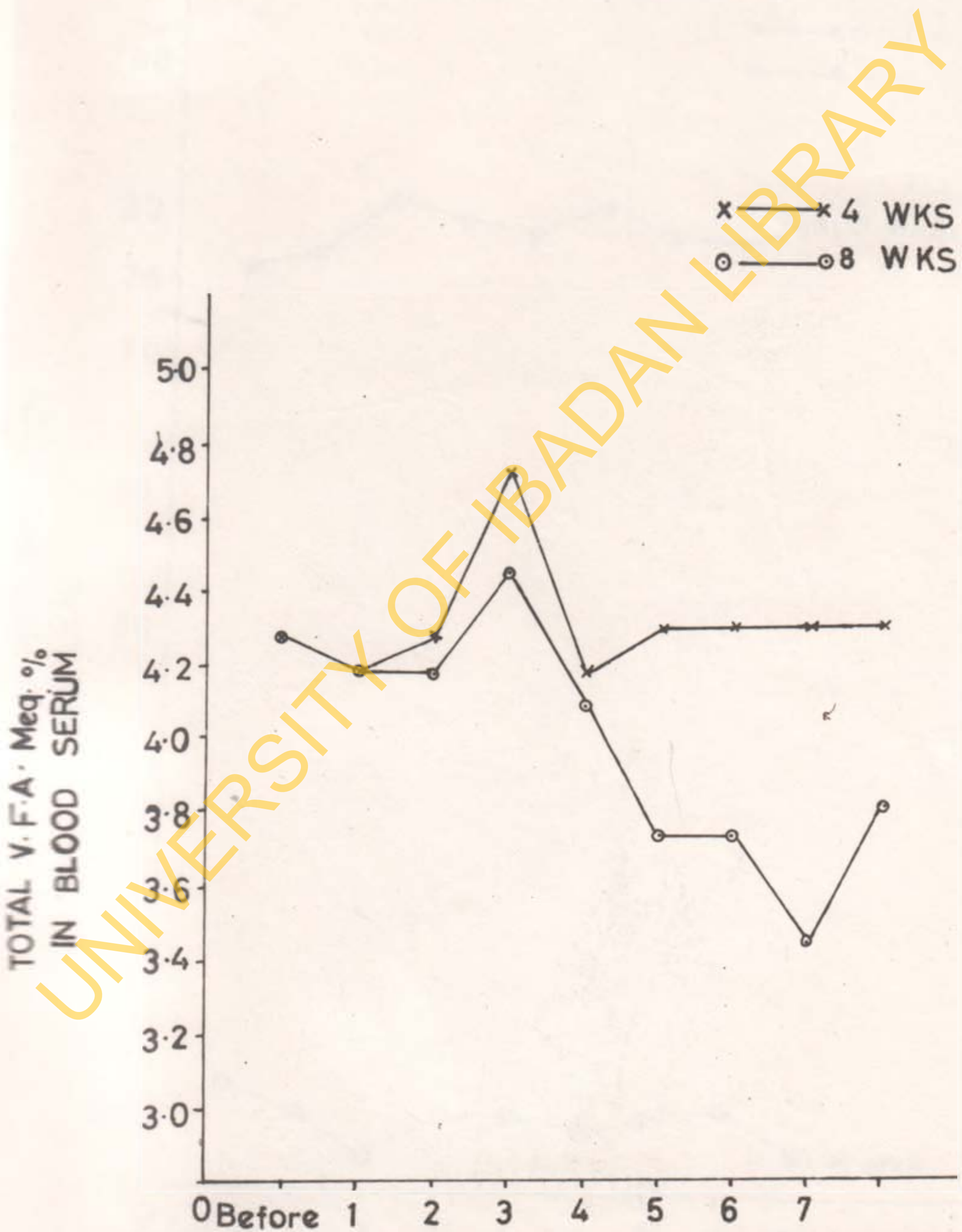

FIG. 7.13 TIME OF SAMPLING (hrs) AFTER FEEDING.

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (H) AT 8 WKS. OF AGE TO ZEBU CATTLE ON ACETIC AND PROPIONIC ACIDS. (MOLAR %)

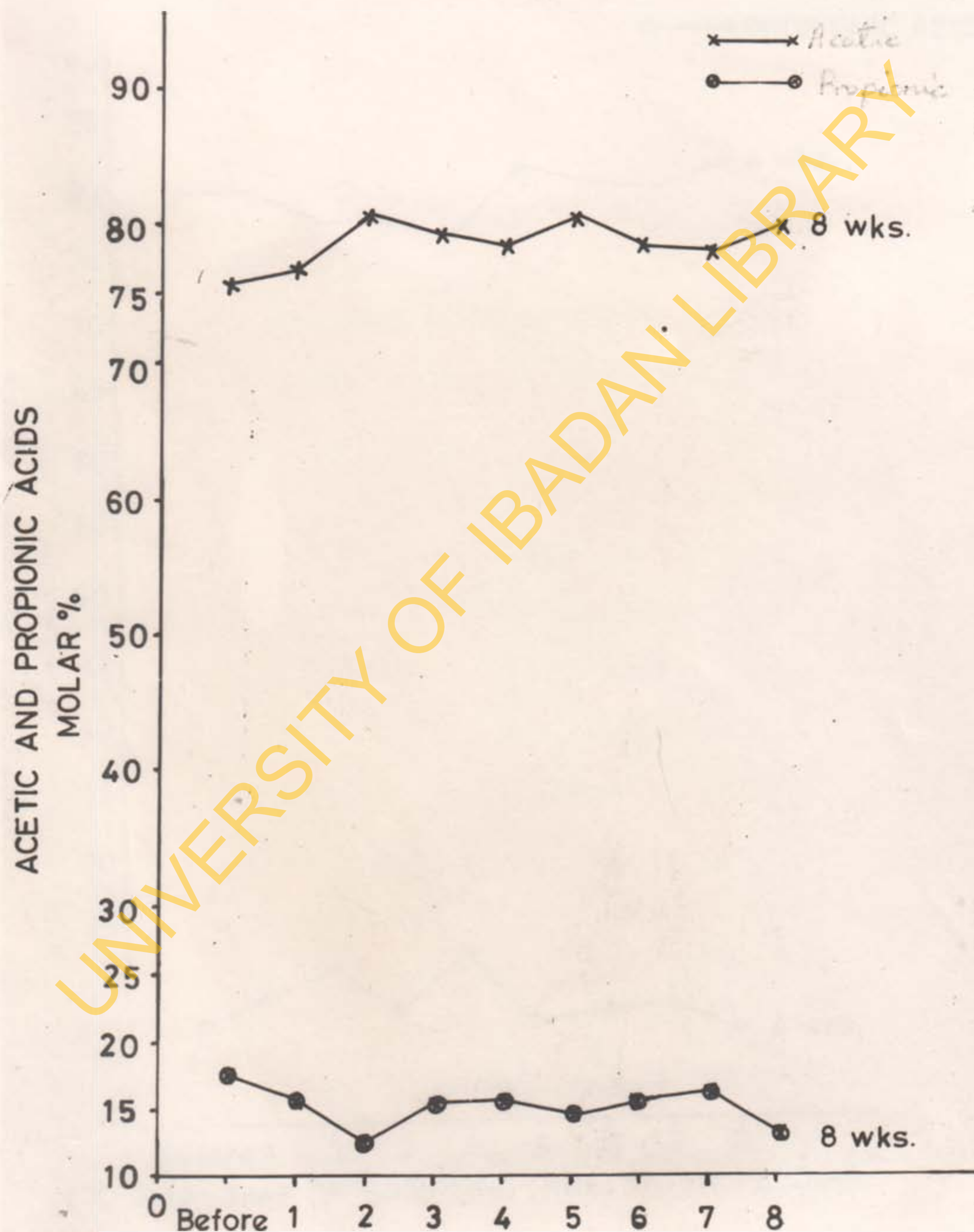

FIG. 7.14 TIME OF SAMPLING (hrs.) AFTER FEEDING.

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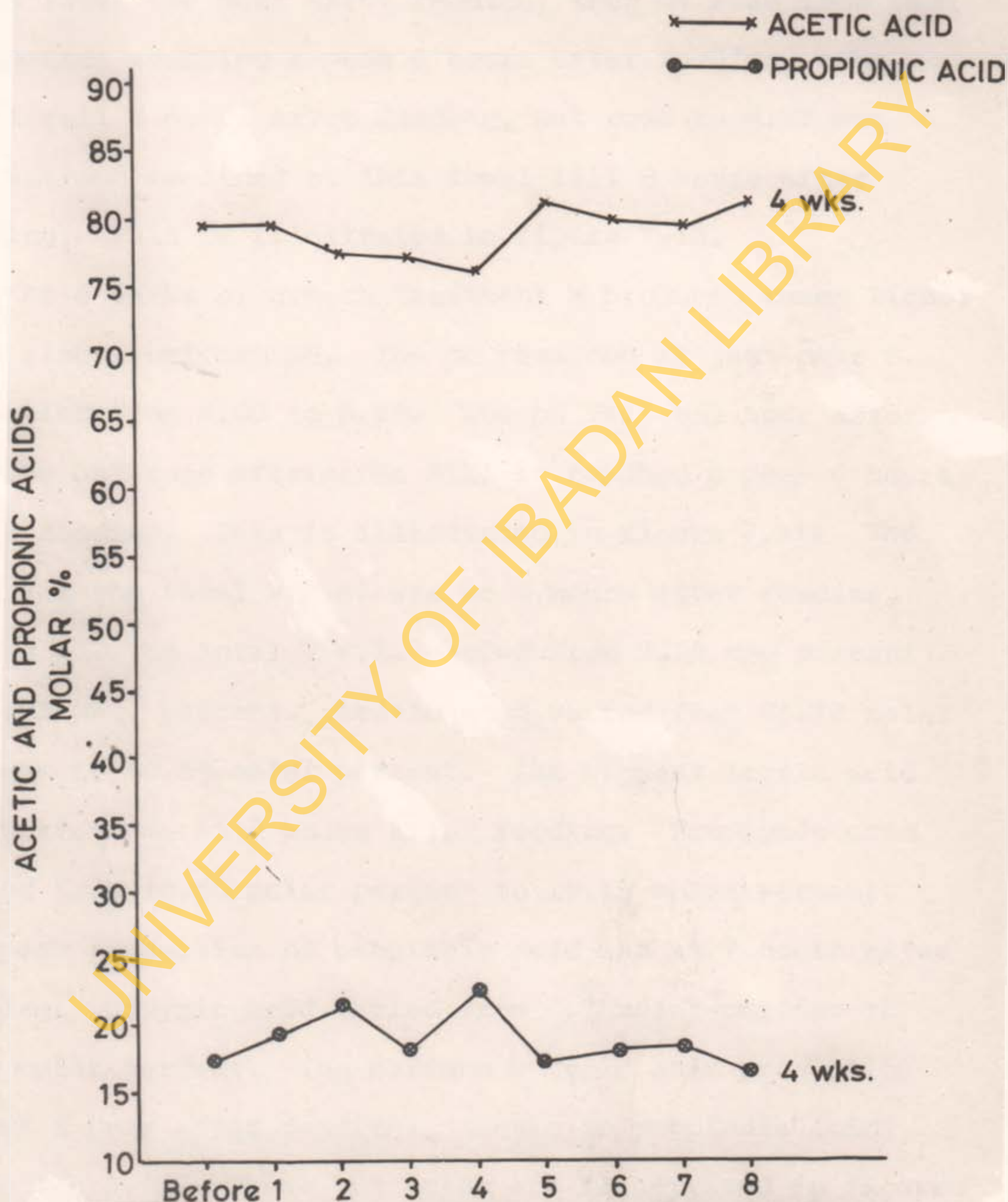

FIG. 7·15 TIME OF SAMPLING (hrs.) AFTER FEEDING.

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The total V.F.A. in blood serum did not vary much. The level dropped a bit from the prefeeding value to a lower level one hour after feeding, then it rose from then onwards reaching a peak 3 hours after feeding. The level fell 4 hours after feeding, but rose to 4.37 meq percent and remained at this level till 8 hours after feeding. This is illustrated in figure 7.13.

At 8 weeks of growth treatment H produced rumen liquor with almost uniform pH. The pH remained at just over 8. It varied from 8.00 to 8.70. The pH fell one hour after feeding but rose afterwards till it reached a peak 6 hours after feeding. This is illustrated in figure 7,11. The peak for the total V.F.A. was at 4 hours after feeding, figure 7.12. The total V.F.A. varied from 5.72 meq percent to 8.85 meq. percent. Acetic acid varied from 76.78 molar percent to 80.69 molar percent. The highest acetic acid production was at 2 hours after feeding. Propionic acid varied from 12.50 molar percent to 16.15 molar percent. The peak production of propionic acid was at 7 hours after feeding. Butyric acid varied from 4.7 molar percent to 7.24 molar percent. The maximum butyric acid production was at 1 hour after feeding. Acetic to propionic acid ratio was 5.25. These two acids are illustrated in figures 7.15 and 7.14.

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The total V.F.A. in blood serum remained at just over 4 up to 4 hours after feeding. Its level dropped from 5 hours after feeding to 7 hours after feeding. By 8 hours after feeding the level rose almost to the prefeeding level. The graphical presentation of the total V.F.A. in blood serum is seen in figure 7.13.

Table 7.5 showed the pH, total V.F.A., individual V.F.A. Acetic to propionic acid ratio, and total V.F.A. in blood serum, when treatment J was fed at 4, 8 and 12 weeks of growth. The pH variation over 9 hours of sampling, and the total V.F.A. in rumen and blood serum acetic to propionic acid ratio, over the same sampling periods are also illustrated in figures 7.16, 7.17, 7.18, 7.19, 7.20 and 7.21 respectively. At 4 weeks of growth the pH remained at just over 7. There was a fall in pH level after feeding and it rose till it reached a peak 4 hours after feeding. At 8 weeks of growth the pH rose after feeding but dropped after one hour. There was a rise from 3 hours onwards till it reached the peak 6 hours after feeding. At 12 weeks of growth the pH remained at just over 7. The pH increased after feeding but the highest level was at 8 hours after feeding.

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (J) AT DIFFERENT STAGES OF GROWTH TO ZEBU CATTLE ON RUMINAL PH

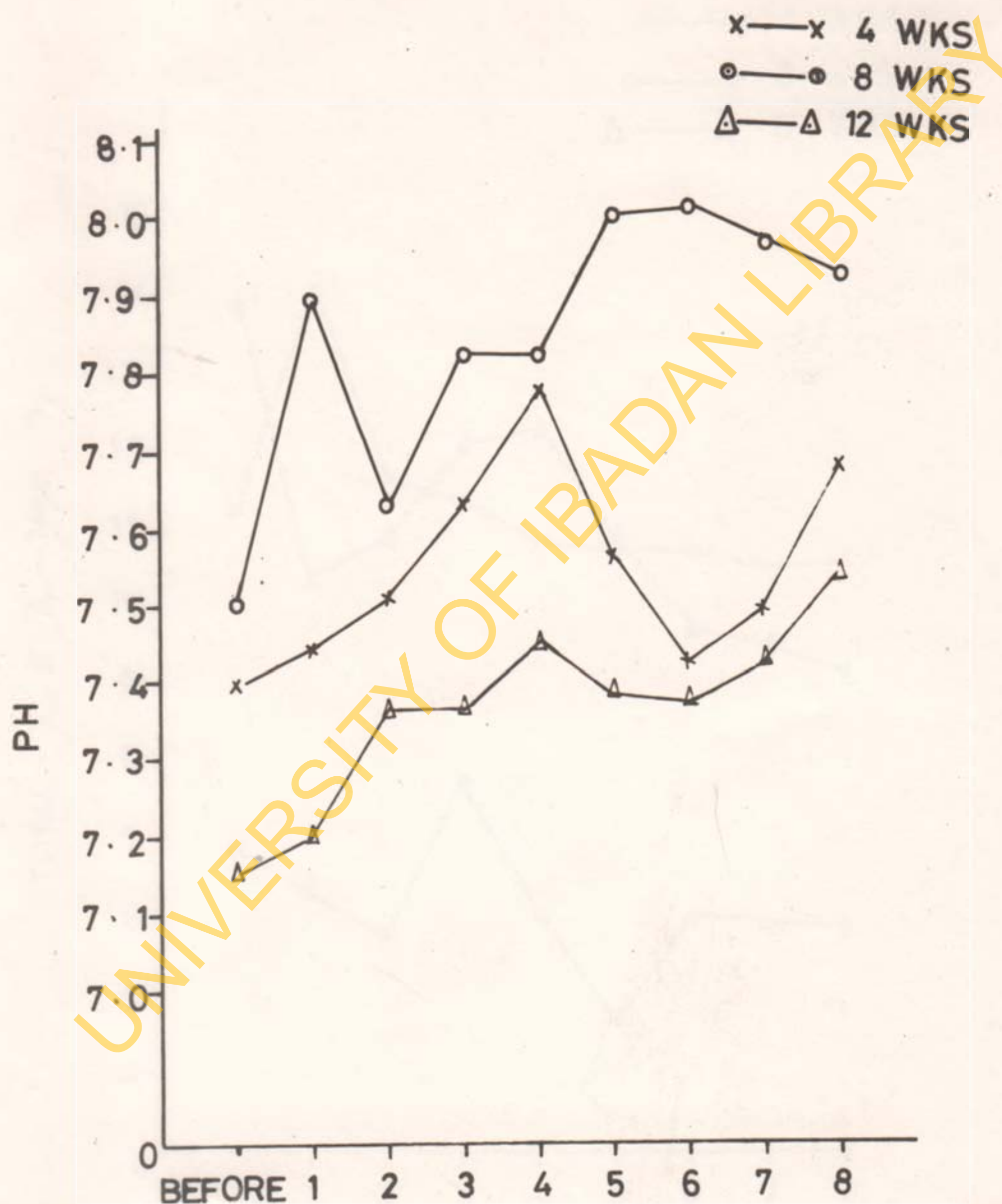

FIG. 7.16 TIME OF SAMPLING (hrs) AFTER FEEDING

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (J) ON THE TOTAL VOLATILE FATTY ACID PRODUCTION BY ZEBU CATTLE.

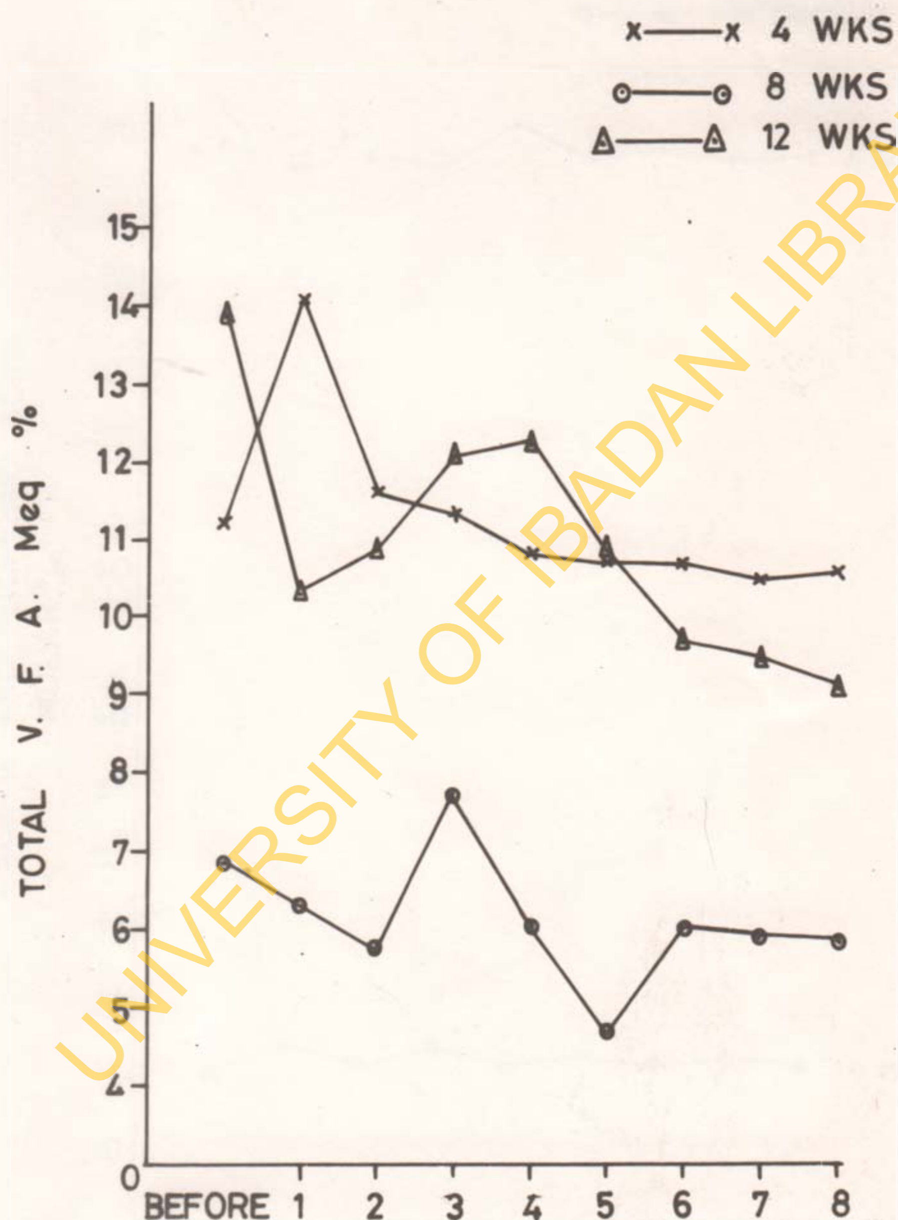

FIG.7.17 TIME OF SAMPLING (hrs) AFTER FEEDING

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (J) AT 4 WKS. OF AGE TO ZEBU CATTLE ON ACETIC AND PROPIONIC ACIDS (MOLAR %)

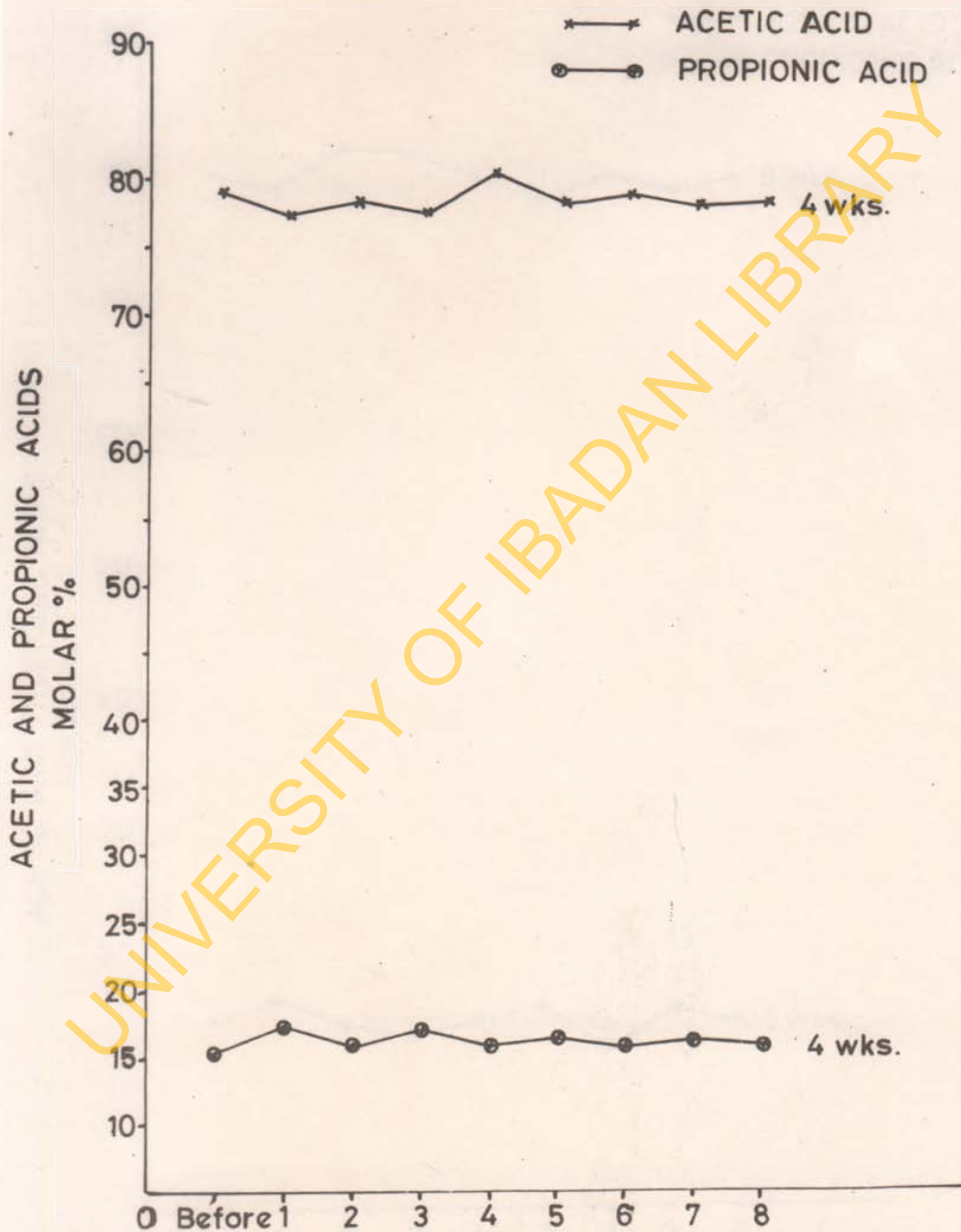

FIG.7.18 TIME OF SAMPLING (hrs.) AFTER FEEDING.

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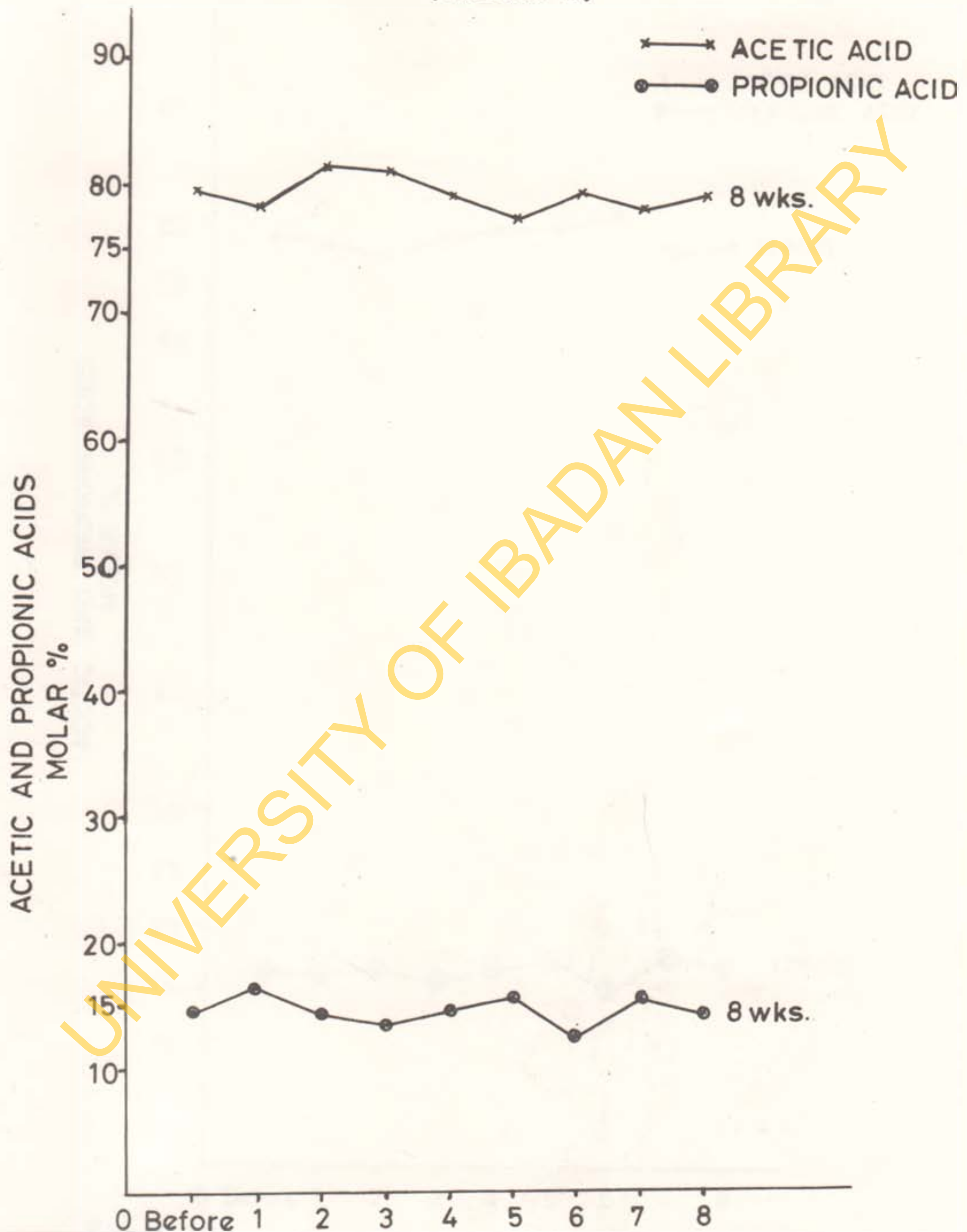

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FIG. 7.19 TIME OF SAMPLING (hrs.) AFTER FEEDING.

EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (J) AT 12 WKS. OF AGE TO ZEBU CATTLE ON ACETIC AND PROPIONIC ACIDS (MOLAR %)

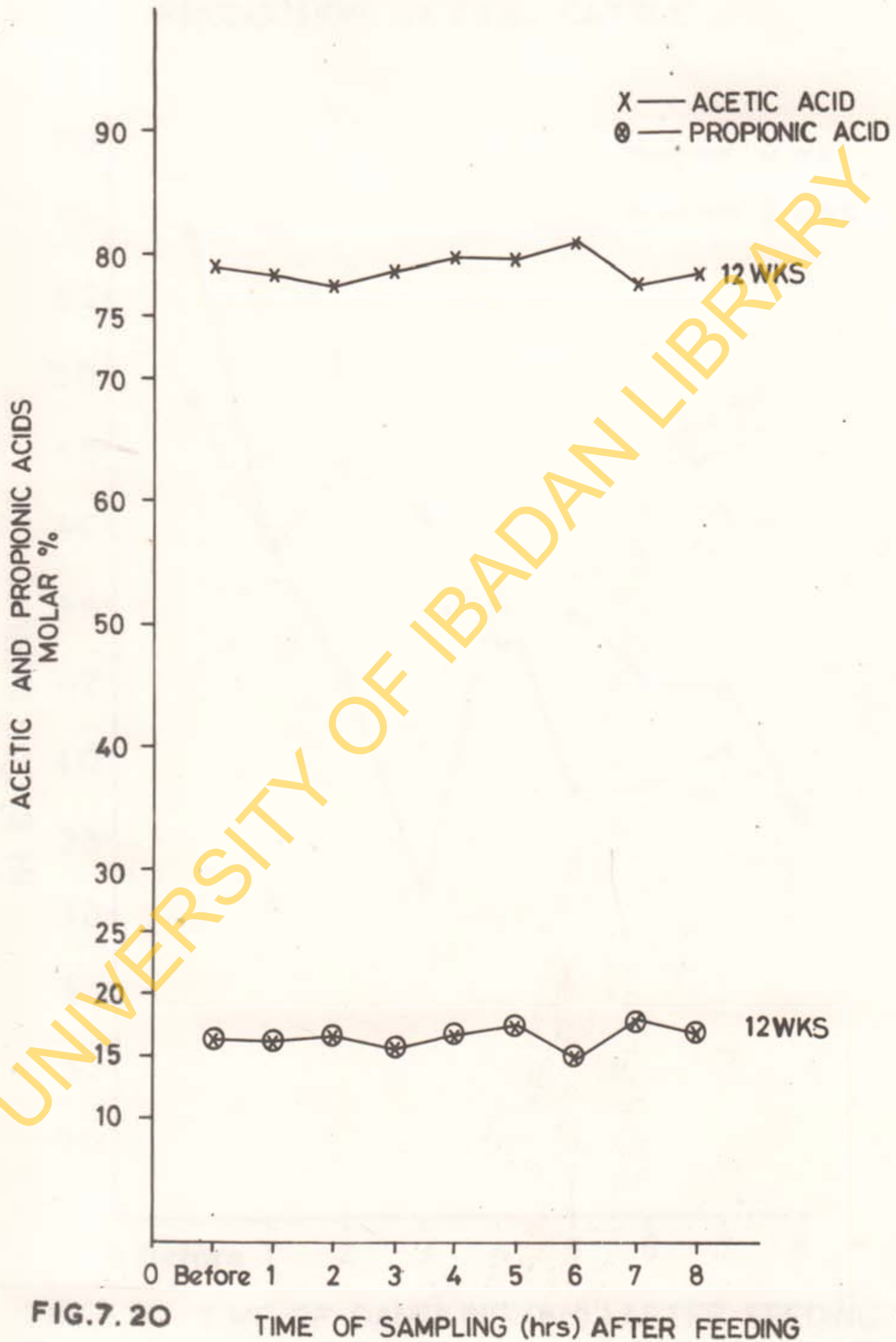

FIG.7.20

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (J) ON THE TOTAL BLOOD VOLATILE FATTY ACID PRODUCTION BY ZEBU CATTLE.

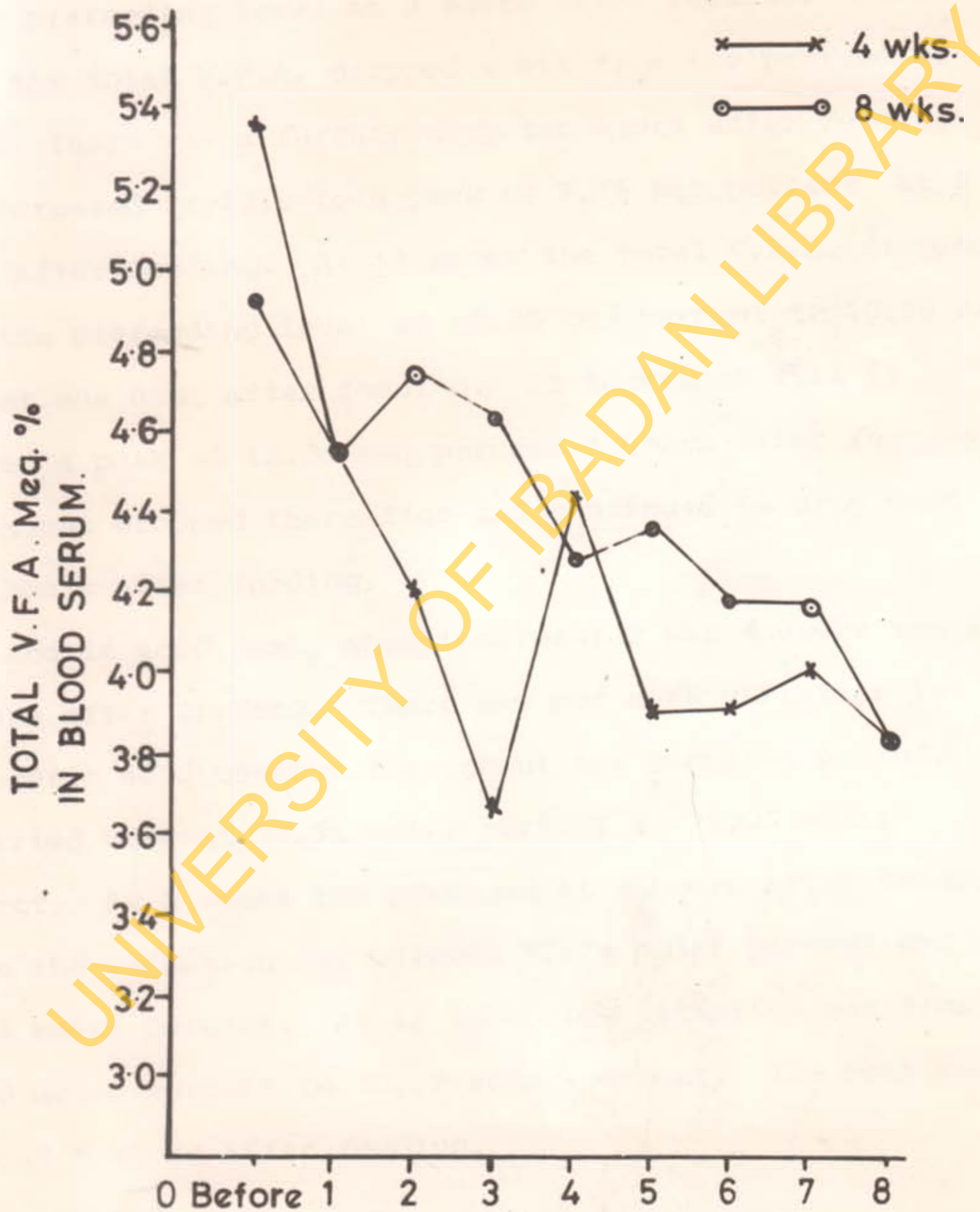

FIG.7. 21 TIME OF SAMPLING (hrs.) AFTER FEEDING.

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At 4 weeks the total V.F.A. increased after feeding to a maximum of 14.13 meq percent one hour after feeding. The total V.F.A. content dropped afterwards and came close to the prefeeding level at 8 hours after feeding. At 8 weeks the total V.F.A. dropped a bit from the prefeeding level. There was a further drop two hours after feeding but increased rapidly to a peak of 7.76 meq percent at 3 hours after feeding. At 12 weeks the total V.F.A. dropped from the prefeeding level of 13.89 meq percent to 10.39 meq percent one hour after feeding. It increased till it reached a peak of 12.34 meq percent 4 hours after feeding. The amount dropped thereafter and continued to drop even at 8 hours after feeding.

Acetic acid peak, when treatment J was 4 weeks was at 6 hours after feeding. There was not much variation in the acetic acid content throughout the sampling period. It varied between 77.91 molar percent to 79.05 molar percent. At 8 weeks the peak was at 2 hours after feeding, while the variation was between 77.74 molar percent and 81.55 molar percent. At 12 weeks the variation was from 77.38 molar percent to 80.17 molar percent. The peak was also at 6 hours after feeding.

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At 4 weeks of growth the propionic acid content varied between 15.27 to 17.12 molar percent. There was a sharp rise after feeding to a peak one hour after feeding. The content rose and fell afterwards and did not vary much from the prefeeding level at 8 hours after feeding. At 8 weeks of growth the propionic acid peak was also at one hour after feeding. The level fell afterwards and came close to the prefeeding level 8 hours after feeding. At 12 weeks of growth propionic acid peak was at 7 hours after feeding. The variation was from 14.93 to 17.78 molar percent.

Butyric acid content when treatment J was at 4 weeks varied from 4.97 molar percent to 6.01 molar percent. The peak of 6.01 molar percent was at 4 hours after feeding. The level fell to nearly the prefeeding level. At 8 weeks butyric acid content varied from 4.23 to 7.60 molar percent. The peak of 7.60 molar percent was at 6 hours after feeding. At 12 weeks butyric acid content varied between 4.16 and 6.07 molar percent at 2 hours after feeding. The amount dropped till it came close to the prefeeding amount.

The acetic to propionic ratio varied from 4.55 to 5.06. The mean was 4.89 when treatment J was 4 weeks. The ratio also varied from 4.72 to 6.39 when the treatment

was 8 weeks. The mean was 5.48. At 12 weeks the variation was from 4.36 to 5.37. The mean was 4.77. The ratios for the treatment at 4 and 12 weeks were close while the ratio when the treatment was 8 weeks was higher.

The total V.F.A. in blood serum did not show much variation throughout the sampling period. The amount dropped after feeding and thereafter rose and fell. At 8 weeks the amount also fell after feeding but rose and fell slightly. The amount at 8 hours after feeding was the same as when the feed was at 4 weeks. The means for both growth periods are close. At 12 weeks the total V.F.A. in blood serum remained fairly constant till 3 hours after feeding before it started to fluctuate. The mean was 4.23 meq/100 ml.

Table 7.6, and figures 7.22, 7.23, 7.24 and 7.25 showed the effect of time of sampling on the pH, total V.F.A., individual V.F.A., acetic to propionic acid ratio and the total V.F.A. in blood serum by Zebu cattle fed treatment K at 4, 8 and 12 weeks.

When treatment K was 4 weeks and fed to Zebu cattle the pH of the rumen liquor was above 7 but below 8. The variation was not much since the range was between 7.19 and 7.55 during the sampling period. The highest was

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (K) AT DIFFERENT STAGES OF GROWTH TO ZEBU CATTLE ON RUMINAL PH.

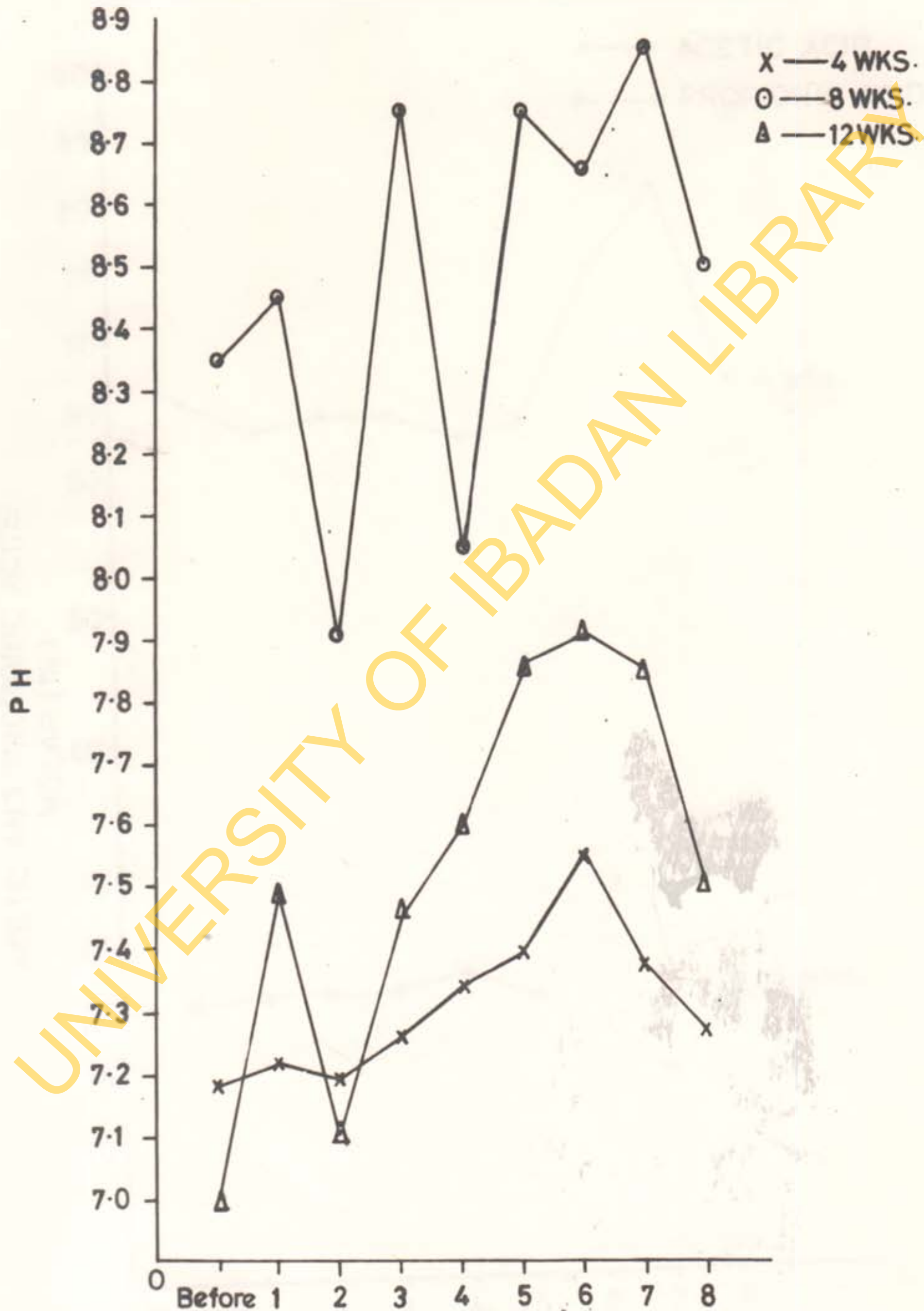

FIG.7.22 TIME OF SAMPLING (hrs) AFTER FEEDING

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGE (K) AT 4 WKS. OF AGE TO ZEBU CATTLE ON ACETIC AND PROPIONNC ACIDS (MOLAR %)

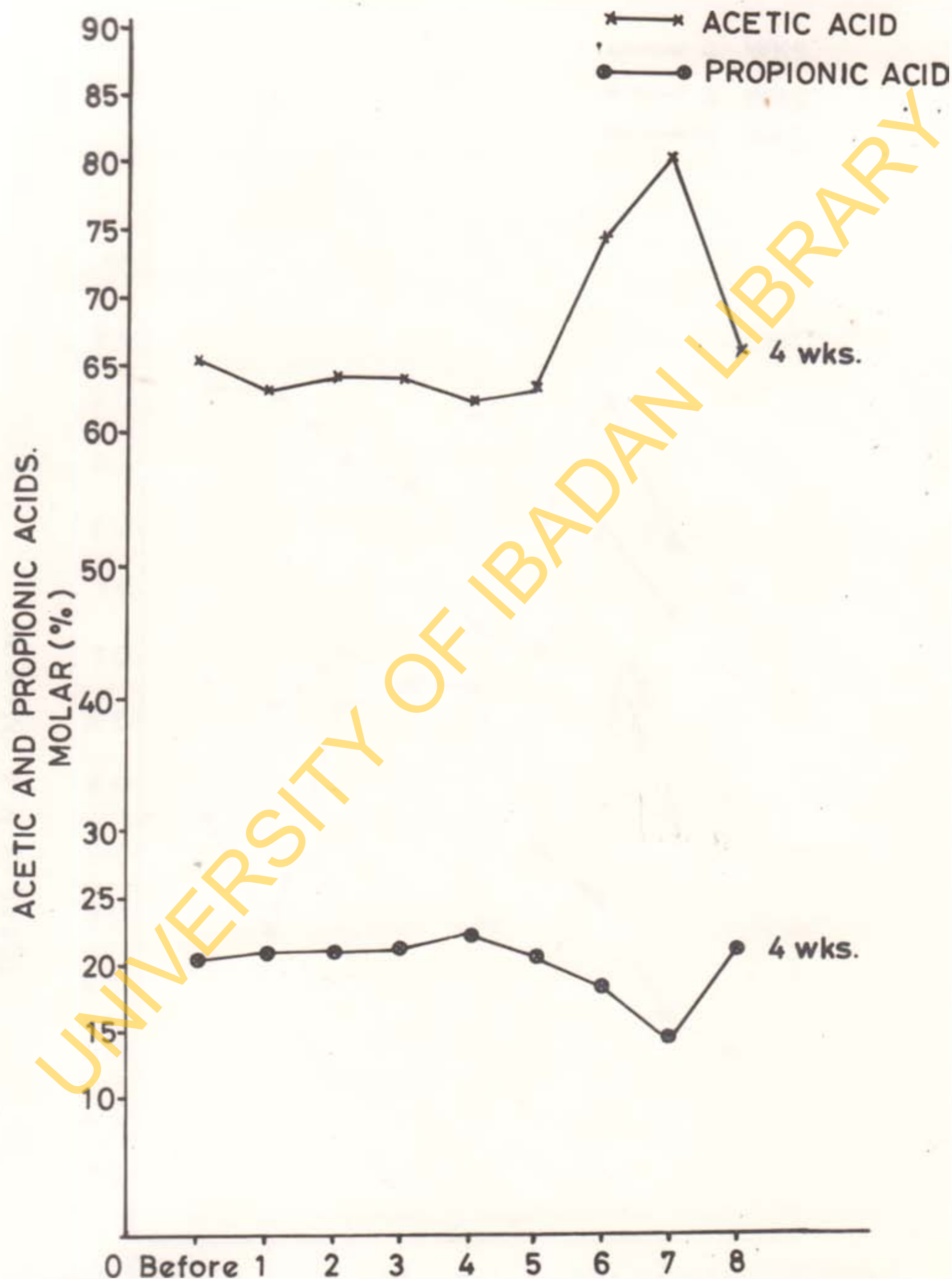

FIG.7.23 TIME OF SAMPLING (hrs.) AFTER FEEDING.

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (K) ON THE TOTAL VOLATILE FATTY ACID PRODUCTION BY ZEBU CATTLE.

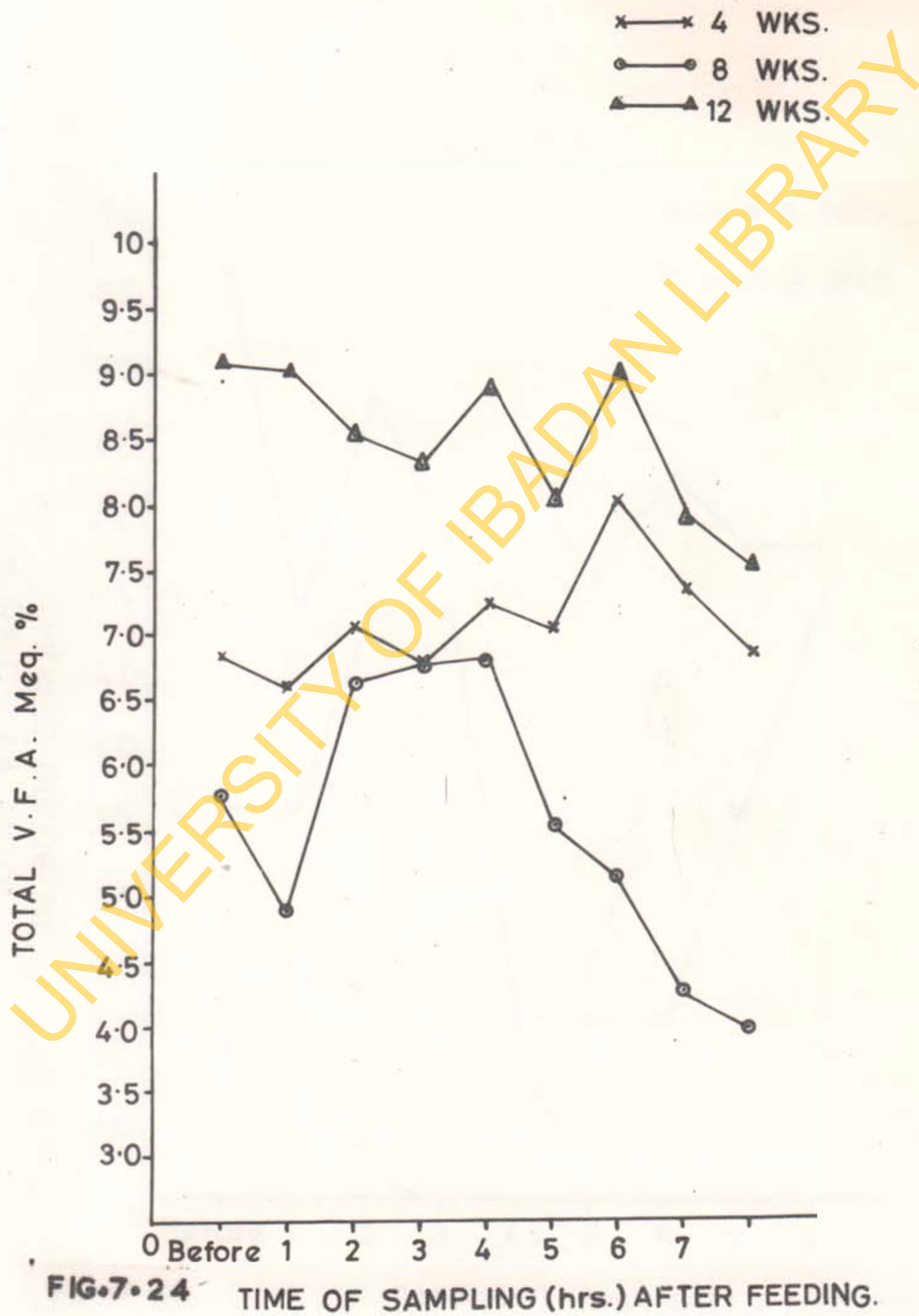

FIG.7.24

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (K) ON THE TOTAL BLOOD VOLATILE FATTY ACID PRODUCTION (Meq.%) BY ZEBU CATTLE.

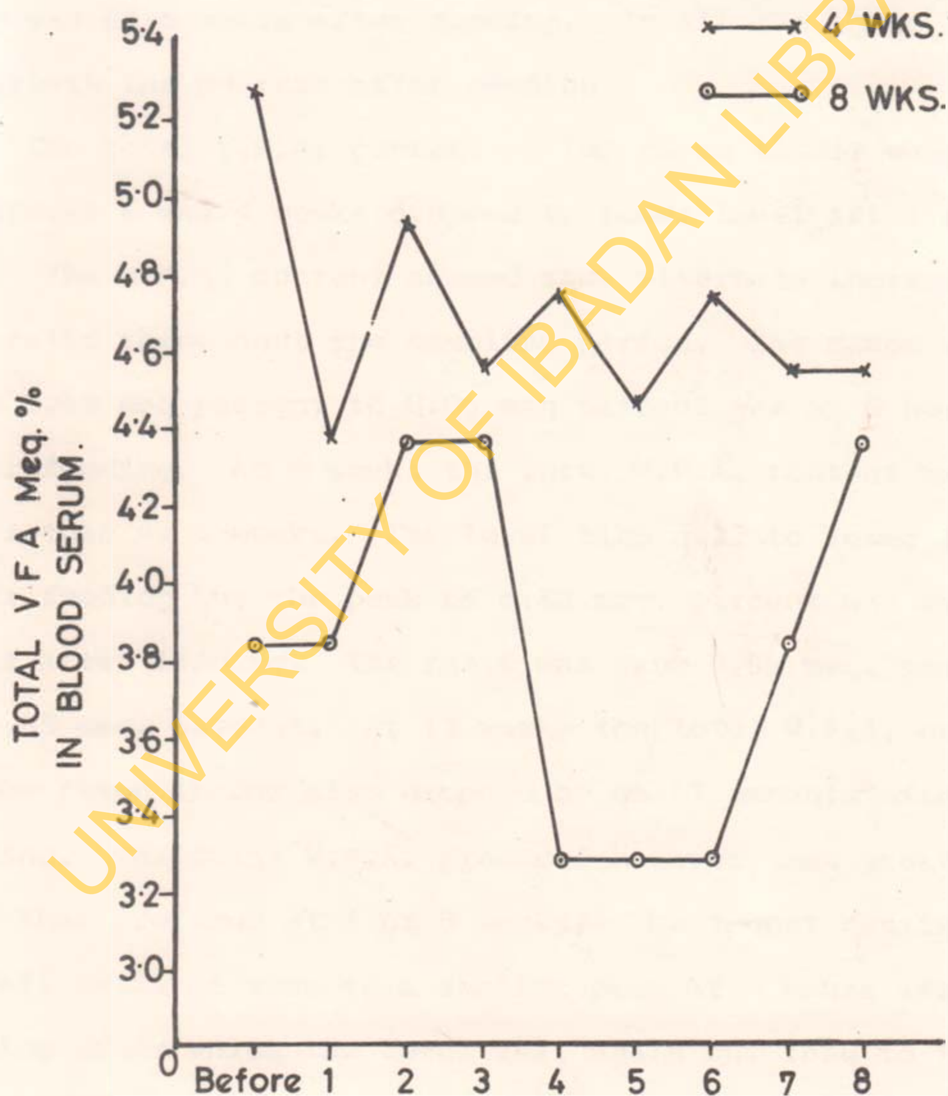

FIG.7.25 TIME OF SAMPLING (hrs.) AFTER FEEDING.

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7.55 and it was at 6 hours after feeding. At 8 weeks the pH was higher throughout the sampling periods. The pH was above 7 but below 9. The peak was at 7 hours after feeding and the pH range was between 7.90 and 8.85. At 12 weeks the pH range was from 7.10 to 7.90. The peak of 7.90 was at 6 hours after feeding. In all the three stages of growth the pH rose after feeding.

The total V.F.A. content of the rumen liquor when treatment K was 4 weeks dropped to lower level after feeding. The V.F.A. content showed some alternate increases and falls throughout the sampling period. The range was from 6.60 meq percent to 8.06 meq percent was at 6 hours after feeding. At 8 weeks the total V.F.A. content was lower than at 4 weeks. The level also fell to lower level after feeding but the peak of 6.83 meq. percent was at 4 weeks after feeding. The range was from 3.99 meq. percent to 6.83 meq. percent. At 12 weeks the total V.F.A. content of the rumen liquor also dropped by small amounts after feeding. The total V.F.A. produced however, was greater than that produced at 4 or 8 weeks. The amount continued to fall until it rose to a smaller peak at 4 hours after feeding after which the level fell again but rose to the larger peak 6 hours after feeding.

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Separation into individual V.F.A. by GLC

The chromatographic separation of the V.F.A. in the rumen liquor into the individual V.F.A. showed acetic acid as the major acid in all the three stages of growth. At 4 weeks of growth the acetic acid content dropped to lower level after feeding. It rose to the peak 7 hours after feeding. The level then dropped again. On the average it formed 67.17 molar percent of the total V.F.A. separated. The range was from 62.57 molar percent to 80.40 molar percent. On the other hand propionic acid content increased after feeding until it got to the peak of 22.57 molar percent 4 hours after feeding. The level then dropped. The lowest amount of 14.68 molar percent coincided with the acetic acid peak. The mean propionic acid during the sampling period was 20.08 molar percent.

Butyric acid content was the smallest in all cases. It rose immediately after feeding but fell 2 hours after feeding and remained almost constant until 5 hours after feeding, when the peak production of 15.10 molar percent was obtained. The amount dropped sharply after 5 hours. The mean butyric acid content over the sampling period was 12.75 molar percent.

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At 8 weeks the acetic acid content rose immediately after feeding but rose and fell alternately afterwards. The peak was at 1 hour after feeding. The range was from 78.11 molar percent to 82.33 molar percent. The average was 79.82 molar percent. The acetic acid content was higher at 8 weeks than at 4 weeks.

Propionic acid level dropped immediately after feeding, but rose steadily afterwards till it got to a peak 6 hours after feeding. Propionic acid content was lower at 8 weeks than at 4 weeks. The average amount was 14.23 molar percent and the range was from 12.48 molar percent to 15.59 molar percent.

Butyric acid range was between 5.18 molar percent and 7.46 molar percent. The amount was lower than at 4 weeks. The peak was at 8 hours after feeding, and the mean was 5.95 molar percent.

At 12 weeks the acetic acid content rose up to two hours after feeding, before the level then dropped to lower levels and continued so until the end of sampling period. The peak was at 2 hours after feeding. The average of 78.20 molar percent was greater than the value at 4 weeks but lower than the value at 8 weeks.

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Propionic acid level dropped immediately after feeding and continued to drop but rose sharply to a peak 4 hours after feeding and the level dropped again. The range was from 12.59 molar percent to 15.24 molar percent. The average was 13.97 molar percent, a value less than that at 4 or 8 weeks.

Butyric acid level fell after feeding and continued so until 2 hours after feeding. After 2 hours the level rose till it reached a smaller peak 5 hours after feeding. The level fell after this period but rose to the larger peak 8 hours after feeding. The range was from 5.30 molar percent to 9.87 molar percent. The mean was 7.83 molar percent and this value was less than that at 4 weeks but more than the value at 8 weeks.

Acetic to propionic acid ratio range when treatment K was 4 weeks old was from 2.77 to 5.48. The mean was 3.43. At 8 weeks the range was from 5.06 to 6.60 and the mean was 5.64. At 12 weeks the ratio varied from 4.97 to 6.26, while the mean was 5.62. This value was less than the value at 8 weeks but greater than the value at 4 weeks.

Blood V.F.A.

The total V.F.A. in blood serum dropped immediately after feeding, but rose to a peak 2 hours after feeding.

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The level showed some alternate rises and falls after 2 hours. The amount remained constant as from 7 hours after feeding. The range was from 4.37 to 4.92 meq percent, and the mean was 4.68 meq percent.

At 8 weeks the total V.F.A. in the blood serum was lower than the amount during 4 weeks of growth. The peak was at 2 hours after feeding and it remained at this level till 3 hours after feeding. The level dropped after 3 hours and remained constant up till 6 hours after which it rose sharply till it reached the second peak 8 hours after feeding. The mean was 3.89 meq percent and the range was from 3.28 meq percent to 4.37 meq percent. The lower V.F.A. level of the blood serum may be as a result of the lower V.F.A. level of the rumen liquor when treatment K was 8 weeks.

At 12 weeks the total V.F.A. in blood serum remained fairly constant until 4 hours after feeding before falling. The mean was 3.93 meq/100 ml.

Table 7.7, figures 7.26, 7.27, 7.28, and 7.29 showed the effect of sampling on the pH, total V.F.A., individual V.F.A., Acetic to propionic acid ratio and total V.F.A. in blood serum by Zebu cattle fed treatment L at 4, 8 and 12 weeks of growth.

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (L) AT DIFFERENT STAGES OF GROWTH TO ZEBU CATTLE ON RUMINAL PH.

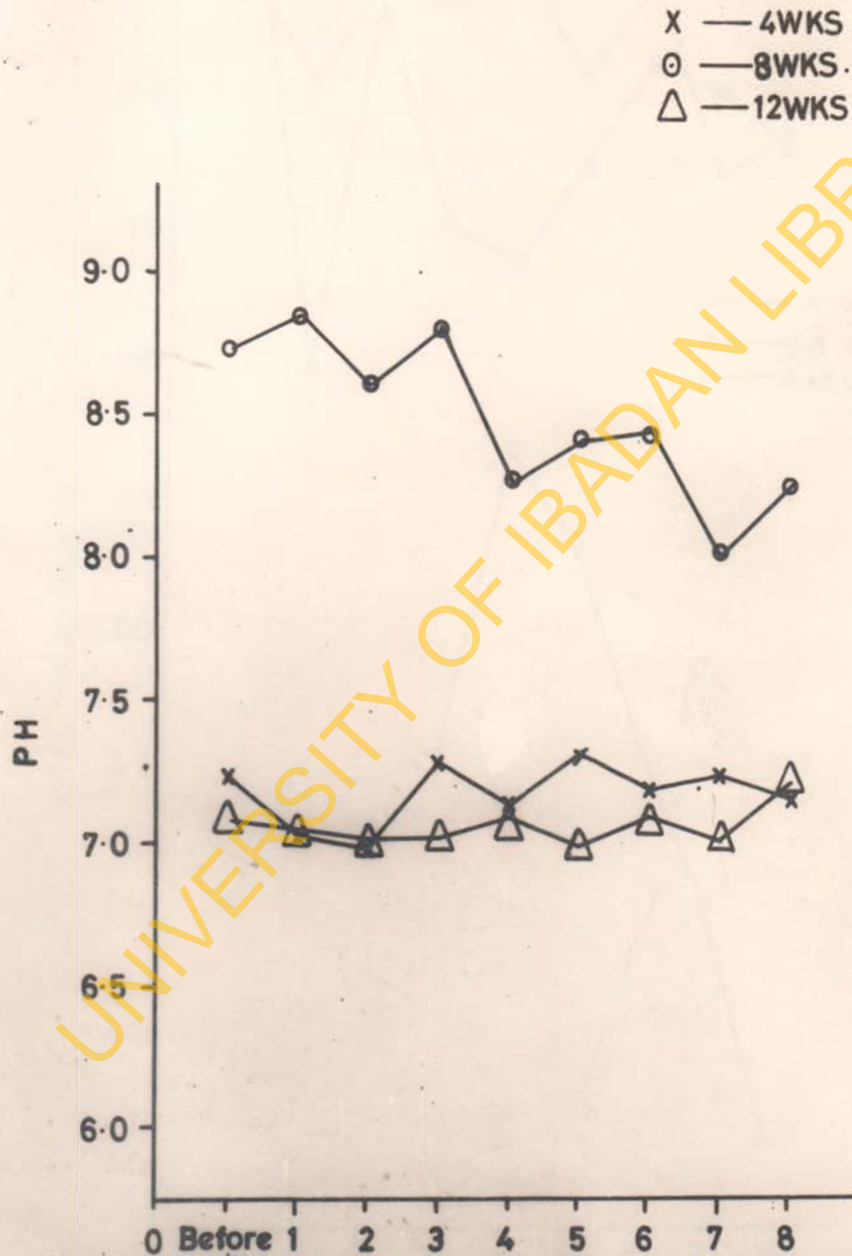

FIG.7.26 TIME OF SAMPLING (hrs) AFTER FEEDING

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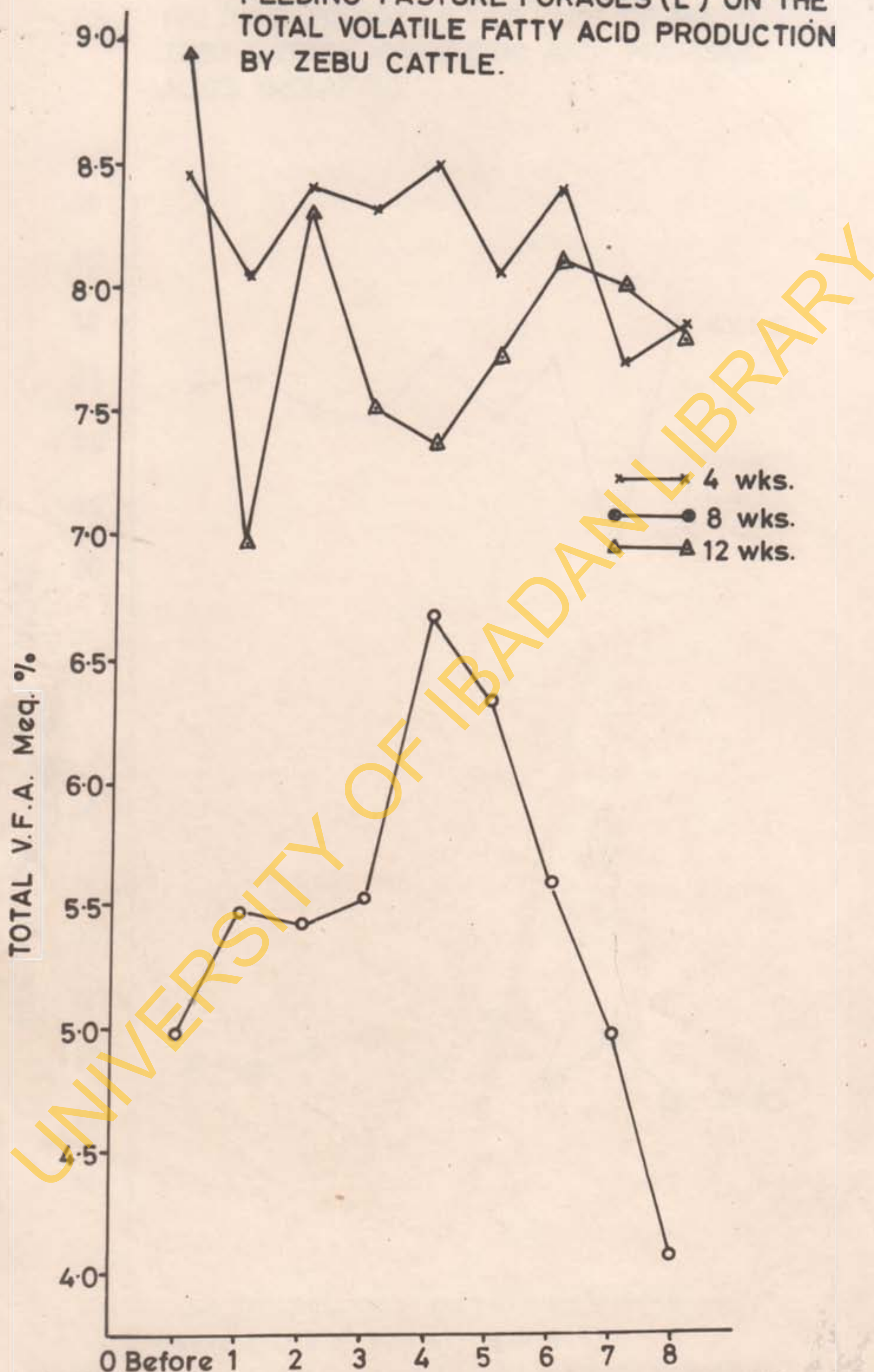

FIG.7.27
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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (L) AT 4WKS OF AGE TO ZEBU CATTLE ON ACETIC AND PROPIONIC ACIDS (MOLAR %)

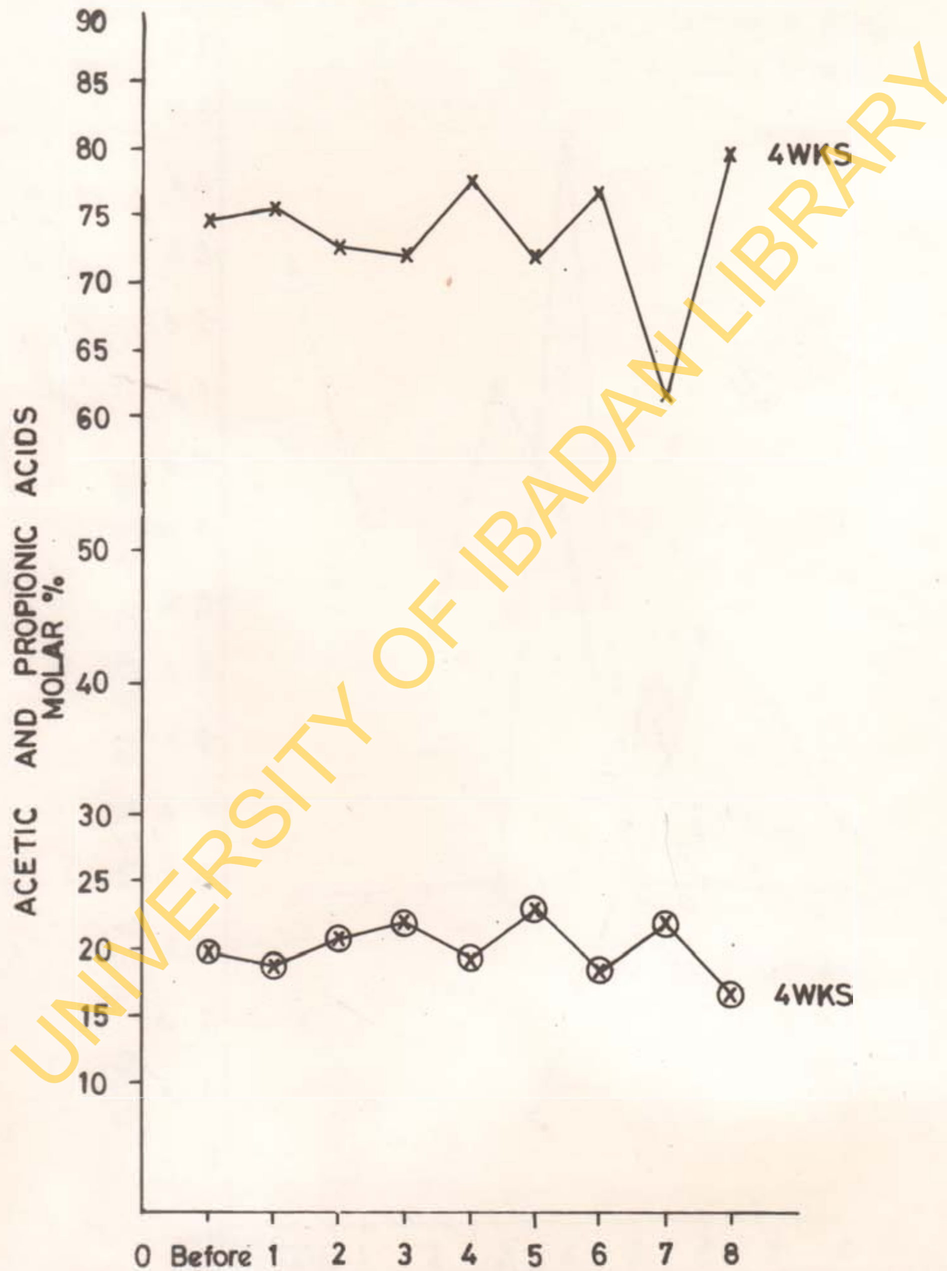

FIG.7.28 TIME OF SAMPLING (hrs) AFTER FEEDING

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EFFECT OF TIME OF SAMPLING AFTER FEEDING PASTURE FORAGES (L) ON THE TOTAL BLOOD VOLATILE FATTY ACID PRODUCTION (Meq)%) BY ZEBU CATTLE.

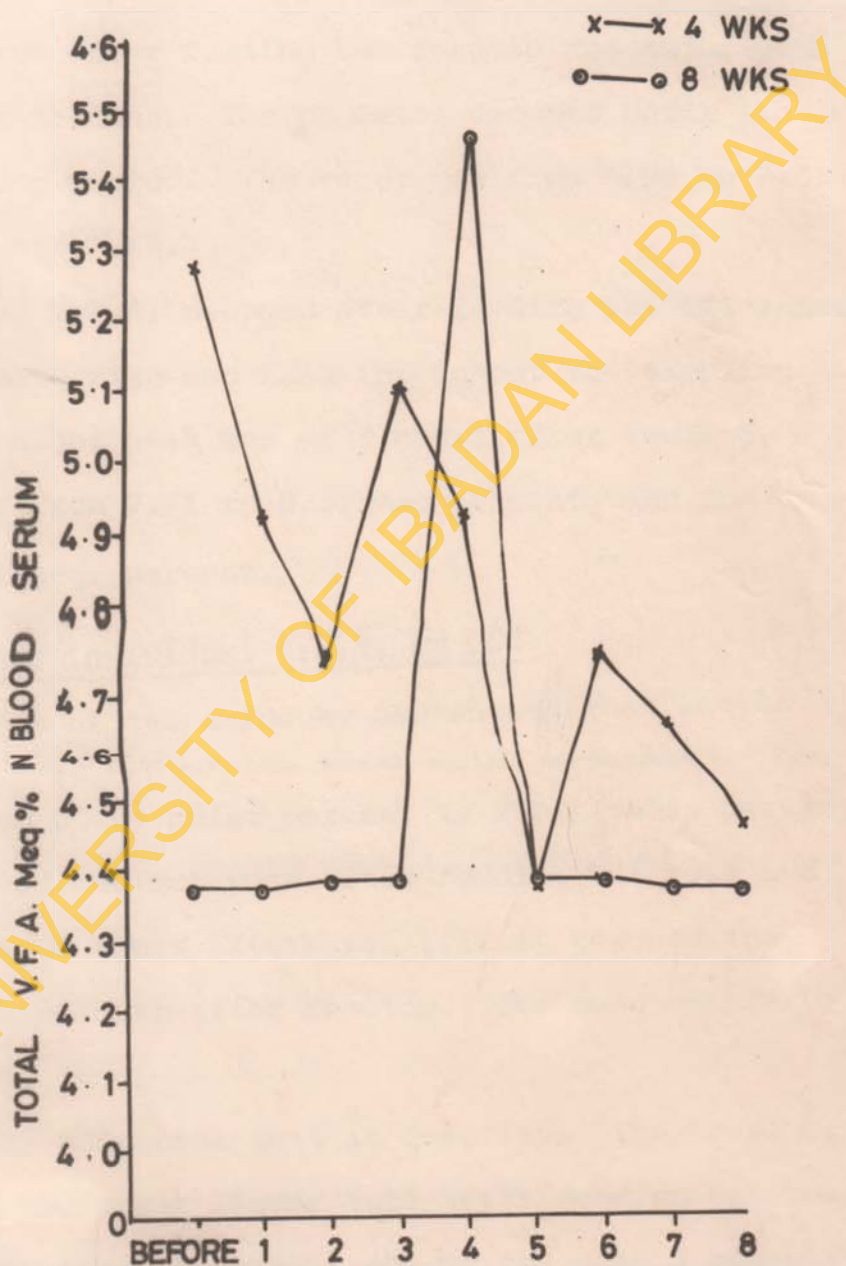

FIG.7.29 TIME OF SAMPLING (hrs) AFTER FEEDING

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At 4 weeks the pH of the rumen liquor dropped immediately after feeding and continued to drop slowly, until it rose to a minor peak 3 hours after feeding. The level dropped 4 hours after feeding but rose to the major peak 5 hours after feeding. The pH later dropped until the end of sampling period. The range was from 6.96 to 7.31 and the mean was 7.17.

The total V.F.A. dropped after feeding but afterwards showed alternate rises and falls throughout the sampling period. The major peak was at 4 hours after feeding. The range was from 7.71 to 8.51 meq percent, and the mean was 8.21 meq. percent.

Separation into individual V.F.A. by GLC

Separation of the acids by GLC showed that acetic acid made up over 60% of the total acids separated. The range was from 60.94 molar percent to 79.11 molar percent. The acetic acid content rose after feeding but rose and fell a number of times afterwards till it reached the highest level 8 hours after feeding. The mean was 73.06 molar percent.

Propionic acid came next in quantity. The level of this acid in the rumen liquor fell after feeding but rose and fell a few times before it got to the peak 5 hours after feeding. The range was from 16.83 molar percent to 22.54 molar percent.

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Butyric acid formed the smallest proportion of the V.F.A. separated. The level of this acid rose after feeding and continued to rise slowly until it rose very sharply to a peak 7 hours after feeding and it immediately fell sharply also one hour after. The range was from 4.06 molar percent to 17.32 molar percent, and the mean was 6.99 molar percent.

Acetic to propionic acid ratio varied between 2.80 and 4.70, while the mean was 3.71.

Blood V.F.A.

The total V.F.A. in blood serum fell after feeding but rose to a peak 3 hours after feeding. The V.F.A. content then dropped by very small amounts until the end of sampling. The variation was from 4.37 meq percent to 5.10 meq percent, while the mean was 4.80 meq. percent.

At 8 weeks pH of treatment L remained high throughout the sampling period. The pH was above 8 but below 9. The peak was attained 1 hour after feeding. It later dropped by small units. The range was from 8.0 to 8.85. The mean was 8.48, and the pH was higher than that at 4 weeks.

Total V.F.A. rose after feeding and it rose slowly to a peak 4 hours after feeding. The level dropped sharply

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after 6 hours, but the range was from 4.05 meq. percent to 6.67 meq percent, and the mean was 5.43 meq. percent. The peaks for 4 and 8 weeks treatment were obtained at 4 hours after feeding. But the total V.F.A. content of the rumen liquor was lower when the feed was 8 weeks.

Individual V.F.A.

Acetic acid formed over 70 percent of the total V.F.A. separated by GLC. The range was from 75.43 molar percent to 78.50 molar percent. The variation was small, but the mean was 76.65 molar percent and the highest amount was obtained 8 hours after feeding.

Propionic acid came next in production. The propionic acid level fell by small amount after feeding. The amount remained fairly constant until 5 hours after feeding when it reached a minor peak, but the level fell at 6 hours after feeding but rose sharply to the major peak 7 hours after feeding. The mean was 14.23 molar percent. Propionic acid level at 8 weeks was smaller than the level at 4 weeks.

Butyric acid showed a lot of variation at 8 weeks. The level rose after feeding but continued to rise and fall throughout the sampling period. The peak was attained 3 hours after feeding. The range was from 7.11 molar

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percent to 10.36 molar percent. The mean was 9.12 molar percent, a level higher than that at 4 weeks.

A/P

Acetic to propionic acid ratio remained fairly constant. The ratio was above 5 but below 6 except for the ratio 7 hours after feeding. The range was from 4.73 to .76.

Blood V.F.A.

Total V.F.A. in blood serum at 8 weeks did not vary much also. The level of V.F.A. remained fairly constant throughout. There was however a peak 4 hours after feeding after which the level dropped sharply to the prefeeding level. The peak was reached one hour later than at 4 weeks.

12 Weeks

At 12 weeks treatment L produced rumen liquor with fairly constant pH. The pH range was from 6.98 to 7.20, but the peak was at 8 hours after feeding. The pH level dropped after feeding, and the mean was 7.05. The total V.F.A. dropped immediately after feeding but rose again 2 hours after feeding. The V.F.A. content rose and fell a number of times during the sampling period. The range was from 6.97 meq percent to 8.32 meq percent, while the peak

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production was at 2 hours after feeding. The total V.F.A. content of the rumen liquor at 12 weeks was higher than at 8 weeks.

Individual V.F.A.

Chromatographic separation of the fatty acid showed acetic acid as the major acid, since it formed over 70% of the total acids separated. The acetic acid level increased after feeding and rose to a minor peak 4 hours after feeding. The major peak was at 7 hours after feeding, but the range was from 76.15 molar percent to 79.02 molar percent, while the mean was 76.55 molar percent.

Propionic acid came next in quantity and this acid also increased after feeding. Its level however dropped after one hour and came close to the prefeeding level at the end of the sampling period. The peak production was at one hour after feeding, and the range was from 13.98 molar percent to 16.42 molar percent. The mean was 14.96 molar percent.

Butyric acid level dropped after feeding but rose to a minor peak 3 hours after feeding, before the level then dropped sharply one hour after but rose sharply also to the major peak 5 hours after feeding. The level then dropped till the end of sampling, and the range was from

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6.38 molar percent to 12.35 molar percent. The mean was 8.48 molar percent.

A/P

Acetic to propionic acid ratio varied between 4.66 and 5.65, and the mean was 5.13. The highest ratio was at 7 hours after feeding. The ratios for 8 and 12 weeks treatments were quite close.

Blood V.F.A.

The level of blood V.F.A. remained about the same throughout the experimental period. The mean was 4.47 meq/100 ml.

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TABLE 7.4

Effect of time of sampling on the pH, Total V.F.A., individual V. F. A. Acetic/Propionic acid ratio and Total V.F.A. in blood serum by Zebu cattle fed treatments (H) at two stages of growth.

Time of clipping forage (weeks)	Time of sampling	pH of liquor	Total V.F.A. Meq per 100 ml Rumen liquor	Individual V.F.A. of rumen liquor Molar %			A/P	Total V. F. A. in blood serum meq/100 ml
				Acetic Acid	Propionic Acid	Butyric Acid		
H 4	Before feeding	7.42	7.07	79.61	17.29	3.10	4.60	4.28
	Hours after feeding							
	1	7.05	4.69	79.65	19.27	1.08	4.61	4.19
	2	7.38	4.82	77.62	21.60	0.78	3.59	4.28
	3	7.15	3.90	77.48	18.20	4.32	4.26	4.74
	4	7.00	5.38	76.23	22.74	1.03	3.35	4.19
	5	6.96	5.08	81.46	17.03	1.51	4.78	4.37
	6	7.16	4.75	80.15	18.11	1.74	4.43	4.37
	7	7.26	5.08	79.81	18.43	1.76	4.33	4.37
	8	6.93	4.42	81.73	16.54	1.73	4.94	4.37
	Mean*	7.15	5.02	79.30	18.80	1.89	4.32	4.37
8	Before feeding	7.70	8.60	75.09	17.78	7.13	4.22	4.28
	Hours after feeding							
	1	8.27	8.20	76.78	15.98	7.24	4.80	4.19
	2	8.00	7.62	80.69	12.50	6.81	6.46	4.19
	3	8.33	7.61	79.81	15.10	5.71	5.24	4.46
	4	8.20	8.85	78.76	15.28	5.96	5.15	4.10
	5	8.17	7.21	80.62	14.68	4.70	5.49	3.74
	6	8.70	5.87	78.76	15.87	5.38	4.96	3.74
	7	8.30	5.72	78.07	16.15	5.78	4.83	3.46
	8	8.00	5.79	80.35	13.10	6.55	6.13	4.01
	Mean*	8.19	7.27	78.70	15.16	6.14	5.25	4.02

*Each is the mean of 18 determinations.

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TABLE 7.5

Effect of time of sampling on the pH, Total V. F. A., individual V. F. A. Acetic/Propionic acid ratio and Total V.F.A. in blood serum by Zebu cattle fed treatments (J) at three stages of growth.

Time of clipping forage (weeks)	Time of Sampling	pH of liquor	Total V. F. A. Meq per 100 ml Rumen liquor	Individual V.F.A. of rumen liquor Molar %			A/P	Total V. F. A. in blood serum meq/100 ml.
				Acetic Acid	Propionic Acid	Butyric Acid		
J 4	Before feeding	7.39	11.21	79.28	15.23	5.44	5.21	5.37
	Hrs. after feeding							
	1	7.44	14.13	77.91	17.12	4.97	4.55	4.55
	2	7.51	11.60	78.82	15.79	5.38	4.99	4.20
	3	7.63	11.39	77.92	16.98	5.10	4.59	3.65
	4	7.78	10.80	78.71	15.27	6.01	5.06	4.44
	5	7.56	10.66	78.50	16.14	5.35	4.86	3.91
	6	7.42	10.69	79.05	15.71	5.54	5.03	3.92
	7	7.49	10.55	78.22	16.35	5.42	4.78	4.01
	8	7.68	10.60	78.67	15.93	5.12	4.94	3.83
	Mean	7.54	11.29	78.56	16.06	5.37	4.89	4.21
8	Before feeding	7.50	6.85	79.75	14.65	5.59	5.44	4.92
	Hrs. after feeding							
	1	7.90	6.27	78.39	16.53	5.08	4.72	4.55
	2	7.63	5.79	81.55	14.22	4.23	5.73	4.74
	3	7.83	7.76	81.33	13.49	5.17	6.03	4.64
	4	7.83	6.05	79.42	14.88	6.69	5.34	4.28
	5	8.03	4.74	77.74	15.52	6.74	5.01	4.37
	6	8.07	6.05	79.89	12.50	7.60	6.39	4.19
	7	7.97	5.97	78.28	15.50	6.21	5.05	4.19
	8	7.93	5.90	79.38	14.12	6.49	5.62	3.83
	Mean	7.85	6.15	79.53	14.60	5.87	5.48	4.41
12	Before feeding	7.15	13.89	79.00	16.40	4.60	4.82	4.62
	Hrs. after feeding							
	1	7.20	10.39	78.30	16.34	5.36	4.79	4.20
	2	7.36	10.86	77.38	16.55	6.07	4.68	4.20
	3	7.36	12.03	78.77	15.88	5.35	4.96	4.10
	4	7.45	12.34	78.87	16.64	4.49	7.74	3.98
	5	7.38	10.92	78.73	17.11	4.16	4.60	4.20
	6	7.37	9.81	80.17	14.93	4.89	5.37	3.96
	7	7.42	9.48	77.44	17.78	4.78	4.36	4.41
	8	7.54	9.17	78.39	16.91	4.70	4.64	4.42
	Mean	7.36	10.98	78.56	16.50	5.04	4.77	4.23

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TABLE 7.6

Effect of time of sampling on the pH, Total V. F. A., individual V. F. A. Acetic/Propionic acid ratio and Total V.F.A. in blood serum by Zebu cattle fed treatments (K) at three stages of growth.

Time of clipping forage (weeks)	Time of sampling	pH of liquor	Total V. F. A. Meq per 100 ml Rumen liquor	Individual V.F.A. of rumen liquor Molar %			A/P	Total V. F. A in blood meq/ 100 ml.
				Acetic Acid	Propionic Acid	Butyric Acid		
K 4	Before feeding	7.18	6.83	65.05	20.21	14.75	3.22	5.28
	Hrs. after feeding							
	1	7.22	6.60	63.17	21.02	14.56	3.06	4.92
	2	7.19	7.08	64.42	21.02	14.56	3.06	4.92
	3	7.28	6.79	64.14	21.04	14.82	3.05	4.55
	4	7.34	7.27	62.57	22.57	14.86	2.77	4.74
	5	7.39	7.04	64.09	20.81	15.10	8.08	4.46
	6	7.55	8.06	74.66	18.32	7.02	4.08	4.74
	7	7.37	7.39	80.40	14.68	4.92	5.48	4.55
	8	7.27	6.88	66.02	21.05	12.92	3.14	4.55
	Mean	7.31	7.10	67.17	20.08	12.75	3.43	4.68
8	Before feeding	8.35	5.68	80.86	13.84	5.29	5.29	3.83
	Hrs. after feeding							
	1	8.45	4.86	82.33	12.48	5.18	6.60	3.83
	2	7.90	6.61	78.80	14.99	6.21	5.26	4.37
	3	8.75	6.78	78.95	14.85	6.20	5.32	4.37
	4	8.05	6.83	78.69	15.07	6.25	5.22	3.28
	5	8.75	5.57	80.84	13.38	5.78	6.04	3.28
	6	8.65	5.17	78.95	15.59	5.46	5.06	3.28
	7	8.85	4.27	80.84	13.44	5.72	6.01	3.83
	8	8.50	3.99	78.11	14.43	7.46	5.41	4.37
	Mean	8.47	5.53	89.82	14.23	5.95	5.64	3.89
12	Before feeding	7.00	9.10	79.27	14.85	5.88	5.34	4.73
	Hrs. after feeding							
	1	7.48	9.06	80.71	13.83	5.46	5.84	4.74
	2	7.10	8.56	81.19	13.51	5.30	6.01	4.83
	3	7.45	8.32	78.86	12.59	8.55	6.26	4.55
	4	7.60	8.91	75.81	15.24	8.95	4.97	4.32
	5	7.85	8.07	75.71	15.19	9.10	4.98	3.83
	6	7.90	9.06	78.10	13.23	8.67	5.90	3.28
	8	7.50	7.53	76.34	13.79	9.87	5.54	3.83
	Mean	7.53	8.51	78.20	13.97	7.83	5.62	3.93

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TABLE 7.7

Effect of time of sampling on the PH, Total V. F. A., individual V.F.A. Acetic/Propionic acid ratio and Total V.F.A. in blood serum by Zebu cattle fed treatments (L) at three stages of growth.

Time of clipping forage (weeks)	Time of sampling	pH of liquor	Total V.F.A. Meq per 100 ml Rumen liquor	Individual V.F.A. of rumen liquor Molar %			A/P	Total V. F. A. in blood serum meq/100 ml
				Acetic Acid	Propionic Acid	Butyric Acid		
L 4	Before feeding	7.24	8.48	74.49	19.79	5.72	3.76	5.28
	Hrs. after feeding							
	1	7.02	8.05	75.37	18.83	5.80	4.00	4.92
	2	6.96	8.43	72.56	20.71	6.73	3.50	4.74
	3	7.26	8.32	71.73	21.99	6.28	3.26	5.10
	4	7.12	8.51	75.26	19.12	5.62	3.94	4.92
	5	7.31	8.09	71.80	22.54	5.66	3.19	4.37
	6	7.19	8.42	76.30	18.01	5.70	4.24	4.74
	7	7.24	7.71	60.94	21.74	17.32	2.80	4.64
	8	7.15	7.89	79.11	16.83	4.06	4.70	4.46
	Mean	7.17	8.21	73.06	19.98	6.99	3.71	4.80
8	Before feeding	8.73	4.97	76.97	13.91	9.12	5.53	4.37
	Hours after feeding							
	1	8.85	5.47	76.25	13.42	10.33	5.68	4.37
	2	8.60	5.41	76.98	13.52	9.50	5.69	4.38
	3	8.80	5.52	75.97	13.67	10.36	5.56	4.38
	4	8.25	6.67	75.43	14.73	9.84	5.12	5.47
	5	8.40	6.34	76.52	14.93	8.55	5.13	4.38
	6	8.43	5.58	77.12	13.39	9.49	5.76	4.38
	7	8.00	4.87	76.12	16.08	7.80	4.73	4.37
	8	8.25	4.05	78.50	14.39	7.11	5.46	4.37
	Mean	8.48	5.43	76.65	14.23	9.12	5.41	4.50
12	Before feeding	7.07	8.96	74.70	15.36	9.95	4.90	4.80
	Hrs. after feeding							
	1	7.05	6.97	76.50	16.42	7.08	4.66	4.37
	2	7.00	8.32	76.15	14.57	9.29	5.23	4.50
	3	7.00	7.52	74.59	14.79	10.62	5.04	4.37
	4	7.08	7.38	78.88	14.74	6.38	5.35	4.74
	5	6.98	7.72	73.60	14.05	12.35	5.24	4.37
	6	7.07	8.12	77.76	15.74	15.74	4.94	4.37
	7	7.00	8.02	79.02	13.98	7.00	5.65	4.46
	8	7.20	7.82	77.79	15.03	7.18	5.18	4.38
	Mean	7.05	7.87	76.55	14.96	8.48	5.13	4.47

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TABLE 7.8

Ruminal V.F.A., pH, and blood V.F.A. of treatments H, J, K, and L at 4 weeks of age fed to Zebu cattle.*

		H	J	K	L
pH of Rumen liquor		7.15	7.54	7.31	7.17
Total V.F.A. of Rumen liquor Meq %		5.02	11.29	7.10	8.21
Individual V.F.A. of Rumen liquor molar %	Acetic Acid	79.30	78.56	67.17	73.06
	Propionic Acid	18.80	16.06	20.08	19.95
	Butyric Acid	1.90	5.38	12.75	6.99
Acetic/Propionic Ratio (A/P)		4.32	4.89	3.43	3.71
Total V.F.A. in blood serum Meq %		4.37	4.21	4.68	4.80

* Each figure is the mean of 18 determinations.

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TABLE 7.8 continued

Ruminal V.F.A., pH, Acetic/Propionic Ratio, (A/P) and blood V.F.A. of treatments H, J, K and L at 8 weeks of age fed to Zebu cattle*

		H	J	K	L
pH of Rumen liquor		8.19	7.85	8.47	8.48
Total V.F.A. of Rumen liquor Meq. %		7.27	6.15	5.53	5.43
Individual V.F.A. of Rumen liquor Molar %	Acetic	78.70	79.53	79.82	76.46
	Propionic Acid	15.16	14.60	14.23	13.94
	Butyric Acid	6.14	5.87	5.95	9.60
Acetic/Propionic Ratio (A/P)		5.25	5.48	5.64	5.50
Total V.F.A. in blood serum Meq. %		4.02	4.41	3.89	4.50

* Each figure is the mean of 18 determinations.

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TABLE 7.8 continued

Ruminal V.F.A., pH, Acetic/Propionic Ratio (A/P) of treatments H, J, K and L at 12 weeks of age fed to Zebu Cattle*

		J*	K*	L*
pH of Rumen liquor		7.36	7.53	7.05
Total V.F.A. of Rumen liquor Meq. %		10.98	8.51	7.87
Individual V.F.A. of Rumen liquor Molar %	Acetic Acid	78.56	78.20	75.13
	Propionic Acid	16.50	13.97	14.80
	Butyric Acid	5.04	7.83	10.07
Acetic/Propionic Ratio (A/P)		4.77	5.62	5.13

* Each figure is the mean of 18 determinations.

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TABLE 7.9

Ruminal V.F.A., pH, Acetic/Propionic ratio, (A/P) and blood V.F.A. of treatments H, J, K and L at different stages of growth fed to Zebu cattle.

Forage stage of growth (weeks)	pH of liquor	Individual Rumen liquor V. F. A. Molar %			A/P	Total V.F.A. Meq/ 100 ml	Total V.F.A. in blood serum Meq %
		Actic Acid	Propionic Acid	Butyric Acid			
			H				
4	7.15	79.30	18.80	1.90	4.32	5.02	4.37
8	8.19	78.70	15.16	6.14	5.25	7.27	4.02
			J				
4	7.54	78.56	16.06	5.37	4.89	11.29	4.21
8	7.85	79.53	14.60	5.87	5.48	6.15	4.41
12	7.36	78.56	16.50	5.04	4.77	10.98	4.23
			K				
4	7.31	67.17	20.08	12.75	3.43	7.10	4.68
8	8.47	79.82	14.23	5.95	5.64	5.53	3.89
12	7.53	78.20	18.97	7.83	5.62	8.51	3.93
			L				
4	7.17	73.06	19.95	6.99	3.71	8.21	4.80
8	8.48	76.46	13.94	9.60	5.50	5.43	4.50
12	7.05	75.13	14.80	10.07	5.13	7.87	4.47

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Tables 7.8 and 7.9 showed the summary of ruminal pH, V.F.A., acetic to propionic acid ratio and blood serum V.F.A. of treatments H, J, K and L at 4, 8 and 12 weeks of growth fed to Zebu cattle.

Separation of blood serum V.F.A. by GLC showed that acetic acid was the only detectable acid in the blood serum under the operating conditions. The acetic acid content was however very low.

Discussion and Summary

PH levels

The pH of the rumen liquor of the Zebu cattle was above 7 but below 8 in most cases. Whether at 4 weeks, 8 or 12 weeks of growth the pH remained high. These high pH values below 8 are seen in tables 2, 3, 4, 5, 6-9, 10-11, and 12-15. However the pH was below 7 in tables 6-9 when L was 4 weeks and treatment J was 12 weeks.

The pH levels for all the treatments were high when the treatments were 8 weeks. In most cases the pH levels were higher than the values at 4 or 12 weeks. In most cases the pH levels were higher than 8, and high values are seen in tables 12-15.

The pH levels showed a tendency to rise or fall to the prefeeding level. This tendency is shown in tables

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2-7, table 8 when the treatment was 4 weeks, table 9 when then treatment was 12 weeks, tables 10, 11, and 13, 14 and 15, and table 12 when the treatment was 8 weeks.

High pH levels are characteristic of fibrous and unsupplemented feeds.

The pH values recorded by Steward, Stewart and Schultz (1958) did not rise up to 7. They worked with four rations one of which was solely alfalfa hay. This and the supplemented feeds produced rumen liquor with pH less than 7. Raymond and Terry (1966) discovered that there is a wide range of pH values "*in vivo*". The pH range was said to be between 5.5 and 8.0, depending on the feed. In this experiment the pH of over 8 was recorded in some cases.

Total V.F.A.

The total V.F.A. content of the rumen liquor of Zebu cattle fed H, K and L was between 7 and 10 meq per 100 ml, while the content of the liquor when J was fed was between 13 and 15 meq per 100 ml. This is shown in table 7. In some cases the total V.F.A. content of the rumen liquor showed little difference between the treatments at 4 and 12 weeks. This little difference is shown in tables 7, 7.6 and 7.7. There were times when the total V.F.A. content of the rumen liquor was higher at 4 than at 12 weeks as is

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shown in table 7, and more at 12 weeks than at 4 weeks as is shown in table 7.6.

In table 7, the total V.F.A. was lowest when the treatments were 8 weeks. However, in table 7.4 the total V.F.A. content was lower at 4 than at 8 weeks. There was a tendency here also for the total V.F.A. content of the rumen liquor to rise or fall to the prefeeding level. This was shown in table 7.7 when the feed was 8 weeks; table 7.6 when the feed was 4 weeks, and table 7.5 when the treatment was 4 weeks.

Stewart, Steward and Schultz (1958) reported increases in total V.F.A. production after feeding for each of the four rations used in their experiment. In these experiments, the total V.F.A. content of the rumen liquor did not show such a regular pattern. In some cases the amount increased, while the level dropped in others. After 6 hours the level dropped appreciably (Stewart _et al._,1958). This was also observed in the present work. The level dropped six hours after feeding, but in a few cases the peaks occured after six hours. The reason for some inconsistencies in the pH and total V.F.A. levels in the present work may be due to the feeding habits of the animals. The time interval from the field to the laboratory may also be one of the causes, since the microflora continuedto ferment actively.

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Individual V.F.A.

Whether the feed is supplemented or not acetic acid formed the major proportion of the acids in the rumen liquor. The only difference was that the acid increased in proportion if the feed was fibrous and of low digestibility and was unsupplemented. The acetic acid levels in these studies were over 70 molar % and even in certain cases went up to over 80 molar %. Propionic acid came next in quantity and butyric acid made up the lowest.

Acetic acid to propionic acid ratio (A/P)

The ratio of acetic acid to propionic acid was high. The ratio ranged between 3 and 6 on the average, but the highest of 5.64 was obtained for treatment K when 8 weeks. The ratio was higher for the treatments when 8 and 12 weeks. The high ratio is peculiar to fibrous and unsupplemented feeds. When the acetic acid content rises and propionic and butyric acid levels decrease, the ratio increases. This was what was observed in these studies. A wide A/P ratio means high concentration of acetic acid and low concentration of propionic acid. The indication is that such feed would favour fat deposition either in milk or in the body; in order words such rations are lipogenic. Whereas a narrow ratio means higher concentra-

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tion of propionic acid and such ration is glucogenic and may favour tissue protein synthesis. That is the signicicance of this ratio. These treatments with A/P ratio of more than 4 can favour more fat deposition in the milk.

Blood V.F.A.

Total bllod serum V.F.A. did not vary much. The almost constant V.F.A. content of the blood tend to show that the liver may be converting excess V.F.A. to other substances and the liver regulates the amount that enters the blood stream.

Chromoatographic separation of the V.F.A. in the blood serum showed acetic acid as the only acid detectable to a large extent under the operating conditions. The very small amount of acetic acid, and the minute amounts of the other acids may be an indication that small amounts of digested and absorbed volatile fatty acids are used in the synthesis of milk and body fats. To give a clearer view, tracer techniques need to be employed so that the lebelled carbon atom in the feed or substance infused may be traced within the animal.

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CHAPTER 8

MILK CONSTITUENTS

8.1 INTRODUCTION

Milk is a very important food to both young and old all over the world. There are established dairy farms owned by private and government agencies in advance countries, and these farms own high yielding Holstein or Freisian cows which produce good quality milk for sale as fresh milk or as dairy products like butter, cream and cheese. A lot of research has been carried out in Europe and America on the nutritional aspects, health, breeds, production and quality of the dairy animals.

In Nigeria, little is known, and little work has been done on the selection, breeding, nutrition, health, management, production and quality of the dairy animals. The Government dairy farms and some University faculties of Agriculture have tried to improve milk production by importing high yielding cows from Europe or America and by crossing the imported breeds with the local White Fulani cattle.

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This chapter therefore deals with the effect of feed and the volatile fatty acid production in the rumen, on the milk composition of the White Fulani cows.

8.2 MILK COMPOSITION

Milk fat and protein are the two constituents that show a lot of variation (Table 8.2).

		Percent
Water		87.25
Dry Matter		12.75
Fat	3.8	
Protein	3.5	
Sugar	4.8	
Ash	0.65	
Total	12.75	100.00

Detailed composition (Ling, 1946; Jenness and Patton, 1959; Kon and Cowie, 1961; and Ling, Kon and Porter, 1961) showed that water content of milk is over 80%. Water helps to hold in solution the soluble constituents of the milk, and the percentage of water varied from 84.0 to 89.0%. Milk composition according to Jenness and Patton (1959) is shown in table 8.1

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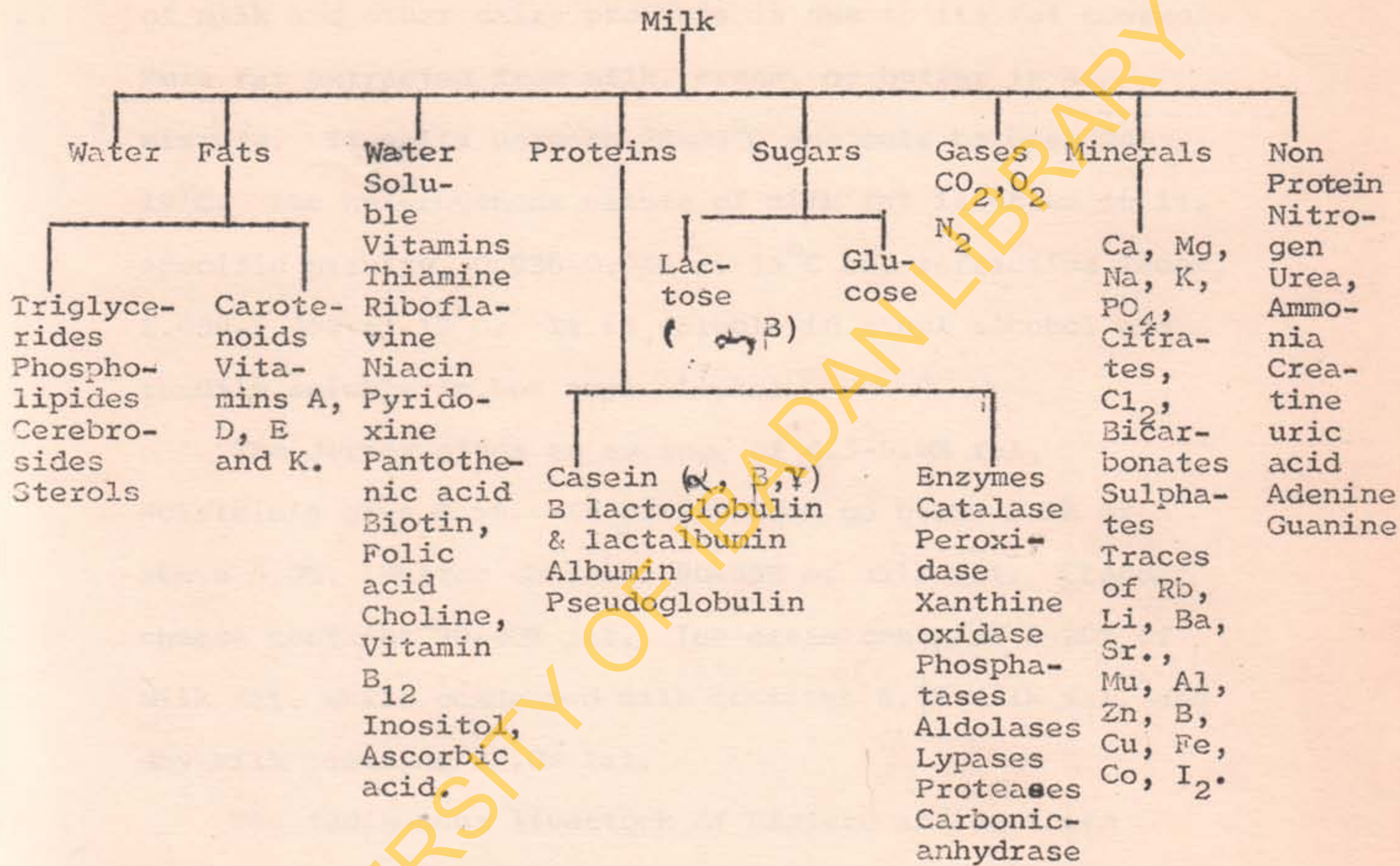
TABLE 8.1

Cow's Milk Constituents according to Jenness and Patton (1959).



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Milk Fat:

Milk fat is also called butter fat. It is commercially the most valuable constituent of the milk. The flavour of milk and other dairy products is due to its fat content. Pure fat extracted from milk, cream, or butter is a mixture. It melts between 28-33°C and sets between 24-19°C. The heterogenous nature of milk fat is shown in its specific gravity, 0.936-0.946 at 15°C and refractive index, 1.459-1.462 at 15°C. It is soluble in ethyl alcohol and readily soluble in hot amyl alcohol.

The Jersey gives an average of 4.5-5.0% fat, Holstein's give 3.5%. It will seldom go below 2.5% or above 6.0%. Butter contains 80-85% of milk fat. Cheddar cheese contains 30-40% fat. Ice cream contains 8-20% of milk fat, while condensed milk contains 8.0% milk fat, and dry milk contains 27.0% fat.

The indigenous livestock of Eastern and Southern Africa as well as West African Zebu breeds give milk fat that ranges from 3.4% to well above 6%, (Mason and Maule, 1960). The Kanana Zebu breed gives 4.73% fat and 9.25% S.N.F. The Red Butana Zebu gives 5.5% fat; the Abyssinian

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(Ethiopian) shorthorned Zebu gives 3.4% to 6.5%; the Gasara (small Somalia Zebu) gives 5.5%; the Garre Zebu gives 5.4% and the Kenya Boron Zebu gives 5-6.8%.

TABLE 8.2

The Composition of milk of Various Species according to Eckles et al (1943)

Species	Water %	Fat %	Protein %	Lactose %	Ash %
Human	88.30	3.11	1.9	7.18	0.21
Cow	87.25	3.80	3.50	4.80	0.65
Goat	87.88	3.82	3.21	4.54	0.55
Sheep	80.82	6.86	6.52	4.91	0.89
Mare	90.70	1.20	2.00	5.70	0.40

Lactose or milk sugar:

Lactose is formed exclusively in the milk of mammals from glucose (Folley, 1956). Milk from various breeds of cattle contain variable amount of lactose. Jersey gives 5%, Holstein gives 4.6% while the human milk is about 6.3%. Lactose is in the true solution in the serum. On crystal-

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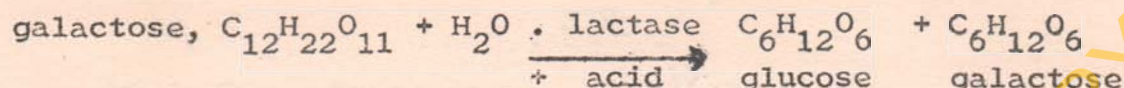
lization from water it forms hard, gritty crystals $C_{12}H_{22}O_{11}.H_2O$. By the action of lactase in the intestines and mineral acids, lactose is hydrolysed to glucose and galactose,

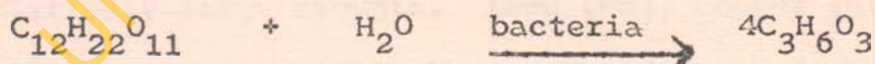


$$C_{12}H_{22}O_{11} + H_2O \xrightarrow[\text{+ acid}]{\text{. lactase}} \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{galactose}}{C_6H_{12}O_6}$$

Aqueous solution of lactose reduces Fehling's solution and ammoniacal silver nitrate solution. After hydrolysis, the reducing power is doubled. Under slightly acid condition it is oxidised to formic acid and laevulinic acids. Oxidation with concentrated HNO_3 gives oxalic acid and carbonic acid. When heated between 110-130°C, lactose hydrate crystals lose their water of crystallization. Above 150°C they turn yellow, and at 175°C they turn brown forming lacto caramel.

Lactose is used in the preparation of infant and invalid foods. It is the only sourse of galactose for human and animals in the brain and nerves. The chief source of lactose is whey.

Bacteria in the milk can decompose lactose to lactic acid at 37°C. The acid gives the milk its sour taste.



$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{bacteria}} 4C_3H_6O_3$$

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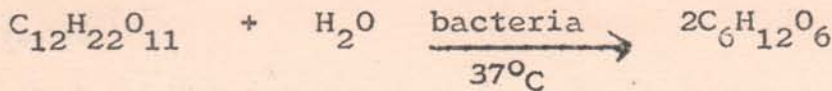
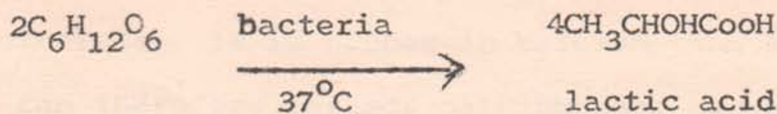
The activity of the micro organisms is stopped at an acidity of 1%.



$$C_{12}H_{22}O_{11} + H_2O \xrightarrow[37^oC]{bacteria} 2C_6H_{12}O_6$$



$$2C_6H_{12}O_6 \xrightarrow[37^oC]{bacteria} 4CH_3CHOHCooH$$

lactic acid

When lactic acid concentration increases, the casein which is normally united with calcium is withdrawn and unites with lactic acid to form calcium lactate. Some of the colloidal calcium phosphate is converted to soluble calcium phosphate.

<u>Mineral matter or Ash content of Milk</u>:

When milk is dired first, and then the residue ashed in a furnace, a white powder is obtained. This is the ash. It forms about 0.7-0.76%, but the total mineral salts in milk will exceed this figure. This ash contains a lot of metallic elements as those found in the animal body. Potassium (k), Sodium (Na), Calcium (Ca), Magnessium (Mg), Chlorin (Cl), Phosphorus (P), and Sulphur (S) are present in relatively large amounts. Iron (Fe), Copper (Cu), Zinc (Zn), Alluminum (Al), Manganese (Mn) and lodine (1_2)

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are present in small amounts, while silicon, boron, titanium, vanadium, rubidium, lithium and strontium are present in traces.

Much of the nutritive properties of milk are due to its minerals. It is richer in calcium than lime water. Milk can therefore correct calcium poor cereal diets of man and animals and it is a very important source of calcium in human nutrition.

All the mineral elements in milk are essential for nutrition, but the iron and iodine are very low.

Other constituents of milk

Phospholipids These are lecithin and cholesterol. Pigments are also present. The carotenoids are fat soluble, and the carotene is responsible for its yellow colour. It is absent in the milk of goat, ewe and camel, so they produce white or colourless milk. The carotene is not synthesized in the cow but finds its way from the feed into the milk via the blood.

The water soluble pigment is the riboflavin and it is found in the serum. It is about 0.05-0.1% and occurs in the milk of most mammals. There is some relationship bet-

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ween the riboflavin content of milk and high protein feeds.

Enzymes of milk

Enzymes of milk are normal constituents of milk. Galactase is a proteolytic enzyme for cheese ripening, while lipase is a fat splitting enzyme that causes rancidity in butter. Catalase, an oxidising enzyme, and reductase, a reducing enzyme are also present. Raw milk contains phosphatase which is inactivated by pasteurization.

Vitamins

The vitamins present are vitamin A, vitamin B_1 in the form of thiamine hydrochloride, B_2 or G (riboflavin), nicotinic acid (nicotinamide), B_6 (pyridoxin), Pantothenic acid, vitamin C (ascorbic acid), D and vitamin E (alpha tocopherol).

Gases:

These make up 7-10% by volume of milk. Carbon dioxide is present as it comes from the udder, but nitrogen and oxygen are taken up by the milk during milking.

Factors affecting the composition of milk:

Many factors affect the yeild and composition of milk. These are the type of feed given to the animal, the age of

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the animal, its breed, the time of milking, the stage of lactation, disease prevailing at the time, the season of the year and hormonal control.

8.3 ANALYTICAL METHOD

During the experimental period, the cows were milked in the morning and evening. Samples were taken from the cows in the morning during the last three days of the experiment. The samples of milk were taken to the laboratory for milk fat, lactose, ash, solid matter and protein analyses. The milk samples were stored at 1° to $2^{\circ}C$ to prevent them from getting sour and from separating out.

Milk Fat

The milk fat content was determined according to the Gerber method. This method is quick and accurate. It involves the use of sulphuric acid specially made for milk testing and amyl alcohol.

Milk Lactose

This was determined colorimetrically. The method of Barnett and Tawab (1957) was adopted.

Water content was determined by drying to constant weight in an oven at $65^{\circ}C$. The dry matter was calculated

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from the residue left in the dish after drying. Milk ash was calculated after ashing the dry residue, and the milk protein was determined by the Kjeldahl method, and the result multiplied by 6.38.

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RESULTS

Tables 8.21 and 8.22 showed the milk constituents of the milk samples taken from cows fed treatments H, J, K and L at four weeks. The water content of each milk sample was over 80%. The range was between 83.08% and 85.98%, and this variation in water content was small. The fat content was also fairly high. The differences in the fat contents of all the milk samples were very small since the range was between 5.91% and 6.73%.

Milk protein varied from 3.70% to 4.38%, while lactose varied from 4.14gm % to 5.09gm %. Milk ash varied from 0.68% to 0.83 and total solids varied from 14.02% to 16.92%. The solid-not-fat content varied from 8.11% to 10.38%.

Treatment H produced milk with more protein, total solids and solid-not-fat. It also produced milk with the least water content compared with treatments J, K and L.

Tables 8.31 and 8.32 showed the milk constituents of cows milk when the cows were fed treatments H, J, K and L at 12 weeks. Water formed the major part of the milk in each case. It made up over 82% of each milk sample. The

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TABLE 8.21

Comparison of milk constituents of cows fed H, J, K and L when four (4 weeks)

(%)

Milk Constituent	H		J		K		L	
	Cow 195	Cow 183	Cow 195	Cow 183	Cow 195	Cow 236	Cow 322	Cow 236
Water	83.08	85.06	64.60	85.53	84.60	85.98	85.62	84.86
Fat	6.54	6.14	6.70	6.58	6.73	5.91	6.13	5.99
Protein	4.38	3.98	3.84	3.94	3.71	3.70	3.94	4.02
Lactose	4.14	4.94	4.86	4.81	5.07	5.09	5.07	4.90
Ash	0.69	0.74	0.83	0.78	0.72	0.68	0.69	0.69
Total solids	16.92	14.94	15.40	14.47	15.40	14.02	14.38	15.14
Solid-not-Fat (s.n.f.)	10.38	8.80	8.70	7.89	8.67	8.11	8.25	9.15

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TABLE 8.22

Mean % milk constituents of cows fed treatments H, J, K and L at 4 weeks

Milk Constituent	H	J	K	L
Water	84.07	85.07	85.29	85.24
Fat	6.34	6.64	6.32	6.06
Protein	4.18	3.89	3.71	3.98
Lactose	4.54	4.84	5.08	4.99
Total solids	15.93	14.94	14.71	14.76
Ash	0.72	0.81	0.70	0.69
S.N.F.	9.59	8.30	8.39	8.70

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range was from 82.44% to 88.19%. Treatments L produced milk samples containing the least amount of water. The fat content varied more than for the samples taken when the treatments were four weeks. The range was from 4.33% to 7.91%. Treatment K produced milk samples with the least butter-fat content while treatment L produced milk with the highest butterfat content. The protein content did not vary much since it made up between 3.29% and 4.67% of the milk. Treatment L produced milk with the highest milk protein content. The lactose varied from 4.43% to 5.47gm %, while milk ash varied from 0.68% to 0.83%. Since most of the milk samples contained more water than those obtained when the treatments were four weeks, the total solids and solid-not-fat contents were also low. The total solids varied from 11.81% to 17.56%, while solid-not-fat varied from 6.67% to 9.65%. Treatment L produced milk with the greatest amount of solids-not-fat and total solids.

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TABLE 8.31

Comparison of milk constituents of cows fed treatments H, J, K and L at twelve weeks (12 wks.)

(%)

Milk Constituent	H		J		K		L	
	Cow AD11	Cow 220	Cow AD11	Cow 256	Cow AD11	Cow 256	Cow 282	Cow 258
Water	86.21	81.10	85.96	86.18	88.19	85.79	84.84	82.44
Fat	6.28	6.18	7.37	6.50	4.33	5.97	6.30	7.91
Protein	3.36	4.11	3.29	3.68	3.39	3.46	4.65	4.67
Lactose	4.69	4.59	4.59	4.45	4.62	5.47	4.92	4.43
Ash	0.70	0.79	0.74	0.71	0.83	0.71	0.82	0.68
Total solids	13.79	12.90	14.04	13.82	11.81	14.21	15.16	17.56
Solid-not Fat	7.51	6.72	6.67	7.32	7.48	8.24	8.86	9.65

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TABLE 8.32

Mean % milk constituents of cows fed treatments H, J, K and L at 12 weeks

Milk Constituent	H	J	K	L
Water	88.66	86.07	86.99	83.64
Fat	6.23	6.94	5.15	7.11
Protein	3.74	3.49	3.43	4.66
Lactose	4.64	4.52	5.05	4.68
Ash	0.75	0.73	0.77	0.75
Total solids	13.35	13.93	13.01	16.36
S.N.F.	7.12	7.00	7.86	9.26

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Tables 8.41 and 8.42 showed the milk constituents of cows fed treatments H, J, K and L simultaneously when they were four weeks. The water content of the milk samples varied from 85.28% to 88.43%. These milk samples contained more water than the samples of the previous experiment. The fat content also showed more variation this time. It ranged between 3.70% and 6.51%. Treatment L produced milk with more butterfat content. The milk protein remained fairly constant in all the samples. It varied from 3.06% to 3.83%. The milk protein content was lower than that obtained in the previous experiment. Milk lactose varied from 3.84% to 6.40%, and the milk ash varied from 0.62% to 0.68%. The ash content was lower this time. Total solids and solid-not-fat contents were low too. Total solids varied between 11.72% and 14.72% while solid-not-fat varied between 5.71% and 14.72% while solid-not-fat varied between 5.71% and 8.47%

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TABLE 8.41

Comparison of milk constituents of cows fed H, J, K and L at four weeks (4 wks.)

(%)

Milk Constituent	H			J			K			L		
	Cow AD11	Cow 195	Cow 118	Cow 69	Cow 142	Cow 183	Cow AD11	Cow 118	Cow 195	Cow 69	Cow 142	Cow 183
Water	82.28	86.33	86.06	87.82	87.23	85.28	88.43	88.29	86.88	85.96	86.23	85.92
Fat	3.70	5.20	6.02	5.41	5.90	6.51	3.70	6.00	4.66	6.37	5.49	6.16
Protein	3.06	3.65	3.55	3.21	3.39	3.69	3.11	3.61	3.39	3.53	3.44	3.83
Lactose	5.61	5.71	3.84	5.16	5.89	4.62	5.20	4.69	4.06	6.40	4.82	4.67
Ash	0.67	0.67	0.66	0.64	0.67	0.62	0.67	0.68	0.65	0.68	0.70	0.68
Total solids	11.72	13.67	13.94	12.18	12.77	14.72	11.57	11.71	13.12	14.04	13.77	14.08
Solid-not Fat (s.n.f)	8.02	8.47	7.92	6.77	6.87	8.21	7.87	5.71	8.46	7.67	8.28	7.92

TABLE 8.42

Mean % milk constituents of cows fed treatments H, J, K and L at 4 wks.

Milk Constituents	H	J	K	L
Water	84.89	86.78	87.87	86.04
Fat	4.97	5.94	4.79	6.01
Protein	3.42	3.43	3.34	3.60
Lactose	5.05	5.22	4.65	5.30
Ash	0.67	0.64	0.67	0.69
Total solids	13.11	13.22	12.13	13.96
S.N.F.	8.14	7.28	7.35	7.96

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Tables 8.51 and 8.52 showed the milk constituents of cows fed treatments H, J, K and L when eight weeks. Water formed over 80% of the milk samples. The water content varied between 83% and 87.62%. The butterfat content varied between 4.44% and 7.37%. Treatment L produced milk with the greatest butterfat content, and the lowest milk protein content. The milk protein varied from 2.97% to 3.88%, while milk lactose varied from 3.48% to 5.94%. Treatment L produced more lactose than the rest. The ash content varied from 0.50% to 0.73% and Treatment J produced more ash. Total solids varied from 12.38% to 16.68% and t treatment L produced more total solids and solids-not-fat. The solids-not-fat varied between 6.87% and11.18%.

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TABLE 8.51

Comparison of milk constituents of cows fed treatments H, J, K, and L at eight weeks (8 wks)

(%)

Milk Constituents	H			J			K			L		
	Cow AD11	Cow 118	Cow 195	Cow 69	Cow 142	Cow 183	Cow AD11	Cow 118	Cow 195	Cow 69	Cow 142	Cow 183
Water	87.62	85.76	87.20	86.11	87.03	86.26	86.30	85.48	86.42	84.53	86.25	83.32
Fat	4.77	7.37	6.48	5.44	5.44	5.63	4.68	6.97	4.77	7.00	5.83	5.50
Protein	3.73	3.41	3.54	3.72	3.58	3.88	3.10	3.50	3.43	3.63	3.66	2.97
Lactose	4.06	3.48	3.58	3.98	3.83	3.79	5.40	5.37	5.31	5.52	5.94	5.49
Ash	0.67	0.69	0.68	0.66	0.73	0.71	0.63	0.56	0.50	0.61	0.52	0.59
Total solids	12.38	14.24	12.80	13.89	12.97	13.74	13.70	14.52	13.58	15.47	13.75	16.68
Solids-not Fat (s.n.f)	7.61	6.87	8.36	7.05	7.53	8.11	9.02	7.55	8.81	8.47	7.92	11.18

TABLE 8.52

Mean % milk constituents of cows fed treatments H, J, K, and L at 8 wks.

Milk Constituent	H	J	K	L
Water	86.86	86.47	86.07	84.70
Fat	5.53	5.85	5.47	6.11
Protein	3.56	3.73	3.34	3.42
Lactose	3.71	3.87	3.36	5.65
Ash	0.67	0.70	0.56	0.57
Total solids	13.14	13.53	13.93	15.30
S.N.F.	7.61	7.56	8.46	9.19

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Table 8.61 showed the comparison of milk produced by Zebu cattle fed treatments H, J, K and L when four weeks. The milk produced by the Nigerian Zebu contained more total solids than the other breeds listed in the table. The milk also contained more butterfat. Milk protein, lactose and ash contents were very similar.

Table 8.62 also exhibited the same trends noted in table 8.61 except that the differences in butterfat and total solids were not as marked as in table 8.61. The lactose content was more in the Nigerian Zebu than in the other breeds.

Table 8.63 showed that the eight week old treatments produced less milk ash in the Nigerian Zebu than the other breeds compared with it. The milk contained more butterfat and in some cases more lactose than the other breeds.

In Table 8.64 treatment L stood out as a good forage producing good quality milk. The milk produced from it contained less water and more of the other constituents. The prominent feature again is the high butterfat content of the Zebu milk.

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TABLE 8.61

Milk composition of different breeds compared with the Nigerian Zebu Cows

Breed	Treat-ment	Age of forage	Water	Fat	Pro-tein	Lac-tose	Ash	Total solids
Nigerian Zebu	H	4 wks.	84.07	6.34	4.18	4.54	0.71	15.93
Nigerian Zebu	J	4 wks.	85.07	6.64	3.89	4.84	0.81	14.94
Nigerian Zebu	K	4 wks.	85.29	6.27	3.71	5.08	0.70	14.71
Nigerian Zebu	L	4 wks.	85.24	6.06	3.98	4.99	0.69	14.76
Indian Cows			86.48	4.83	2.78	4.56	0.74	13.52
Holstein			88.07	3.45	3.15	4.65	0.68	11.93
Guernsey			85.45	4.98	3.84	4.98	0.75	14.55
Jersey			85.43	5.14	3.80	4.87	0.76	14.57
Ayrshire			87.28	3.85	3.32	4.90	0.65	12.72
Shorthorn			87.19	3.80	3.32	4.99	0.70	12.81

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TABLE 8.62

Milk composition of different breeds compared with the Nigerian Zebu Cows

(%)

Breed	Treat-ment	Age of forage	Water	Fat	Pro-tein	Lac-tose	Ash	Total solids
Nigerian Zebu	H	4 wks.	86.89	4.97	3.42	5.05	0.67	13.11
Nigerian Zebu	J	4 wks.	86.78	5.94	3.43	5.33	0.64	13.22
Nigerian Zebu	K	4 wks.	87.87	4.79	3.37	4.65	0.67	12.13
Nigerian Zebu	L	4 wks.	86.04	6.01	3.60	5.30	0.69	13.96
Indian Cows			86.48	4.83	2.78	4.56	0.74	13.52
Holstein			88.07	3.45	3.15	4.65	0.68	11.93
Guernsey			85.45	4.98	3.84	4.98	0.75	14.55
Jersey			85.43	5.14	3.80	4.87	0.76	14.57
Ayrshire			87.28	3.85	3.32	4.90	0.65	12.75
Shorthorn			87.19	3.80	3.32	4.99	0.70	12.81

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TABLE 8.63

Milk composition of different breeds compared with the Nigerian Zebu Cows

(%)

Breed	Treat-ment	Age of forage	Water	Fat	Pro-tein	Lac-tose	Ash	Total solids
Nigerian Zebu	H	8 wks.	86.86	5.53	3.56	3.71	0.68	13.14
Nigerian Zebu	J	8 wks.	86.48	5.97	3.73	3.87	0.70	13.52
Nigerian Zebu	K	8 wks.	86.07	5.47	3.34	5.36	0.56	13.93
Nigerian Zebu	L	8 wks.	84.70	6.11	3.42	5.65	0.57	13.30
Indian Cows			86.48	4.83	2.78	4.56	0.74	13.52
Holstein			88.07	3.45	3.15	4.65	0.68	11.93
Guersey			85.45	4.98	3.84	4.98	0.75	14.55
Jersey			85.43	5.14	3.80	4.87	0.76	14.57
Ayrshire			87.28	3.85	3.32	4.90	0.65	12.72
Shorthorn			87.19	3.80	3.32	4.99	0.70	12.81

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TABLE 8.64

Milk composition of different breeds compared with the Nigerian Zebu Cows

(%)

Breed	Treatment	Age of forage	Water	Fat	Protein	Lactose	Ash	Total solids
Nigerian Zebu	H	12 wks.	86.66	6.23	3.74	4.64	0.74	13.34
Nigerian Zebu	J	12 wks.	86.07	6.93	3.49	4.52	0.72	13.93
Nigerian Zebu	K	12 wks.	86.99	5.15	3.42	5.04	0.77	13.01
Nigerian Zebu	L	12 wks.	83.64	7.10	4.66	4.68	0.75	16.36
Indian Cows			86.48	4.83	2.78	4.56	0.74	13.52
Holstein			88.07	3.45	3.15	4.65	0.68	11.93
Guernsey			85.45	4.98	3.84	4.98	0.75	14.55
Jersey			85.43	5.14	3.80	4.87	0.76	14.57
Ayrshire			87.28	3.85	3.32	4.90	0.65	12.72
Shorthorn			87.19	3.80	3.32	4.99	0.70	12.81

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Table 8.7 showed the live-weight changes of the cows and steers used in this experiment. When H at 4 weeks was fed to the animals, all the animals lost weight. Cow 94 lost more weight than the other animals. On the average 4.31 kilograms were lost. When treatment J was fed, one of the animals did not gain or lose weight while one gained some weight, the rest lost weight. On the whole 4.14 kilograms were lost. All the animals lost weight when fed treatment K too and on the whole they lost 6.64 kilograms. All animals gained weight when fed treatment L and the gain on the whole was 4.14 kilograms.

When the treatments were twelve weeks and fed to the animals all the animals on treatment H lost weight and the loss was of the same magnitude as when the treatment was four weeks. With treatment J one animal did not gain or lose weight and on the average less weight, 1.49 kilograms, was lost this time. All the animals also lost weight on treatment K but the loss was less than when the treatment was four weeks. No animal lost weight on treatment L and the gain was more than when the treatment was four weeks.

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RESULT

TABLE 8.7

Live weight changes of Zebu cattle during the experiment

TREATMENT H. 13-18/12/69

Stage of Growth	Animal No.	Initial Live weight lb.	Initial Live weight kg.	Final Live weight lb.	Final Live weight kg.	Live weight gain or loss per day lb.	Live weight gain or loss per day kg.	Live weight gain or loss per week kg.
4 weeks	195	822	372.86	810	367.41	-12	-5.44	-3.97
4 weeks	183	800	362.88	790	358.34	-10	-4.54	-3.31
4 weeks	198	758	343.83	746	338.38	-12	-5.44	-3.97
4 weeks	94	800	362.88	782	354.71	-18	-8.16	-5.96
Mean		795	360.61	782	354.71	-13	-5.90	-4.31
12 weeks	AD11	632	286.67	630	285.77	-2	-0.91	-0.66
12 weeks	220	830	376.49	822	372.86	-8	-3.63	-2.65
12 weeks	114	906	410.96	878	398.26	-28	-12.70	-9.27
12 weeks	194	764	346.55	750	340.20	-14	-6.35	-4.64
Mean		783	355.17	770	349.27	-13	-5.90	-4.31

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RESULT

TABLE 8.7 continued

Live weight changes of Zebu cattle during the experiment.

TREATMENT J. 21-26/1/70

Stage of Growth	Animal No.	Initial Live weight lb.	Initial Live weight kg.	Final Live weight lb.	Final Live weight kg.	Live weight gain or loss per day lb.	Live weight gain or loss per day kg.	Live weight gain or loss per week kg.
4 weeks	195	760	344.73	760	344.73	0	0	0
4 weeks	183	732	332.03	740	335.66	8	3.63	2.65
4 weeks	198	760	344.73	764	338.38	-14	-6.35	-4.64
4 weeks	94	080	366.51	780	353.81	-28	-12.70	-9.27
Mean		765	347.00	756.5	343.15	-12.5	-5.67	-4.14
12 weeks	AD11	600	171.16	592	268.53	-8	-3.63	-2.65
12 weeks	256	642	291.21	634	287.58	-8	-3.63	-2.65
12 weeks	114	832	377.39	830	376.40	-2	-0.91	-0.66
12 weeks	194	674	305.72	674	305.72	0	0	0
Mean		687	311.62	682.5	309.58	-4.5	-2.04	-1.49

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RESULT

TABLE 8.7 continued

Live weight changes of Zebu cattle during the experiment.

TREATMENT K 2-7/3/70

Stage of Growth	Animal No.	Initial Live weight lb.	Initial Live weight kg.	Final Live weight lb.	Final Live weight kg.	Live weight gain or loss per day lb.	Live weight gain or loss per day kg.	Live weight gain or loss per week kg.
4 weeks	195	744	351.08	762	345.64	-12	-5.44	-3.97
4 weeks	236	656	297.56	642	291.21	-14	-6.35	-4.64
4 weeks	198	856	388.28	818	371.04	-38	-14.52	-10.60
4 weeks	94	822	372.86	806	365.60	-16	-7.26	-5.29
Mean		777	352.44	757	343.37	-20	-9.1	-6.64
12 weeks	AD11	612	277.60	602	273.07	-10	-5.54	-3.31
12 weeks	256	638	289.39	636	288.49	-2	-0.91	-0.66
12 weeks	194	732	332.03	712	322.96	-20	-9.1	-6.64
Mean		660.7	299.69	647	293.48	-10.7	-4.85	-.345

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RESULT

TABLE 8.7 continued

Live weight changes of Zebu cattle during the experiment

TREATMENT L 2-7/3/10

Stage of Growth	Animal No.	Initial Live weight lb.	Initial Live weight kg.	Final Live weight lb.	Final Live weight kg.	Live weight gain or loss per day lb.	Live weight gain or loss per day kg.	Live weight gain or loss per week kg.
4 weeks	322	482	218.63	508	230.43	26	11.79	8.61
4 weeks	236	632	286.67	640	290.30	8	3.63	2.65
4 weeks	198	672	304.82	674	305.72	2	0.91	0.66
4 weeks	94	808	366.51	822	372.86	14	6.35	4.64
Mean		648.5	294.16	636	288.49	12.5	5.67	4.14
12 weeks	282	644	292.12	686	311.17	42	19.05	13.91
12 weeks	258	668	303.00	700	317.52	2	0.91	0.66
12 weeks	194	744	337.48	746	338.38	2	0.91	0.66
Mean		685.3	310.85	710.7	322.37	15.3	6.94	5.07

Treatment L stood out as a good forage for our Zebu cattle in that all the animals gained weight. This may be due to the fact that treatment L consists of a combination of all the grasses and legumes, it comprised of grass/legume in H, J and K. Treatment L offered more C.P., and these levels (fig. 6.1) at four and twelve weeks may be adequate for maintenance and growth requirements of the animals. The other treatments offered submaintenance nutrients and therefore caused loss in weight.

8.5 DISCUSSION AND CONCLUSION

Looking through the results of this experiment, it is observed that the Nigerian Zebu cows produce more butterfat (5-7%) than the Indian Zebu (4.83%) or the European breeds. The Jersey cows produce high butterfat (5.14%) but the Nigerian Zebu exceeds this limit. The Zebu cows of Eastern and Southern Africa produce high butterfat which falls in line with the Nigerian Zebu. Examples are the Red Butana Zebu which produces 5.5% fat, the Abyssinia (Ethiopia) shorthorned Zebu which produces 6-6.5% fat, the Gasara (Small Somalia) Zebu which produces 5.5% fat and the Kenya Boran Zebu which produces 5% to 6.8% fat, (Mason et al.,

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1960). The Inidan cows which live under similar tropical conditions like the Nigerian Zebu produce lower milk fat, although the fat content is higher than most European cows (except Jersey), and American cows (Mahaderam, 1958; Eckles _et al_., 1943). In some investigations carried out by Olaloku (1968), Adeneye, Oyenuga and Olaloku (1970), and Olaloku, Egbuiwe and Oyenuga (1971) high milk fat contents were recorded for the White Fulani Cows.

The milk protein in the Zebu used in this experiment is between 3.34% and 4.66%. Most of the cows produced less than 4% protein. Similar results have been recorded by Adeneye _et al_. (1970). Since the Indian cows produce less than 3% milk protein and the other breeds of cows produce over 3% but less than 4% milk protein, it may be said that the Zebu cows used in these experiments produced high quality milk. Protein is a very important nutrient needed by man and animal. Milk protein is a very good source of human protein, so its production in large quantities is therefore necessary to avoid malnutrition.

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These cows produced higher milk lactose and total solids than the other breeds under comparison. The other breeds produce over 4% but under 5% lactose, but these cows produced (except in two cases) between 4% and 6% and lactose. The lactose level is however higher than that recorded by Adeneye et al. (1970). Milk is the only source of lactose, so the production of higher quantity of lactose by the Nigerian Zebu cows puts the breed in a favourable position. Lactose may play important role in nutritional studies in the future, and a good source may be of commercial importance in the future. The total solids did not at any one time fall as low as 11.92% which is the figure for Holstein cows. The value in these experiments were between 13% and 16%, Similar results have been recorded by Adeneye et al. (197); and Olaloku et al. (1971). This showed that these cows secrete more dry matter than the other breeds. The quantity of the milk (in lb.) may be less than those of the European or American breeds, but the quality is surely better.

The ash content is less than 1.0% in all cases. The other breeds produce less than 1% milk ash also. The

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greatest variation in the milk analysed occured here. The ash content varied from 0.56% to 0.81%. Definitely the ash content is above average.

The four feeds are quite good for the production of high quality milk. For greater milk production, high milkers should be selected and bred, so that their projeny may produce more milk under better animal husbandry.

Treatment L under all conditions appeared to be the best for high quality milk since the cows on this treatment produced the highest total solids, lowest water, highest fat, protein, lactose and ash in most cases.

Table 8.8 shows the summary of mean milk yield and chemical constituents.

The cows in advanced stages of lactation on treatments H and J at 4 weeks produced less milk per day and less butter fat per day. The S.N.F. content too was lower for the cows in advanced stages of lactation. The crude fibre and crude protein content of treatment H at 4 weeks were higher than those for treatment J at the same stage of growth. The cows in advanced stages of lactation on treatments K and L produced more milk per day, more butter-

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fat, and more S.N.F. Treatment K however contained higher crude fibre and lower crude protein at 4 weeks while treatment L contained lower crude fibre and higher crude protein at 4 weeks.

The cow in advanced stage of lactation on treatment H at 12 weeks produced less milk per day, more butter fat, and more solid-not-fat. The crude fibre was lower and crude protein was higher in treatment H at 12 weeks than at 4 weeks. On the average the lower crude fibre and higher crude protein treatment H produced less milk per day, less butterfat and less S.N.F. Treatment J at 12 weeks contained more crude fibre and less crude protein than at 4 weeks. This treatment increased milk production per day, and the butterfat content, but the S.N.F. content was lower than at weeks.

Treatment K at 12 weeks decreased milk yield per day, and it also decreased butter fat, and S.N.F. contents. The crude fibre and crude protein were lower than at 4 weeks.

Treatment L contained less crude fibre and crude protein at 12 than at 4 weeks. At 12 weeks the cows

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produced more milk per day, more butterfat and more S.N.F.

Comparing all the treatments at 4 and 12 weeks, the milk production per day for cows on treatment H was consistently higher than those of the others. Production per day of the cows on J, K and L followed in that order downwards.

Brown, Stull, and Stott, (1962) reported that low rouphage diet plus cotton seed oil significantly depressed total milk production, 4% fat corrected milk (FCM), percent fat and the average daily fat production. On the low and high roughage diets the addition of fat did not significantly alter the protein or lactose content of the milk. Joseph Edwards (1950) compared milk production and butter fat in two breeds of cows. He observed that from the 45th day of lactation the rates of milk production for both breeds of cows fell at a very regular rate. About the 7th month of lactation the rate of fall becomes more rapid. In the early stages of lactation, the rate of secretion is twice as great as in the last stages. The fat content rises from 5.8% at the start of lactation to 6.51 at the end. Banicoat, Logan, and Grant, (1949)

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observed a decrease in milk fat of one ewe on six consecutive days.

Johnson, Fourt, Hibbs, and Ross, (1961) reported the lowest solids-not-fat (S.N.F.) content during the second month of lactation for both Hosteins and Jersey cows. The fat content was lowest the third month of lactation for the Holsteins, and during the second month for the Jerseys. There was less variation in solids-not-fat than in milk fat for both breeds of cows. The relationship between fat and solids-not-fat was not linear, but there was a gradual yet irregular decrease in both fat and solids-not-fat content with age for both breeds. Nickerson (1961) in his own work on milk constituents reported that most individual constituents of milk change positively with changes in total solids. Some constituents show no significant correlation to changes in total solids of milk. Table 8.8 showed that treatment L at 12 weeks which produced milk with the highest total solids also produced milk with the highest butter fat and high S.N.F. Fat has a higher correlation with total nitrogen than with any other constituent measured, but table 8.8 showed that the

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treatment with the highest protein does not necessarily produce milk with the highest butter fat. The treatment with the lowest crude protein however produced milk with the lowest butter fat content.

Casein, Lactalbumin, and lactoglobulin increase as the total nitrogen content of the milk increase, but at respectively slower rates. Total nitrogen is highly correlated to fat and total solids, therefore casein should show a similar high correlation.

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TABLE 8.8

Summary of Mean Yield and Chemical composition of milk

(%)

Treatment	wks.	Cow No	Days in Lactation	Milk Production lb.	Milk Production per day lb.	Butter fat	Butter fat (mean)	S. N. F.	S. N. F. (mean)	Total solids	Total solids (mean)	C. F.	C. P.
H	4	195	198	2,925	14.8	6.54	6.34	10.38	9.59	16.92	15.93	35.33	9.54
		183	207	2,139	10.3	6.14		8.80		14.94			
	12	AD11	227	2,135	9.4	6.28	6.23	7.51	7.12	13.79	13.35	34.52	9.81
		220	170	1,786	10.5	6.18		6.72		12.90			
J	4	195	233	3,236	13.9	6.70	6.64	8.70	8.30	15.40	14.94	32.49	8.98
		183	242	2,259	9.3	6.58		7.89		14.47			
	12	256	185	986	5.3	6.50	6.94	7.32	7.00	13.82	13.93	33.77	8.60
		AD11	205	2,332	11.3	7.37		6.67		14.04			
K	4	195	275	3,468	12.8	6.73	6.32	8.67	8.39	15.40	14.71	33.67	8.25
		236	107	670	6.2	5.91		8.11		14.02			
	12	256	227	1,191	5.2	5.97	5.15	8.24	7.86	14.21	13.01	31.76	7.20
		AD11	247	2,406	9.8	4.33		7.48		11.81			
L	4	322	107	524	5.0	6.13	6.06	8.25	8.70	14.38	14.78	23.53	10.94
		236	156	858	5.5	5.99		9.15		15.4			
	12	282	169	1,070	6.3	6.30	7.11	8.86	9.26	15.16	16.36	31.01	9.87
		258	147	965	6.5	7.91		9.65		17.56			

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CHAPTER 9

Ammonia nitrogen and urea nitrogen produced in the rumen liquor and blood serum of the Zebu cattle on treatments H,J,K and L cut at different stages of growth.

9.1 INTRODUCTION

The ruminants are able to digest most of the fibrous parts of their feeds (cellulose, hemicellulose) and in some cases the simple carbohydrates due to the activities of the numerous microorganisms in the rumen, and the accompaying forestomachs. Other feed components utilized by the ruminants only are the non-protein nitrogen of the feeds. The mixed rumen organisms have great power to decompose amino acids to ammonia, and other products like carbon dioxide. This power is increased greatly when the amount of soluble protein in the feed is increased.

El-shazly (1952) in his work on degradation of protein in the rumen of the sheep noted that volatile fatty acids, ammonia and carbon dioxide were the end products of amino acids decomposition.

Owen, Smith, and Wright (1943) used urea as a partial protein substitute in the feeding of dairy cattle. They found blood meal and urea to be equally good. The milk yield fell appreciably when no urea or

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blood meal was fed and the yield did not come up to the original level when urea or blood meal was re-introduced. McDonald (1952; 1954) fed casein, gelatine, and zein to sheep and measured the ammonia nitrogen formed. Ammonia nitrogen formed from casein represented about 20% of the total nitrogen added. Amide nitrogen consists of 9.3% of the total nitrogen of casein, and the rise in ammonia is not due solely to removal of amide groups. This shows that deamination and deamidation reactions were responsible for the formation of ammonia. Similar results were obtained from gelatine which contains only a trace of amide nitrogen. Direct addition of zein to the rumen unlike the former two did not produce any change in the concentration of ammonia nitrogen in the rumen. This was because zein was very insoluble in aqueous media and was resistant to proteolysis. However zein was digested to a considerable extent in the rumen, and this showed that its rate of digestion was too slow to allow an accumulation of ammonia. Casein is a readily attacked protein and its hydrolysis produces ammonia and other non-protein nitrogen. The rise in residual nitrogen was of brief duration and after two hours the amount decreased and the level of ammonia nitrogen continued to rise for four hours after feeding. In the presence of readily

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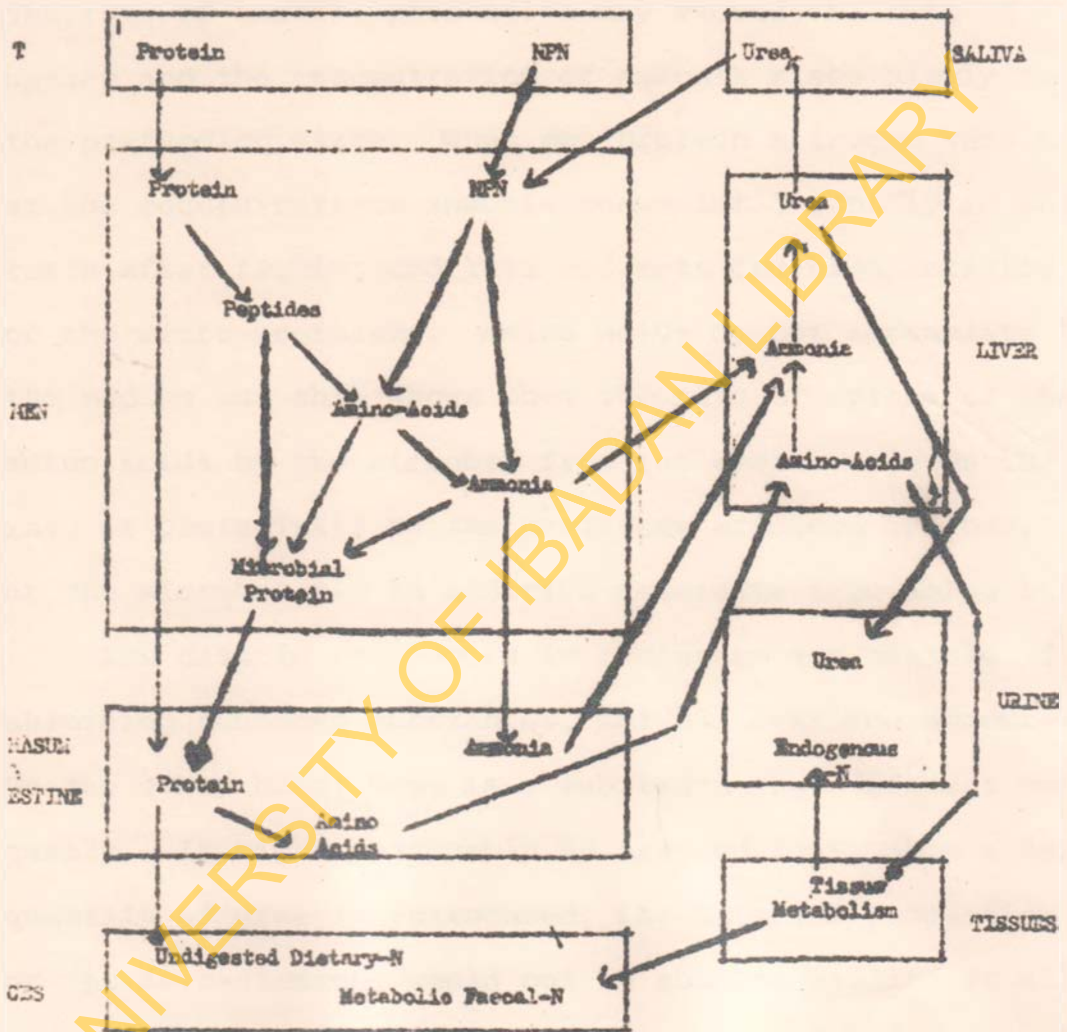
NITROGEN METABOLISM IN THE RUMINANT



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available sources of energy (glucose and starch) the organisms use the ammonia as a source of nitrogen for growth. When the growth rate of the microbes declined, the rate of ammonia production may exceed the rate of uptake and the concentration of ammonia rises slowly to the prefeeding stage. When non-protein nitrogen were fed at low concentrations ammonia accumulated rapidly in the rumen after feeding and this reflects the high activity of the micro-organisms. Amino acids do not accumulate in the medium and this shows that the rate of uptake of the amino acids by the microbes from the medium exceeds the rate of proteolysis by the proteases produced by them, or the microbes may in addition deaminate free amino acids.

The gastric reservoirs in ruminants are capable of absorbing numerous substances, notably urea and ammonia. On the other hand, urea is a substance that diffuses very easily. It might reasonably be assumed that, when a large quantity of urea is introduced, the baterial population of the fore-stomachs would not be able to utilize it all immediately, therefore most of the urea and ammonium salts would pass into the blood to be eliminated by the kidneys.

McDonald (1948); Dinning (1948); Bouckaert and Oyaert (1952); Chalmers (1954); Annicolas (1956); and

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Lewis (1957); demonstrated the absorption of ammonia. The absorption of ammonia is increased by increase of pH in the rumen (Bouckaert and Oyaert, 1952).

In general, ammonia is found in only small quantities in the peripheral blood because the liver converts ammonia to urea (Le Bars, 1957). Labouche (1960) observed that blood ureas were higher in N'damas than in Zebus, and that blood ureas are higher in old animals than in young cows, but seasonal variations, linked with the nature of the food can be observed, whatever the age.

The urea in the blood is not excreted solely by the kidneys; a considerable quantity is returned to the gastric reservoirs, either in the saliva or directly through the epithelium of the rumen. Somers (1961) estimated that under normal conditions, urea represented 60-70% of total nitrogen in sheep saliva, which contained no ammonia. When 3gm urea was given intra - arterially to sheep which had been fasting for 24 hours, the salivary content remained high for six hours, and fluctuations therein did not run parallel to fluctuations of the urea in the blood. Under normal conditions, it has been observed that fluctuations in the salivary content of urea in the course of the twenty four hours are dependent on blood levels

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which themselves are a function of the ammonia content of the rumen. Two phases are evident in the twenty four hour salivary nitrogen cycle. The first is an immediate response to the act of feeding, in which there is increase both in the volume of saliva secreted and in the nitrogen content of the saliva. The second phase is the intensity of the fermentation processes in the rumen and masticaction which stimulates salivary secretion.

There appears to be competition between kidneys and salivary glands in connection with excretion of blood urea. The rumen may also play some role in urea excretion. When there is increased need of nitrogen, salivary excretion is increased and urinary excretion reduced. Schmidt-Nielsen (1957) have shown that in the camel receiving a ration with a normal nitrogen content, about 40% of the urea filtered out in the glomerulus is excreted in the urine, but this percentage falls to a mere 1-2% when the feed is poor in nitrogen. A comparable phenomenon has been observed in the sheep.

Schaadt and Johnson (1969) in their feeding experiments found that when urea was fed to sheep along with corn silage, the pH decreased after feeding and did not increase until four hours after feeding. The total volatile fatty acids produced increased rapidly during

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the first hour after feeding and only slightly more to a maximum at two hours after which they declined. Somers (1961) investigated a few of the factors that affect the nitrogen fractions in the parotid saliva of sheep. The concentration of ammonia nitrogen in the rumen fluid increased at a relatively rapid rate reaching a maximum of 24 mg/100ml 4 hours after feeding. It declined to the prefeeding level at the 24 hr. sampling period. The rise in the concentration of blood urea nitrogen is an indication that part of the ammonia produced in the rumen was absorbed, metabolised to urea in the liver and the urea discharged into the systemic circulation. The rise was progressive and continued until it reached the maximum concentration of 27mg/100ml of blood at 8¾hr. after feeding. The concentration of urea nitrogen in blood and saliva increased and did so for the next 7 hours. But the rate of increase in the concentration of urea nitrogen in the saliva exceeded that in the blood. This showed that the salivary urea nitrogen secretion rate was limited by the blood urea nitrogen level. Urea nitrogen in blood continued to rise for a further two hours before reaching the maximum concentration. The concentration of urea nitrogen and total nitrogen in the saliva started to fall after the seventh (7th) hour.

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Lewis (1957) sampled the blood from the jugular vein and the blood from the carotid artery of the sheep. The ammonia lost from the rumen was proportional to the concentrations of ammonia in the rumen liquor. The rate of increase in the ammonia of portal vein was not as great as that of the rumen liquor. Blood urea level was relatively constant in all cases but was clearly dependent on the diet of the animal. As rumen ammonia increased there was a greater return of nitrogen to the rumen via the saliva. There were no significant differences between the concentrations of urea in venous and arterial blood. The concentrations of rumen ammonia varied considerably during twenty four hours. Rapid changes in rumen ammonia concentrations produced by variations in the diet are paralleled by equally rapid changes in blood urea level. Considerable ammonia passes along the portal vein which results in a higher blood urea six to seven hours after feeding.

9.2 DETERMINATION OF UREA AND AMMONIA

Different methods were employed by different workers to determine urea in blood and ammonia in the rumen liquor. In most cases the enzyme urease in buffer solution was used in these determinations (Howell, 1939a;b). Pearson

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THE NITROGEN CYCLE IN NATURE

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and Smith (1943) compared three different methods of urea analyses and obtained good and similar results. Conway and Cooke (1939) used a titrimetical method in their ammonia determinations. Donald Van Slyke (1927) used urease and measured the urea in the blood by the ammonia concentration and by the carbon dioxide produced from the ammonium carbonate formed by the action of urease on the urea. Lebiboff and Kahn (1929) used a colorimetric method for their urea determination. Other people who used colorimetric methods for urea and ammonia determinations were Fawcett and Scott (1960) and Chaney and Marbach (1962).

9.3 ANALYTICAL PROCEDURE

The blood urea concentrations and rumen ammonia were determined using the methods of Fawcett and Scott (1960) and Chaney and Marbach (1962). The optical density was read at 625µm and the concentration in mg. per 100ml rumen liquor or 100ml blood calculated from standard curves prepared from ammonium sulphate solutions for ammonia and from urea solutions for urea.

9.4 RESULTS

Table 9.1 showed the ammonia nitrogen expressed in mg. per 100ml rumen liquor produced by the Zebu cattle. The

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treatments were at four weeks of growth when cut and fed to the animals. The ammonia level before feeding was fairly high. The level was higher than that present one or two hours after feeding in case of treatments H and K. But the level increased after feeding in treatments J and L. The increase in both treatments remained almost constant for the two hours after feeding. The greatest amount of ammonia production was by treatment L, and the least was by treatment K.

Table 9.2 showed the ammonia nitrogen produced by Zebu cattle fed treatments K and L at 12 weeks. The ammonial levels of the two rumen liquors were not very high. The levels increased after feeding, although the increase was small in the case of treatment K while it was abrupt in case of treatment L. Treatment L also gave the greatest ammonia production at this stage of growth.

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TABLE 9.1

Ammonia nitrogen (mg/100ml rumen liquor) produced by Zebu cattle fed four treatments cut at 4 weeks.

Time of Sampling	H	J	K	K
Before feeding	16.80	17.75	12.69	19.89
Hours after feeding 1	15.20	20.00	9.21	26.58
2	12.85	19.25	10.52	26.64
Mean	14.95	19.00	10.81	24.37

Each is the mean of 20 determinations

TABLE 9.2

Ammonia nitrogen (mg/100ml rumen liquor) produced by Zebu cattle fed two treatments cut at 12 weeks

Time of Sampling	K	L
Before feeding	10.52	8.60
Hours after feeding 1	10.75	15.60
2	11.15	14.53
Mean	10.81	12.91

Each is the mean of 10 determinations

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In table 9.3 the level of ammonia in the rumen liquor increased one hour after feeding only when treatment J was fed. All the other treatments caused a decrease in the ammonial level of the rumen liquor after feeding. The level started to rise in the case of treatments H and K three hours after feeding and got to the maximum four hours after feeding. The peak however was reached two hours after feeding in the case of treatments J and L. On the average treatment H produced more ammonia than the rest and treatment K produced the least.

Table 9.4 showed the ruminal ammonia production by Zebu cattle sampled hourly up to eight hours after feeding. All the treatments caused a decrease in the rumen liquor ammonia level. In treatments H and J the rumen ammonia level did not rise to the pre-feeding level throughout the sampling periods. But treatments K and L produced rumen ammonia levels higher than the prefeeding levels. Treatment K produced a peak production five hours after feeding while treatment L produced a peak level four hours after feeding. On the average there was little difference in ammonia production by treatments H,J and K. Treatment L produced the least amount of ammonia.

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TABLE 9.3

Ammonia nitrogen (mg/100ml rumen liquor) produced by Zebu cattle fed four treatments cut at 4 weeks.

Time of Sampling	H	J	K	L
Before Feeding	17.90	17.75	14.80	15.55
Hours after feeding 1	17.30	20.10	9.65	12.50
2	14.50	20.25	8.50	20.00
3	18.15	9.69	14.00	16.20
4	24.60	19.00	20.50	17.20
Mean	18.49	17.34	13.91	16.29

Each is the mean of four determinations

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TABLE 9.4

Ammonia nitrogen (mg/100ml rumen liquor) produced by Zebu cattle fed treatments H, J, K, and L at 4 weeks.

Time of Sampling	H	J	K	L
Before feeding	21.48	21.63	14.56	13.83
Hours after feeding 1	16.68	17.97	11.58	13.59
2	11.70	18.72	12.79	15.87
3	13.68	13.27	16.02	16.08
4	17.08	11.32	13.75	17.25
5	17.08	16.42	19.65	13.08
6	16.37	15.84	17.60	11.48
7	12.52	17.43	15.77	10.73
8	15.96	11.28	17.75	13.01
Mean	15.84	15.99	15.50	13.88

Each figure is the mean of six determinations

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Table 9.5 showed the data for ammonia produced in the rumen liquor by Zebu cattle when the treatments were eight weeks. Both treatments caused an increase in the ruminal level one hour after feeding, and both reached their peak production one hour after feeding. The levels then dropped slowly till they reached almost the prefeeding levels eight hours after feeding. On the average the difference in ammonia level was small. There was a lot of fluctuations during the sampling periods. The levels varied from 10.75 mg/100ml rumen liquor to 19.58 mg/100ml for treatment H and they varied from 12.69 mg/100ml rumen liquor to 21.45 mg/100ml rumen liquor for treatment J.

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TABLE 9.5

Ammonia nitrogen (mg/100ml rumen liquor) produced by Zebu cattle fed H and J at 8 weeks

Time of Sampling	H	J
Before feeding	14.75	16.92
Hours after feeding 1	19.58	21.45
2	17.05	14.65
3	19.45	15.27
4	12.15	12.69
5	9.93	15.00
6	10.75	17.17
7	14.02	16.10
Mean	14.46	15.79

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Table 9.6 showed the ruminal ammonia production when the treatments were twelve weeks. With treatment J the ruminal ammonia level increased after feeding and continued to increase till it reached a peak four hours after feeding. The level was maintained for the next one hour before it started to fall, but the level was still higher than the prefeeding level. With treatment L the ruminal ammonia level decreased after feeding and did not rise to the prefeeding level throughout the sampling period. The level rose to a peak three hours after feeding and the level showed alternate rises and falls throughout the sampling period.

On the average, treatment L produced significantly more ruminal ammonia than treatment J. The fluctuations in the levels were high. With treatment J the levels varied from 9mg/100ml rumen liquor to 20mg/100ml rumen liquor, while with treatment L the levels varied from 20mg/100ml rumen liquor to 31mg/100ml rumen liquor.

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TABLE 9.6

Ammonia nitrogen (mg/100ml rumen liquor) produced by Zebu cattle fed two treatments cut at 12 weeks.

Time of Sampling	J	L
Before feeding	9.00	31.60
Hours after feeding 1	14.00	21.75
2	16.50	26.00
3	18.30	28.70
4	20.20	27.10
5	20.20	26.80
6	18.00	27.10
7	16.20	25.70
8	16.20	20.25
Mean	16.54	26.11

Each is the mean of duplicate samples.

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Table 9.7 showed urea nitrogen produced by Zebu cattle fed treatments H and J cut at four weeks. The sampling was carried out up to four hours after feeding. After feeding the urea level in blood serum increased in both cases. The peak blood serum level was reached one hour after feeding with treatment H, but the peak was reached four hours after feeding with treatment J. The blood urea level in both cases reached almost the same level four hours after feeding. The fluctuations in the urea levels were not as great as fluctuations in ammonia levels in the rumen liquor.

Table 9.8 showed the sampling of blood up to eight hours after feeding. Treatments H, J and L produced the highest level of urea seven hours after feeding. Treatment K produced the peak urea five hours after feeding. The fluctuations in all cases were not much within the sampling periods.

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TABLE 9.7

Urea nitrogen (mg/100ml blood serum) produced by Zebu cattle fed two treatment cut at 4 weeks

Time of Sampling	H	J
Before feeding	17.50	15.88
Hours after feeding 1	20.58	16.25
2	17.20	15.50
3	20.00	15.90
4	18.63	18.65
Mean	18.78	16.44

Each is the mean of four determinations.

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TABLE 9.8

Urea nitrogen (mg/100ml blood serum) produced by Zebu cattle fed four treatments cut at 4 weeks

Time of Sampling	H	J	K	L
Before feeding	21.00	20.78	22.18	15.83
Hours after feeding 1	22.96	17.70	21.73	16.98
2	23.26	19.54	22.10	17.29
3	24.20	18.54	22.39	17.97
4	22.90	18.74	22.38	18.86
5	24.33	20.29	24.27	21.95
6	23.88	20.80	23.83	21.09
7	25.55	21.51	22.87	23.11
8	21.81	18.77	17.67	16.77
Mean	23.32	19.63	22.16	18.87

Each is the mean of 12 determinations.

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Table 9.9 showed the urea levels of the Zebu cattle blood when the treatments were 8 weeks. The fluctuations in the urea levels did not vary much for treatments H and J, but there were wide fluctuations in the blood urea levels for treatments K and L. On the average the urea production from treatments H and J were similar, treatments K and L produced less blood urea.

9.5 DISCUSSION AND CONCLUSION

Since the ammonia released into the rumen liquor reached the peak at different times, it may be that the microbial activities varied a lot within the rumen. The microbial population may also be a factor. The more they are the more rapid the ammonia production. Ammonia is absorbed into the blood system and some of it changed to urea in the liver. The rate of absorption will also depend on the amount in the rumen liquor.

The fibrous feeds are expected to take longer time for digestion and absorption. The resuts indicate very little difference in the rate of ammonia production for the feeds clipped when four, eight or twelve weeks. The herbage was as fibrous at four weeks as at twelve weeks.

Urea is the diamide of carbonic acid, and it is the principal end product of nitrogen metabolism in mammals.

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TABLE 9.9

Urea nitrogen (mg/100ml blood serum) produced by Zebu cattle fed four treatments cut at 8 weeks

Time of Sampling	H	J	K	L
Before feeding	18.70	20.82	23.22	19.25
Hours after feeding 1	18.19	19.76	23.70	18.25
2	21.30	20.53	26.85	22.40
3	21.32	19.95	15.95	19.94
4.	20.91	21.77	10.45	14.55
5	22.25	22.99	18.63	11.03
6	23.72	25.51	8.89	13.13
7	23.80	25.61	19.98	8.89
8	18.60	19.71	12.45	8.40
Mean	20.98	21.85	17.79	15.09

Each is the mean of 12 determinations

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The ammonia produced from the microbial breakdown of protein and non-protein compounds is used partly by the microflora for their growth, and some are absorbed into the blood stream. A lot of the absorbed ammonia is quickly converted to urea by the liver and is excreted as such. Some of the ammonia is also used in the synthesis of non-essential amino acids by amination, and some of the urea reenters the rumen through the saliva. When the micro organisms are not synthesising microbial protein, the ammonia produced tend to accumulate and a lot is absorbed; converted to urea and lost to the body. Urea can safely replace a part of the protein in selected rations for beef, dairy and growing cattle. But ammonia produced from urea is toxic so it can only be used to a certain extent.

Ruminal ammonia level in this experiment rose in some cases to 26mg/100ml rumen liquor, a value higher than what Somers (1961) recorded in his sheep experiment.

Blood serum urea levels also showed almost the same pattern as ruminal ammonia levels. The rises or falls indicate that part of the ammonia produced in the rumen was absorbed, converted to urea in the liver and excreted into the blood. The highest level in this experiment

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was just over 27mg/100ml blood serum, a value quite close to that obtained by Somers (1961). Somers work indicated however that it was possible to get an increase in blood urea level and the peak production after eight hours after feeding.

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CHAPTER 10

"In vitro" determination of digestibility coefficient of treatments according to Tilley and Terry, modified by Alexander.

10.1 INTRODUCTION

In animal nutritional studies it is not always possible to get enough animals to complete a stastically sound experiment. A lot of information is needed concerning the digestion, absorption and the metabolism of different feeds. It is not possible to increase the number of animals in a head as one wishes because a cow can only produce a calf in one to one and a half years. The heifer calf has to grow up to two years or more before it is ready for reproduction. Research workers on ruminants have therefore devised a technique whereby a lot of information is obtained within a short time from experiments carried out under conditions controlled in such a way as to simulate what obtains in the animal.

One of such techniques is the "in vitro" technique where feeds are digested in tubes using rumen liquor withdrawn from the donor animal. A lot of information has been obtained in the past, and a lot is being obtained presently.

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The ruminants such as cattle, sheep and goats are needed for beef and milk production. The present output of these animals in Nigeria is very low. One of the factors causing low yield is feed utilization. We want to know the feed value of a feed and how much of it is used for a particular function. The nutritive value of a treatment is a function of chemical composition and digestibility. The type of the end product produced during digestion is therefore very important.

Factors which influence the extent of "in vitro" digestibility of a treatment are many. Some of these are concentration of the feed, the freshness of the feed, its fineness, the composition and buffering capacity of the medium, the amount and preparation of the rumen fluid inoculum and the diet of the donor animal.

The "in vitro" determination of digestibility is of considerable use in predicting the value of dried grasses as sources of energy for ruminants. The determination of digestibility has the added advantage of providing a measure of the faecal energy loss which is not only the major determinant of the energy per unit of food, but also of great importance as a factor controlling voluntary feed intake by ruminants.

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There is increasing interest shown in beef and dairy production in Nigeria, but there is little information available on indigenous pasture. This has therefore necessitated the use of rapid methods for pasture evaluation. The "in vivo" digestibility trials, though give the actual digestibility by the use of animals, requires a considerable use of time, requires large quantities of uniform treatment, and the collection of large number of samples from treatments and faeces. Collection of faecal samples requires a lot of care in order to avoid contamination.

However "in vitro" methods are capable of handling large numbers of samples in a short period of days. In "in vivo" experiments only one treatment digestibility can be carried out and completed in a 3 - 4 week experimental period.

10.2 RUMEN LIQUOR

Rumen liquor for inoculation was obtained from a fistulated steer. The liquor was immediately stored in a thermos flask and taken to the laboratory. The liquor was strained through thin cloth and then used for inoculation. 10ml of rumen liquor was used for each grass sample. The pH of the liquor was measured before use.

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10.3 ANALYTICAL PROCEDURE

Large boiling tubes (20cm x 3 cm) were washed and dried at 100°C in the oven. The tubes were cooled and duplicate samples, 0.5gm, were weighed. The samples were kept in a water bath at 39°C until needed. 640 ml artificial saliva buffer solution was poured into a flask and kept in the water bath, and CO_2 was passed through it until it was saturated and clear (15 minutes). The pH was adjusted to between 6.7 - 6.9, before 160 ml rumen liquor was added, and 16 ml $(NH_4)_2\ SO_4$ solution was also added. CO_2 was passed through the mixture again and the temperature kept at 39°C.50 ml of this mixture was added into each tube and left in the water bath at 39°C. Each tube was shaken gently by swirling, and CO_2 passed through to displace any gas in the tube. The tubes were left in the bath for 24 hours.

After 24 hours the pH of each tube was read and adjusted to pH 6.9 by adding N Na_2CO_3. The tubes were left for another 24 hours. Then 1.5 ml followed by 2.5 ml HCl (20% v/v) was run down the side of each tube as it sits in the bath. The tubes were not disturbed at this time to avoid frothing. The pH of the solution was adjusted to 1.2.

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Iml of aqueous pepsin solution (1.2g/5ml) was added to each tube and left to digest for 48 hours. CO_2 was passed through the tubes after pepsin addition.

After 48 hours the contents of each tube was filtered, using fine celite as filter aid, through whatman filter paper 541 under suction. The residue was washed several times with hot water, dried at 100°C overnight, weighed, ignited at 480°C and weighed again.

<u>Calculation</u>

"<u>in vitro</u>"

$$\text{Digestibility of organic Matter} = \frac{\text{O.M in 0.5gm sample} - (\text{O.M. in residue} - \text{O.M. in control})}{\text{O.M in 0.5 gm sample.}} \times 100$$

O.M. = Organic Matter.

10.4 RESULTS

Table 10.1 showed that in 1967 treatment H had the lowest digestibility at one week. The digestibility increased at two weeks and then rose to the highest value at t hree weeks before declining at four weeks. The digestibilities at three and four weeks were higher than those at one and two weeks.

In 1968 treatment H showed a higher digestibility than in the previous year. The digestibility was greater

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at one week than at two weeks. The treatment was most digestible at three weeks. The digestibility fell to nearly the same level as was in the previous year when four weeks old. Treatment H showed the greatest digestibility at three weeks during the two years. The means for both years were 57.74%, 53.49%, 60.05% and 52.87% at one, two, three and four weeks respectively.

Treatment J also had the lowest digestibility during the first week of growth in 1967. The digestibility increased by about 4% in the second week but dropped by a few units the third week before reaching the highest digestibility at four weeks.

However in 1968 treatment J had almost the same digestibility at one, three and four weeks. It was least digestible at two weeks. The average for both years showed that the treatment was more digestible at four weeks, and the digestibilities at two and three weeks were very close, while the value for one week was the lowest. Treatment K was most digestible in its first week of growth in 1967. It dropped by about 3% in the second week and rose slightly the third week but it dropped again in the fourth week. The lowest digestibility was obtained during the second week.

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In 1968 the digestibility was fairly good in the first week. The value was higher than in the previous year. This value was higher than that of the second week. Treatment K was more digestible in the second week than in the previous year. The treatment was most digestible in the third week. The digestibility fell by almost 4% in the fourth week. The digestibilities were higher at the four stages of growth during the second year. The means for both years showed the third week to be the period with the highest digestibility, 59.23%. 57.38%, 55.02%, and 36.78% were the digestibilities for the first, second, and fourth weeks respectively.

In 1967 treatment L had almost the same digestibilities during the four stages of growth. The least was in the third week while the highest was in the fourth week. There was little difference between the first and second week.

In 1968 the digestibilities were higher in every case except in the fourth week. The highest digestibility was obtained in the first week and this declined gradually from then on till it fell to 51.11% in the fourth week. This value was lower than that of the previous year.

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The mean values also showed the treatment to be most digestible in the first week with the digestibility declining till the fourth week.

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TABLE 10.1

Comparison of "in vitro" Organic Matter digestibilities of treatments H, J, K, and L at four stages of growth with Zebu cattle.

Treatment	Stages of growth (weeks) 1	2	3	4	
H					
1967	42.52	46.15	56.51	52.72	
1968	62.96	60.83	63.59	53.02	
Mean	57.74	53.49	60.05	52.87	56.04
J					
1967	51.71	55.40	54.84	57.62	
1968	60.76	58.67	60.66	60.16	
Mean	56.24	57.04	57.75	58.89	57.48
K					
1967	55.56	52.02	53.93	52.74	
1968	59.19	58.01	64.53	60.82	
Mean	57.38	55.02	59.23	56.78	57.10
L					
1967	51.53	51.80	50.95	52.29	
1968	60.54	58.25	56.32	51.11	
Mean	56.04	55.03	53.64	51.70	54.10

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Comparing the means of the four treatments, treatments H and K had the same digestibilities at one week, and treatments J and L also had about the same digestibilities at one week. Treatment H however was the most digestible of them all at one week. When two weeks old, treatment J had the highest digestibility, while treatments K and L were identical in their digestibilities. Treatment H was the least digestible of the treatment at this time of growth. At three weeks treatment H had the highest digestibility followed closely by treatment K. Treatment J was next to treatment K in digestibility, and the least digestible was treatment L. At four weeks treatment L was the least digestible followed by treatment H with slightly higher digestibility, but treatment J was the most digestible followed by treatment K.

The mean digestibilities of the four treatments over the four periods of growth did not vary much. Treatment L was the least digestible in all cases and it had a mean of 54.10% while treatments J and K were almost identical. Treatment H was lower in value by about 1%. Except for treatment J, all the treatments were less digestible at four weeks than at one week. The difference was about 1%

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for treatment K but the difference was about 5% for treatments H and L.

The lower digestibility at four weeks may be due to increased crude fibre, cellulose, lignin and other less digestible cell wall constituents in the fodders. When plants grow the crude fibre, lignin and cell wall constituents increase. This increase with maturity was also reported by Oyenuga (1958 1960), Ademosun <u>et al.</u> (1967), Ademosun (1970), Tilley and Terry (1964), Maynard and Loosli (1962), VanSoest (1966, 1967).

Tables 10.2 and 10.3 showed the chemical composition of some grasses planted as sole plants and clipped at 4 and 8 weeks. Their "<u>in vitro</u>" digestibilities are also shown. At four weeks all the grasses contained over 90% dry matter (D.M.). <u>Paspalum scrobilatum</u> had the highest D.M. content followed very closely by <u>Chloris gayana</u>. The variation in D.M. % among the grasses was very narrow, being from 90.68% to 93.66%. The organic matter (O.M.) content too was fairly high for every grass. Each grass contained over 80% organic matter and the amount varied from 80.25% to 86.90%. <u>Setaria sphacelata</u> contained the

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lowest amount of D.M. and O.M. while Chloris gayana had the highest organic matter content. There was much variation in the ash content of the grasses. The lowest amount, 5.7%, was found in Andropogon tectorum while the highest amount 10.44%, was produced by Setaria sphacelata.

All the grasses except Paspalum Scrobilatum contained over 8% crude protein (C.P.). IB8 Giant Star grass contained the highest amount of C.P., 14.73%, whilst Paspalum scrobilatum contained just 7.57% C.P. Setaria sphacelata and Andropogon gayanus also contained fairly good amount of C.P.

At this tender age the crude fibre (C.F.) content of all the grasses was about 30%. This is typical of tropical grasses. Digitaria decumbens was the most fibrous with 35.9%C.F. followed very closely by Setaria sphacelata. The C.F. content varied from 30.51% to 35.90%.

The ether extractives were above 1% in all the grasses. IB8 Giant Star contained fairly high ether extracts and it is followed by Andropogon gayanus and Setaria sphacelata. The Nitrogen free extracts (N.F.E.) varied widely. The lowest amounts were found in Setaria sphacelata and IB8 Giant Star, but Chloris gayana and Paspalum scrobilatum contained the highest amounts of N.F.E.

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It is interesting to note that these grasses showed a tendency to produce high protein along with high crude fibre content. Setaria sphacelata, Andropogon gayanus, and IB8 Giant Star grasses contained high protein and high crude fibre contents. Paspalum scrobilatum also contained the lowest amount of crude fibre and crude protein.

At 8 weeks the grasses contained higher amounts of dry matter, the variation however was very small. The variation was from 93.17% to 94.98%. The organic matter content was also higher in all the grasses than at 4 weeks. The ash content decreased slightly at this stage of growth than at 4 weeks except IB8 Giant Star grass which showed an increament in ash content.

The crude protein content, ether extracts, and the crude fibre contents also decreased. IB8 Giant Star still maintained the lead in having the highest amount of protein and fat, followed by Setaria sphacelata and Andropogon gayanus. The crude fibre content of Melinus minutiflora increased at 8 weeks and it had the highest amount of crude fibre at this stage of growth, but IB8 Giant Star contained the lowest amount of crude fibre.

Nitrogen free extract varied very widely this time. The range was from 40.44% to 58.76%. Paspalum scrobilatum

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contained the highest amount of N.F.E., whilst IB8 Giant Star grass contained the lowest amount of N.F.E.

The "*in vitro*" digestibility of organic matter of these grasses at 4 weeks showed that *Setaria sphacelata*, *Andropogon gayanus* and *Melinus minutiflora* have very low digestibilities. They all gave less than 50% digestibilities. *Setaria sphacelata* was the least digestible. *Andropogon tectorum*, *Chloris gayana*, *Digitaria decumbens* and *Paspalum scrobilatum* gave digestibilities above 50%. IB8 Giant Star showed a very good degree of digestibility since it was the only grass having a digestibility of over 60% at this stage of growth.

At 8 weeks, the "*in vitro*" digestibilities increased, and the increase was appreciable in the case of *Setaria sphacelata*, *Digitaria decumbens*, *Andropogon gayanus* and *Melinus minutiflora*. The digestibility of *Paspalum scrobilatum* decreased at 8 weeks, whereas IB8 Giant Star showed the same digestibility as at 4 weeks; it also had the highest digestibility at 8 weeks. The very wide variation in digestibility shown at 4 weeks was reduced greatly at 8 weeks. All the grasses had over 50% digestibility and IB8 Giant Star was the only one having digestibility above 60%.

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TABLE 10.2

Chemical composition and "in vitro" digestibility of organic matter of single stand grasses at 4 weeks planted on University of Ibadan Farm

(% Dry matter)

	4 weeks							
Fodder	D. M.	O. M.	Total Ash	C. Protein	C. F.	Ether Extract	N. F. E.	"In Vitro" Dig. %
Setaria sphacelata	90.68	80.25	10.44	11.70	35.29	2.00	40.57	43.07
Andropogon tectorum	91.19	85.49	5.70	9.30	33.73	1.60	49.67	52.84
Chloris gayana	93.56	86.90	6.68	8.77	31.65	1.25	51.65	50.54
Digitaria decumbens	90.97	82.41	8.55	9.71	35.90	1.10	44.74	51.86
Paspalum scrobilatum	93.66	85.17	8.48	7.57	30.51	1.56	51.88	54.50
IB8 G. Star	90.90	81.09	9.87	14.73	32.06	2.68	40.66	62.68
Andropogon gayanus	91.83	85.72	6.10	10.00	34.46	2.00	47.44	44.89
Melinus minutiflora	92.48	86.12	6.41	9.11	33.04	1.75	49.69	45.71

Each figure is the mean of duplicate samples

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TABLE 10.3

Chemical composition and "in vitro" digestibility of organic matter of single stand grasses at 8 weeks planted on University of Ibadan Farm.

(% Dry Matter)

	8 weeks							
Fodder	D.M.	O. M.	Total Ash	C. Protein	C. F.	Ether Extract	N. F. E.	"In Vitro" Dig. %
Setaria sphacelata	93.17	83.73	9.44	9.95	29.39	1.96	49.26	50.13
Andropogon tectorum	94.01	88.65	5.35	8.12	27.37	1.00	57.16	54.00
Chloris gayana	94.98	90.01	4.91	7.50	28.81	0.99	57.79	52.51
Digitaria decumbens	93.94	85.83	8.11	8.00	28.59	0.75	54.55	57.65
Paspalum scrobilatum	93.22	86.01	7.20	6.91	26.29	0.84	58.76	51.73
188 G. Star	94.15	82.23	11.91	12.08	23.16	2.41	40.44	62.57
Andropogon gayanus	93.32	88.15	5.62	8.84	27.49	1.86	56.19	56.78
Melinus minutiflora	94.31	88.80	5.51	8.21	35.47	1.16	49.65	50.61

Each figure is the mean of duplicate samples.

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10.5 DISCUSSION

In an early investigation by French (1940), Zebu and Ayrshire cattle were fed hay and green grass. The digestibility of organic matter for hay was 60.45% for Zebu and 55.91% for Ayrshire. When green grass was fed the digestibility coefficient of organic matter for the Zebu was 48.85% and 49.34% for Ayrshire. The higher digestibilities of the feeds used in this experiment may be due to the legumes planted along with the grasses. Some of the results here showed that the digestibility of grasses or grass legume/mixtures will not necessarily be high when they are very young.

Of all the eight grasses analysed only *Digitaria decumbens*, and IB8 Giant Star grass showed higher digestibility coefficients in their early stages of growth.

Todd (1956) in his work on tropical grasses obtained 63.46% as the digestibility coefficient of organic matter for *Chloris gayana* in its early flowering stage, while the digestibility fell to 55.34% in the early dry season. *Pennisetum clandestinium* also had a higher digestibility coefficient of organic matter (72.68%) during the younger stage of growth and a lower digestibility, 59.10% when it was older.

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Woodman, Evans and Norman (1934) in their investigations on the nutritive value of lucerne, obtained highest digestibility at the earliest stage of growth. This is similar to the digestibility of grass. At this stage also the dry matter of lucerne was richest in starch equivalent and digestible protein.

Todd (1956) investigated the digestibility and nutritive value of Chloris gayana (Rhodes grass) and Pennisetum clandestinum (Kikuyu grass) at medium altitudes in the tropics. Chloris gayana was found to be more digestible in the early flowering stage than in the early dry season. Pennisetum clandestinum was more digestible at the vegetative young growth stage than at the high vegetative stage. Okorie, Hill and Mcllroy (1965) obtained higher digestibility coefficients with the dwarf sheep and N'dama cattle during the first year than in the second year of grazing tropical grass/legume pasture. French (1960) compared the digestive powers of Zebu and high grade European cattle (Ayrshire) using different feeds. The digestibility coefficients obtained for both animals in most cases were very close. He obtained no significant difference between the average digestibility coefficients in the Zebu and the Ayrshire crosses.

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CHAPTER 11

DIGESTIBILITY TRIALS

11.1 INTRODUCTION

The urgent need for a method or methods for assessing the digestibility of tropical pastures has been felt in recent times. Some work has been carried out on many of the local species of grasses and legumes of which little or nothing was known about their digestibilities. Some investigators assessed the nutritive value of pasture forages by the live weight gains of grazing animals (Stobbs, 1969a, b; c; d) or by their chemical composition (Long et al., 1969; Schneider et al., 1951; Raymond et al., 1960;) or by "in vitro" digestibility studies (Raymond et al., 1966; Barns, 1965; Stewart et al., 1958; Sutton, 1968).

Faeces voided by grazing or stall fed animals can be collected attaching collection bags to harnesses fitted on to the animals. Many animals do not like being harnessed because of discomfort. This method is very good, particularly with male animals, in that there is no contamination by urine, feed or soil. Digestibility of pasture herbage by grazing animals can also be carried out without harnesses, but by the administration of markers such as chromic oxide (Cr_2O_3). This substance has been used in

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digestibility trials all over the world for ruminants as well as non-ruminants like pigs and poultry. There are at times diurnal variations and uneven voiding of the marker due to feed and method of administration (Moore, 1958; Corbett *et al.*, 1958).

Digestibility trials using Cr_2O_3 as indicator involves the measurement of amount of Cr_2O_3 in the faeces voided, and the amount of the oxide administered every day during the preliminary and collection periods.

Collection of grass samples and faeces and their chemical analyses have been described in chaper 3.

11.2 DETERMINATION OF CHROMIC OXIDE IN FAECES

Coup and Lancaster (1952) used arsenious acid and Postassium permanganate along with other oxidising agents in their chromix oxide recovery determinations. Christian and Coup (1954) used Postassium bromate and phosphoric acid along with other oxidising agents in their chromic oxide recovery determinations. A modified form of the method of Christian and Coup (1954) was used to estimate the chromix oxide in the faeces (C.A.B. 1962)

11.3 ANALYTICAL PROCEDURE

The proximate analysis of the feeds and faeces were

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carried out according to the method of the A.O.A.C. (1964). Chromic oxide recovery was carried out using the modified method of Christian and Coup (1954). This method was modified a little in that ferrous ethylene di-ammonium sulphate was used instead of ferrous ammonium sulphate. Digestibility coefficient for each animal was calculated as:

$$D = 100 \times \frac{(\text{Wt. of D.M. in herbage eaten}) - (\text{Wt. of D.M. in faeces voided})}{(\text{Wt. of D.M. in herbage eaten})}$$

Where D. = Percentage digestibility of dry matter or organic matter in the herbage.

$$\text{Ib of faeces D.M. or O.M. voided by animal under grazing condition} = \frac{\text{gm } Cr_2O_3 \text{ fed per day}}{453.6 \times \text{gm } Cr_2O_3 \text{ voided per day}}$$

$$\text{D.M. or O.M. of herbage consumed in the field} = \frac{\text{wt of faeces voided} \times 100}{100 - \text{percentage digestibility}}$$

$$\%\ \text{Recovery of } Cr_2O_3 = \frac{\text{gm } Cr_2O_3 \text{ voided}}{\text{gm } Cr_2O_3 \text{ consumed.}} \times 100$$

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11.4 RESULTS

The digestibility coefficients of treatments H,J,K, and L at 4 weeks by Zebu cattle are shown in table 11.1. With treatment H, ether extract was the least digestible of the nutrients. At this stage the crude fibre was highly digestible, being only slightly less digestible than the crude protein. It was more digestible than the nitrogen free extracts. The first two rows (Appendix XI) showed the mean digestibility values for the samples taken during the first six days of the experiment, while the last two rows showed the mean values for the last six days of the experiment. The first two readings were higher in most cases than the last two readings and the difference was sharp in the case of dry matter digestibility. The result showed that the digestibility of a treatment can change from day to day during the experimental period, lasting for more than three days. The digestibilities in this case dropped after the sixth day.

Treatment J did not show as much variation in digestibility as treatment H. The ether extract was also the least digestible in this case. The crude fibre and the crude protein were digested to almost the same extent. The crude

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TABLE 11.1

Mean Digestibility coefficients of four treatments cut at 4 weeks by Zebu cattle

Treatment	D.M.	O.M.	C.F.	C.P.	E.E.	Total Ash	N.F.E.
H	61.1	60.4	67.9	69.3	21.2	57.8	55.2
J	55.0	58.1	57.0	56.7	21.3	40.5	56.8
K	43.6	47.5	52.1	42.3	10.5	26.7	42.2
L	51.6	55.2	57.6	58.6	9.0	23.2	54.0

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fibre had almost the same digestibility as nitrogen free extract. Ether extracts and total ash had the lowest digestibilities. All the nutrients in treatment J except N.F.E. were less digestible than those in treatment H. The tendency for the digestibility to fall as the experiment (Appendix XI) progressed was shown, although less clearly in this treatment.

Treatment K had very low dry matter digestibility, particularly during the last six days of the experiment, when there was a sharp drop. The crude fibre was more digestible than the crude protein and nitrogen free extract. The digestibility of the nutrients dropped after the first six days, while total ash and ether extract were the least digestible of the nutrients.

In treatment L the ether extract had the lowest digestibility. The crude fibre was more digestible than the nitrogen free extracts and organic matter. The digestibility of the nutrients fell after the first six days of the experiment.

Comparing the four treatments at 4 weeks, treatment H had the highest dry matter and organic matter digestibility coefficients, followed by treatment J, while treatment K had the lowest digestibility. Treatments H

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and L had the highest crude fibre digestibility coefficients followed by treatment J and lastly by treatment K. The crude protein digestibility was very high in treatment H followed by treatments L and J while K had a lower value of 42.3%. Treatment L was least digestible as to ether extract constituent. Treatment H had the highest total ash digestibility followed by treatments J, K and L in that order respectively.

Generally, treatment H showed the highest digestibility of each nutrient.

Table 11.2 showed the digestibility coefficients of the four treatments at twelve weeks of growth. With treatment H, the ether extract had the lowest digestibility coefficient, but the crude fibre was digested to a greater extent than nitrogen free extract. Ether extract and total ash had the lowest digestibilities, although the crude protein was digested to a fairly good extent in treatments H and L. The digestibility of each nutrient dropped after the first six days (Appendix XI).

Treatment J also had the ether extract having the lowest digestibility. The crude fibre was more digestible than the crude protein and nitrogen free extract.

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The digestibility of each nutrient decreased during the last six days of the experiment.

The ether extract had the lowest digestibility in treatment K. The crude fibre was more digestible than crude protein or nitrogen free extract, and as the case with treatments H and J, ether extract and total ash were the least digestible. The digestibility of each nutrient decreased during the last six days of the experiment.

The ether extract of treatment L was the least digestble just as was the case with the other treatments. The organic matter, crude fibre, crude protein and nitrogen free extract had very close digestibilities. The nutrients in most cases had lower digestibilities during the last six days of the experiment.

Considering the four treatments at twelve weeks, treatments H and L seemed to be the better of the treatments since some of their nutrients were digested to a greater extent than those in J and K with treatment K having the least digested nutrients.

Comparing the four treatments at both stages of growth it was clear that they all showed very low dry

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TABLE 11.2

Mean Digestibility coefficients of four treatments cut at 12 weeks by Zebu cattle

Treatment	D.M.	O.M.	C.F.	C.P.	E.E.	Total Ash	N.F.E.
H	55.3	55.2	62.8	62.9	33.5	37.5	51.1
J	51.0	53.5	59.7	47.9	13.4	40.3	50.7
K	46.1	49.9	53.8	39.3	34.5	38.0	43.9
L	57.9	58.2	58.7	61.2	27.3	52.5	58.8

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matter digestibilities. Treatment K showed the lowest digestibility at both stages of growth. The organic matter digestibility remained very close to D.M. digestility. The crude fibre digestibility increased at twelve weeks except for treatment H which had a decrease in digestibility. The crude protein and N.F.E. digestibilities also decreased at twelve weeks except for treatment L which showed an increase in its digestibility.

<u>Chromic Oxide Recovery</u>

Recovery of Cr_2O_3 from faeces organic matter for the digestibility trials is given in table 11.3.

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TABLE 11.3

Mean % recovery of Cr_2O_3 from faeces organic matter of Zebu cattle

Treatment	4 weeks	12 weeks
H	97.57 ± 7.29	99.31 ± 4.7
J	97.63 ± 4.02	98.83 ± 2.88
K	99.84 ± 4.34	99.31 + 0.02
L	95.69 ± 2.06	94.09 ± 0.72

For treatment H at 4 weeks, (Table 11.3,) the mean recovery was 97.57 ± 7.29, while the range for this treatment was from 92.20% to 101.18%. At 12 weeks the mean for this treatment was 99.31 ± 4.7, while the range was from 97.16% to 103.22%. The variations in these cases were not large. For treatment J the ranges at 4 and 12 weeks were also small. The range at 4 weeks was from 94.66% to 100.21%. The mean was 97.63 ± 4.02%, whereas at 12 weeks the range was from 96.92% to 100.27% and the mean was 98.83 ± 2.88%. Treatments K and L did

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not show much variation. The means for both were 99.84 ± 4.34%, 99.31 ± 0.02, 95.69 ± 2.06, and 94.09 ± 0.72%.

Corbett, GreenHalgh, Gwynn and Walker (1958) observed variation in Chromic Oxide recovery of the same grass cut at different times of the year. They also did not get any variation in chromic oxide recovery of more than 10%. Olubajo (1969; 1970) obtained good Cr_2O_3 recovery in his digestibility trials.

11.6 CORRELATION AND REGRESSION EQUATIONS

Correlation and regression equations for dry matter digestibility trials of stall-fed and grazing animals using faecal nitrogen as the index are shown in appendix XI.

The correlation between faecal nitrogen and dry matter digestibility in these trials was very low. The correlation for treatment H at 4 weeks was 0.4. When the regression equation was used to calculate the digestibility of the treatment by the grazing animals the mean value was very high. A correlation of 0.5 was obtained for treatment H at 12 weeks, and the regression equation when applied this time also gave very high mean digestibility. Treatment J at both stages of growth gave

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negative correlations between faecal nitrogen and dry matter digestibility. The regression equations used in both cases gave digestibility values less than those for the stall-fed animals.

Treatment K gave a very good correlation between faecal nitrogen and dry matter digestibility at 4 weeks. The regression equation gave very good digestibility value for the grazing animals. Whereas the 12 weeks cut gave a negative correlation and the regression equation produced lower digestibility value for the grazing cattle.

Treatment L gave a very low positive correlation at 4 weeks and the equation used produced digestibility value almost the same for the two groups of animals. There was also a positive correlation for the 12 week cut and the regression equation produced almost similar digestibility value for the grazing animals.

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Regression equations from percent digestibility of organic matter of stall fed animals during the experimental period.

Treatment	Weeks	Regression equation	Correlation Coefficient
H	4	$Y = 62 X - 8.21 \pm 6.93$	$r = 0.4$
	12	$Y + 102.9X-78.08 \pm 11.98$	$r = 0.5$
J	4	$Y = 54.94 - 0.01 X \pm 0.65$	$r = -0.05$
	12	$Y = 94.47 - 29 X \pm 2.53$	$r = -2.23$
K	4	$Y = 109 X - 105.92 \pm 7.66$	$r = 0.71$
	12	$Y = 48.16 - 1.5 X \pm 0.18$	$r = 0.47$
L	4	$Y = 47.61 + 2.77 X \pm 0.74$	$r = 0.11$
	12	$Y = 40.39 + 12 X \pm 0.45$	$r = 0.55$

X = % nitrogen in faeces organic matter from gazed herbage.

Faecal nitrogen has been used successfully by various workers, Topps (1962), GreenHalgh and Corbett (1960a; 1960b) as an index to predict the dry matter digestibility of treatments. Others have met with difficulties in obtaining good correlation for most of the feeds.

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The correlations obtained for the treatments in these trials showed that faecal nitrogen can be used to predict digestibility, but that it does not give good correlation all the time. It can be used along with other indi ces like lignin, methoxyl groups, to predict the digestibility of treatments by grazing animals (Sullivan, 1959; Richards and Reid, 1952).

11.7 DISCUSSION

In these experiments faecal nitrogen or crude protein was more for the grazing animals than for the stall-fed animals. Topps (1962) obtained significantly more digestible crude protein in herbage grazed by cattle than that collected by hand. GreenHalgh _et al._ (1960b) observed that single sward regressions were considerably more precise than those which combine results from many treatment types. Grazing animals may have lower O.M. digestibility than the stall-fed animals if they consume more of the treatment, since high forage intake depresses digestibility. This may be the cause of the lower organic matter digestibility shown by some grazing animals in some of the treatments. Other causes may be the health of the animals or intestinal infestations.

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These observations were also expressed by Minson (1958), and Raymond, Minson and Harris (1956).

Differences in the equation for the faecal nitrogen used for predicting digestibility of the treatments, and the different digestibilities obtained can be attributed to analytical methods used or to differences in animals used, or to the type of herbage. In these experiments the two groups were used for all the treatments, so that the differences in herbage were likely to be the major cause of different digestibilities obtained.

French (1940) used Zebu cattle as well as Zebu x Ayrshire crosses in his digestibility trials. The green grass he fed the animals gave very low digestibility values for each of the nutrients as compared with the values of the present work. Dry matter digestibility was the lowest except for N.F.E. for the Zebu cattle. Ether extract was the least digestible nutrient. Ether extract and total ash were the least digestible nutrients in the present trial. The increased digestibility of each nutrient in the present work may be due to the presence of the legume centrosema.

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Okorie, Hill and Mcllroy (1965) grazed grass legume mixture with N'dama cattle, and they obtained fairly high digestibility coefficient for each nutrient. Crude protein was the most digestible while ether extract was the least digestible nutrient. The second year digestibility coefficients were lower than the first year values. The fall in digestibility coefficients was noticed in each of the trials during the experimental period of the present trial.

The crude fibre of the treatments were digested to greater extent than some of the nutrients in the feed, particularly the nitrogen free extract and crude protein in some cases. Nitrogen free extract is supposed to be highly digestible but its digestibility has not been as high as that of crude fibre, in some cases since the crude fibre was digested very well at four weeks and also at the advanced stage of twelve weeks, it seems that the Zebu cattle possess a good ability to digest crude fibre. These four mixtures have proved that there are some selected tropical pasture treatments that can be very digestible.

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Analyses of variance for the nutrients in the treatments H,J, K and L cut at 4 weeks (Appendix XI) showed that there were significant differences between the treatments ($P > 0.05$) for the dry matter, organic matter and ash contents. The difference in crude fibre contents were very low. There were significant differences between days of cutting the samples for organic matter and ash contents. There were no significant differences for N.F.E., crude protein and ether extracts.

The analysis of variance for the dry matter content of fresh pastures showed significant differences among the treatments.

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APPENDIX V

Microbiology of the rumen liquor

It is possible to count all the bacteria present in the liquor, or to count only the viable bacteria, or the numbers of the individual species present. In this experiment all the bacteria present were counted.

Collection of Specimen for the Total Bacterial Count

The liquor was collected through a permanent fistula, using rubber tubing with special valves and connected to a thermos flask. Slight pressure was exerted using a metal syringe to draw out the liquor. The liquor was taken to the laboratory and 200 ml to 300 ml of it mixed in a blendor or a shaker with small quantity of surface active agent such as Tween 80. This prevent clumping and so encourages good separation of the organisms from the food particles. CO_2 was bubbled through the liquor and then filtered through muslin.

1 - 2 ml of the liquor was diluted with Cysteine hydrochloride and sodium carbonate and then strained with Resazyrin and Fuchsin, safranin or Gentian violet. The sample was pured into a tube and autoclaved. The sample

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was cooled at 45^0 - 50°C and then CO_2 bubbled through to replace any oxygen present and to adjust the pH.

Counting the Bacteria

The diluted specimen was shaken and a portion of it dropped on the Neubauer Counting Chamber, preventing overflow of the fluid into the Channels. The cover slip was placed on carefully avoiding air spaces. The chamber was left on the bench for 2-3 minutes to settle properly. The chamber was mounted and focussed on the microscope. All the bacteria on the four corner squares and the centre of the central ruled area were counted. All the bacteria on the treble lines were also counted. For quick calculation it is enough to multiply the number of cells counted by 5×10^1.

Total Count of Protozoa

A portion of the fluid in the flask was diluted with Cysteine hydrochloride, sodium carbonate and then stained with Resazyrin. The solution was poured into a tube and autoclaved. It was then cooled to 45°C - 50°C. CO_2 was bubbled through it to replace the oxygen present.

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The specimen was diluted for easy counting and 1-2ml of the diluted specimen was taken and 1-2 ml of Grams iodine were added to kill or immobilize the protozoa. The iodine solution will also stain the protozoa for easy recognition. It was mixed thoroughly and left for five minutes. It was shaken again and only 0.01ml of it was mounted for counting. Counting was started from the upper left corner of the cover slip. The counting was done horizontally until the right corner was reached. Counting continued until the bottom line of the cover slip was reached. The number of protozoa counted was multiplied by the dilution factors.

The sizes of the protozoa varied from 20 - 200 μ and they had highly and specialized internal structures which were readily shown by the iodine stain.

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APPENDIX VI

Analysis of variance for Dry Matter (D.M.) content of treatments H, J, K and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	23	86.76			
Replication	5	3.23	0.646	0.7 N.S.	2.90
Variety	3	69.86	23.29	25.51**	3.29
Error	15	13.70	0.913		

Analysis of variance for Organic Matter (O.M.) content of treatments H, J, K and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	23	94.15			
Block	5	15.19	3.04	1.88 N.S.	2.90
Varieties	3	54.73	18.24	11.26**	3.29
Error	15	24.23	1.62		

** = Significant at 1% level

N.S. = Not significant

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Analysis of variance for organic matter contents of treatments H, J, K and L cut at 4 and 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	31.34			
Block	3	4.65	1.55	22.1**	9.28
Varieties	1	26.49	26.49	378.4**	10.13
Error	3	0.20	0.07		

** = Significant at 5% level

N.S. = Not significant

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Analysis of variance for crude fibre contents of treatments H, J, K and L cut at 4 and 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	14.62			
Block	3	1.10	0.37	0.37 N.S.	9.28
Variety	1	10.48	10.48	10.48 N.S.	10.13
Error	3	3.04	1.01		

Analysis of variance for crude protein levels of treatments H, J, K and L cut at 4 and 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	9.25			
Block	3	0.62	0.21	1 N.S.	9.28
Variety	1	8.02	8.02	40.1**	10.13
Error	3	0.61	0.20		

** = Significant at 5% level

N.S. = Not significant

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Analysis of variance for crude fibre (C.F.) content of treatments H, J, K and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	23	83.35			
Block	5	13.32	2.66	1.06 N.S.	2.9
Variety	3	32.22	10.74	4.26*	3.29
Error	15	37.81	2.52		

Analysis of variance for crude protein (C.P.) content of treatments H, J, K and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	23	81.35			
Block	5	3.20	0.64	0.18 N.S.	2.9
Variety	3	23.42	7.81	2.14 N.S.	3.29
Error	15	54.73	3.65		

* = Significant at 5% level

N.S. = Not significant

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Analysis of variance for Nitrogen free extracts (N.F.E.) content of treatments H, J, K, and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	23	148.08			
Block	5	53.20	10.64	1.7 N.S.	2.9
Variety	3	1.84	0.613	0.09 N.S.	3.29
Error	15	93.04	6.20		

N.S. Not significant

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APPENDIX VI

Chemical Composition of Pasture treatments cut at 4 and 12 weeks of growth. (Dry matter %)

4 Weeks								12 Weeks							
Treatment	D. M.	O. M.	Ash	C. F.	C. P.	Ether Extract	N. F. E.	Treatment	D. M.	O. M.	Ash	C. F.	C. P.	Ether Extract	N. F. E.
H	92.05	84.30	7.05	32.94	10.22	0.87	48.92	H	92.25	83.20	8.01	31.36	10.68	1.02	48.93
	88.84	80.96	7.88	37.81	11.15	1.15	42.01		88.35	81.67	6.68	35.43	10.90	1.13	45.86
	91.51	84.80	6.71	35.57	10.52	1.13	46.07		90.45	84.10	6.72	36.02	9.92	1.08	46.26
	89.90	82.73	7.18	37.01	8.13	0.93	46.75		89.32	81.90	7.43	35.37	10.29	1.13	45.78
	91.71	84.00	7.71	33.56	7.39	1.10	50.24		88.88	80.17	8.17	33.87	7.75	1.11	48.56
Mean	90.93	83.31	7.50	35.33	9.54	1.05	46.58	Mean	89.76	82.25	7.40	34.52	9.81	1.10	47.17
J	94.28	83.21	10.09	31.90	9.51	1.05	47.45	J	93.15	82.59	11.31	33.93	9.89	1.12	43.75
	94.25	84.53	9.72	29.63	9.28	1.08	50.29		92.89	80.77	12.12	30.95	9.66	1.01	46.26
	93.04	82.28	10.03	33.34	9.17	1.11	46.35		91.76	81.09	11.29	34.58	7.39	1.08	45.63
	92.65	83.42	9.22	32.90	9.68	1.08	47.12		93.88	80.35	13.53	32.19	7.46	1.11	45.71
	93.12	81.75	11.37	33.43	8.46	0.99	45.75		90.53	81.34	9.19	33.82	8.95	1.16	46.88
	92.85	82.26	11.05	33.72	7.78	1.07	46.38		93.21	81.27	11.20	37.16	8.22	1.14	42.28
Mean	93.37	82.91	10.25	32.49	8.98	1.06	47.22	Mean	92.57	81.25	11.44	33.77	8.60	1.10	45.09

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Treatment	D. M.	O. M.	Ash	C. F.	C. P.	Ether Extracts	N. F. E.
K	93.21	84.10	9.11	34.38	5.28	1.12	50.11
	93.08	84.24	10.03	34.84	8.23	1.04	45.86
	93.58	83.26	10.28	32.74	9.82	1.06	46.10
	92.97	84.36	9.80	33.90	8.48	1.10	46.72
	93.89	83.13	10.76	32.63	8.85	1.13	46.63
	92.95	84.00	9.77	33.58	8.82	1.11	46.72
Mean	93.28	83.85	9.96	33.68	8.25	1.09	47.02
L	88.94	80.39	8.55	32.17	12.31	1.08	54.44
	90.09	81.84	8.20	33.41	9.34	0.88	48.17
	90.49	81.84	8.65	35.53	7.02	0.78	48.02
	89.39	80.72	10.68	32.10	11.25	0.92	45.05
	89.43	78.95	10.68	32.10	11.25	1.16	44.81
	87.44	76.09	11.35	29.84	14.49	1.06	43.26
Mean	89.30	79.97	9.69	32.53	10.94	0.98	45.86

Treatment	D. M.	O. M.	Ash	C. F.	C. P.	Ether Extract	N. F. E
K	95.06	82.95	12.11	31.29	5.42	1.05	50.13
	95.01	83.06	12.00	31.70	7.37	1.02	47.91
	94.86	83.62	12.88	32.50	8.64	1.09	44.89
	94.64	83.20	11.83	32.38	7.86	1.10	46.83
	93.84	81.81	11.99	32.20	7.46	1.03	47.32
	94.78	82.99	12.04	30.50	6.47	1.10	49.89
Mean	94.70	82.94	12.14	31.76	7.20	1.07	47.83
L	89.32	78.95	10.37	29.49	12.25	1.21	46.68
	88.89	77.59	11.30	30.80	9.86	1.06	46.98
	89.28	78.36	10.92	32.21	7.85	0.96	48.06
	88.60	76.61	11.99	30.84	9.63	0.97	46.57
	88.66	76.77	11.89	32.05	10.37	1.13	44.56
	89.58	76.69	12.89	30.66	9.27	1.12	46.06
Mean	89.06	77.50	11.56	31.01	9.87	1.08	46.48

* Each figure is the mean of duplicate samples.

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Analysis of variance for Dry Matter (D.M.) content of fresh pastures H, J, K, and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	23	1881.56			
Block	5	306.79	61.16	0.97 N.S.	2.9
Variety	3	632.90	210.97	3.36 N.S.	3.29
Error	15	941.87	62.79		

N.S. = Not significant

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APPENDIX VII

Rumen liquor Sampling

Rumen liquor was obtained by suction through a rubber tubing fitted with special valves. The pressure was applied by using a metal syringe fitted to one end of the rubber tubing. The perforated end of the rubber tubing was dipped into different parts of the rumen, and the rumen liquor brought up went down straight into a thermos flask fitted into another end of the rubber tubing. This devise was used to prevent loss of temperature. The liquor was carried in the stoppered falsk to the laboratory immediately for the various analyses.

Removal of protein and carbohydrate

The rumen liquor was strained through two layers of thin cloth to remove most of the food residues and bacteria and other materials. 5 ml of the strained liquor in duplicate was poured into a beaker or centrifuge tube, and an equal volume of normal sulphuric acid (N H_2SO_4) saturated with magnesium sulphate ($MgSO_4$) was added to precipitate the carbohydrates and proteins present in the fluid. The mixture was shaken gently and

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allowed to stand for ten minutes (10 mins). It was then centrifuged for ten minutes (10 mins) at 2,500 r.p.m. The supernatant was decanted and centrifuged again at 3,500 r.p.m. for ten minutes (10 mins.) to further remove the proteins and carbohydrates in the liquor. The very cloudy supernatants were filtered through Whatman No.4, or 41, or 541 filter paper before centrifuging at 3,500 r.p.m. 3 µ.l. of the supernatant were injected into the column and the samples not needed immediately were kept in the refrigerator (below $0^{o}C$) until needed.

When the samples gave poor separations and poor base line, 25% ortho-phosphoric acid in 5N sulphuric acid was used. 1 ml per every 5ml of rumen liquor was added and left for 30 minutes before centrifuging only once at 1,500 r.p.m. for ten minutes.

Barium hydroxide $Ba(OH)_2$, zinc sulphate ($ZnSO_4$), sodium sulphate (Na_2SO_4) may also be used to saturate the normal sulphuric acid and used to precipitate proteins and carbohydrates in rumen liquor.

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Centrifuge

Any good centrifuge calibrated above 4,000 r.p.m. will serve the purpose. For this work a dual purpose MSE centrifuge calibrated from 1,000 to 6,500 r.p.m. was used. There was an automatic timer to the centrifuge. The high centrifugal force applied was used to separate the colloidal particles in the liquor. Not all the colloidal particles will come down in the first centrifugation, but the remaining precipitate will come down during the second centrifugation.

Chromatograph

One of the Pye Series 104 Chromatographs was used. Model 24 which is a dual flame ionisation detector programmed chromatograph was used. This model provides routine and research analytical facilities over a wide range of sensitivity. The analyser oven enables the coiled column to be held at the controlled operating temperature of 125°C.

Calibration graphs for Argon, hydrogen and compressed air were prepared according to the instructions in the manual. The hydrogen flow rate was 31.6ml/min, Argon was

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61.5ml/min. and compressed air was 923.1ml/min. The column was purged at the end of each days run by passing through the column the carrier gas (Argon) overnight at 125°C.

Preparation of standard V.F.A. solutions

Acetic Acid:- 99.6% acetic was used. The weight per ml is 1.048.

Molecular weight is 60.05 and equivalent weight is 60.05.

IN solution of 99.6% contains $\frac{60.05}{1.048}$ = 57.3ml/litre

IN solution of 100% Acetic acid contains $\frac{100}{99.6}$ x 37.3ml/litre

This comes to 57. ml/litre.

57,3ml/litre is equivalent to 60.05 gm

IM/litre = 60.05 ml/litre of 99.6% Acetic acid

IM/50 ml = $\frac{60.05}{1000}$ x 50 = 3.0025 ml

2M/50 ml of 100% acetic acid = $\frac{57.5}{1000}$ x 50 x 2 = 5.75ml

Propionic acid: 99% propionic acid was used. The weight per ml is 0,0003. Molecular weight is 74.08 and the equivalent weight is 74,08.

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1 Normal Solution = $\frac{74.6}{0.993}$ ml/litre

100% Propionic acid = $\frac{100}{99}$ x 74.6 = 75.4 ml/litre

IM/50ml = 3.77ml

2M/50ml = 7.54ml

Iso-Butyric Acid:- 99% iso butyric acid was used

Weight/ml = 0.947. Molecular weight = 88.11 and equivalent weight = 88.11.

IN solution = $\frac{88.11}{0.947}$ = 93.04 ml/litre

100% iso butyric acid = $\frac{100}{99}$ x 93.04 = 93.98 ml/litre

1Mol/50 ml = $\frac{93.98}{1000}$ x 50 = 4.70 ml

2M/50 ml = 9.40 ml

N-Butyric Acid:- 99.7% n-butyric acid was used.

Weight/ml = 0.957. Molecular weight = 88.11 and equivalent weight = 88.11.

IN solution = $\frac{88.11}{0.957}$ = 0.92 ml/litre

100% n-butyric acid = $\frac{100}{99.7}$ x 92.07 = 93.0 ml/litre

1Mol/50 ml = $\frac{93.0}{1000}$ x 50 = 4.65 ml

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Fatty acid mixture:- Acetic, propionic and n-butyric acids were mixed in molar proportions of 2: 1: 1 respectively. The volume was made up to 100 ml with distilled water. A known volume, 0.3 μ.l., of this standard fatty acid mixture was injected into the coiled column using PEG 20M as the packing material. The working termperature was 125°C and Argon was the carrier gas. Nine runs of this standard solution were made and the estimated standard error, and recovery were calculated. Acetic acid had a recovery rate of 98.77%, propionic acid had a recovery of 98.13%, 97.99% for n-Butyric acid and 106.91% for n-Valeric acid. The iso acids were only present in trace amounts and were disregarded in this present work. The total V.F.A. in the samples were determined by steam distilation.

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APPENDIX VII

Analysis of variance for pH levels of treatments H, J. K and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	0.68			
Block	1	0	0	0 N.S.	6.61
Variety	1	0.65	0.65	108***	6.61
Error	5	0.03	0.006		

Analysis of variance for total V.F.A. production of H, J, K and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	79.90			
Block	1	0.37	0.37	7.40*	6.61
Variety	1	79.42	72.42	158.8***	6.61
Error	5	0.11	0.05		

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
N.S. = Not Significant

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Analysis of variance for Acetic acid production of H, J, K and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	16.47			
Block	1	0.03	0.03	0.01 N.s.	6.61
Variety	1	5.25	5.25	2.34 N.s.	6.61
	5	11.19	2.24		

Analysis of variance for Propionic acid production of treatments H, J, K and L cut at weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	10.90			
Block	1	2.52	2.52	3.04 N.S.	6.61
Variety	1	4.21	4.21	5.07 N.S	6.61
Error	5	4.17	0.83		

N.S. = Not significant

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Analysis of variance for Butyric acid production of treatments H, J, K and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.5}$
Total	7	12.33			
Block	1	3.12	3.12	3.15 N.S.	6.61
Variety	1	4.32	4.32	4.36 N.S.	6.61
Error	5	4.89	0.99		

Analysis of variance for pH levels of Treatments H,J, K and L cut at 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	1.56			
Block	1	0.19	0.19	3.0 N.S.	6.61
Variety	1	1.06	1.06	17.6*	6.61
Error	5	0.31	0.06		

* = Significant at 5% level

N.S.= Not significant

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Analysis of variance for total V.F.A. production of treatments H, J, K and L cut at 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	55.08			
Block	1	0.90	0.90	7.5*	6.61
Variety	1	53.46	53.46	445.5***	6.61
Error	5	0.72	0.12		

Analysis of variance for Acetic acid production of H, J, K and L cut at 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	35.62			
Block	1	3.02	3.02	0.6 N.S.	6.61
Variety	1	9.55	9.55	2.07 N.S.	6.61
Error	5	23.05	4.61		

* = Significant at 5% level
** = " " 1% "
*** = " " 0.1% "
N.S. = Not significant

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Analysis of variance for Propionic acid production of treatments H, J, K and L cut at 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	20.22			
Block	1	1.75	1.75	3.65 N.S.	6.61
Variety	1	15.94	15.94	33.2**	6.61
Error	5	2.42	0.48		

Analysis of variance for Butyric acid production of treatments H, J, K and L cut at 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	42.01			
Block	1	0.17	0.17	0.06 N.S.	6.61
Variety	1	28.21	28.21	10.37*	6.61
Error	5	13.63	2.72		

* = Significant at 5% level

** = " " 1% "

N.S. = Not significant

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Analysis of variance for total V.F.A. production from treatments J and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	9	74.14			
Block	4	6.18	1.54	2.41N.S.	6.39
Variety	1	65.38	65.38	102.16*	7.71
Error	4	2.58	0.64		

Analysis of variance for total V.F.A. production

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	9	111.24			
Block	4	5.87	1.47	7.74 N.S.	6.39
Variety	1	104.59	104.59	5.50 N.S.	7.71
Error	4	0.78	0.19		

* = Significant at 5% level

N.S. = Not significant

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Analysis of variance for Acetic acid production from treatments J and L cut at 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	9	11.15			
Block	4	8.06	2.01	2.76 N.S.	6.39
Treatment	1	0.19	0.19	0.23 N.S.	7.71
Error	4	2.90	0.72		

Analysis of variance for Butyric acid production from treatment J and L cut at 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	9	28.73			
Block	4	11.40	2.85	0.9 N.S.	6.39
Treatment	1	5.68	5.68	1.95 N.S.	7.71
Error	4	11.65	2.91		

N.S. Not significant

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Analysis of variance for propionic acid production from Treatments J and L cut at 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	9	22.59			
Block	4	5.42	1.36	0.7 N.S.	6.39
Variety	1	9.57	9.57	5.04 N.S.	7.71
Error	4	7.60	1.90		

Analysis of variance for Acetic acid production from treatments J and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	9	81.51			
Block	4	34.08	8.52	0.7 N.S.	6.39
Treatment	1	4.54	4.54	0.3 N.S.	7.71
Error	4	42.89	10.72		

N.S. = Not significant

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Analysis of variance for butyric acid production from treatments J and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	9	30.63			
Block	4	8.56	2.14	0.0 N.S.	6.39
Variety	1	12.97	12.97	5.76 N.S.	7.71
Error	4	9.10	2.25		

Analysis of variance for Propionic acid production from treatments J and L cut at 4 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	9	27.66			
Block	4	10.76	2.7	2.3 N.S.	6.39
Variety	1	2.14	1.14	1.8 N.S.	7.71
Error	4	4.76	1.19		

N.S. = Not significant

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APPENDIX VII

Analysis of variance for the pH leve of rumen liquor produced by animals fed treatments H, J, K and L cut at 4 and 8 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	2.19			
Block	1	1.83	1.83	18.30*	10.13
Variety	3	0.07	0.02	0.20 N.S.	9.12
Error	3	0.29	0.10		

Analysis of variance for the pH level of rumen liquor produced by animals fed treatments J, K, and L cut at 4, 8, and 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	8	2.21			
Block	2	1.76	0.88	9.78*	6.94
Variety	2	0.07	0.03	0.03 N.S.	6.96
Error	4	0.38	0.09		

* = Significant at 5% level

N.S. = Not significant

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Analysis of variance for the total V.F.A. of rumen liquor produced by animals fed treatments J, K, and L cut at 4, 8 and 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	8	26.86			
Block	2	11.75	5.88	1.68 N.S.	6.94
Variety	2	1.21	0.60	0.02 N.S.	6.94
Error	4	13.90	3.50		

Analysis of variance for the total V.F.A. of rumen liquor produced by animals fed treatments H, J, K and L at 4 and 8 weeks.

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	29.22			
Block	1	6.55	6.55	1.37 N.S.	10.13
Variety	3	8.38	2.79	0.58 N.S.	9.12
Error	3	14.29	4.76		

N.S. = Not significant

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Analysis of variance for the Acetic acid content of rumen liquor produced by animals fed J, K and L cut at 4, 8 and 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	8	17.97			
Block	2	2.87	1.44	4.00 N.S.	6.94
Variety	2	13.64	6.82	18.44**	6.94
Error	4	1.46	0.36		

Analysis of variance for Acetic acid content of rumen liquor produced by animals fed H, J, K and L cut at 4 and 8 weeks

Source	D.F.	S.S.	M.S.	F.	Fo.05
Total	7	135.96			
Block	1	34.49	34.49	2.62.N.S.	10.13
Variety	3	48.86	19.29	1.47.N.S.	9.12
Error	4	52.61	13.15		

** = Significant at 1% level

N.S. = Not significant

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Analysis of variance for the Propionic acid of rumen liquor produced from treatments H, J, K and L at 4 and 8 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	45.96			
Block	1	34.86	34.86	16.14*	10.13
Variety	3	4.63	1.54	0.71 N.S.	9.12
Error	3	6.47	2.16		

Analysis of variance for Propionic acid of rumen liquor produced from treatments J, K and L cut at 4, 8 and 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	8	46.13			
Block	2	32.27	16.14	4.91 N.S.	6.94
Variety	2	0.68	0.34	0.01 N.S.	6.94
Error	4	13.18	3.29		

* = Significant at 5% level

N.S. = Not significant

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Analysis of variance for Butyric acid Produced by animals fed treatments H, J, K and L cut at 4 and 8 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	7	68.99			
Block	1	0.00	0.00	0 N.S	10.13
Variety	3	34.44	11.48	0.99 N.S.	9.12
Error	3	34.55	11.52		

Analysis of variance for Butyric acid produced by animals fed treatments J, K and L cut at 4, 8 and 12 weeks

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
Total	8	47.18			
Block	2	3.54	1.51	0.02 N.S.	6.94
Variety	2	19.79	9.89	1.24 N.S.	6.94
Error	4	23.85	7.95		

N.S. = Not significant.

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APPENDIX VIII

Milk Fat:

The milk fat was determined according to the Gerber method which was chosen because it is a quick and accurate method.

Reagents used:

H_2SO_4 (for milk testing) S.P. 1.815g. per ml. at 20^oC. This acid must be colourless. Amyl Alcohol.

Apparatus:

Standard butyrometer

Standard rubber stopper

Standard pipette to deliver 10.94 ml of milk

Standard pipette to deliver 10 ml H_2SO_4

Shaking stand for the butyrometers

Centrifuge specially made for milk testing

This centrifuge should go up to 1,100 r.p.m. and the diameter is between 18" - 20".

The centrifuge has a timer and heat regulating devise which keeps the temperature at 70^oC.

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Water bath for the butyrometers The water bath has a standard stand to hold the butyrometers. The temperature can go as far as 100°C. The water bath has an automatic thermometer built in it. This gives the temperature as the water is heated.

10 ml H_2SO_4 specially made for milk testing was poured into the Gerber butyrometer from an automatic Gerber dispenser. 10.94 ml of warm (30°C) well mixed milk was pipetted from a standard pipette into the butyrometer. 1 ml amyl alcohol was then added. The butyrometer neck was closed with a special stopper and the samples were shaken in a protected stand until no white particles were seen. The butyrometers containing the samples were centrifuged at 1,100 r.p.m. at 70°C for four minutes. They were removed and placed in the water bath maintained at 65°C for three minutes. The fat content was read from the graduated neck of each butyrometer. The fat content is a percentage by weight.

A special key was used for the Cerber butyrometer. This was used to push the sample up the tube whenever the solution fell below the graduated neck.

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Milk Analysis:

There are three methods for milk fat analysis. These are the

a. Rose-Gottlieb method which is the determination of milk fat by weight.

b. The Gerber method which is good for routine laboratory analysis. It is also a quick method. It is a rapid commercial method for milk fat determination.

c. The Babcock method is another rapid commercial method. It is a specific method.

Milk Lactose:

This can be determined:

a. By difference. 100 - (% water, + % protein, + % fat + % Ash).

b. By using a lactometer. Lactose will rotate the plane of polarized light, so it can be determined using a polarimeter.

c. By the Chloramine - T method of Hinton and Macara.

d. By the reduction of Fehling's solution.

e. Colorimetrically.

Water content:

This was determined by drying a known weight of homogenised milk at 65°C for 24 hours. The difference in weight is the water content.

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Dry Matter:

The weight of the residue left in the dish after drying is the dry matter of the milk.

Milk Ash:

The dish containing the residue after drying is ashed in a muffled furnace at 500°C for 2 hours. The difference between the weights before and after ashing is the weight of the ash.

Milk Protein:

The total milk protein content is determined by the Kjeldahl method. The nitrogen content is multiplied by 6.38 to give the protein content in milk.

Lactose:

The lactose content of milk was determined colorimetrically. Phenol was used as the colouring agent. This method needs only a small volume and it is quick. The method of Barnett and Tawab (1957) was adopted.

Procedure:

The milk was warmed and well mixed. 0.1 ml of this well mixed sample was pipetted using a micro pipette, into a 5 ml volumetric flask. This was made up to the mark

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with distilled water. 0.5 ml of this solution was pipetted into another 5 ml volumetric flask, and made up to the mark with distilled water. 0.25 ml of the last solution was taken and poured into a 20 ml volumetric flask. 0.5ml of 80% phenol (A.R.) plus 6 ml concentrated H_2SO_4 (A.R.) were added respectively. The flask was left for ten minutes to allow the reaction to end and the solution to develop the colour fully. The flask was cooled to room temperature and the optical density read at 490 μM from an SP 500. Duplicate samples of each milk sample were determined.

Blank solution was prepared using distilled water instead of milk sample.

Standard curve was prepared by plotting the optical density against the different standard lactose solutions (concentrations) used.

Calculation

$$\text{Reading (mg) from standard curve} \times \frac{100 \times 200}{1000 \times 0.25} = \text{gm lactose \%}$$

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Preparation of Reagents:

1 gm lactose was weighed, dissolved in distilled water and poured into a 100 ml volumetric flask. More distilled water was added and made to the mark. The solution was kept in the refrigerator until needed.

80% Phenol:

80 gm phenol crystals were weighed into a 100 ml volumetric flask. Warm distilled water was added to reach the mark. The phenol solution was stored in the refrigerator until needed.

Sulphric Acid:

Analar grade of sulphric acid was used. Ordinary grades are good too, but their use will involve the preparation of new standard curves each time the stock solution finishes.

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APPENDIX IX

Urea and Ammonia determinations

Reagents:

Aqueous sodium hypochlorite

Ammonium sulphate, Urea, and Mercuric iodide

O.IN sodium thiosulphate

Sulphuric Acid

Starch solution

Potassium iodate solution, Potassium iodide solution

Urease tablets to prepare 1gm/litre or 1 mg/ml.

Ethylene diaminetetra acetic acid (EDTA) 1% solution

Sodium nitropruside

Phenol, and Chloroform

Sodium hydroxide

Glacial Acetic Acid.

Preparation of solutions

Aqueous sodium hypochlorite. 1.74N

32gm NaOH(A.R.) were dissolved in 15ml distilled water. It was cooled to room termperature and 150gm crushed ice were added. The container was immersed in ice blocks

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plus crushed ice and a rapid stream of chlorine gas, generated from a Kipp's apparatus containing $KMnO_4$ into which concentrated hydrochloric acid was poured, was passed into it. This was continued until the total weight increased by 21 - 24 gms. Under this condition about 4gm of the alkali remained unchanged and the final solution was 1.74N. or approximately 2N. The excess alkali present will suppress hydrolysis which would have given rise to hypochlorous acid.

0.1N Sodium thiosulphate solution:- $Na_2S_2O_3$:-

Weigh 6.25gm sodium thiosulphate H_2O (A.R.). Dissolve in deionised or carbon dioxide free water. Transfer to 250 ml standard flask. Add 0.025gm sodium carbonate or 0.0025 gm mercuric iodide or 1 drop of chloroform and make up to the mark with carbon dioxide free water.

Standadization of Sodium thiosulphate solution with potassium iodate

0.1N $K10_3$ Solution:- Dry $K10_3$ at 100 - 120°C and weigh 0.8918 gm dry $K10_3$. Dissolve in carbon dioxide free water and transfer to 250 ml volumetric flask. Make up to the mark. Pipette out 25ml 0.1N $K10_3$ solution into a 25ml conical flask, and 10 ml 10% iodate free KI solution and

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3ml 2N H_2SO_4 solution. Titrate the liberated iodine with thiosulphate solution with constant stirring. When the colour of the liquid is pale yellow, dilute to 200 ml with distilled water, add 2 ml of starch solution, and continue to titrate until the colour changes from blue to colourless. Repeat three times and find the mean.

1mI N $Na_2S_2O_3$ = 0.03567g $K1O_3$

∴1mI 0.1N $Na_2S_2O_3$ = 0.003567g $K1O_3$

Starch solution:

Weith 1gm starch and make to a paste with a little water. Pour with constant stirring into 70ml boiling water and boil for 1 minute. Transfer to 100ml flask and cool. Add 0.005gm mercuric iodide or 2-3gm KI.

Standadization of sodium Hypochlorite solution

Pipette out 5ml of the sodium hypochlorite solution into 100 ml flask, add some distilled water, shake well and make up to the mark. Pipette 25ml of this solution into a 250 ml conical flask, add 25ml distilled water, followed by 20ml 10% KI solution and 10ml glacial acetic acid. Titrate the liberated iodine with standard 0.1N sodium thiosulphate solution. Add 2ml starch solution

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when the colour is pale yellow, continue to titrate until the blue colour disappears. Repeat two times and find the mean.

$1ml\ N\ Na_2S_2O_3 = 0.03546\ gm\ I_2$

$\therefore 1ml\ 0.1N\ Na_2S_2O_3 = 0.003546\ gm\ Cl_2$

Buffered urease solution:-

Dissolve 150mg (1 tablet) urease in 100ml of a 10% solution of ethylene diaminetetra acetic acid (EDTA). Adjust the pH to 6.5. This will keep for one month if kept in the refrigerator.

Phenol-sodium nitropruside solution:

Weigh 50gm phenol and 0.25gm sodium nitropruside into a beaker. Dissolve in distilled water and pour into a litre volumetric flask. Make to the mark. Pour into a brown bottle to avoid direct light and keep in the dark or in the frigidaire. Take 1 ml of this and dilute to 5ml for use.

Sodium hydroxide-sodium phypochlorite solution:

Dissolve 25gm sodium hydroxide plus 2.1gm sodium hypochlorite in water. Pour into 1 litre volumetric flask and make to the mark. Pour into a brown bottle to protect

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it from direct light. Take 1 ml and dilute to 5 ml for use. Keep the solution in the refrigerator.

Urea standard solution:

Weigh 1 gm urea and dissolve in 100ml distilled water. This 1gm/litre solution gives 1mg/ml.

Standard Ammonium sulphate solution:

Weigh 4.7gm ammonium sulphate (A.R.) and dissolve in distilled water. Pour into 1 litre volumetric flask and make up to the mark with distilled water. This gives 1 gm ammonium nitrogen per litre or 1mg/mI.

Determination of Ammonia in rumen liquor

Procedure:

The rumen liquor was strained through two layers of cloth. 1 ml of this was poured into a 10ml volumetric flask or graduated test tube, and made to the mark with distilled water. 0.1ml of this solution was taken up in a pipette and poured into 50ml standard flask in duplicate. 5ml of dilute phenol-sodium nitropruside solution and 5ml dilute sodium hydroxide-sodium hypochlorite solution were added respectively, and the volume was made up to 50ml. The blank was prepared using distilled water instead of

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the rumen in water. Pour into 1 litre volumetric flask and make to the mark. Pour into a brown bottle to protect it from direct light. Take 1 ml and dilute to 5 ml for use. Keep the solution in the refrigerator.

Urea standard solution:

Weigh 1 gm urea and dissolve in 100ml distilled water. This 1gm/litre solution gives 1mg/ml.

Standard Ammonium sulphate solution:

Weigh 4.7gm ammonium sulphate (A.R.) and dissolve in distilled water. Pour into 1 litre volumetric flask and make up to the mark with distilled water. This gives 1 gm ammonium nitrogen per litre or 1mg/ml.

Determination of Ammonia in rumen liquor

Procedure:

The rumen liquor was strained through two layers of cloth. 1 ml of this was poured into a 10ml volumetric flask or graduated test tube, and made to the mark with distilled water. 0.1ml of this solution was taken up in a pipette and poured into 50ml standard flask in duplicate. 5ml of dilute phenol-sodium nitropruside solution and 5ml dilute sodium hydroxide-sodium hypochlorite solution

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were added respectively, and the volume was made up to 50ml. The blank was prepared using distilled water instead of the rumen liquor. The flasks were left for 30 minutes for maximum colour development. On some occasions the flasks were warmed in a water bath at 37°C for 5 minutes. The blue colour that developed was stable for twenty four hours. The optical density of each sample in duplicate was read from an SP 500 colorimeter at 625 m.μ.

A standard curve was prepared using ammonium sulphate (A.R.).

Calculation:

$$\text{mg } NH_3\text{-N \%} = \frac{\text{Reading of sample}}{\text{Reading of standard}} \times 100 \times \text{dilution factor}$$

Determination of blood urea

Procedure:

Blood samples from the Jugular vein of the Zebu animals were kept in stoppered bottles and left for 24 hours in a cold room. During this time the blood plasma seperated out from the blood cells and other substances in the blood. The blood was poured into centrifuge tube and centrifuged. The serum was decanted into a clean

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bottle. 1ml of each blood sample was diluted to 100ml. 1ml of this dilute blood solution was poured into 50ml standard flask and 2ml buffered urease solution was added. The flasks with their contents were warmed in a water bath at 37°C for 5 minutes. Then 5ml dilute phenol-sodium nitropruside plus 5ml dilute sodium hydroxide-sodium hypochlorite were added respectively. The flasks were left at room temperature for 30 minutes for maximum blue colour development and the optical density read on an SP 500 colorimeter at 625 m.μ.

Blank solution was prepared using distilled water instead of blood.

A standard curve was prepared using urea.

<u>Calculation</u>:

$$\text{mg Urea-N\% in blood} = \frac{\text{Reading of sample}}{\text{Reading of standard}} \times 100 \times \text{dilution factor}$$

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APPENDIX X

"In Vitro" Digestibility Experiments

Boiling tubes

Large Pyrex boiling tubes, 20cm x 3cm were used for the experiment. This was to give enough room for shaking. Each tube was stoppered with a rubber bung through which passed a 6mm glass tubing with 1mm internal diameter bore. This tube was connected to another 6mm glass rod by rubber tubing. A small hole of about 4mm was cut vertically in the rubber tubing to release the gases produced during the anaerobic fermentation.

Artificial Saliva (buffer) solution

A large amount of buffer solution, 40ml was used. This was to maintain the pH level of the samples within the limits usual for rumen digestion. This was to make sure the acid concentration did not exceed that found in the animal.

The buffer solution was made according to the formula proposed by McDougall. $CaCl_2$ was added last. The solution was warmed to dissolve some of the insoluble chemicals. CO_2 was passed through the solution at 39°C until it was saturated. A clear solution was obtained.

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Composition of the buffer solution

$NaHCO_3$	9.8	gm per litre
$Na_2HPO_4\ 12H_2O$	9.3	gm " "
NaCl	0.47	gm " "
KCL	0.57	gm " "
$CaCl_2$ (anhydrous)	0.04	gm " "
$MgCl_2$ (anhydrous) or	0.06	gm " "
$MgCl_2 . 6H_2O$	0.13	gm " "

Gasing

It has been found preferable by many workers to digest in glass tubes and to rely on the gas produced to maintain anaerobic conditions. But in this experiment the tubes were opened every 24 hours and CO_2 passed over the sample to make sure that all oxygen (O_2) was removed.

Nitrogen supplementation

Alexander (1964) observed that unsupplemented rumen liquor gave variable digestibility coefficients. The digestibilty of the ration is affected by the level of crude protein in the ration. To reduce or remove this dietary effect, $(NH_4)_2SO_4$ was used by Alexander. This

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procedure is adopted in this experiment. 1 ml $(NH_4)_2SO_4$ was added to 50 ml buffer liquor mixture.

Pepsin solution

Aqueous pepsin solution was used (0.12 gm per 5 ml distilled water). Fresh samples were prepared for each experiment. The solution was kept in the refrigerator until needed. The pepsin powder was also stored in a cold room. The pepsin may be dissolved in normal HCl, but the former method is better and quicker. The pepsin used was of the order 1:2,500.

pH Meter

A Pye Ingold pH meter was used. A combined electrode was prefered to the separate electrodes. The combined electrode facilitated easy access to the solutions in the tubes while sitting in the water bath at 39°C. The meter was set with a buffer of pH4 and another buffer of pH7. The buffer solutions were cooled in the refrigerator for a few minutes before use, and the temperature noted before using them to set the meter. The meter was then set at 39°C.

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Water bath

Incubation can be done using an incubator or a water bath. A water bath was used for this experiment because the pH of the solution in each test tube can be read and adjusted while the tube is in the water bath. The samples are to remain at 39°C throughout the experiment. To avoid cooling a water bath was used. If an incubator is used, it will be necessary to take out the tubes for pH reading, and also for the addition of reagents. The temperature of the water was kept constant at 39°C by the continuous stirring of a stirrer. The water bath used was the immersion heater type and it was manufactured by Astell Laboratory Service Company of London.

Filter aid and Filter Paper

In order to avoid loss of the residue during filteration, a filter aid was used. The commonly used aid is the hyflo supercel. Instead of this very fine Celite was used. Celite is ashless and inert so that it has no effect on the result. It can be recovered and used over again. Filteration at the end of the pepsin digestion is difficult if no filter aid is used.

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APPENDIX XI

Recovery of Chromic Oxide from faeces

The recovery of chromic oxide from faeces organic matter for the digestibility trials is given in table below.

Percent recovery of chromic oxide from faeces organic matter of Zebu cattle

Treatment	4 weeks treatment	12 weeks treatment
H	100.66 ± 7.29	97.83 ± 4.7
	92.20 ± 7.29	99.01 ± 4.7
	96.25 ± 7.29	97.16 ± 4.7
	101.18 ± 7.29	103.22 ± 4.7
Mean	97.57 ± 7.29	99.31 ± 7.7
J	100.21 ± 4.02	96.92 ± 2.88
	94.66 ± 4.02	100.27 ± 2.88
	96.72 ± 4.02	100.21 ± 2.88
	97.95 ± 4.02	98.02 ± 2.88
Mean	97.63 ± 4.02	98.83 ± 2.88
K	100.49 ± 4.34	99.66 ± 0.02
	99.22 ± 4.34	99.58 ± 0.02
	96.82 ± 4.34	
	102.82 ± 4.34	
Mean	99.84 ± 4.34	99.31 ± 0.02
L	95.99 ± 2.06	99.70 ± 0.72
	94.89 ± 2.06	95.68 ± 0.72
	94.65 ± 2.06	
	97.25 ± 2.06	
Mean	95.69 ± 2.06	94.09 ± 0.72

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APPENDIX XI

Digestibility Coefficients of four treatments cut at 4 weeks by White Fulani (Zebu) cattle.

(%)

Zebu Animal No	Treatment	D.M.	O.M.	C.F.	C.P.	E.E.	N.F.E.	Total Ash
49	H	71.1	70.7	75.9	80.8	33.3	66.7	63.4
198		73.8	73.3	78.3	83.4	33.3	68.5	70.1
49		52.1	51.2	61.5	57.1	18.2	45.7	50.0
198		47.5	46.5	55.7	56.0	0.0	40.0	47.6
	Mean	61.1	60.4	67.9	69.3	21.2	55.2	57.8
49	J	54.7	56.5	55.1	54.4	29.4	56.2	48.0
198		53.9	58.9	54.5	60.5	11.8	57.2	35.0
49		55.8	58.6	57.6	55.7	37.5	57.9	40.7
198		55.4	58.2	60.6	56.2	6.3	56.0	38.3
	Mean	55.0	58.1	57.0	56.7	21.3	56.8	40.5
49	K	52.0	54.3	58.3	43.2	33.3	52.1	39.6
198		56.8	59.4	66.2	52.5	28.6	55.4	38.4
49		31.7	36.7	35.7	34.6	-20.0	32.7	17.6
198		33.7	39.5	48.0	38.8	0.0	28.6	11.1
	Mean	43.6	47.5	52.1	42.3	10.5	42.2	26.7
49	L	52.5	59.9	60.3	54.2	-22.2	62.5	-4.7
198		53.1	56.3	61.8	55.8	-25.0	55.5	23.7
49		51.9	54.5	55.6	65.1	11.1	50.8	35.1
198		48.9	50.1	52.7	59.1	0.0	47.2	38.5
	Mean	51.6	55.2	57.6	58.6	-9.0	54.0	23.2

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APPENDIX XI

Digestibility Coeeficients of four treatments cut at 12 weeks by White Fulani (Zebu) cattle.

(%)

Zebu Animal No	Treat-ment	D.M.	O.M.	C.F.	C.P.	E.E.	N.F.E.	Total Ash
114	H	53.3	54.1	62.7	62.7	0.0	46.0	31.0
194		56.7	56.4	63.9	68.1	64.7	52.7	45.5
114		55.0	54.2	60.5	62.2	9.1	53.1	38.4
194		56.1	56.1	64.2	58.7	60.0	52.6	35.0
	Mean	55.3	55.2	62.8	62.9	33.5	51.1	37.5
194	J	37.6	40.0	47.3	40.0	-14.3	35.1	23.9
114		60.2	64.3	68.1	51.2	26.7	63.3	65.8
194		47.8	52.4	57.3	46.3	0.0	46.3	32.0
114		58.2	57.3	66.2	54.2	41.2	58.0	39.4
	Mean	51.0	53.5	59.7	47.9	13.4	50.7	40.3
194	K	45.8	50.1	53.6	36.8	27.3	45.8	33.1
194		46.4	49.7	54.0	41.7	41.7	42.0	42.8
	Mean	46.1	49.9	53.8	39.3	34.5	43.9	38.0
194	L	57.0	57.1	58.1	61.2	27.3	57.6	50.5
194		58.8	59.2	59.3	61.2	27.3	60.0	54.5
	Mean	57.9	58.2	58.7	61.2	27.3	58.8	52.5

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APPENDIX XI

Faecal Chromic Oxide Determination

Regagents:

1. Potassium Bromate, ($KBrO_3$):- 4.5 w/v solution.
2. Manganese sulphate, 10% solution.
3. Ortho Phosphoric acid, (H_3PO_4) 85% w/v solution:-

   Mix 30ml 10% $MnSO_4$. $4H_2O$ solution with 1 litre analar 85% H_3PO_4 solution.
4. Sulphoric acid, (H_2SO_4), 50% solution. (A.R.). :-

   Mix 5 ml of 10% $MnSO_4$. $4H_2O$ solution with 1 litre of 50% H_2SO_4.
5. Clearing solution:- Weigh 125 gm. $(NH_4)_2SO_4$ (A.R.) pour into yOml HCl (A.R.) and make to 1 litre.
6. Ferrous ethylene di ammonium sulphate N/20 solution:-

   Weith 100 gm of ferrous ethylene di ammonium sulphate. Make it up to 5 litres with 5% H_2SO_4.
7. H_2SO_4, 5% solution. A.R.
8. Ferroin, 0.025M (Vegel):- Dissolve 1.485 gm. O-phenanthroline monohydrate in 100 ml 0.025M $FeSO_4$. $7H_2O$ solution.

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9. Ferrous sulphate, ($FeSO_4 \cdot 7H_2O$) solution. 0.025M:-
Weigh 0.695 gm. $FeSO_4 \cdot 7H_2O$ and dissolve it in 100 ml distilled water.

10. Potassium dichromate, ($K_2Cr_2O_7$) N/20 solution:-
Dry A.R. $K_2Cr_2O_7$, at 140^oC to 150^oC for 1 hour. Cool. Weigh 2.452 gm. of it and make up to 1 litre with distilled water.

<u>Standardization of Ferrous ethylene di ammonium sulphate solution</u>

To 25 ml N/20 $K_2Cr_2O_7$ solution add 100 ml distilled water. Also add 6 ml A.R. O-H_3PO_4, plus 5 ml A.R. H_2SO_4 plus 2 drops of ferrous ethylene di ammonium sulphate $FeC_2H_4(NH_3)_2$ solution to the reddish brown end point. 1 ml N/20 Ferrous ethylene di ammonium sulphate = 1.266 mg Cr_2O_3.

Method·

Weigh 2 gm dried milled faeces into silica dishes in duplicate, and ash at 600^oC in a muffle furnace for 2 hours. Brush the ash into a 350 ml conical flask. Rinse the dish with 4 ml distilled water into the flask. Add 5 ml $H_3PO_4/MnSO_4$ mixture and 3 ml $KBrO_3$ solution, and digest the mixture immediately on a hot plate until efferves-cence and a purple colour appears. Remove from the hot

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plate and cool for 15 - 30 seconds. Add 40 ml distilled water, from wash bottle, followed by 10 ml $H_2SO_4/MnSo_4$ solution and 4 ml $KBrO_3$ solution. Break any solid material adhering to the flask with a glass rod. Add silica chips to prevent bumping. Boil the solution for 5 minutes or until it turns orange-red. Remove from the hot plate and add 100 ml distilled water and 5ml clearing solution. Boil the mixture until starch iodide paper shows it to be free from bromine (10 minutes). Cool and titrate with N/20 ferrous ethylene di ammonium sulphate solution, using two drops of ferroin as an indicator.

<u>Correlation and regression equations for 'in vivo' O.M. Digestibility trials of stall-fed and grazing Zebu animals using faecal Nitrogen as the index.</u>

X = % Faecal Nitrogen

Y = % Digestibility of Dry Matter

Treatment H - 4 weeks Stall fed Animals

X	X^2	Y	Y^2	XY
1.30	1.2769	53.61	2874.03	60.5793
1.20	1.4400	57.64	3322.37	69.1680
1.05	1.1025	73.80	5446.44	77.4900
1.10	1.2100	59.88	3585.61	65.8680
$\Sigma X = 4.48$	$\Sigma X2=5.03$	$\Sigma Y=244.93$	$\Sigma Y2=15228.45$	$\Sigma XY=274.94$
$\overline{X}=1.12$		$\overline{Y}=61.23$		

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$$\Sigma(X - \bar{X})(Y - \bar{Y}) = \Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N}$$

$$= 274.94 - \frac{(4.48)(244.93)}{4} = 274.94 - 274.32$$

$$= 0.62$$

Regression coefficient

$$b = \frac{\Sigma(X - \bar{X})(Y - \bar{Y})}{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}$$

$$Y = \bar{y} + b(\bar{X} - X) = 61.23 + 62(X - 1.12)$$

$$= 61.23 + 62X - 69.44$$

$$= 62X - 8.21 \pm 6.93$$

Correlation coefficient

$$\Sigma(X - \bar{X})^2 = 0.01$$

$$\Sigma(Y - \bar{Y})^2 = 15228.45 - \frac{(244.93)^2}{4}$$

$$= 230.77$$

$$\Sigma XY = \Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N} = 0.62$$

$$r = \frac{0.62}{\sqrt{0.01 \times 230.77}} = \frac{0.62}{\sqrt{2.31}} = \frac{0.62}{1.52}$$

$$= 0.4$$

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$\Sigma xy = 0.62$

$\Sigma x^2 = 0.01$

Reduction S.S. = $\frac{(0.62)^2}{0.01} = \frac{0.3844}{0.01}$ = 38.44 (I.d.f.)

Residual S.S. for Y = 230.77 - 38.44 = 192.33 (n-2.d.f.)

Analysis of Variance

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
X	1	38.44	38.44	0.4	18.51
Residual	2	192.33	96.17		

$Sy^2.X = \frac{M.S.}{n - 2} = \frac{96.17}{2} = 48.063$

$SyX = \sqrt{48.063} = 6.93$

Treatment H 4 Weeks - Grazing Animals.

Using the regression equation Y = 62X - 8.21 ± 6.93

X	
1.63	92.85
1.44	81.07
1.43	80.45
1.51	85.41

$\Sigma Y = 339.78$

$\bar{Y} = 84.94\%$

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Treatment H 12 weeks - Stall Fed Animals

X	X^2	Y	Y^2	XY
1.24	1.5376	37.55	1410.00	46.5620
1.36	1.8496	56.05	4262.60	76.2280
1.37	1.8769	53.34	2845.16	73.0758
1.25	1.5625	73.67	5427.27	92.0875
EX	EX^2	EY	EY^2	EXY
5.22	6.8266	220.61	13945.03	289.9533

$\bar{X} = 1.305$ $\bar{Y} = 55.15$

$$\Sigma(X - \bar{X})(Y - \bar{Y}) = \Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N}$$

$$= 289.9533 - \frac{1151.5842}{4} = 289.9533 - 287.8960$$

$$= 2.0573$$

Regression coefficient

$$b = \frac{2.0573}{6.8266 - \frac{(5.22)^2}{4}} = \frac{2.0573}{6.836-6.81} = \frac{2.0573}{0.02}$$

$$= 102.86$$

$$Y = \bar{y} + b(X - \bar{X}) = 55.15 + 102.86(X - 1.305)$$

$$= 55.15 + 102.86X - 134.23$$

$$= 102.86X - 79.08 \pm 11.89$$

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Correlation coefficient

$$\Sigma(X-\bar{X})^2 = 6.8266 - \left(\frac{5.22}{4}\right)^2 = 0.02$$

$$\Sigma(Y-\bar{Y})^2 = 13945.03 - \frac{(220.61)^2}{4}$$

$$= 13945.03 = 12167.19 = 777.84$$

$$\Sigma(X-\bar{X})\ (Y-\bar{Y}) = 2.06$$

$$r = \frac{2.06}{0.02 \times 777.84} = \frac{2.06}{15.56} = \frac{2.06}{3.94}$$

$$= 0.5228$$

$$\Sigma XY = 2.06$$

$$\Sigma x^2 = 0.02$$

$$\text{Reduction S.S.} = \frac{(2.06)^2}{0.02} = \frac{4.2436}{0.02} = 212.18 \quad (1 \text{ d.f.})$$

Residual S.S. for Y = 777.84 - 212.18 = 565.66 (n-2 d.f.)

Analysis of Variance

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
X	1	212.18	212.18	0.75	18.51
Residual	2	565.66	282.83		

$$Sy.x = \frac{M.S.}{n-2} = \frac{282.83}{2} = 141.41$$

$$Sy.x = \sqrt{141.42} = 11.89$$

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Treatment H 12 weeks - Grazing Animals

Using the regression equation

$$Y = 102.86X - 79.08 \pm 11.89$$

X	Y
1.67	92.70
1.37	61.84
1.48	73.15
1.44	69.04

$\Sigma Y = 296.73$ $\bar{Y} = 74.18\%$

Treatment J 4 weeks - Stall Fed Animals

X	X	Y	Y	XY
1.47	1.1609	54.44	2963.7136	80.0268
1.31	1.7161	54.18	2935.4724	70.9758
1.38	1.9044	55.66	3098.0356	76.8108
1.35	1.82251.8225	55.53	3083.5809	74.9655
ΣX	ΣX^2	ΣY	ΣY^2	ΣXY
5.51	7.6039	219.81	12080.8025	302.7789

$\bar{X} = 1.38$ $\bar{Y} = 54.95$

$$\Sigma(X-\bar{X})(Y-\bar{Y}) = 302.78 \quad \left(\frac{5.51)(219.81}{4}\right) = 302.78 - \frac{1211.1531}{4}$$

$$= 302.78 - 302.79 = 0.01$$

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Regression coefficient

$$b = \frac{-0.01}{7.61 - \frac{30.3601}{4}} = \frac{-0.01}{7.61 - 7.59} = \frac{-0.01}{0.02} = -0.5$$

$$Y = 54.95 - 0.01X + 0.01 = 54.94 - 0.01X \pm 0.65$$

Correlation coefficient

$$\Sigma(X-\bar{X})^2 = 0.02$$

$$\Sigma(Y-Y)^2 = 12080.80 - \frac{(219.81)^2}{4} = 12080.80 - \frac{48316.44}{4}$$

$$= 12080.80 - 12079.11 = 1.69$$

$$\Sigma(X-\bar{X})(Y-\bar{Y}) = -0.01$$

$$r = \frac{-0.01}{0.02 \times 1.69} = \frac{-0.01}{0.0338} = \frac{-0.01}{0.185} = -0.054$$

$$\Sigma XY = \frac{(\Sigma X)(\Sigma Y)}{N} = -0.01$$

$$\Sigma x^2 = \Sigma X^2 - (\Sigma X)^2 = 0.02$$

$$\text{Reduction S.S.} = \frac{(-0.01)^2}{0.02} = -\frac{0.0001}{0.02} = -0.005 \text{ (I d.f.)}$$

$$\text{Residual S.S. for } Y = 1.69 - (-0.005) = 1.695 \text{ (n-2 d.f.)}$$

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Analysis of Variance

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
X	1	-0.005	-0.005	0.005	18.51
Residual	2	1.695	0.848		

$Sy^2.x = \frac{M.S.}{n - 2} = \frac{0.848}{2} = 0.424$

$Sy.x = \sqrt{0.424} = 0.65$

Treatment J   4 weeks - Grazing Animals

Using the regression equation

$Y = 54.94 - 0.01X \pm 0.65$

X	Y
1.50	54.90
1.57	54.92
1.37	54.93
1.52	54.92
1.72	54.92
1.58	54.92
1.66	54.92
1.39	54.93
1.35	54.93
1.58	54.92
1.29	54.93
1.62	54.92

$\Sigma Y = 659.08$

$\bar{Y} = 54.92\%$

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Treatment J 12 weeks - Stall Fed Animals

X	X^2	Y	Y^2	XY
1.56	2.4336	52.69	2776.42	82.1964
1.61	2.5921	45.17	2040.33	72.7237
1.42	2.0164	54.74	2996.47	77.7308
1.40	1.9600	51.26	2627.59	71.7640
ΣX	ΣX^2	ΣY	ΣY^2	ΣXY
5.99	9.0021	203.86	10440.63	304.4149

$\bar{X} = 1.50$ $\bar{Y} = 50.97$

$$\Sigma(X-\bar{X})(Y-\bar{Y}) = \Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N}$$

$$= 304.41 - \frac{(5.99)(203.86)}{4} = 304.41 - 305.28$$

$$= -0.87$$

<u>Regression coefficient</u>

$$b = \frac{-0.87}{9.00 - \frac{(5.99)^2}{4}} = \frac{-0.87}{9.00 - 8.97} = \frac{-0.87}{0.03} = -29$$

$$Y = \bar{y} + b(X-\bar{X}) = 50.97 + (-29)(X-1.50)$$

$$= 50.97 - 29X + 43.50$$

$$= 94.47 - 29X \pm 2.53$$

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Correlation coefficient

$\Sigma(X-\bar{X})^2 = 0.03$

$\Sigma(Y-\bar{Y}) = 10440.63 - \frac{(203.86)^2}{4} = 10440.63 - 10389.72$

$= 50.91$

$\Sigma(X-\bar{X})(Y-\bar{Y}) = -0.87$

$r = \frac{-0.87}{0.03x50.91} = \frac{-0.87}{1.53} = \frac{-0.87}{0.39} = -2.23$

$\Sigma XY = -0.87$

$\Sigma X^2 = 0.03$

Reduction S.S. $= \frac{(-0.87)}{0.03} = \frac{0.7569}{0.03} = 25.23$ (1 d.f.)

Residual S.S. for Y = 50.91 - 25.23 25.68 (n-2d.f)

Analysis of Variance

Source	D.F	S.S.	M.S.	F.	$F_{0.05}$
X	1	25.23	25.23	1.96	18.51
Residual	2	25.68	12.84		

$Sy^2.x = \frac{M.S.}{n-2} = \frac{12.84}{2} = 6.42$

$Sy.x = \sqrt{6.42} = 2.53$

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Treatment J 12 weeks - Grazing Animals

Using the regression equation

$$Y = 94.47 - 29X \pm$$

X	Y
2.06	35.73
1.84	41.11
2.01	46.18
1.71	44.88
1.23	58.80
2.00	36.47
1.74	44.01
1.63	47.20
1.46	52.13
1.42	53.29
1.41	53.58
1.38	54.45

$\Sigma Y = 567.83$

$\bar{Y} = 47.32\%$

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Treatment K 4 weeks - Stall Fed Animals

X	X^2	Y	Y^2	XY
1.50	2.2500	55.24	3051.4576	82.8600
1.37	1.8769	53.64	2877.2496	73.4868
1.35	1.8225	33.07	1093.6249	44.6445
1.33	1.7689	32.41	1050.4081	43.1053
ΣX	ΣX^2	ΣY	ΣY^2	ΣXY
5.55	7.7183	174.36	8072.7402	244.0966

$\bar{X}$ = 1.39 $\qquad$ $\bar{Y}$ = 43.59

$$\Sigma(X-X)(Y-Y) = \Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N} = 244.10 - \frac{(5.55)(174.36)}{4}$$

$$= 233.10 \quad \frac{976.6980}{4} = 244.10 - 241.92 = 2.18$$

Regression Coefficient

$$b = \frac{2.18}{\Sigma X^2 - \frac{(\Sigma X)^2}{N}} = \frac{2.18}{7.72-\frac{(5.55)^2}{4}} = \frac{2.18}{7.72-\frac{(30.8025)}{4}} = \frac{2.18}{7.72-7.70} = \frac{218}{0.02} = 109$$

$$Y = \bar{y} + b(X - \bar{X}) = 45.59 + 109 \quad (X - 1.39)$$

$$= 45.59 + 109X - 151.51$$

$$= 109X - 105.92 \quad \pm 7.66$$

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Correlation coefficient

$\sum(X-\bar{X})^2 = 0.02$

$\sum(y-\bar{Y}) = 8072.74 - \frac{(174.36)^2}{4} = 8072.74 - \frac{30401.4096}{4}$

$= 8072.74 - 7600.35 = 472.39$

$\sum(X-\bar{X})(Y-\bar{Y}) = 2.18$

$r = \frac{2.18}{0.02 \times 472.39} = \frac{2.18}{9.45} = \frac{2.18}{3.07} = 0.71$

$\sum XY = 2.18 \quad \sum X^2 = 0.02$

Reduction S.S. $= \frac{(2.18)^2}{0.02} = \frac{4.7524}{0.02} = 237.62$ (1.d.f.)

Residual S.S. for Y $= 472.39 - 247.62 = 234.77$ (n-2d.f.)

Analysis of Variance

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
X	1	237.62	237.62	2.02	18.51
Residual	2	234.77	117.38		

$Sy^2.x = \frac{M.S.}{n-2} = \frac{117.38}{2} = 58.69$

$sy.x = \sqrt{58.69} = 7.66$

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Treatment K 4 weeks - Grazing Animals

Using the regression equation.

$$Y = 109X - 105.92 \pm 7.66$$

X	Y
1.40	46.68
1.45	52.13
1.40	46.68
1.46	53.22
1.31	36.87
1.52	59.76
1.35	41.23
1.55	63.02
1.46	53.22
1.57	63.21
1.30	35.78
1.44	51.04

$\Sigma Y = 604.85$

$\bar{Y} = 50.40\%$

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Treatment K 12 weeks - Stall Fed Animals

X	X^2	Y	Y^2	XY
1.41	1.9881	45.84	2101.31	64.6344
1.51	2.2801	45.84	2101.31	69.2184
1.31	1.7161	45.84	2101.31	60.0504
1.37	1.8769	46.35	2148.32	63.4995
1.26	1.5876	46.35	2148.32	58.4010
1.35	1.8225	46.35	2148.32	62.5725
ΣX	ΣX^2	ΣY	ΣY^2	ΣXY
8.21	11.2718	276.57	12748.89	378.3762

$\bar{X} = 1.37$ $\bar{Y} = 46.10$

$$\Sigma(X-X)(Y-Y) = 378.38 - \frac{(276.57)(8.21)}{6} = 378.38 - \frac{2270.6397}{6}$$

$$= 378.38 - 378.44 = -0.06$$

Regression Coefficient

$$b = \frac{-0.6}{11.27-\left(\frac{8.21}{6}\right)^2} = \frac{-0.06}{11.27-\left(\frac{67.4041}{6}\right)} = \frac{-0.06}{11.24-11.23} = \frac{-0.06}{0.04} = -1.5$$

$$Y = \bar{y} + b(X - \bar{X})$$

$$Y = 46.10 + (-1.5X) - 2.06 = 46.10 - 1.5X + 2.06$$

$$= 46.10 - 1.5(X-1.37) = 46.10 - 1.5X + 2.06 = 48.16 - 1.5X$$

$$Y = 48.16 - 1.5X \pm 0.18$$

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Correlation Coefficient

$\sum(X-\bar{X})^2 = 0.04$

$\sum(Y-\bar{Y})^2 = 12748.89 - \frac{(276.57)^2}{6} = 12748.89 - \frac{76490.9649}{6}$

$= 12748.89 - 12748.49 = 0.40$

$\sum(X-\bar{X})(Y-\bar{Y}) - 0.06$

$r = \frac{-0.06}{0.04 \times 0.40} = \frac{0.06}{0.0160} = \frac{0.06}{0.127} = 0.47$

$\sum XY = 0.06$

$\sum X^2 = 0.04$

Reduction S.S. = $\frac{(-0.06)^2}{0.04} = \frac{.0036}{0.04} = -0.09$ (1.d.f.)

Residual S.S. for Y = 0.40 - (-0.09) = 0.49 (n-2 d.f.)

Analysis of Variance

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
X	1	-.0.09	-0.09	-0.75	18.51
Residual	4	0.49	0.12		

$Sy^2.x = \frac{M.S.}{n-2} = \frac{0.12}{4} = 0.03$

$Sy.x = \sqrt{0.03} = 0.18$

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Treatment K 12 weeks - Grazing Animals

Using the regression equation.

$$Y = 48.16 - 1.5X \pm 0.18$$

X	Y
1.54	45.85
1.54	45.85
1.49	45.92
1.45	45.98
1.38	46.09
1.41	46.04
1.44	46.00
1.41	46.04
1.52	45.88
1.62	45.73
1.36	46.12
1.44	46.00

$\sum Y = 551.50$

$\bar{Y} = 45.96\%$

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Treatment L   4 weeks - Stall Fed Animals

X	X^2	Y	Y^2	XY
1.40	1.9600	53.10	2819.61	74.3400
1.26	1.5876	53.52	2864.39	67.4352
1.62	2.6244	53.10	2819.61	86.0220
1.60	2.5600	53.52	2864.39	85.6320
1.49	2.2201	53.52	2864.39	79.7448
1.44	2.0736	48.88	2389.25	70.3872
1.55	2.4025	51.92	2695.69	80.4760
1.35	1.8225	48.88	2389.25	65.9880
1.52	2.3104	51.92	2695.69	78.9184
1.51	2.2801	48.88	2389.25	73.8088
1.60	2.5600	51.92	2695.69	83.0720
ΣX	ΣX^2	ΣY	ΣY^2	ΣXY
16.34	24.4012	569.16	29487.21	845.8244

$\bar{X} = 1.49 \quad \bar{Y} = 51.74$

$\Sigma(X-\bar{X})(Y-\bar{Y}) = \Sigma XY - \frac{(\Sigma Y)(\Sigma X)}{N} = 845.82 - \frac{(569.16)(16.34)}{11}$

$= 845.82 - \frac{9300.0744}{11} = 845.82 - 845.46 = 0.36$

Regression Coefficient

$b. = \frac{0.36}{24.40-\frac{(16.34)^2}{11}} = \frac{0.36}{24.40-\left(\frac{266.9956}{11}\right)} = \frac{0.36}{24.40-24.27} = \frac{0.36}{0.13} = 2.77$

$Y = \bar{y} = b\ (X-X) = 51.74 + 2.77\ (X - 1.49$

$= 51.74 + 2.77X - 4.13 = 47.61 + 2.77X \pm 0.738$

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Correlation Coefficient

$\Sigma(X-\bar{X})^2 = 0.13$

$\Sigma(Y - \bar{Y})^2 = 29487.21-\frac{(569.16)^2}{11} = 29487.21 - \frac{323943.11}{11}$

$= 29487.21 - 29449.37 = 37.84$

$\Sigma(X-\bar{X})(Y-\bar{Y}) = 0.36$

$r = \frac{0.36}{0.13 \times - 37.84} = \frac{0.36}{11.73} = \frac{0.36}{3.43} = 0.105$

$\Sigma XY = 0.36 \quad \Sigma XY^2 = 0.13$

Reduction S.S. $= \frac{(0.36)}{0.13} = \frac{0.1296}{0.13} = 1$ (1 d.f.)

Residual S.S. for Y = 37.84 - 1 = 36.84 (n -2d.f.)

Analysis of Variance

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
X	1	1	1	0.204	18.51
Residual	9	36.84	4.9		

$Sy^2.x = \frac{M.S.}{n-2} = \frac{4.9}{9} = 0.54$

$Sy.x = \sqrt{0.54} = 0.738$

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Treatment L 4 weeks - Grazing Animals.

Using the regression equation.

$$Y = 47.61 + 2.77X \pm 0.738$$

X	Y
1.55	51.90
1.40	51.49
1.56	51.93
1.36	51.38
1.52	51.83
1.61	52.07
1.39	51.46
1.53	51.85
1.43	51.57
1.49	51.74
1.55	51.90
1.41	51.52

$\Sigma Y = 620.65$

$\bar{Y} = 51.72\%$

Treatment L 12 weeks - Stall Fed Animals

X	X^2	Y	Y^2	XY
1.36	1.8496	57.01	3250.14	77.5336
1.44	2.0736	57.01	3250.14	82.0944
1.51	2.2801	57.01	3250.14	86.0851
1.47	2.1609	58.80	3457.44	86.4360
1.49	2.2201	58.80	3457.44	87.6120
1.49	2.2201	88.80	3457.44	87.6120
ΣX	ΣX^2	ΣY	ΣY^2	ΣXY
8.76	12.8044	347.43	20122.74	507.3731

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$\bar{X} = 1.46 \qquad \bar{Y} = 57.91$

$$\Sigma(X-\bar{X})(Y-\bar{Y}) = \Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N} = 507.37 - \frac{(8.76)(347.43)}{6}$$

$$= 507.37 - \frac{304.4868}{6} = 507.37 - 507.2478 = 0.12$$

Regression Coefficient

$$b = \frac{0.12}{12.80-\frac{(8.76)^2}{6}} = \frac{0.12}{12.80-\frac{76.7376}{6}} = \frac{0.12}{12.80-12.7896} = \frac{0.12}{0.01} = 12$$

$$Y = \bar{y} + b(X - \bar{X}) = 57.91 + 12(X - 1.46)$$

$$= 57.91 + 12X - 17.52 = 40.39 + 12X + 0.45$$

Correlation coefficient

$$\Sigma(X-\bar{X})^2 = 0.01$$

$$\Sigma(Y-\bar{Y})^2 = 20122.74 - \frac{(347.43)^2}{6} = 20122.74 - \frac{120707.6049}{6}$$

$$= 20122.74 - 20117.93 = 4.81$$

$$\Sigma(X-\bar{X})(Y-\bar{Y}) = 0.12$$

$$r = \frac{0.12}{\sqrt{0.01 \times 4.81}} = \frac{0.12}{\sqrt{0.048}} = \frac{0.12}{\sqrt{0.22}} = 0.545$$

$$xy^2 = 0.01$$

$$\text{Reduction S.S.} = \frac{(.12)^2}{0.01} = \frac{0.0144}{0.01} = 1.44 \text{ (1 d.f.)}$$

Residual S.S. for Y = 4.81 - 1.44 = 3.37 (n - 2 d.f.)

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Analysis of Variance

Source	D.F.	S.S.	M.S.	F.	$F_{0.05}$
X	1	1.44	1.44	1.61	18.51
Residual	4	3.37	0.84		

$$Sy^2.x = \frac{M.S.}{n-2} = \frac{0.84}{4} = 0.21$$

$$Sy.x = \sqrt{0.21} = 0.45$$

Treatment L 12 weeks - Grazing Animals.

Using the regression equation.

$$Y = 40.39 + 12X \pm 0.45$$

X	Y
1.31	56.11
1.46	57.91
1.52	58.63
1.41	57.31
1.56	59.11
1.48	58.15
1.40	57.19
1.37	56.83
1.42	57.43
1.41	57.31
1.46	57.91
1.49	58.27

$\Sigma Y = 692.16$

$\bar{Y} = 57.68\%$

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