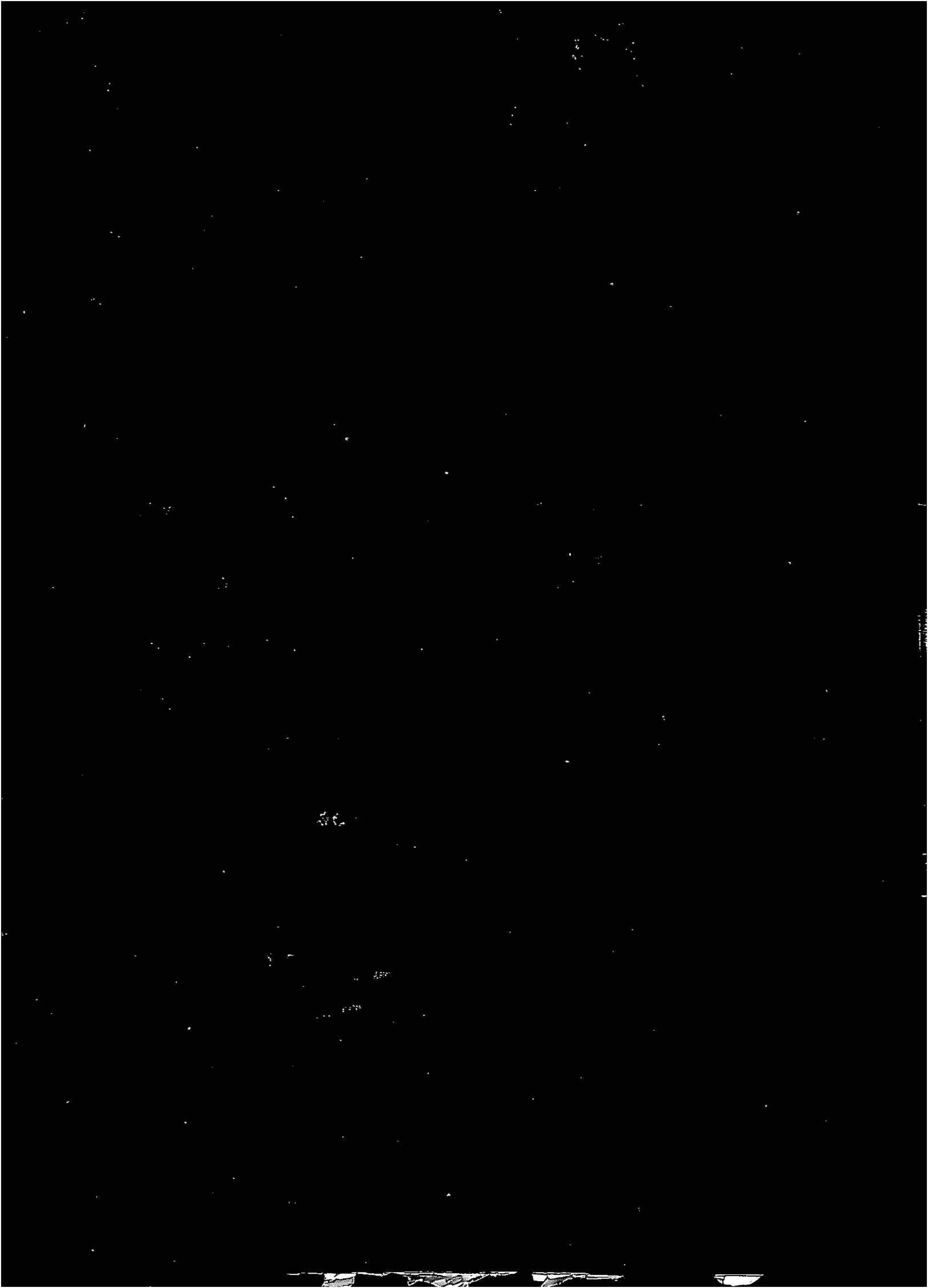

I certify that this Thesis has been prepared under my supervision by Shri Dwipendra Nath Maitra, a candidate for the M.Sc.(A.H.) with Animal Nutrition as major subject, and that it incorporates the results of his independent study.

19.13. Mukherjee

(D.B.Mukharjee)
M.Sc., Ph.D.
Professor of Animal Nutrition,
Bihar Veterinary College, Patna.

STUDIES ON 'IN VITRO' FERMENTATION OF BAGASSE AND ITS UTILISATION BY GOATS:

Thesis

Submitted to the University of Magadh in partial fulfilment of the requirements for the Degree of MASTER OF SCIENCE in Animal Husbandry in the Faculty of Veterinary Science:

...

BIHAR VETERINARY COLLEGE,
OCTOBER, 1962:

D.N.MAITRA,
B.Sc., B.V.Sc.& A.H.

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Shri Sunil Kuma
Research Assistant,
Station, Bihar, Patna
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C O N T E N T S .

A C K N O W L E D G E M E N T S:

I like to express my sincere gratitude to my revere teacher Dr.D.B.Mukherjee,M.Sc.,Ph.D.(Aberdeen), Professor of Animal Nutrition for, suggesting the problem, valuable guidence constant encouragement in the work.

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***

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

Evolutionary findings led people to believe that multicellular organisms originated from unicellular creatures through the ages. Many of the living beings that had their existance on the soil have been eliminated from the earth in course of evolution. Those who proved to be fittest survived. One of such evlutionary creatures are the ruminants- the polygastric animals which present wonderful features among the herbivores.

Ruminants present a volume of peculiarities in the utilization of ingested food which calls for the attention of physiologists, bio-chemists, nutritionists and even of bacteriologists.

The fundamental peculiarities in the process of utilization of ingested food by ruminants are many and some of these are noted below -

1. Ruminants consume food which is mostly cellulose - a complex carbohydrate which cannot be degraded by any mammalian enzymes.

2. They have got no upper incisors and as such mastication of food is always incomplete.

3. There is no carbo-hydrate and protein splitting enzymes in saliva and hence the proximate prindiples cannot be acted by saliva. There is a little lipolytic action in the saliva of cattle.

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nd the last

In the rumen all nted by the microorganisms to produc arides. The food material which escap ms are pres pushed to the lower gut to follow or vat'. s a little monosaccharides are also pr maintaine

ucts of fermentation:

Ruminants are anatomica d in respect to other herbivores because they lose in the rumen followed by ordinary enzymat d again followed by Caecal fermentation.

The carbohydrates irrespective o hemical making up; are degraded to VFA as the endproduct of en fermentation. Caecal fermentation also give rise to th oduction of VFA but the intestinal digestion produces monosac ride.

In investigations on the VFA produced, it has been found that acetic, propionic and butyric acids e the chief acids produced in fermentation. Besides these, mall amounts of other higher fatty acids are also occasionally resent in the rumen sample. Phillipson (1947) pointed out that cellulose is broken down to cellulobiose, glucose, pyruvate, lactate and ultimately to VFA. CH_4 is mainly produced from Acetic, Butybic and valoric acid by rumen bacteria which are biochemically closely related to 'METHANO BACTERIUM SUBOXYDANS' in which butyric and....

4. The simple stomach has been developed into complex stomach having four chambers of which rumen, reticulum and omasum have got no secretion and the last part abomasum acts as simple stomach.

5. Innumerable micro-organisms are present in rumen and reticulum which act as'fermenting vat'.

6. The temperature of rumen is maintained at a higher level than that of the body.

Fermentation of food in rumen:

Food material, strictly speaking the proximate principles are the potential source of chemical energy to the living being. This chemical energy is liberated in three phases. The 1st phase is the hydrolysis of food in digestion; 2nd stage is the stage of anaerobic glycolysis, decarboxylation of carbo hydrate and deamination of protein; followed by the entry of abode products in the TCA cycle to produce CO_2 and H_2O. The hydrolysis energy is very small and the major part of the food energy is produced in the other two phases.

Cellulose being the major part of the ruminants' ration, must be utilised to provide energy to the host in some other way other than hydrolysis, as there is no 'Cellulase' in the mammalian system. Nature has provided means for it.The rumen acts as 'fermenting vat' and large number of functional micro-organisms exists there in symbiotic relationship. These microbes help in the fermentation of crude fibre and the end products thus produced are .....

are available to the host.

In the rumen all the carbohydrates are fermented by the microorganisms to produce VFA instead of monosaccharides. The food material which escapes rumen fermentation is pushed to the lower gut to follow ordinary digestion and thus a little monosaccharides are also produced.

Products of fermentation:

Ruminants are anatomically much more adopted in respect to other herbivores because they ferment in cellulose in the rumen followed by ordinary enzymatic digestion and again followed by Caecal fermentation.

The carbohydrates irrespective of their chemical making up; are degraded to VFA as the endproduct of rumen fermentation. Caecal fermentation also give rise to the production of VFA but the intestinal digestion produces monosaccharide.

In investigations on the VFA produced, it has been found that acetic, propionic and butyric acids are the chief acids produced in fermentation. Besides these, small amounts of other higher fatty acids are also occasionally present in the rumen sample. Phillipson (1947) pointed out that cellulose is broken down to cellulobiose, glucose, pyruvate, lactate and ultimately to VFA. CH_4 is mainly produced from Acetic, Butyric and valoric acid by rumen bacteria which are biochemically closely related to 'METHANO BACTERIUM SUBOXYDANS' in which butyric and....

and valoric acid serve as reductants with CO_2 as the oxidant in 'invivo'condition (Nelson,1958). Multiplication of the rumen microbes resulting in synthesis of starch Glycogen and vitamin of B-complex group take place at the cost of the product of fermentation. The extent of active growth is dependant on available source of energy and amount of nitrogen present.

FACTORS THAT MAINTAIN MICROBIAL POPULATION ACTIVE:

For the normal thrive and efficient working of the microbial population a suitable environment is inevitable. Constant passage of food material to the rumen for 24 hours, the mechanical movement of the rumen, passage of the digesta into lower gut, entrance of the end products of digestion into the general circulation through rumen wall, constant and steady range of P_H , rumination and anarobiosis through the production of various gases by way of fermentation help in constituting an optimum environment which facilitâtes the growth and reproduction of microbes.

RATE OF PRODUCTION OF VFA:

McAnally & Phillipson (1942) as well as Barcroff et.al.(1944) showed that the constant passage of VFA into the circulation take place through rumen wall. Constant absorption, passage into lower gut metabolisation of VFA by rumen wall, passage of saliva into rumen & their interconversion stand on the way of estimation of the rate of production of VFA in 'in-vivo'. The only rational solution of this problem is the application of Radio-active Isotope in this field. Prior to this 'in-vitro' measurements of

of the rate of production formed the only basis of estimation which at best was only approximate. With the help of Isotope dilution technique, the rate of production of the acids have been estimated by Mukherjee (1960) as Acetic, 4.79 ; propionic-1.10 and butyric-0.60 mili Eq-/hour/Kg.of rumen content respectively.

ABSORPTION:

The acids are absorbed by the reticulo-ruminal epithelium. The rate of absorption depends on the PH of the rumen and the concentration of the VFA. The determination of the rate of absorption is also rather difficult because of the elaborate surgical process involved in it. With the Isotope technique Mukherjee (1960) estimated the rate of absorption in fasting sheep to be Acetic-38.8; Propionic-9.66; Butyric-4.22 mili mole/ hour / Kg. rumen contents respectively.

METABOLISM OF THE ACIDS:

Cambridge group of workers established that these acids are extensively metabolised that by the animal as source of energy and also for the synthesis of body fat.

Propionic acid is a Glycogen former. Reid (1950) has shown that insulin hypoglycaemia can be effectively relieved by injecting propionate. Potter (1952) noticed similar effect with butyrate and as such butyrate probably forms carbohydrate. Pennington (1952) showed that it is metabolised by rumen wall to produce ketone bodies.

Acetic acid is metabolised by almost all tissues. Reid (1950) showed that it is metabolised by brain tissue. Acetic acid, as quoted by Phillipson (1947) is regarded as a Glycogen former upto about 40%. This finding was confirmed by Lorber at.al. (1945) by Isotopes studies. Milk fat is synthesised from blood acetate as shown by Popjak at.al. (1949) and lactose originate from acetate was shown by Cowie at.al.(1951). Cholestrol is also formed from acetate.

CALORIFIC CONSIDERATION:

The energy value of VFA calculated by Pfander & Phillipson (1953) was 600 Cal/day and by Schambye (1951a,b,c,and 1955 a,b) to be 600-1200 Cal/day in case of sheep. Maintenance requirement in case of sheep was calculated by Armstrong & Blaxter (1956) to be 1200 Cal/day and on this basis about 85% of the maintenance energy requirement of the sheep is met with from VFA. About 70% of the maintenance energy requirementwas was met in Steers by VFA as shown by Carrol & Hungate (1954)

The accepted calorific value of the fatty acids are 208.4, 373.5,504.8 Cal/m.of acetic, propionic and butyric acid respectively (Carrol & Hungate-1954). Even in fasting sheep where production rate is minimal, Mukherjee (1960) calculated that about 25% of the maintenance need is met from VFA.

In the present work, the fermentation of bagasse by rumen microorganisms was studied in 'in-vitro' under different conditions and the product of fermentation analysed.

A series of digestion trial were undertaken to determine the digestion co-efficient of the dry matter and other organic nutrients in support of the 'in-vitro' findings. The effect of added soluble sugar on the digestibility of crude fibre was also studied.

U.P. and Bihar are the largest sugar producing area. Their share averaging 73% of total production. On this light it can be said that if the bagasse that are derived from sugar cane are utilised as cattle fodder, it will have enormous economic advantages in the less agriculturally advanced country like India.

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CHAPTER- I

REVIEW OF LITERATURE:

Volatile fatty acids in rumen:

Tappeiner (1884) first detected VFA in rumen and Woodman (1938) said that the rumen VFA is the end product of cellulose in rumen fermentation. But the exact significance of VFA was unknown till the beginning of forties - when Elsden and his school said that ruminal VFA namely viz Acetic, propionic and Butyric and traces of other higher fatty acids are available to the host.

Woodman and Evans (1938) found that glucose, puryvic acid, lactic acid and VFA all appear during fermentation of cellulose. Phillipson (1942) showed that hexoses pass to VFA stage via lactic acid. Small amount of formic acid as well as some branched-chain acids are occasionally present (Annison, 1954) but under natural circumstances acetic acid concentration predominates (Elsden, 1946).

The concentration and the proportion of individual VFA production are dependant on the nature of diet, species of animal and even with individuals of the same species (Phillipson, 1947).

Some of the findings of the various workers have been shown in Table I.

Table-I.

Table showing the product of fermentation ('in-vitro') on various substrate:

Substrate	Animal	A	P	B	Reference.
Cellulose.	Sheep	42.10	54.50	3.40	Elsden (1945-46)
Glucose.	Sheep	23.20	51.20	25.60	
Dried grass.	Sheep	52.50	34.50	13.00	
Wheat & Hay.	[illegible]	46.00	41.00	13.00	Gray at.al. (1951)
Luce		59.00	27.00	14.00	
		0.30	21.10	18.60	Carrol & Hungate (1954)
		.40	22.60	14.00	
		.00	16.00	12.00	
					Mukherjee (1960)
			24.40	24.40	

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Wheat & Hay.	Sheep	46.00	41.00	13.00	Gray et.al. (1951)
Lucern hay.	sheep	59.00	27.00	14.00	
Hay.	Steer	60.30	21.10	18.60	Carrol & Hungate (1954)
Grain.	Steer	63.40	22.60	14.00	
Hay & Concentrate.	Fasting sheep.	75.00	16.00	12.00	Mukherjee (1960)
Hay & Concentrate.	Non-fasting sheep.	51.20	24.40	24.40	

In 'in-vitro' studies, Elsden (1945-46) detected greater propyionic acid in case of cellulose, in contrast to 'in-vivo' condition, which was assume to be probably due to microflora survived in 'in-vitro' being not a representative of the mixed flora in rumen.

From the general nature of VFA production as shown in the above table, it is concluded that soluable sugar tend to produce more propyionic acid.

Gray et.al. (1952) observed high amount of propyionic acid from 'in-vitro' fermentation of wheaten hay in sheep on 2 hours of incubation, the percentage composition being acetic, 43; propionic 51; Butyric 6. When the period of incubation was extended upto 48 hours, the percentage composition of VFA was different viz. acetic-52; propyionic -35 and Butyric-13. A considerable part of the higher acid produced in long period incubation, may be due to secondary re-action from both acetic and propionic acid.

The extent of inter-conversion of the three ruminal acids have been studied both in 'in-vivo' and in 'in-vitro' and was found to be not more than 3% excepting in case of ~~by~~ butyrate to acetate (Mukherjee, 1960).

In 'in-vitro' studies the percentage conversion in three hours were 12.8 and 29.3 for non-fasting and fasting animals respectively. Whereas, in 'in-vivo' condition it was in the order of 88.9 and 73.0 ~~percentage~~ percent, the last one being the data for fasting sheep.

Some of the important findings on VFA levels in the 'in-vivo' condition is shown in Table- 1.1:

Table 1.1:

VFA levels in the rumen under different diet:

Diet.	Animal.	A.	P.	B.	Reference
Flaked maize.	Sheep. 1.	52.00	42.00	5.00	Phillipson (1952)
	Sheep. 2.	60.00	35.00	3.00	
	Sheep. 3.	59.00	23.00	14.00	
Wheaten hay.	Sheep. 1.	64.70	23.50	9.20	Gray et.al. (1952)
	Sheep. 2.	65.40	22.30	8.90	
Hay & concentrates.	Cow.	40.60	36.50	10.70	Balch & Rowland (1957)
Hay & concentrates.	Sheep.	75.50	15.30	9.00	Mukherjee(1960)

It is seen from the Table 1 and 1.1 that the proportions of the three acids both in 'in-vivo' and 'in-vitro' are widely different depending upon the conditions of the experiment.

el-Shazly (1952) and Annison (1954) pointed out that the proportion of C_4 and C_5 acids were more when the animals were kept on protein rich diet, for under anaerobic condition, ammonia Co_2 and VFA are produced from protein by bacterial action (El-Shazly, 1952).

The products of fermentation of both carbo-hydarates and protein together give rise to various straight and branched chain fatty acids and all these are utilised by host. Butyric acid and some branch chain acids are utilised by the rumen wall itself (Annison and Pennington, 1954; Pennington, 1954; Pennington and Sutherland, 1956).

RATE OF PRODUCTION OF VFA IN RUMEN:

The rate of production in 'in-vivo' of 7.18 m.moles/kg./hour of VFA was determined by Mukherjee (1960) by the Isotopes technique, cannot be compared with any similar data as no previous work has been done on that line. Comparison can, therefore only be made with 'in-vitro' rate for which considerable data are available. Gray and Piligrim (1952) fermented powdered wheaten hay by rumen liquor from a sheep fed on the same diet and obtained a rate of 8.3 m.moles between 2-4½ hours and 8.7 m.moles between 4½ and 7½ hours. Thereafter, the rate fell off sharply, possibly due to lack of substrate. Fermentation of rumen content for 4 hours from a hay fed steer, drawn 16 hours after feeding, produced acids at a rate of 8.3 m.moles per hour per kilogram while the 13 hours sample gave a rate

rate of 12.5 m.moles. Apparently the rate tended to fall off between 13 and 16 hours.

ARTIFICIAL RUMEN:

The advant in recent years of the rumen fistula and the artificial rumen technique has ~~xmpm~~ provided research tools which have contributed greatly to our knowledge of rumen function. Elsden, Johns and other co-workers first adopted the procedure of incubation of rumen content of mixed micro-biol population to study the rumen fermentation which opened a new chapter in this field. They used 'Washed suspension' of rumen micro-organisms and since then various improvements have been achieved in this field. Gradually, the'Artificial rumen' technique developed which is now adopted universally to conduct 'in-vitro' studies.

Pearson and Smith (1943) used undiluted rumen content in the incubating flasks. Marston(1948) improved the same by attempting to regulate the pH ~~mk~~ with the addition of suitable buffer and maintained anaerobic condition. Flow of artificial saliva at definite rate was definitely an improvement adopted by Burrough et.al. (1950). Louw et.al. (1949) used semipermiable vessel which allowed the elimination of fermented product by diffusion resembling close to 'in-vivo' condition. Warner (1956) used 'Cellophanesse sac' containing rumen liquor and substrate-it is dialysed against a mineral solution similar in composition to rumen liquor and got preditable result even upto 8 hours of incubation.

'In-vitro' studies can be carried out in small scale work under controlled condition, without significant alteration of bacterial population and pure microbiol population free from

from substrate contamination is obtained for critical studies on their enzyme system (Elsden, 1946).

For 'In-vitro' studies, 'in-vivo' condition must be maintained (Hungate, 1954) as the Cellulolytic bacteria (Hungate, 1950) and Protozoa (Hungate, 1942) of rumen are very sesitive to change in its environment.

However, in 'in-vitro' and 'in-vivo' studies quantitative difference rarely occur but quantitative difference may result which may be due to differential absorption of end products, change of P_H , altered bacterial metabolism, inactivation of enzyme in processing cell-suspension, exclusion of protozoa, ommission of important bacteria, lack of co-factors for certain re-action, assimilation and utilisation of VFA in absence of absorption and other 'in-vivo' condition (Hueter et.al. 1958).

In spite of these limitations, the 'in-vitro' studies provide satisfactory result provided the 'in-vivo' environment is maintained and the incubation period is short.

CHROMATOGRAPHY OF FATTY ACIDS.

The application of column chromatography was used in the separation of plant pigments. The process has since been extended to include, Gas-liquid partition chromatography by James et.al. (1952a), Liquid-liquid partition chromatography by Martin et.al. (1941).

For the estimation of ruminal VFA Gas-liquid, liquid-liquid partition chromatography are often used. For column chromatography both the procedure of Elsden (1946) and Peterson (1948) can be aep adopted. James et.al. (1952a, 1952b and 1956) used gas-liquid partition chromatography for separation of higher and lower acids as well as the esters of both saturated and unsaturated fatty acids .

Paper chromatography gives satisfactory result (Reid and Lederer, 1952), Menon et.al. (1962) used a new indicator which is very sensitive but cannot be used for quantitative estimation.

By the use of silica-gel column, Smith (1942) has been able to resolve chloroform plus 1% Butanol solution for formic , acetic propionic, n-butyric, and n-valerianic acid into their componant compounds. Although the butanol- chloroform-water system give satisfactory separation of C_1 - C_4 acids, it is not effective for acids having long chains (Elsden, 1946)and (Ramsey et.al.1945). When silica-gel tube was buffered, such a system may be used for C_2 - C_8 eid acids (Moyle et.al.1948). Peterson & Johnson (1948) were able to isolate the C_2 -C_{10} acids by employing Benzene-aquous-sulphuric acid in celite packed tubes.

HEAT INCREMENT & CALORIMETRIC CONSIDERATION OF VFA:

Kitzman & Benedict (1938) found that the heat increment in ruminant is high. The exact cause is not known but it may be due to rapid formation, absorption and metabolisation of VFA and on their quantitative aspects.

Armstrong & Blaxter (1957a,b; Armstrong et.al.1957c) determined heat increment of the individual acids in fasting and non-fasting sheep.

The heat increment of these acids in fasted animals, determined independantly, was found to be acetic 41 Cal.Propionic 13 Cal.and butyric 16 Cal. per 100 Cal. of metabolisable energy respectively. A mixture of the acids gave a heat increment of 17 Cal which was below per from that of the anticipated result. Therefore, the heat increment of VFA in fasting condition is of little practical importance.

Heat increment of these acids was found to be -acetic 67 Cal; Propionic 44 Cal; Butyric 38 Cal when determined at or above maintenance ration. These values were considerably higher from that of fasting condition.

The high heat increment of these acids may be due to greater part of these acids being used for fat synthesis.

The above findings indicate that VFA are relatively less well utilised for production as compared to in maintenance.

The relative efficiency of feed in maintenance and in fattening in the utilisation of the product of fermentation is on the average is 100:76 and this value is not constant (Armstrong and Blaxter,1957a; Armstrong et.al.1957b). However, the VFA are used with fairly constant efficiency of 85% to meet the energy need. They concluded that for fattening acetic acid is used with an efficiency of 33%, propionic 55%; butyric 62% . Thus the ratio of maintenance to fattening efficiency is calculated to be acetic, 100:39 ; propionic 100:65; butyric 100:73.

Armstrong et.al.(1958) as has pointed out that metabolisable energy of maize is utilised about 17% better way than that of hay for maintenance and 82% better for that of production. This is due to greater propionic acid production in maize.

USE OF BAGASSE AS CATTLE FEED:

Very few industrial ~~bi-products~~ bye products are used in feeding as animal feed in India. Molasses and molasses feed have been tried with success in West (Kellner,1926; Morisson, 1937).

Possibility of the use of bagasse with molasses and other feed stuff has been explored. Molasses feed (Mixture of concentrated molasses, oil cake and bagasse in varying proportion) and bagomolasses (concentrated molasses and bagasse)have been tried extensively.

The use of these feeds in the ration of both growing and milch animal was mainly based on the idea of utilising molasses as a concentrate and not on the use of bagasse as

as a roughage, for which practically no data are available.

Schneider & Rathore (1938) (quoted by Sen), Kothavalla (1942) and Mukteshwar workers (cited by Sen) used 'molasses feed' in place of concentrate both for growth and production without any significant difference in their effect.

Kothavalla (1942) explored the possibility of utilising bagomolasses as a substitute for a part of concentrate on one hand and the roughage on the other in the ration of milch and growing cattle.

In replacing a pound of concentrate by 3 lbs of bagomolasses, the growth rate was lowered but milk production was unaffected.

On replacing 3 lbs of green roughage by a lb.of bagomolasses caused improved rate of growth without any effect on milk production.

Schneider & Rathore (1936-38) successfully replaced wheat-bran by bagomolasses for milk production but not for growth.

The replacement formulae for concentrate with bagomolasses adopted by Kothavalla has been criticised by Roy & Talapatra (1945). According to them the beneficial effect produced in substitution of roughage by bagomolasses is probably due to protein sparing effect of the bagomolasses. This view has been supported by similar finding of Ayyar & Zubary (1945). They concluded that the protein rich concentrate can be replaced successfully by bagomolasses in working animal.

CHAPTER II.

...TERIAL AND METHODS.

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Table 2.1

...le showing the average composition
...ressed in percentage.

...isture	Ash	C.F.	E.E.	N.F.
8.83	2.07	37.55	1.54	48.76
5.50	3.40	8.50	4.20	60.80

...moisture the material was subjected to ...
...rotein was estimated by Kjeldahl's metho...

Bagomolasses did not contain any digestable protein or Ether extract. The digestibility co-efficient of total carbohydrate was 72: The T.D.N. value was found to be 47 (Roy & Talapatra,1945) Molasses feed on the other hand contain 7% D.C.P. and 40.6% S.E. (Lander,1938).

Mahadevan et.al. (1962) used a cattle feed mixture 50% of the bulk of which was the bagasse screening. They also incorporated urea in the ration which maintained the animals in positive nitrogen balance, when used as a sole feed. They also determined the digestibility co-efficient of the whole ration from which the data for bagasse alone is not readily available.

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CHAPTER II.

MATERIAL AND METHODS.

ANALYSIS OF FEED:-

The analysis was done only for two feed viz. Gram and Bagasse.

Bagasse was collected from local crushing centres and the material was pooled and samples from different parts were taken to the laboratory for routine analysis.

A representative sample of gram used in connection with the work was also analysed.

The average composition of both these materials is shown in Table 2.1.

Table 2.1

Table showing the average composition of feed expressed in percentage.

Material	Moisture	Ash	C.F.	E.E.	N.F.E.	Protein	Organic matter.
Bagasse.	8.83	2.07	37.55	1.54	48.76	1.25	89.10
Gram.	5.50	3.40	8.50	4.20	60.80	17.60	91.10

For moisture the material was subjected to 102°C for 10 hours. Protein was estimated by Kjeldahl's method. For crude

crude fibre analysis Weende 's method was employed. Total ash was determined by igniting the sample in muffle furnace at 575°C for 4 hours. For ether extract Seshan's method (1935) was employed i.e. extraction of the material in soxhlet by petrolium ether for 60 hours without any consideration of number of extraction per hour and uniform extraction.

Fistulated Animal:

One goat was fitted with 1" ebonite cannula following the procedure of Ash (1957).

The animal showed no untowards reaction and was feeding normally after about a week of operation.

Rumen liquor used in incubation:-

The rumen content used for incubation throughout this experiment was taken from slaughtered goats from the butcher's shop very near to the college immediately after the slaughter. The ruemn wall was slited by a sharp knife and about a pound of rumen content was taken in a wide-mouth bottle having Carbon dioxide (CO_2) passed into it from the laboratory. The bottle was then taken to the laboratory and CO_2 was again passed for 5 minutes.

The rumen content was strained through folded muslin and rumen liquor was pipetted into incubation flask.

A few samples were also collected from the fistulated goats, fed on concentrate mixture and green leaves, to check the results obtained from samples collected from slaughtered animals.

Incubation:-

The incubation was carried out in flasks having well fitted stoppered provided with both inlet and out let device in it. The inlet tube being well below the outlet one in the incubating flasks. Four c.c. (4 c.c.) of $\frac{M}{2}$ phosphate buffer having p_H 6.6 was added into the flasks before the addition of rumen liquor. 20 ml. of rumen liquor was pipetted into the flask and CO_2 gas was passed along the inlet tube to maintain anaerobic condition which was tested by passing the outlet gas in Barium chloride solution.

The bagasse used in incubation was finely powdered and 0.5 gm was added for 20 ml. of rumen liquor.

Glucose when added for incubation was in the form of 1 ml of 5% solution and was always prepared fresh.

The CO_2 was generated in Kipp's apparatus from marble chips and Hcl (1:1) . A trap was inserted in the circuit to arrest any Hcl if carried by chance by the stream of CO_2 .

The flasks were connected in series and placed in constant temperature water bath of 39-40°C.

Estimation of VFA in Rumen liquor:

The total VFA in rumen liquor was estimated by the method of Elsden et.al. (1946).

The rumen content was strained through double layer of muslin. To a measured volume of the rumen liquor was added an equal volume of 2N H_2SO_4 saturated with magnesium sulphate. A 10 c.c. aliquote of this mixture was taken in "Markham still" and was steam distilled . About 150 c.c. distillate was collected and was titrated with N/10 NaoH solution using Bromo-thymol blue as indicator. Further 25 c.c. of the distillate was also collected and titrated as a precautionery measure.

Care was taken to see that the steam distillation was not too first to make the tip of the condenser hot.

CHROMATOGRAPHIC SEPARATION OF THE VFA AND MEASUREMENT OF THEIR SPECIFIC ACTIVITY:

The whole process consists of the following steps which are described individually below. The Chromatographic procedure followed was on the lines recommended by the Bueding and Yale (1951).

1. Preparation of the VFA sample for chromatography.

The first 100 c.c. of Markham distillate was made distinctly alkaline after titration by the addition of an extra

extra drop of N.NaOH and evaporated down to a volume of about 20 ml. over an open flame. The use of a jet of CO_2 free air bubbling through the liquis, hastened the evaporation process considerably. The small volume of the distillate was then transferred to a round bottom 100 ml. flask and dried down completely on a water bath with the air jet directed on it.

Two different methods were used for the extraction of the acids into chloroform solution which is necessary for chromatography. In the first method 5 ml. of pure chloroform (dried over Na2 SO_4) and enough H_2SO_4 to turn the indicator pink were added to the dry mass and the suspension was transferred to a centrifuge tube. Enough anhydrous Na_2SO_4 was then added to the suspension until dry crystals of Na_2SO_4 appeared. The mixture was well stirred, centrifuged lightly and the supernatent fluid was decanted into a 25 ml. volumetric flask. The round bottom flask and the Na_2SO_4 were washed three times with 3 ml. portions of pure chloroform and then three times with 3 ml. portions of 1% butanol in chloroform, both dried over Na_2SO_4. The volume was then made upto 25 ml. with chloroform.

In the second method due to Elsden (1946a) , the dry powder was desolved in 0.2 ml. water and sufficient anhydrous $KHSO_4$ powder was added to form a dry mass which did not stick to the sides of the flasks. The powder was then extracted 6 times with small volumes of 5% butanol in chloroform dried

dried over Na_2SO_4 and then made upto volume.

Although efficiency of extraction was found to be nearly 100% by both these methods, the second one was found to have distinct advantage in the fact that the reagent blank was constant and negligible compared to the first, in which it was both high and variable probably due to varying traces of H_2SO_4 remaining in the chloroform.

2. Preparation of the Chromatographic column.

The celite - Johns- Manville No.535 (Celite 535) used in the chromatogram was prepared for this purpose by boiling it vigrously in concentrated HCl for 30 minutes, to remove most of the iron in it. The supernatant HCl was decanted off and the celite was repeatedly washed with about 3 litre portion of tap water till nearly free from HCl, when it was washed twice with the same volume of distilled water till completely free from HCl. During the washings a fair amount of the finer particles of celite that failed to settle down within 15 minutes was also decanted off. The celite was then dried in an oven at 110°C. to a constant weight. The dried celite was then washed with ether by pouring it over the celite in a Buchner's funnel and drawing off the solvent by suction. The ether washed celite was finally dried in a desicator under continuous evacuation.

8 gm of this processed celite was thoroughly ground in a morter with 4.8 ml.of 2 M phosphate buffer of p_H 6.5 , so that the buffer was uniformly distributed in the celite.

Sufficient Chloroform equilibrated with the buffer was added to the celite until a slurry was obtained.

The end of the column, of about 12 mm. internal diameter was plugged with glass wool to act as a support for the celite. A few ml. of chloroform was run through the glass wool which was tamped again to remove the air bubble in it. About 0.5 gm. of the celite slurry was introduced into the column with the help of a funnel and sufficient chloroform was poured to make a suspension of it. The suspension was stirred by a slender rod and then tamped down by another rod which had a flattened end almost as large in diameter as the colum. The rod was given a twisting motion as it was lowered and an even downward pressure was exerted on the celite while packing it. The column was ~~exerted on the celite while~~ rotated slowly and the egge of the pack was tamped round with the slender rod. The next charge of another 0.5 gm. of celite was then introduced and all the above mentioned steps were carefully gone through. ~~xxx~~ After the last addition of the celite all floating particles were tamped down so that the column surface was flat and even. Particular care was taken to follow this packing procedure in every details so as to avoid the formation of channels and consequent poor resolution.

3. Separation of the acids on the chromatogram and their titration:

The small volume of the solvent always remaining above the celite column was allowed to flow through until its level

level just reached the surface of the celite. From 3-5 ml. of the solution of the acids in the solvent containing from 100-150 micro moles of total acids was allowed to flow into the celite. The sides of the column were washed twice with 3 ml. portions of pure chloroform (CBO). The column was then filled with CBo and 5 or 10 ml. samples were collected for titration. Very often the first fraction of the eluate was collected in bulk of 50 ml. to save time and error in titration.

When the titration reading of the last sample of the eluate dropped down to blank value, the receiver was changed and after the remaining volume of CBo passed through the column, it was filled up with the next solvent 5% butanol in chloroform (CB_5) and the same procedure as described above, was followed till the titration reading again dropped down to the blank value when the last solvent 20% butanol in chloroform (CB_{20}) was used.

The titration of the eluate was carried out with N/200, methanolic KOH using Bromothymol blue as indicator. Before the titration of the CBo fractions 10 drops of methanol was added to it so as to avoid the false and point that is often noticed when the amount of butyric acid is less than 5 micromoles. This step was found to be essential to avoid confusion in the titration of the CBo fractions as pointed out by Peterson and Johnson (1948).

For titration with aqueous N/200 NaOH, an equal volume of water and 2 drops of bromothymol blue were added to the eluate.

In both the cases the mixtures was aerated by bubbling CO_2 free air through it for 3 minutes before the commencement of titration and also during the process. Particularly vigorous bubbling was needed towards the end point for the removal of the last traces of the acids from the organic to the aqueous phase.

The natural flow of the solvent through the column was often found to be too slow and it was usually necessary to apply increased air pressure to maintain a flow rate of one drop per second. The efficiency of separation of the acids was found to be unaffected by variation in the rate of the flow of the solvents.

For the separation of the C_6, C_5 and C_4 fatty acids the same procedure was exactly repeated excepting that a buffer of pH 7.6 was used as the stationary phase and the solvents were equilibrated with it. Separation of higher acids was not undertaken in this work.

Recovery of CB Waste:

The waste CB mixture was made distinctly alkaline by the addition of few KOH steaks to neutralised any free acids present. The entire bulk was then washed in tap water by bubbling for about 3 hours to remove all the alkalic and methanol from BC waste.

The CB layer was then separated in separating funnel and the separated CB mixture was distilled in a glass distilled apparatus in over an electrical water bath.

The distilled CB mixture was then treated with $CaCl_2$ grannules (5 gm per 1000 ml.) to remove traces of water. It was then filtered through filter paper and absolutely water free CB thus obtained was used to prepare CB_5 and CB_{20} and equibriated to phosphate buffer.

REAGENTS USED :

1. 2M phosphate buffer, pH 6.5- 104 gm. K_2HPO_4 and 190 gm KH_2PO_4 per litre.
2. Butanol-n-butanol, reagent quality.
3. Chloroform - BP grade, washed with distilled water for 2-3 hours to remove the alcohol added to it for es stabilisation.(ICI)
4. Methanol- reagent quality.
5. Solvents- pure chloroform (CB_0), 5% butanol in chloroform (CB_5) and 20% butanol in vhloroform (CB_{20}). All solvents were equilibrated with the same buffer which is used as the stationary phase of the column. 5 ml.of the buffer per 100 ml.of solvent was thoroughly shaken in a separating funnel. After separation of the two layers, the solvent was filtered in order to remove any water remaining in suspension.
6. Bromothymol Blue indicator- 0.04 gm. of the indicator in 100 c.c. of absolute alcohol.

Digestion trial:-

To study the nutritive value a series of digestion trial was carried out in Jamnapari goats(Male).

A pre-experimental period was given for 7-10 days in each set and the collection period was extended for 7-10 days.

The pre-experimental period was extended about 20 days in the first set of experiment so as to make the animals familiar with the new type of ration and the environment. Pre-experimental period in India as suggested by Sen, should be for 7-15 days and the collection period should extend for 10 days. At Pennsylvania State College, the preliminary period often ranges between 4-5 days. Mitchell et.al. (1932) considered that for digestion trial a short preliminary period was sufficient whereas a long preliminary period was inevitable for balance studies.

In the present work no balance studies was adopted and as such a moderate preliminary and experimental period was planned.

A total quantitative collection of faeces was made for 7-10 days as per the experimental set up. The food consumption was recorded on dry matter basis daily. Account for the loss of moisture in the residual feed in the next day was also made.

A certain aliquote of fresh faeces voided was taken which was mostly 1/3 of the total quantity. In some cases ¼

of the fresh faeces was taken as aliquote to determine the dry matter present in the total faeces. The dried faeces was kept in stock and in this way the dried faeces for whole of the experimental period was collected for each individual experimental animal.

The composite dried faecal sample thus obtained was then analysed at the end of the collection period.

Throughout the preliminary period as well as in the experimental period care was taken not to change the plane of nutrition at all. A protein source i.e. a concentrate feed viz. gram was given to these animal. In the first set of experiment 55 gm. of Gram was given throughout the both period and in the second set onward 88 gm. of Gram was given.

Considering vitamin A requirement, Cod liver oil, one tea-spoonful per week per head was administered.

An attempt was made to determine the effect of soluble sugar on the utilisation of the crude fibre in the bagasse. Molasses was added to the ration on the basis of 5% of total feed consumption and next it was increased to 10% of the dry matter consumption.

In these two cases the composite dry faecal sample was nalysed only for crude fibre.

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CHAPTER III.

ESTIMATION OF ERRORS IN VARIOUS EXPERIMENTAL PROCEDURES:

Error involved in steam distillation of ruminal VFA:-

The method adopted in this experiment invariably gave highly reproducable recovery.

The efficiency of 150 c.c. distillate to remove 100% of the VFA was tested in the following experiment.

A known amount of standard acid was steam distilled in 'Markham still' in the usual way and 4 successive 50 c.c. distillate was separately collected and titrated against standard alkali. The result is shown in table 3.1 :

Table-3.1:

Table showing the recovery of VFA in successive fractions of distillate (in percentage):

No.of observations	1st fraction.	2nd fraction.	3rd fraction.	4th fraction
4	76.2	14.7	9	Nil

It is seen from Table 3.1 that the last 50 c.c. invariably contain traces, if any of the acids and that 100% of the acid was invariably recovered in 150 c.c. distillate. As such 150 c.c. of the distillate was collected as a routine procedure. The efficiency of distillation - both too slow and two fast rate produced errors. A period of 15-20 minutes was found to be optimum and was regularly followed.The result is also shown graphically in figure-1.

The efficiency of the recovery in this method was tested by distilling the standard acid solution and titrating the 150 c.c. distillate against standard alkali. The recovery test was done at different room temepratures. The close agreement of duplicate recovery from rumen liquor which was invariably obtained also testify the accuracy of the full process.

The result of the recovery with standard acid is shown in Table 3.2:

Table-3.2:

Table showing the efficiency of distillation of VFA in 'Markham still' (m.mole of acid):

No.of observation	Total acid used for distillation.	Total acid recovered.	Percentage recovery.	S. E.
4	0.4	0.406	101.5	± .001
4	0.8	0. 79	97.5	± .002
4	1.0	0. 99	99.0	± .001

It is seen from the above table that recovery from the distillation process adopted in this work invaiably recovered nearly 100% of the VFA.

Loss of VFA on exposure to room temperatures:-

The room temperature varied from 25-40°C during the period through which the experiment was comducted. As such it was necessary to assess the error which may crop up due to exposure of VFA within that range of temperature.

This point was investigated in the following experiment.

A known amount of standard acid was exposed to room temperature at 37°C and at 40°C in hot air oven for half an hour and the loss of acid determined. The results of this experiment is shown in Table 3.3:

Table- 3.3:

Loss of vFA on exposure to 37° and 40°C for half an hour:

Temp.to which exposed.	No.of observations	VFA in m.mole Before exposure	VFA in m.mole After exposure	Percentage loss.
37°C	4	0.5	0.5	Nil
40°C	4	0.5	0.49	2

From the above table it was clear that between 32-37°C there was no loss. The loss was only 2% even at the high temperature of 40°C. As such no percipitable error would arise from this source.

Error due to exposure of rumen content:-

The microbial population in the rumen are very sensitive and any change in temperature, moisture and its atmosphere is detremental for their normal thrive. As some amount of exposure in air will invariably occur between collection and incubation, it was necessary to find out the maximum time of exposure which won't cause any effect on subsequent microbial activity.

Rumen liquor from a rumen sample was obtained in the normal way. The beaker containing the rumen liquor was exposed to atmosphere. 20 ml. of this liquor was taken in the incubation flasks after 0 hour, 15 minutes and 30 minutes and 1 hour exposure and the fermentation rate of each sample was determined in the normal way in four hours incubation. The result is shown in Table-3.4:

Table-3.4:

Table showing the VFA produced on 4 hours incubation of rumen samples exposed to air for different periods. (m.mole of acid/1000 ml.of rumen liquor)

No.of observations	VFA produced			
	No exposure.	15 minutes exposure.	30 minutes exposure.	1 hour exposure.
12	86.00	84.00	58.00	52.00

It is seen from the above table that no perciptable adverse effect was observed even after exposure of the rumen liquor to atmosphere for 15 minutes. On statistical analysis it was found that there was no significant difference between the VFA produced after immediate incubation and 15 minutes exposure. Un-to-wards effects was observed on 30 minutes and 1 hour exposed samples.

Error involved in Chromatographics separation:-

A Standard solution of all the three acids were prepared in chloroform and a mixture of the three solution were prepared in the ratio of 1:2:6 in CB_5 so that the concentration of 5 c.c. of the mixture did not exceed 150 micromoles of VFA which is guarded a safe load for the column.

5 c.c. of the mixture containing ~~xx~~ known amount of the acid was used in Chromatogram and recovery of the each of the acids was tested by the normal way. The result is shown in Table-3.5.

Table-3.5.

Table showing the percentage recovery of VFA in Chromatography.

Elaute fraction.	No. of obs.	N/200 Methanolic alkali		Percentage recovery.
		Initial	Recovered	
CB_0	4	3.3	3.1	96
CB_5	4	7.2	7.1	98
CB_{20}	4	18.0	17.9	99

It is seen from the above table that the recovery of all the ~~thxee~~ acids by chromatographic separation process ~~xxx~~ adopted in this work was nearly 100 percent in each case.

In a preliminary trial run, the volume of elauent, needed to elaute completely the related acids, successive ~~xxxXXXXXXXXX~~ 10 ml. of the elaute was titrated with alkali. The process was continued till a particular 10 c.c. elaute showed no trace of acid in it. The volume of CB_0 , CB_5 and CB_{20} to elaute butyric , propionic, and acetic respectively from the column was found in these preliminary trials to be 80 ml., 70 ml. and 120 ml.

In experimental run, the process was shorten by collecting bulk samples of 50 c.c. in case of CB_0 and CB_5 and 80 c.c. in case of CB_{20}. and subsequent 10 c.c. samples till the reading dropped down to blank value.

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CHAPTER-IV.

INVITRO INCUBATION OF BAGASSE:

This part of the investigation was undertaken to dertermine the extent of fermentation of bagasse by the cellulolytic orgnisms present in rumen.

Effect of varying periods of incubation:-

The rumen samples were collected from slaughtered goats as described under material and method. An experiment was run in which the effect of varying periods of incubation on bagasse fermentation was studied to determine the total VFA produced as well as the rate of production at different time interval. In this experiment a zero hour control, was kept and rumen liquor was incubated alongwith bagasse. The period of incubation was 4 hours, 8 hours, 12 hours and 24 hours. The result is shown in Table 4.1:

Table-4.1:

VFA produced on fermentation of bagasse at different length of incubation.
(mM acid /1000 ml. of rumen liquor)

Period of incubation.	No.of observations	VFA at zero hour	VFA after incubation	VFA produced	Rate per hour.
4 hrs.	10	75.4	120.7 ± 4	45.3	11.3
8 hrs.	10	75.4	164.8 ± 7	89.4	11.1
12 hrs.	10	75.4	168.6 ± 7	93.2	7.7
24 hrs.	10	75.4	177.4 ± 5	102.0	4.2

The above table indicates that the maximum VFA content was on 8 hours of incubation. A steady range of VFA was maintained

maintained throughout upto 24 hours of incubation.

In the table the rate of fermentation at various time period has also been indicated. The rate of fermentation was maximum in 4 & 8 hours of incubation. The fermentation rate decreased between 8-12 hours and was minimum in 24 hours. The rate in respect of incubation period is represented graphically in figure-2.

Fermentation of bagasse:

Several experiments were undertaken in which the fermentation of rumen liquor alone, fermentation of bagasse with rumen liquor and fermentation of bagasse in presence of glucose were studied. The result is shown in table 4.2

Table-4.2

Result of incubation of bagasse for 4 hours with different added substrate (m.moles of VFA/1000 ml.of rumen liquor)

Sources	No.of observations	VFA produced Average	VFA produced Range	Rate per hour Average	Rate per hour Range
From rumen liquor.	15	23.4 ±4.4	4-62	5.7 ±0.89	1-15.5
From Glucose.	14	32.0 ±6.6	12-56	8.0 ±1.50	3-14.0
From Bagasse.	15	39.0 ±5.4	6-92	9.8 ±1.60	1-26.0
From bagasse in presence of Glucose.	10	35.8 ±10.6	3-120	8.9 ±10.6	3-30

From the table it appears that bagasse is well fermentable. Rumen liquor alone on incubation yielded 23.4 m.moles of acids whereas VFA produced on fermentation of bagasse was 39.0 m.moles.

This high VFA production out of bagasse clearly suggests that the organic nutrient in it are well fermentable.

Effect of addition of Glucose:

Although bagasse contains 48% NFE, it was thought worth while to study the effect of soluble sugar on the rate of fermentation of bagasse. The effect of added glucose to rumen liquor served as control. The result is shown in table 4.2.

It is seen from the table that rumen liquor alone produced 23.4 m.moles of VFA on fermentation whereas on fermentation of glucose 32.0 m.moles of acids was produced. On the other hand 39.0 m.moles of acids were produced out of bagasse fermentation. It indicates that 0.5 gm. of bagasse produced almost equal amount of VFA as from 50 mgm. of glucose. The VFA produced from bagasse in presence of glucose was calculated by substrating the VFA produced on fermentation of rumen liquor with glucose from that produced out of rumen liquor, glucose and bagasse and was found to be 35.8 m.moles, which is in close agreement as that found without glucose.

Therefore, the VFA produced from bagasse with or without glucose were found to be 35.8 and 39.0 m.moles respectively. The addition of glucose to bagasse, however, had no significant effect on the fermentation of the later, perhaps due to availability of sufficient soluble sugar from bagasse itself.

Rate of fermentation:

The rate of fermentation of bagasse with or without glucose was calculated from incubation results. It is shown in table 4.2 From the table 4.2, the fermentation rate for incubated rumen liquor was found to be 5.7 m.moles/ 1000 ml. of rumen liquor whereas rate of bagasse as well as for glucose was in the order of 9.8 and 8.0 m.moles respectively. The rate of fermentation of bagasse in presence of glucose was ~~ela~~ calculated to be 8.9 m.moles, which, however, did not show any beneficial effect of the added soluble sugar on the rate of fermentation.

The range of the rate of production of VFA per hour per 1000 c.c. of rumen liquor has also been indicated in table 4.2. It shows that there was enormous fluctuations in the rate of production between samples. The range for the rate for rumen liquor was found to be 1-15.5 m.moles per hour and 3-14.0 m. moles was the range for the glucose. Wide fluctuation was noted for bagasse in presence and in absence of glucose which were 3-30.0 and 1-26.0 m.moles per hour respectively.

Fermentation of bagasse by rumen liquor drawn from fistulated goat:

With a view of compare the rate of production of VFA from 'in-vitro' fermentation of bagasse by more physiological rumen samples with that obtained from samples from slaughtered goats, fermentations experiments were undertaken with rumen liquor from a fistulated goat maintained on wheatbran, gram and green leaves. The individual VFA was not separated but ~~the~~

but the total acid production was estimated. The result of the finding is tabulated in table ~~4.3~~ 4.3.

Table-4.3

Rate of production of VFA from bagasse with samples drawn from fistulated animals on 4 hours incubation. (m.moles of VFA per 1000 ml. of rumen liquor).

Sources.	No. of obs.	VFA produced.	Rate per hour Average	Range.
Rumen liquor.	4	25.2	6.3 ± 0.7	5-8.5
Bagasse.	4	43.0	10.7 ± 0.6	9.5-12.0

The total acid produced on fermentation of rumen liquor was 25.2 and hence the rate was calaculated to be 6.3 with a range of 5-8.5 m.moles. On the other hand the VFA production of bagasse fermentation was 43.0 m.moles. The rate was determined to be 10.7 with a range of 9.5 to 12.0.

Comparing the table 4.2 and 4.3 it was found that the rate of bagasse fermentation did not differ materially within the samples from slaughtered animals and fistulated goats. The rate was found to be bit higher in the latter case. The fluctuation in the rate as seen from the range is rather wide in cases from slaughtered goats whereas the range was noted to be rather narrow in the samples from fistulated animal.

Composition of VFA produced on fermentation:-

The individual fatty acid produced on fermentation were analysed and the percentage composition of the three acids viz.

acetic, propionic and butyric acids present at zero hour, on 4 hours of incubation and produced with the addition of bagasse have been shown in table 4.4

Table-4.4

Individual fatty acids obtained under different conditions: (Expressed as percentage):

Material	No. of obs.	Acetic Ave-rage	Acetic Range	Propionic Ave-rage	Propionic Range	Butyric Ave-rage	Butyric Range
Un-incubated rumen liquor.	5	68.9	68.3-69.8	16.7	15.8-17.5	14.4	12.8-15.6
Incubated rumen liquor.	4	69.7	68.7-70.1	16.2	15.2-16.8	13.1	11.8-14.3
Rumen liquor with baggase.	5	66.4	65.1-68.3	23.5	23.1-23.8	10.1	7.7-11.2

The table 4.4. above indicated that the proportions of the 3 acids did not change much within the samples.and between the first two treatments viz. Incubated and un-incubated rumen liquor.

On the other hand, the samples containing bagasse showed a higher amount of propionic acid and a little fall of butyric acid but the acetic acid composition remaining almost the same.

It is interesting to find that the percentage composition of VFA in the rumen liquor obtained from slaughter animal did not materially differ from that of the acid produced from its 'in-vitro' fermentation.

A probable reason for this agreement may be found in the fact that both the 'in-vitro' and 'in-vivo' fermentation pattern could be similar to a great extent.

The rate of production of individual fatty acids from bagasse was calculated. The result is shown in table 4.5

Table-4.5.

Rate of production of individual fatty acids on fermentation of bagasse.(m.moles of VFA per 1000 ml. of rumen liquor).

Acids	Total VFA produced in Rumen liquor & bagasse.	Incubated rumen liquor	VFA produced from bagasse	Rate/ hours
Acetic.	78.4	54.37	24.0	6.60
Propionic.	27.3	12.63	15. 1	3.77
Butyric.	11.9	10.20	1.7	0.42

The total VFA produced obtained from totalling the three acids is 40.8 m.moles per 1000 ml. of rumen liquor which agrees very well with that of the experimentally observed total VFA production of 39.0 m.moles (Table-4.2).

The amount and the percentage composition of the three acids produced from fermentation of rumen liquor as also from bagasse were calculated and the data presented in table 4.6.

Table-4.6

Composition of VFA produced on Incubation:

Material	Amount of acid(m.mole per 1000 ml.)			Percentage composition.		
	A	P	B	A	P	B
Incubated rumen liquor.	10.27	1.93	1.00	77.00	14.62	7.70
From bagasse.	24.00	15.10	1.70	58.80	37.00	4.20

From the table 4.6 it appears that the rumen liquor on incubation for four hours produced 77 percent acetic, 14.6 percent propionic and 7.7 percent ~~butyric~~ butyric acid, whereas the fermentation of bagasse in percentage composition of the acids are acetic-58.8 percent, propinic-37 percent and butyric acid-4.2 percent. Propionic acid concentration was much higher on bagasse fermentation at the cost of both acetic and butric acids. No data for the percentage composition of the acids produced from fermentation of glucose by rumen liquor was obtained as this point has no direct bearing on this work.

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CHAPTER - V.

D I G E S T I O N T R I A L.

Digestion trial was conducted in Jamunapari goats (male) to determine the nutritive value of the different organic nutrients present in bagasse.

The trial was conducted in four sets- In the first set the animals recived low level of protein, in the second set the animals received 12 grms. of digestible protein which was presumed to be sufficient for maintenance. In the third and fourth sets, the animals were given some molasses along with the concentrate which was 10 gms. and 20 gms (On fresh weight basis) respectively. Common salt was added to the ration half ounce per head per day in all the sets.

In the first set of the experiment, the animals were kept on 55 gms. of gram and bagasse adlib. and the collection period was for 10 days.

The result of the first set of trial is shown in table 5.1.

From the table 5.1 it is seen that the digestibility co-efficient of the dry matter as well as other nutrients is fairly high. Individual variations in the digestibility were also noted.

In the second set of trial, the animals were given 88 gms. of gram and bagasse adlib. This was expected to supply 12 gms. of digestible crude protein which was sufficient for the maintenance

of these animals on the basis of Morrison's standard assuming the DCP and TDN requirements to be 0.06 lb. and 0.75 lb per 100 pound body weight. The collection period ~~xx~~ in the second set was for 7 days. The result of the 2nd trial is shown in Table-5.2.

Table-5.1

Data of digestion trial of bagasse with low protein intake.

Nutrient	Animal	Intake in Gram gms.		Excreted in gms.		Amount digested from bagasse.	Digestibility co-efficient.	Average digestibility.
		Bagasse.	Gram	Bagasse.	Gram			
Dry matter	1.	167.38	50.00	58.18	20.00	109.20	65.00	61.6
	2.	156.49	50.00	73.75	20.00	82.74	52.00	
	3.	135.77	50.00	43.48	20.00	92.29	67.90	
C.F.	1.	62.85	5.00	21.99	2.00	40.86	65.00	61.3
	2.	58.76	5.00	26.51	2.00	32.25	54.00	
	3.	50.98	5.00	17.44	2.00	33.54	65.00	
NFE.	1.	81.61	31.09	31.85	12.40	49.76	60.90	56.7
	2.	76.30	31.09	41.47	12.40	34.83	45.60	
	3.	66.20	31.09	23.96	12.40	42.24	63.70	
EE.	1.	2.58	2.11	0.79	0.34	1.78	68.90	64.7
	2.	2.41	2.11	0.97	0.34	1.44	59.80	
	3.	2.09	2.11	0.72	0.34	1.37	65.50	
Total carbo-hydrate:	1.	144.46	~~xx~~-	53.84	-	90.62	62.70	59.0
	2.	135.06	-	67.98	-	67.08	49.60	
	3.	117.18	-	41.40	-	75.78	64.70	

N.B.:- Dry matter and crude fibre digestibility of gram were assume to be 60 percent and other values were taken from I.C.A.R. Bulletine No.25 by Sen (1952).

Table-5.2.

Result of second set of digestion trial:

Nutrient	Animal	Intake in gms. Basse.	Intake in gms. Gram.	Excreated in gms. Basse.	Excreated in gms. Gram	Amount digested from (B)	Digestibility co-eff.	Average dig. coeff.
Dry matter:	1.	199.70	80.00	71.50	32.00	128.20	64.00	
	2.	188.30	80.00	63.12	32.00	125.18	66.00	63.5
	3.	120.70	80.00	45.75	32.00	75.13	60.50	
C.F.	1.	75.00	8.00	26.80	3.20	48.20	64.00	
	2.	70.70	8.00	23.68	3.20	47.02	66.40	62.8
	3.	43.30	8.00	19.01	3.20	26.29	58.00	
N.F.E.	1.	97.39	50.40	42.90	20.20	54.49	55.00	
	2.	91.81	50.40	35.37	20.20	56.44	61.00	58.0
	3.	58.83	50.40	24.65	20.20	34.18	58.00	
E.E.	1.	3.07	3.40	0.95	0.54	2.12	69.00	
	2.	2.99	3.40	0.68	0.54	2.31	77.00	71.6
	3.	1.80	3.40	0.55	0.54	1.25	69.00	
Total CHO.	1.	172.39	-	69.70	-	102.69	59.70	
	2.	162.51	-	59.05	-	103.46	63.60	60.4
	3.	104.13	-	43.66	-	60.47	58.10	

N.B.:- Dry matter and crude fibre digestibility of gram were assume to be 60 percent and other values are taken from I.C.A.R.Bulletin No.25 by Sen (1952).

From tables 5.1 and 5.2 above, it was found that the dry matter digestibility was 61.6 percent on the average in the 1st trial. The values for crude fibre, N.F.E. and E.E. and total carbo-hydrate were 61.3 , 56.7, 67.7 and 59.0 respectively in the 1st trial wereas, in the 2nd set the digestibility of dry matter, crude fibre ~~xxx~~, N.F.E., total carbo-hydrate and E.E. was found to be 63.5, 62.8, 58.0, 60.4 and 71.6 respectively.

On comparison between the two sets of trial from the above tables, it was also found that the digestibility of all the nutrients including the dry matter, in the 2nd set of trial tended to show a slight increase from that of the 1st set. On further calculation it was found that the digestibility co-efficient of crude fibre, NFE and total carbo-hydrate improved by 2.4, 2.2 and 2.3 percent respectively, whereas the value was as high as 10.6 percent in case of ether extract.

Whether this improvement of digestibility was due to excess of protein or some other factor was not investigated.

The digestible nutrients as calculated from the trial is shown in table-5.3 and 5.4. [of the 1st and 2nd set of trial respectively.]

Table-5.3.

Digestible nutrient of bagasse:

Animal	Digestible nutrients in 100 gm.			T D N.	Average T D N.
	C.P.	Carbohydrate	Ether extract		
1.	-	53.00	2.38	55.38	
2.	-	42.52	2.07	44.59	52.54
3.	-	55.40	2.25	57.65	

Table-5.4.

Digestible nutrient of bagasse as calculated from second set of trial:

Animal	Digestible nutrients in 100 gm.			T D N.	Average T D N.
	C.P.	Carbohydrate	Ether extract		
1.	-	50.87	2.38	53.25	
2.	-	56.66	2.67	59.31	55.00
3.	-	50.07	2.38	52.45	

The average TDN of bagasse was calculated to be 52.54 from the 1st set of trial (Table-5.3) and was calculated to be 55.00 from the 2nd set of trial (Table-5.4).

The effect of addition of molasses on the utilisation of crude fibre have been studied in the 2nd set of trial. In this case the animals received 88 gms. of gram, 10 gms. (fresh weight basis) of molasses (5% of dry matter consumption, taking 200 gms. as the average dry matter consumption per day) and bagasse ad adlib. In this set only the digestibility of crude fibre was determined. The average intake and outgo of crude fibre as well as the digestibility co-efficient is shown in table-5.5

Table-5.5.

Data of digestibility of crude fibre of bagasse with 10 gms. (fresh weight) of molasses.

Animal	Intake (gms)	Excreted (gms)	Amount digested	Digestibility coef.	Av. digestibility
1.	78.19	22.31	55.88	71.00	
2.	53.97	19.77	34.20	63.00	68.6
3.	58.57	15.99	42.58	72.00	

It is seen from table-5.5 that the addition of molasses has improved the digestibility co-efficient of crude fibre . The digestibility co-efficient of crude fibre increased to 68.6 from 62.8, which is an increase of 10.64% over the 2nd set of trial.

The reason for this beneficial effect of addition of molasses may be due to the readily available source of energy from it (Soluble sugar) for the rumen micro-organisms.

The effect of further increase of molasses on the digestibility of crude fibre of bagasse was studied in the last set. In this set, the animals received the same amount of concentrates as that in the 3rd set but the molasses consumption was increased to 20 gms. The result of 'in-take and out-go of crude fibre from bagasse is shown in table-5.6.

Table-5.6.

Data of digestibility of crude fibre of bagasse with 20 gms. of molasses.

Animal	Intake in gms.	Excreated in gms.	Digested	Digestibility co-eff.	Average digest.
1.	73.48	31.88	41.60	56.00	
2.	73.65	29.13	44.52	60.00	58.30
3.	49.35	19.94	29.41	59.00	

The digestibility of crude fibre as seen from the Table-5.6 was found to be diminished in all the three animals. The digestibility was 62.8 in second set whereas it was only 58.3 in the fourth set. Thus, it was found that addition of 20 gms. (fresh weight) of molasses reduced the digestibility co-efficient of crude fibre by 5.9% . This reduction may be due to excess of soluble carbo-hydrate which would be attacked by the micro-organisms preferentially to the crude fibre of the diet.

However, the effect of added molasses on the digestibility of crude fibre needs further and fuller investigation before any firm conclusion on the lines suggested above could be drawn.

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CHAPTER- VI:

D I S C U S S I O N:

Estimation of VFA:

As the ruminal fatty acids are volatile and the room temperature is generally high in India, in the direct steam distillation method of Elsden et.al. (1946), some of the fatty acids may escape condensation or be lost by evaporation. It is probable that this method may not work as efficiently as in the West due to high tropical temperature.

Keeping this in view, a modification of the direct method was tried as a preliminary part of the work. In this modification, a system of back titration was introduced. A known amount of standard alkali (NaOH) was taken in a beaker and the collecting tube of the condenser was dipped into the standard alkali. After the collection of about 150 c.c. distillates, the excess of alkali was titrated against standard acid solution. From the difference, the VFA aboserbed in the alkali was determined.

This modified method, when used in recovery test proved to be an utter failure. It always gave high recovery irrespective of the indicators used in the titration.

The reasons for the impracticability of this modified method are innumerated below.

NaOH being strong alkali, will absorb CO_2 from the atmosphere in course of distillation and thereby some of the alkali will be neutralised even in the absence of VFA. This error can, however, be eliminated by using methyle orange or bromo-phenol blue instead of bromo-thymol blue as indicator. However, the methyl orange and bromo phenol blue having the pK 3.7 and 4.0 respectively, cannot be used in VFA titration due to the fact that Sodium and Potassium salts of fatty acids are alkaline in reaction and as such the end point of VFA titration should be in the alkali range.

This point was further clarified in the following point. To 5 c.c. of N/10, NaOH , 5 c.c. of N/10 VFA mixture was added, using different indicators. The expectations was that these two acid-alkali reaction will neutralised each other and as such no further acid to be added to have the neutral point. But, actually it was not so. It is shown in the table-6.1.

Table-6.1

NaOH and VFA titration with different indicators.

ml.of N/10 NaOH used.	N/10 VFA added(ml.)	Indicator used.	N/10 acid required to produce acid colour of the indicator.
5 c.c.	5 c.c.	Methyle orange.	5.0 c.c.
5 c.c.	5 c.c.	Bromo phenol Blue.	4.3 c.c.
5 c.c.	5 c.c.	Bromo thymol Blue.	0.1 c.c.

The table 6.1 indicates that additional 5 c.c., 4.3 c.c. of N/10 acid was required to have the acid colour when methyl orange and Bromo phenol blue were used respectively.

The titration with indicators having pK in the acid region will invariably give higher and misleading value. Although it may eliminate the error from absorption of carbon dioxide from atmosphere. It may be mentioned here that the error from CO_2 absorption by the usual direct titration method will be negligible because of the very low solubility of CO_2 in acid solution.

Anaerobiosis of incubation flasks:

Maintenance of anaerobic condition in the incubation flask is of paramount importance in the 'in-vitro' studies. This particular point was studied and was found to be quite satisfactory in maintaining anaerobic condition in the flasks by the CO_2 generated in Kipp's apparatus. 20 ml. of water was taken in the 4 incubation flasks which were connected in series with Kipp's apparatus. The extent of displacement of the air of the flask by CO_2 was ascertained by noting the change of pH of water in the flask and as also by the effect on a lighted tapper held at the end out-let. The table-6.2 given below will speak for itself.

The saturation point of CO_2 in water of the flask or its minimum pH, indicated complete expulsion of air from the flasks by CO_2 which were attained in 5 minutes when taken

singly and after 15 minutes when 4 were in series. When further tested with a lighted tapper, it was found that it extinguished after 3 minutes from the 1st flask, 5 minutes from the 2nd 8 minutes from the 3rd and 10 minutes from 4th and it took 15 minutes when all were connected in series.

Table-6.2.

Effectiveness of displacement of air of the flask by CO_2.

Flask No.	Specification	Initial pH.	Final pH	Effect on lighted tapper
I.	Flasks connected in series and CO_2 passed for 15 minutes.	7.8	5.8	Tapper extinguished on 15th minute.
II.	"	"	5.8	"
III.	"	"	6.0	"
IV.	"	"	5.9	"
I.	CO_2 passed for 5 minutes.	7.8	5.8	Tapper extinguished.
I.& II.	Connected in series and CO_2 passed for 10 minutes.	7.8	5.8	Tapper extinguished.

Buffering effect:-

In 'in-vitro' studies an optimun pH should be maintained by means of a suitable buffer so that excess of VFA produced on fermentation do not inhibit the bacterial activity. The buffer used in the present work was M/2 phosphate buffer having pH 6.6.

The pH was 6.6 when 4 c.c. of these buffer was added to 20 ml. of rumen liquor. There was a change in pH on 4 hours of incubation. The changes in pH under various conditions of incubation have been shown in Table-6.3.

Table-6.3

Chanage in pH on incubation for 4 hours with 4 c.c. of M/2 buffer (pH 6.6.)

Incubated rumen liquor	Rumen liquor with glucose	Rumen liquor with bagasee	Rumen liquor with bagasse & glucose.
6.6	6.1	6.1	5.8
(6.9-6.5)	(5.9-6.4)	(5.6-6.5)	(5.3-6.1)

Figures within brackets indicates the Range and the other one is the average.

From the table it is seen that the pH changed towards acidity. The maximum fall in pH was noted when the rumen liquor bagasse and glucose were incubated. It was also noted that fall in pH was always associated with increased VFA content. The fall of pH upto 5.8 should not arrest 'in-vitro' fermentation since a pH of 5.6 is of common occurrence in the rumen content of animals (Pfander & Phillipson, 1952). Therefore it can be said that the amount and the stregth of the buffer used prevented a sufficient fall of pH to be deliterious for ruminal fermentation.

Composition of bagasse:

The composition of bagasse as per analysis done in connection with this work is in agreement with those of common straws available in this country. No data, so far known to the author, is available regarding the composition of this material. A comparative chemical composition is shown ~~in~~ here in Table-6.4.

Table-6.4:

Table showing the composition of straws as compared to bagasse(Percentage composition on dry matter basis).

Name	Reference.	Ash %	CF %	NFE %	CP %	EE
Wheat straw.	Sen et.al. (1942)	10.2	39.4	47.2	2.7	0.47
Rice straw.	Chatterjee (1939-40)	12.1	32.9	50.7	3.7	0.96
Bagasse.	-	2.07	37.55	48.76	1.25	1.54

The above table indicates that the organic material present in bagasse is more or less the same as that in other straws. The bagasse itself contains very little amount of total ash. The high NFE content may perhaps be due to incomplete extraction of sugar in country processing. The percentage of reducing sugar in bagasse was determined and found to be about 1% on dry matter basis. The coldwater soluble constituents as determined in connection with the present work was

found to be more than 20%. As the total ash content of the material is only 2.07%, this water-soluble portion may represent a part of soluble sugar. The work was left at this stage and no further investigation was undertaken. If the assumptions made above hold good, then there is a high probability that 38-40% of NFE comprises the soluble sugar.

DIGESTIBILITY CO-EFFICIENT OF BAGASSE:

Comparative data for straw:

The digestion trial in connection with the work were undertaken by using goats as experimental animals. Comparatively few digestibility trials have been made with goats, such data as are available suggest that there is little difference between goats and sheep (Mazumdar, 1960). Das Gupta (1940) did't noticed any difference in digestibility of cow-heifers and buffalo-heifers. The nitrogen metabolism in milch cows and buffaloes did not differ as recorded by Singh (1933).

It is probable that the digestibility co-efficient of feeds should differ within species of animals. The result obtained from trial with bovine may not represent identical picture for ovine and caprine. A few digestibility data carried out in sheep and goat have been shown here in table-6.5.

Table-6.5.

Table showing the digestibility coefficient of some feeds in sheep & goats:

Food.	Species	CF	NFE	EE	CP
Grass mixed.	sheep	69	71	60	74
	Goat.	66	79	64	70
Meadow hay.	Sheep.	62	64	52	60
	Goat.	62	60	53	52

In the present work the digestibility co-efficient even in low level of protein was found to be rather high as compared in table 6.5 (Data adopted from "Animal Nutrition and Veterinary Dietetics", 1960 by J.T.Abram & R.G.Linton-page-694). The data obtained by other workers on certain common straws carried out in cattle have been shown in table-6.6 to compare the values obtained in present work with goats.

Table-6.6.

Digestibility co-efficient of some straws as compared to that of bagasse:

Feeds.	C F	E E	NFE	Total CHO	TDN	Reference
Wheatstraw.	53.0	4.0	52.0	-	45.5	Sen et.al. (1942).
Ricestraw.	57.9	55.1	49.2	-	46.0	Chatterjee (1939-40)
Bagasse.	62.8	71.6	58.0	60.4	55.0	-

The table 6.6 above reveals that the digestibility co-efficient of all the nutrients in bagasse is similar to those of straws in cattle for all the constituents. The TDN is slightly higher than that of straw.

A point to be noted that the dry matter consumption was about 1% as compared to the usual 2% consumption in cattle.

Effect of addition of molasses:

It is an established fact that the soluble sugar in small amount furnish available source of energy to the micro-organism in the rumen and the bacterial population is thus increased , resulting into improved utilisation and break down of crude fibre. But if the ration contains enough of soluble sugar, then the utilisation of crude fibre is hampered as the microbes attack the soluble sugar preferentially to crude fibre. Commonly, molasses is used as a source of soluble sugar in feeding livestock for this purpose. Too much addition of the material is not physiological so far the digestion of crude fibre is concerned.

In the present case the bast utilisation was obtained when the total molasses added to the ration was only 10gms. (fresh weight). The normal value of the digestibility of crude fibre was assumed to be 62.8 from the 2nd set of trial as in this trial it is presumed that, the animals received 12 gms of DCP, which was sufficient for maintenance. The addition of molasses increased the digestibility to 68.6 from 62.8. Experiment on the other hand indicates that the addition of 20 gms. (fresh weight) of molasses impaired crude fibre digestion. In this case the digestibility of CF was reduced to 58.3 from 62.8.

It has been reported by various workers from time to time that addition of molasses in the feed of cattle & sheep reduce the digestibility of protein as well as all other

other nutrients including CF. But the beneficial effects of addition of small quantities of molasses have been reported by Morrison (1959). Workers in the Missouri as well as in other places (cited by Morrison, 1959) have reported increased digestibility of protein and all other nutrients including crude fibre due to addition of small amount of molasses.

A point to be noted however, is that the total NFE intake was not materially increased by the addition of 10 or even 20 gms. of molasses as the NFE contributed from molasses on rough estimate would not exceed 4 & 8 gms respectively while that from other ingredients were 132.7 g and 132.1 gm ~~respectively~~ .

Nutritive Ratio:

The nutritive status of the animals in 1st & 2nd set of experiment have been shown in Table-6.7.

Table-6.7

Table showing the N.R. of the ration used in two sets of trial.

Set	Animal	D.C.P. consumed		TDN consumed		Total DCP consumed	Total TDN consumed	N.R.
		B.	G	B	G			
1st.	1.	-	7.16	87.95	41.00	7.16	128.95	17.0
	2.	-	7.16	82.23	41.00	7.16	123.23	16.2
	3.	-	7.16	71.34	41.00	7.16	112.34	14.6
2nd.	1.	-	11.46	109.83	65.60	11.46	175.43	14.3
	2.	-	11.46	103.56	65.60	11.46	169.16	13.7
	3.	-	11.46	66.38	65.60	11.46	131.98	10.5

N.B:- B- for Bagasse and 'G' stands for Gram.

It will be seen from the above table that in the 1st set of experiment, the animals were kept on a very wide range of NR of 1:15.6, while in the 2nd set it was 1:12.8; which compares favourably with that of 1:10, 1:12 in case of maintenance in cattle.

The maintenance requirement of ~~digestibility~~ digestible crude protein was calculated to be 12 gms. on the basis of recommendation of Morrison for cattle at the rate of ~~xxxx~~ 0.06 lb. of DCP per 100 lbs. live weight. It will be seen, therefore, that the animals were receiving this amount of protein in all sets of experiments excepting in the ~~fast~~ 1st set in which it received only 7 gms of digestible protein.

The TDN requirement was calculated to be 162 gms. on the basis of recommendation of Morrison for cattle at the rate of 0.75 lb per 100 lbs liveweight. It is seen therefore, that the animals were receiving this amount of energy in all sets excepting in the first one.

Palatibility:

The feed consumption is largely dependent on the palatibility of the feed. The animals comsumed nearly half a pound of dry matter per head per day which works out to be little above one percent as compared to the usual 2% level in cattle.

The dry matter consumption per head per day in all the 4 sets of experiments have been shown in Table-6.8.

Table-6.8:

Table showing daily food consumption on different sets of experiments(Expressed on dry matter basis in gms.)

Animal.	Gram consumed.	Bagasse consumed	Total dry matter consumed.	Bodyweight of animal in lbs.	Food consumed in gm/100lb body weight.	Av.food consumption in gm/100lb. body wt.
			1st set.			
1.	50	167.4	217.4	42	510	
2.	50	156.5	206.5	42	490	500
3.	50	135.8	185.8	37	500	
			2nd Set.			
1.	80	199.7	279.5	42	670	
2.	80	188.3	268.3	42	640	616
3.	80	120.7	200.7	37	540	
			3rd set.			
1.	80	208.3	288.3	42	690	
2.	80	143.8	223.8	42	530	616
3.	80	155.9	235.9	37	630	
			4th set			
1.	80	195.7	275.7	42	650	
2.	80	196.2	276.2	42	650	623
3.	80	131.4	211.4	37	570	

The animals having an average body weight of 50-60 lbs. should coms me about 1-2 lbs of dry matter per head per day. In the present case the animals consumed less than half a lb of dry matter for 40 lbs body weight (Average weight). For the maintenance of normal physiological function of the organisms 3 things are essential. These are Protein, energy and bulk. The animals receiving diet of the 1st set of trial showed an average decrease in body weight of 4 lbs per head in 30 days. This was naturally due to less protein, energy and the bulk. At the end of the 2nd trial, the animals showed a further loss of one lb in body weight per head in course of the next 15 days.

Effect of period of incubation:

The effect of period of incubation on the VFA production rate of bagasse was studied. The rate of production of VFA was determined at 4 hours, 8 hours, 12 hours and 24 hours of incubation. The rate was found to be decreased as the period of incubation was increased. The rate of production of VFA was estimated to be 11.3, 11.1, 7.7 and 4.4 m.moles/per 1000 ml.of rumen liquor on 4 hours, 8 hours, 12 hours and 24 hours of incubation respectively.This findings is in close agreement with those of Gray and Pilgrim (1952). Gray and Pilgrim incubated powdered wheaten hay by rumen liquor from a sheep fed on the same diet and observed a rate of 6 m.moles at 4½ hours, 7.1 on 7½ hours and 3.6 m.moles on 19½ hours of incubation. The rate of fermentation in our case is rather high as compared to the data but it definitely follows the same

same trend. The maximum rate was obtained on 4 hours of incubation and this rate was maintained upto 8 hours and after that the rate fell off gradually to 7.7 on 12 hours and 4.4 on 24th hours of incubation. Gray et.al. also found the maximum rate of production on 7½ hours and on 19½ hours the rate was found to be minimum.

The solid matter in our incubation flask, though not determine, was known to be very small because of the straining of the rumen content. The falling off of the 'in-vitro' rate may be due to either lack of substrate or lack of optimum 'in-vitro' condition or due to both in long period of incubation.

Rate of production of VFA: in 'in-vitro':

The rate of production of VFA was calculated in 'in-vitro'. The average rate of production of VFA from rumen liquor used from slaughtered goats was found to be 5.7 whereas from that of the fistulated goat was found to be 6.3 m.moles per 1000 ml. per hour. The total acid production on average from bagasse in presence of glucose on 4 hours of incubation from samples from slaughtered goats was found to be 35.8 m.moles, the 39 m.moles of acid were produced when bagasse alone was incubated. 43.0 m.moles of acid was produced out of bagasse fermentation from samples from fustulated goats. Thus, 35.8 and 39.0 m.moles of acid were produced on fermentation of bagasse in presence and in absence of glucose respectively.

On the other hand 32.0 m.moles of acid was produced when glucose alone was fermented. The rate of production of VFA in both the cases viz. fermentation of bagasse and glucose proceeded in the same rate. Our findings in this respect indicate that bagasse was fermented as fast as that of glucose. The rate of fermentation of glucose was found to be 8.0 m.moles per hour whereas rate of bagasse was 9.8. The rate of production of acid on fermentation of bagasse in presence of glucose was 8.9 m.moles which, however, indicated that the rate of production of acids was not imperoved due to addition of glucose. The rate of bagasse fermentation was found to be 10.7 m.moles from samples from fistulated goats.

Data in the present work compared favourably with those obtained by other workers from Steer & Sheep which is shown in table-6.9.

Table-6.9.

Rate of production of VFA in 'in-vitro' fermentation of rumen content with various materials as compared to bagasse on 4 hours of incubation.

Materials	Rate /hour m.moles of VFA	Reference
Whole rumen content from hay fed steer.	11.8*	Carrol & Hungate(1954)
Rumen liquor from sheep fed on wheaten hay.	8.7	Gyay & Pilgrim (1952)
Whole rumen content from sheep fed on hay & concentrate.	8.2*	Mukherjee(1960).
Whole rumen content from hay and conc.fed sheep fasted overnight.	4.1*	
Bagasse fermented with rumen liquor from slaughtered goats.	9.8	-
Bagasse with rumen liquor from fistulated goats.	10.7	-

* Rate per Kg.per hour.

The rate of production mf from bagasse with rumen content from fistulated goats showed less variation as evidenced from the narrow range (Table 4.3) and the rate was found to be rather more than that from slaughtered animals. The average rate of fermentation of rumen liquor was found to be 6.3 m.moles per 1000 c.c. per hour with a xmxxxxmf range of 5.0 -8.5 m.moles per 1000 ml. per hour. The average rate of production of VFA on fermentation of bagasse was found to be 10.7 m.moles with a range of 9.5-12.0 m.moles per hour. Much more variation in the rate of production of VFA on fermentation of all the material were noted with samples from slaughtered goat which is shown in Table-4-2 in the text. The fluctuation in the rate of production within the xxxx replicates and within animals have been reported by Carrol & Hungate (1954) and Mukherjee (1960).

The rate of production of VFA varies considerably between animals, different samples from the same animals, replicates of the same samples. This variation may be due to either lack of optimum condition for 'in-vitro' fermentation or to the inherent variation in the factors controlling the xx rate of fermnetation such as type and number of micro-flora present, the concentration and the nature of the substrate.

So, the variation oBserved in the present case is not any thing unusual which is supported by the xxx similar finding of other workers.

Composition of the VFA of rumen liquor and of the fresh acids produced by 'in-vitro' fermentation:

The composition of the VFA produced by ruminal fermentation depends upon the diet of the animal specially the nature and amount of its carbo-hydrate content. According to Elsden (1945-46) 'in-vitro' fermentation of pure cellulose in sheep produced on an average 42% acetic, 56% propionic and only 2% Butyric acid; while glucose produced 26% acetic, 52% propionic and 22% Butyric acid. Thus the soluble sugar of the feed tend to produce relatively more of propionic and butyric acid, while the insoluble fraction tend to yield more of acetic acid. Gray et.al. (1951) obtained two different composition of the VFA from'in-vitro' fermentation of wheaten hay and Lucern hay, the latter yielding relatively more of acetic and the former more of propionic acid. All these fermentation experiments were, however, of long duration ranging from 48-112 hours and as such the overall fermentation pattern might have been some what different from that occurring in rumen due to changes in the microbiol population. Incubation experiments for a shorter period of 4 hours by Carrol & Hungate (1954) using steer, however, did not show any appreciable difference in the composition of VFA produced from hay alone and from high grain diet. A probable reason for this similarity in the composition of VFA peoduced may be that all these experiments were conducted on rumen samples of fed animals and as such may have contained appreciable proportions of the more readily fermentable carbo-hydrate fractions.

In this present experiment the percentage composition of VFA of rumen liquor at zero hour and of rumen liquor on incubation did not show any appreciable difference. More over the results obtained alao did not vary to a great extent withing the replicates of a treatment. The average percentage composition of VFA of un-incubated and of incubated rumen liquor as shown in the table-4.4 in the text was acetic 68.9%, Propionic-16.7% , butyric-14.4% and acetic-69.7%, propionic-16.2%, butyric-11.8% respectively.

The composition of VFA varies considerably between animals difference samples from the same animals and replicates from the same sample. This variation may be due to either lack of optimum conditions for 'in-vitro' fermentation or to the inherent variation in the factors controlling the fermentation.

Inspite of all the sources of variations mentioned above results obtained in these experiments as shown in table-4.4. were of surprising agreement. This is of particular importance because the samples analysed for separation was from different animals, whose feeding habits before collection of the rumen samples were not known. The close agreement in the proportion of the three acids in the rumen contents of these animals probably may be due to the fact that the animals did not get any concentrate ration prior to the day of slaughter, but were allowed to graze only. So naturally, all the animals from which the present sample were analysed, perhaps, received practically similar feed resulting in similar proportions of

proportions of the fatty acids.

The close agreement in the composition of the VFA between the replicates of rumen liquor incubated with bagasse indicates the maintenance of similar incubation conditions which probably were optimum in all the incubations. This may further be explained by the fact that there was probably a considerable similarity in micro-biol population of the rumen due their more or less similar feeding conditions.

The percentage composition of the individual fatty acids in incubated rumen liquor was found to be 77%acetic, 14.62% propionic and 7.7% butyric acid whereas VFA produced on fermentation of bagasse on 4 hours of incubation was found to be acetic-58%, propionic-37% and Butyric-4.2%.The result obtained by Carrol & Hungate (1954) on analysis of rumen content of steer under various dietary condition may be mentioned here. The percentage composition of the VFA produced on 4 hours of incubation of rumen content from hay fed steer was acetic-60.3%, propionic-21.1% and butyric-18.6%. The percentage composition of individual VFA produced on incubation or 1 hour with rumen content of animals on pasture was found to be 60.2% acetic, 23.3% propionic and 16.5%butyric acid.

The proportion of propionic acid in case bagasse fermentation was found to be much more as compared with other cases. This increse in the proportion of the acid was at the cost of both acetic and butyric acids.The high propionic acid

acid production suggests the possibility of the fermentation of soluble sugar of bagasse during the short period of 4 hours incubation. It is well known that fermentation of cellulose and hemi-cellulose occur at a latter stage. The lower production of butyric acid from fermentation of bagasse may be due to lack of protein in it.

Extent of re-producibility in 'in-vitro' incubations:

Many workers have observed good deal of variation as an inherent characteristic of'in-vitro' incubation. The work of Carrol & Hungate (1954) is being cited for this purpose. In four samples, collected from the same animal at different times of the day, the rate of production of VFA fluctuated from 7.6-25.3 m.moles per Kg.per hour in 1st hour of incubation; 7.8-24.4 in 2nd hour; "0"-6.0 in 3rd hour and 5.4-20.0 in 4th hour.

Mukherjee (1960) observed good deal of variations even in the replicates of the same sample. His data showed that there were instances in which the replicates show close agreement in the VFA content, while in some instances, replicate differed to the extent of about 200%. Similar fluctuations in the VFA content have been reported by Carrol & Hungate(1954).

Although, no data on VFA production from replicates of the same samples are available from our work, the acids produced from incubation of rumen liquor and rumen liquor and

and bagasse from the same fistulated animal, show close agreement with one another, even when incubated on different days. This close agreement does not tally with the findings of Carrol & Hungate (1954) and Mukherjee(1960) quoted above. A probable reason for this disagreement may be found in the fact that rumen liquor and not whole rumen content was used in our work, the former probably furnishing comparatively uniform microbiol population and substrate content.

It is pertinent to mention here that our 'in-vitro' findings cannot be used to predict 'in-vivo' pattern as only rumen liquor and not whole rumen content was used by us in the 'in-vitro' fermentation. Obviously, a large percentge of bacterial population was retained in the coarser materials of the rumen content, those were left over after straining. Straining of rumen content was necessitiated by the consideration of eliminating as much of externious substrate of the rumen content as possible, So as to reveal the effect of added substrate more clearly.

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CHAPTER- VII.

S U M M A R Y.

1. Liquid-liquid partition chromatography with celite 535 as inert base, 2 M phosphate buffer of pH 6.5 as the stationary phase and chloroform-butanol mixture as the mobile phase, was found to separate mixture of acetic, propionic and butyric acid quantitatively, even when the acids were present in amounts of 5-10 micro-moles each. It worked efficiently under Indian conditions and the recovery was found to be almost cent percent.

2. Critical estimates of the probable experimental errors of the different steps in the VFA estimation from rumen liquor was made and was found to be negligible.

3. Rumen content on exposure to air for 15 minutes did't produce any untowards effect on VFA production rate whereas rate was decreased on exposure for 30 minutes and one hour.

4. Bagasse was fouhd to contain about 37% crude fibre, 48% NFE, 1.54% E.E., 1.25% protein and 2% ash and judging from these organic and inorganic constituents it could be easily placed in the group of roughages such as straws.

5. The digestibility co-efficient of the nutrients of bagasse including dry matter were found to be 61.3 for C.F., 56.7 for NFE, 64.7% for E.E. and 59 for total carbo hydrate and 61.6 for dry matter on low level of protein (NR 1:15.6). On the other hand, when the NR was 1:12.8, the digestibility co-efficient were CF-62.8, NFE-58, EE-71.6 , total carbo-hydrate-60.4 and dry matter-63.5. No explanation for the high value for E.E. could be indicated.

6. The digestibility co-efficient of crude fibre was 68.6 when 10 gms. of molasses was given. It was an improvement over the value obtained with NR of 1:12.8. The overall increase was estimated to be 10.6%. The digestibility of crude fibre appeared to be diminished slightly on increasing the amount of molasses to 20 gms. presumably due to availability of easily fermentable sugar.

7. The fermentation studies reveal that bagasse is well fermentable and gives rise to the production of VFA. The rate of VFA production out of bagasse was 9.8 m.moles/ hour/1000 ml. of rumen liquor. The addition of glucose did not appear to affect the fermentation rate. The rate also did not appear to vary between 4-8 hours, after which period a definite declined in rate was noted.

8. The percentage composition of voletile fatty acid (VFA) from bagasse was found to be 58 percent acetic acid, 37 percent-propionic acid, and 4.2 percent butyric acid, whereas the fermentation of rumen liquor for four hours yielded seventy seven percent (77%) acetic acid, 14.62 percent propionic acid and 7.7% butyric acid. Higher percentage of propionic acid from bagasse may indicate the relative usefulness as source of energy to the animal.

acid

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R E F E R EN C E S:

Annison, E.F.,(1954) . Biochem.J., 57 , 400.

Annison,E.F. & Pennington,R.J. (1954): Biochem.J.,57 ,685.

Armstrong,D.G. & Blaxter,K.L. (1956): Nature, 177, 1183.

Armstrong,D.G. & Blaxter,K.L. (1957a):Brit.J.Nutr.,11, 247.

Armstrong,D.G. & Blaxter,K.L. (1957b):Ibid .,11, 413

Armstrong,D.G.;Blaxter,K.L. & Graham,N.McC. (1957a):Ibid., 11, 392.

Armstrong,D.G.;Blaxter,K.L. & Graham,N.McC. (1957b):Proc.Brit.Soc.Anim.,Prod,2,3.

Armstrong,D.G.; Blaxter,K.L.& Graham,N.McC and Wainman,F.W. (1958): Brit.J.Nutr.,12, 177.

Ash,R.W.. (1957): J.Physiology.,139, 6.

Ayyar,N.K. & Zubairy,A.W. (1945): Ind.J.vet.Sci.,15,40.

Barcroft,J., Mc.Anally,R.A.& Phillipson,A.T. (1944): J.expt.Biol.,20, 120.

Balch,D.A. and Rowland,S.J. (1957): Brit.J.Nutr.,11, 288.

Burroughs,W.,Headley,K.G., Bethke,R.N.& Gerlaugh,P. (1950): J.Ani.Sci.,9, 513.

Bueding,E.,and Yale,H.W. (1951): J.Biol.Chem.,193,411.

Carrol,E.J. and Hungate,R.E. (1954): Appl.Microbiol.,2, 205.

Chatterjee,I.B. (1939-40):Ann.Rep.Physiol.Chem.,Anim. Nut.Sch.Imp.Coun.Agric. Res.Bengal.

Cowie,H.T.;Folley,S.J.; French,T.H.and Popjak,G. (1951): Biochem.J.,49,610.

Das Gupta,N.C. (1940): Prog.Rep.Cattle Feeding, Res.Sch.Imp.Coun.Agric.Res, United Province.

Elsden,S.R. (1945-46):J.Expt.,Biol.22,51.

Elsden,S.R. (1946): Biochem.J., 40,252.

Elsden,S.R.;Hitchcock,M.W.S.; Marshall,R.A.and Phillipson, A.T. (1946): J.Exptl.Biol.,22, 191.

el.Shazly. (1952): Biochem.J., 51, 640.

Gray,F.V.; Piligram,A.F. and Weller,R.A. (1951): J.exptl.Biol.,28, 74.

Gray,F.V. and Pilgrim,A.F. (1952): Ibid. 29, 54.

Gray,F.V.; Pilgrim,A.F.; Rodda,H.J.and Weller,R.A. (1952): ibid., 29, 57.

Hungate,R.E., (1942): Biol.Bull., 83,303.

Hungate,R.E. (1950): Bacteriol.Review.,14,1.

Hueter,F.G.,Gibboas,R.J. Shaw,J.C. and Doetschrn. (1958): J.Dairy Sci.,41.,651.

James,A.T.and Martin,A.J.P. (1952): Biochem.J., 50,679.

James,A.T.;Martin,A.J.P and Smith,G.H. (1952): Ibid., 52,238.

James,A.T., Martin,A.J.P. (1956): Ibid., 63, 144.

Kellner,O. (1926): The Scientific Feeding of Animdals. Translated by W.Goodwin, Duckworth,London.

Lander,P.E. (1938): Rep.Feeding Trials,Sch.Manuf., Cattle feed from molasses.

Lorber,V.; Lifson,N.and Wood,H.G. (1945): J.Biol.Chem.,161.,411.

Louw,J.G.,Williams,H.H. & Maynard,L.A. (1949): Science.,110.,478.

Martin,A.J.P.and Synge, R.L.M. (1941): Bio.Chem.J.,35.,1358.

Marston,H.R. (1948): Ibid., 42.,564.

Mazumdar,B.M. (1960): J.Agric.Sci.,54.,385.

Mahadevan,V.,Joyal,M.M. and Gupta,P.S. (1962): Proceedings Ind.Sci.Congr., Pt.III.

MacAnally,R.A.,& Phillipson, A.T. (1942): J.Physiol.,101,13.

Menon,U.K.; Krishnamurti, C.R.and Miss Thankam Bastin. (1962): Ind.vet.J.,38, 380.

Mitchell,H.H.;Hamilton,T.S. McClure,E.J.,Haines,W.T., Beadles,J.R.and Morris,H.P. (1932): J.Agric.,Res.,45.,163.

Morrison,F.B. (1937): Feeds & Feeding.,Morrison Publishing co.,New York.

Morrison,F.B. (1959): Feeds & Feeding.

Moyle,V.,Baldwin,E.,& R.Scarisbriskr,R. (1948): Biochem.J., 43., 308.

Mukjerjee,D.B. (1960): Application of Isotope technique in the study of fermentation in the rumen of sheep,Ph.D.thesis (Aberdeen).

Nelson,W.O.,Opperman,R.A. Brown,R.E. (1958): J.Dairy Sci.,41.,545.

Pearson,R.M.and Smith,J.A.B (1943): Biochem.J.,37.,153.

Peterson,M.H.and Johnson, M.J. (1948): J.Biol.Chem.,174.,775.

Pennington,R.J. (1952): Biochem.J..,51.,251.

Pennington,R.J. (1954): Ibid., 56.,410.

Pennington,R.J.and Sutherland,T.M. (1956): Ibid., 63.,618.

Pflander,W.H.and Phillipson,A.T. (1953): J.Physiol.,122.,102.

Phillipson,A.T. (1942): J.exptl.Biol.,19.,186.

Phillipson,A.T. (1947): Ibid., 23.,246.

Phillipson,A.T., (1952): Brit.J.Nutr.,6.,190.

Popjak.,G.,Folley,S.J. and French,T.H. (1949): Arch.Biochem.,23.,508.

Potter.,R.J., (1952):-Nature-London.,1770.,541.

Ramsey,L.L.and Wipatterson,W.I. (1945): J.AoAC.,28.,644.

Reid,R.L. (1950): Nature,165.,448.

Reid,R.L.and Lederer,M. (1951): Biochem.J.,50.,60.

Ritzman,E.G.and Benedict, F.G. (1938): Nutr.Physiol.,adult.ruminant. Carnegic.,Inst.,No.494.

Roy,S.C.and Talapatra,S.K. (1945): ind.J.vet.Sci.,15.,133.

Schambye,P. (1951a): Nord.vet.med.,3.,555.

Schambye, P (1951b): Ibid., 3.,748.

Schambye, P. (1951c): Ibid., 3.,1003.

Schambye, P. (1955a) Ibid., 7, 961.

Schambye,P. (1955b): Ibid., 7.,1001.

Sen, K.C.,Roy,S.C.& Talapatra,S.K. (1942): Ind.J.vet.sci.,12.,263.

Sen, K.C. (1946): Imp.coun.agric.,Res.,Misc.,bul. 25.

Sen, K.C. (1953): Animal Nutrition Research in India,Macmillian & Co.,ltd.

Seshamp P.A. (1935): Ind.J.vet.Sci.,5., 355.

Singh.,B. (1933): Agric.Livestock,India,3.,411.

Tappeiner,H. (1884): Ztschr.Biol., 20.,52.

Warner.,A.C.I. (1956): J.gen.microbiol.,14.,733.

Woodman,H.E.,and Evans,R.E., (1938): J.Agric.Sci.,28.,43.

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