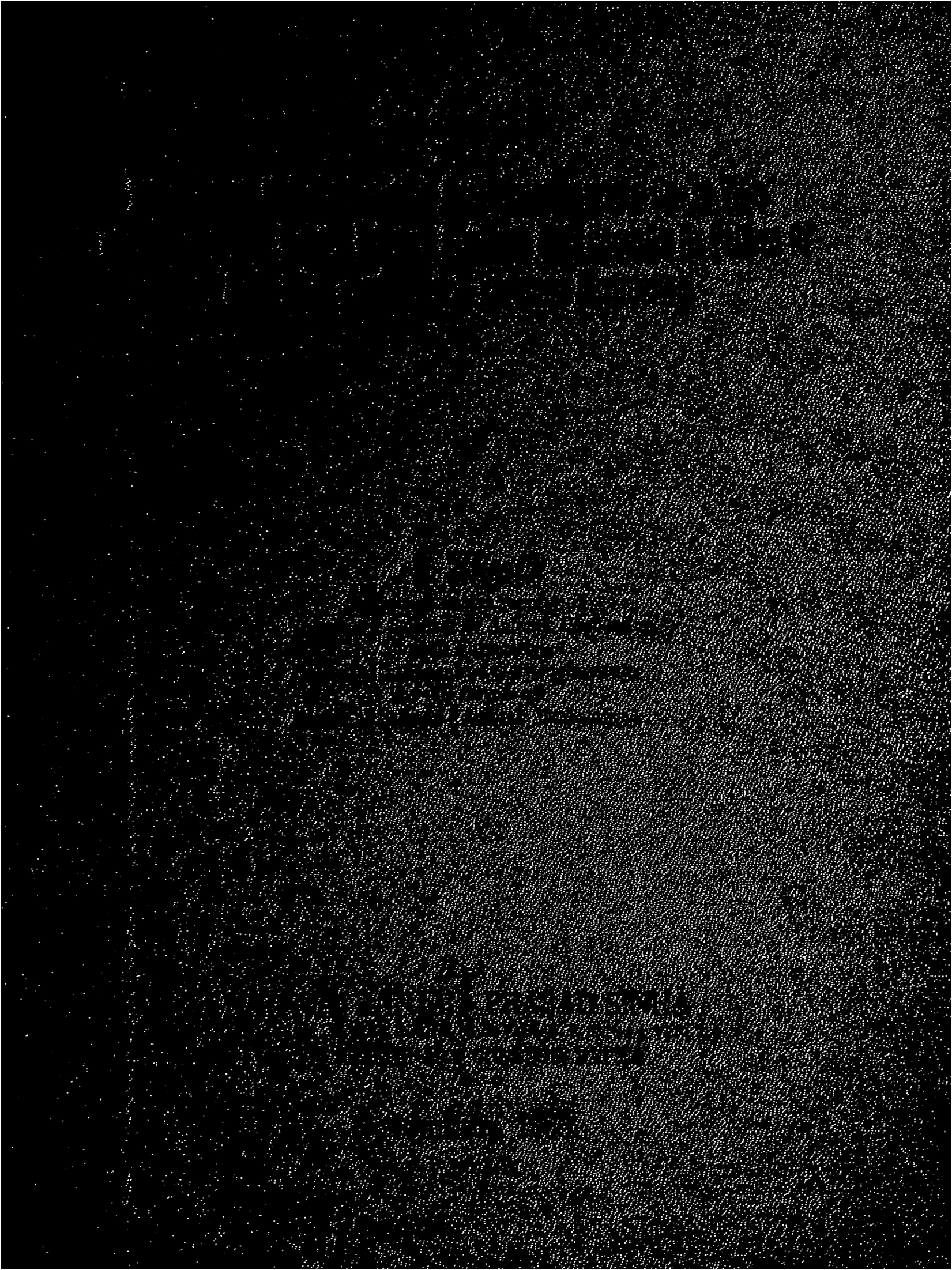

Studies On
The
Influence Of Chlortetracycline And Oxytetracycline On 'In Vitro' Fermentation Of Goat's Rumen Content With Regards To Pattern Of Fatty Acid Production & Carotene Destruction

A Thesis

Submitted to the Faculty of
Veterinary Science & Animal Husbandry,
Magadh University,
In Partial fulfilment of the Requirements
For the Degree of
Master of Science (Animal Husbandry)

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November, 1965

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

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C O N T E N T S.

C H A P T E R - I

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

The greatest problem of most of the countries of the world today is that of food and population. In the production of food from animal sources and from plant sources as well, animal husbandary plays an important role. Human foods from animal sources are of great value and their inclusion in the daily human diets especially in the diets of infants is a necessity.

Proteins of animal origin are biologically superior to those derived from plants. Appropriate balance of important aminoacids and the presence of the unidentified substance known as ' Animal-protein factor ' in animal products are recognised to be responsible for the special virtue of animal protein.

The contribution of ruminants in varied forms to national health and wealth is of immence value. They supply quality foods like milk and meat for human consumption and wool for their use. In India and many other tropical countries, cattle also provides main labour for cultivation, transport of goods from rural to urban areas, thrashing and irrigation work. Besides, farm yard manure forms the main bulk of fertilizer for enriching the soil. Thus agricultural operations

for the production of foods from plant sources also are almost exclusively dependent on cattle.

The value of ruminants is still more raised due to their ability in utilizing feeds like straws, grasses(fresh or ensiled), oilcakes, brans, husks, urea, and other by- products not only for their maintenance but for converting them into milk, meat and wool. As the bulk of food consumed by them can not be used by man or even by swine and poultry, they are the greatest benefactors to human beings as they render such yeoman service to man kind without competing very much with the latter for their sustenance.

This ability of ruminants is due to the remarkable symbiosis between the host and the vast population of microflora which thrive in the rumen, the largest of the four compartments.

PECULIAR FEATURES OF RUMEN DIGESTION

The possession of this complex stomach at the beginning of the alimentary canal is the most characteristic features of the suborder Ruminatia. It has been found that70 to 80 percent of the digestible dry matter disappears during its passage through rumen (Gray, 1947) & fermentation here overshadows entirely the rest of the digestion in the alimentary canal (Blaxter,1954). The rumen is essentially an anaerobic highly reducing system at a slightly acid but buffered pH, at a temperature of 39^{o}C and under a gas phase composed mainly of

carbondioxide, methane a
suitable environment fo
microbiota.

Digestion of Carbohydra

With the ai
cellulose, hemicellulos
the feeding stuffs are
carbondioxide and metha
the rumen liquors, as t
propionic and n- and is
valeric acid. Formic ac
traces. Lactic acid is
rich rations are fed. P
cellulose was broken do
lactate and ultimately
extent of 50 percent a
glucuronic acid and are
formation of short chai
Cuthberson, 1956).

The relati
formed though varying
diet, age of the animal
individuality of the a
that acetic acid forms
acid the next and n-bu

chief volati vides a
in rumen liq ivity of the
o 70 percent,
id 10 to 20 pe
less than one otozoa, the
drates of
ttyacids,
found in
he rumen microflo re acetic,
minant animal is n of
and hemicelluloses ent in
organisms, ruminant carbohydrate
tion purposes, such hat
and ammonium salts vate ,
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men contents to be st h the
th (1943) obtained cl
ich are readily attac
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carbondioxide, methane and nitrogen. Thus it provides a suitable environment for the maintenance and activity of the microbiota.

Digestion of Carbohydrates

With the aid of these bacteria and protozoa, the cellulose, hemicelluloses and even simpler carbohydrates of the feeding stuffs are fermented to short chain fettyacids, carbondioxide and methane. The volatile fatty acids found in the rumen liquors, as the products of fermentation are acetic, propionic and n- and iso-butyric acids, also isomers of valeric acid. Formic acid and succinic acid are present in traces. Lactic acid is also likely to be found when carbohydrate rich rations are fed. Phillipson (1947) pointed out that cellulose was broken down to cellubiose, glucose, pyruvate , lactate and ultimately to VFA. Pentosans are digested to the extent of 50 percent and the simple components xylose, glucuronic acid and arabinose are rapidly fermented with the formation of short chain fatty acids (Phillipson and Cuthberson, 1956).

The relative proportions of the three main acids formed though varying substantially with the nature of the diet, age of the animal and possibly with the species and individuality of the animal, always follow the same trend, in that acetic acid forms the largest concentration, propionic acid the next and n-butyric acid the smallest concentration

of the products among these three chief volatile fatty acids. The proportion of the acids found in rumen liquor of the goat on all roughage diet are acetic 50 to 70 percent, propionic acid 20 to 40 percent and n-butyric acid 10 to 20 percent and other fatty acids together constitutes less than one percent of the total.

Digestion of nitrogenous compounds

The part played by the rumen microflora in the nutritional physiology of the ruminant animal is not restricted to the utilisation of cellulose and hemicelluloses. With the symbiotic action of these microorganisms, ruminant can also use for both maintenance and production purposes, such simple nitrogenous compounds like urea and ammonium salts compounds which are useless in simple stomached animals as source for body or milk proteins.

Sym (1938) found rumen contents to be strongly proteolytic and Pearson and Smith (1943) obtained clear evidence of propeolysis with proteins which are readily attackable. McDonald (1948 a,b) showed that large quantities of ammonia were produced in the rumen under normal feeding conditions. The amount formed was shown to be dependent upon the type of protein and carbohydrate materials present. The intermediates of this

protein break down are the aminoacids which are partly synthesized into microbial protein and partly metabolised to ammonia and the short chain fatty acids. The branched chained isomers of C_4 and C_5 fatty acids of rumen are atributed to this source (El-Shazly, 1952). Lewis (1951) also reported liberation of ammonia as the first stage in the utilisation of nitrogenous compounds.

Simulataneously certain rumen microflora converts the liberated ammonia into their body protein provided sufficient energy yielding substance of proper quality are also present. The microbial proteins thus formed are afterwards enzymically digested lower down and supply the host with necessary amminoacids. The liberated ammonia escaping microbial attack, is absorbed through the rumen wall and is conveyed by the portal blood to the liver where it is converted into urea which is mostly excreted in the urine and some of it used in the synthesis of non-essential aminoacids by amination of appropriate carbon chains and some of it reenters rumen through the saliva.

The microbial protein formed from urea in the presence of starch has a high biological value of about 88 percent(Reed et al, 1949), indicating the capability of microorganisms to synthesize essential aminoacids from ammonia. The microbial poteins thus help the ruminants to be largely independent of the aminoacid composition of the diets.

Thus rumen microorganisms produce fatty acids and ammonia by their activity on nitrogenous compounds. McDonald

(1954) and McDonald and Hall (1957) demonstrated also that the rapid degradation of casein and the much slower degradation of zein was accompanied by the formation of ammonia and fatty acids. El-Shazly (1952 a) showed that casein hydrolysates are acted upon by washed rumen microorganishms to produce ammonia and fatty acids. Sirotnak et al (1953) found that all he 23 amino-acids tested had been deaminated with the production of fatty acids.

Vitamins and microbial activity.

The adult ruminants is in the happy position that it is to a great extent, not dependent on its diet for a large number of vitamins. The microorganisms of the rumen can synthesize all the B-Complex vitamins as well as Vitamin K provided sufficient cobalt is also simultaneously present. Vitamin C is synthesized within the body tissues as calves can be reared on Vitamin C free diet for over a year without the blood content of the vitamin being even slightly affected (Ray, et al, 1941). Injection of paraldehyde or barbitone is found to enhance the synthesis of vitamin C in ruminants (Ray,1942). In tropical countries , vitamin D is not required. Under Indian conditions of management, vitamin E deficiency has not been reported.

Of all the vitamins, vitamin A is the most important. 30 microgram of carotene per kg body weight are required daily

by ruminants inorder to prevent night blindness, whereas,double this quantity is needed for maintaining a normal level of vitamin A in blood. This high requirement in ruminants is due to the fact that only 30-40 percent of feed carotene can be absorbed from their alimentary canal (Ray, 1958).

Destruction was observed when carotene and vitamin A were incubated'in vitro' in rumen fluid (King et al, 1962). Klatte et al (1964) also reported highly significant destruction of vitamin A by ruminal and abomasal fluids. Results obtained with autoclaved ruminal fluid suggested that destruction by ruminal fluid was dependent on microbial activity.

Factors affecting rumen digestion.

Due to their peculiarity of metabolism,ruminants are of sufficient biological interest in themselves. In the last 20 years, much work on ruminant metabolism was carried out and many of the mysteries of their peculiar metabolism were brought to light.

As the end products of microbial digestion are short chain fatty acids mostly, much emphasis was given to their rate of production and pattern of production under different conditions. The proportion of the acids formed varies with the nature of the ration, the organism present and other factors and thus effects the over all performance of the animal.

Recently, Shaw et al (1957) stated that the fat content of milk could be decreased by 50 percent when ruminants (cows in this case), were fed a ration composed

predominantly of bread. They found that the rumen liquor of such animals is low in acetic and high in propionic acid.

The digestion of cellulose and other carbohydrates in the rumen has been studied in detail both by 'in vitro' and in vivo' methods. Arias et al (1951) reported that high levels of carbohydrates decreased cellulose digestion 'in vitro'.

A great deal remains to be done concerning nutritional requirements of microflora and effect of deffciencies and supplementations on over-all rumen function. Hence we should learn more about the conditions which promote the maximum activity of rumen microorganisms, together with the knowledge to take fuller advantage of the rumen processes on which the digestion of the higher carbohydrates depends.

It is evident that research on these microbial processes is important to enable us to make the most effective use of roughage and other by- products in feeding ruminants. As detailed above , in addition to cellulose digestion rumen microorganisms contribute the following to nutrition of the host animal: protein synthesis , B vitamins synthesis, and soluble carbohydrate digestion. Nutritional factors which modify the above machanisms may well improve over all productive efficiency of the ruminant animals.

In short , any knowledge about the ruminants nutritional physiology that can be harnessed for making them more and more profitable is of immence value for humen welfare.

ANTIBIOTICS FEEDING IN LIVESTOCK

The constant effort to produce human foods from animal sources at lower cost to the consumer has spearheaded a continuing search for more suitable combinations of known nutrients and for new chemical substances, with the hope of increasing the efficiency and rate of growth and production. These wide spread efforts have led to the present use of antibiotics and other chemicals in animal feeds.

The feeding of antibiotics to farm animals has brought about a new era in livestock production. The idea of adding small quantities of antibiotics to animal feeds to improve the growth rate , and efficiency of utilization is, relatively , new . The practical and economical implications of the discovery are considerable and since 1950,much of the research on nutritional problems has been devoted to the study of the effects of adding antibiotics to the feeds of domestic and experimental animals and the mode of the action of the antibiotics.

It has been well established now that various antibiotics,namely aureomycin (Chlortetracycline),terramycin

(oxytetracycline),penicillin,bacitracin,and streptomycin (dihydrostreptomycin),stimulate the growth rate of monogastric farm animals,such as chick,turkey,and pig.

The influence of chlortetracycline as a growth stimulant for young dairy calves was demonstrated at about the same time by Bartley et al Kansas State College, Rusoff at Louisiana State University,and Loosli and Wallace at Cornell University (quoted in Animal Nutrition by Maynard and Loosli). Terramycin (oxytetracycline)was also reported by Mackey et al, (1953) to be as effective as aureomycin in stimulating growth of calves. According to Maynard and Loosli,chlortetracycline and oxytetracycline have proved of greatest value for calves.

It is now accepted that inclusion of antibiotics in rations of young calves results in increased rates of growth , over all improved condition and much decreased in the incidence of calf scours. Higher dressing percentages were reported in lambs fed antibiotics.

The observations with mature ruminant animals are less conclusive. Some of the workers have reported beneficial effect when lower levels of aureomycin were fed (Jordan,1952; Bridges et al,1953;Perry et al ,1954). Others have reported detrimental effects of high levels of aureomycin fed to either sheep or cattle (Coly et al,1950; Bell et al,1952). Some workers concluded neither beneficial nor deleterious effect

from feeding lactating animals with 300 to 500 mg of aureomycin daily per cow in form of Aurofac 2 A (Bartley et al,1953). Significant decrease in apparent crude fibre digestibility have been reported when higher levels of chlortetracycline was included.

In a low concentrate ration chlortetracycline was reported to exert an effect on rumen fermentation (Raun et al, 1962) while Hungate et al(1955a) concluded that the capacity of rumen organisms to form fermentation products had not changed by antibiotics addition.

Indirect evidence that the microbial population was changed by feeding aureomycin was reported by Hungate et al(1955a) while others (Chance et al ,1951;Neuman et al, 1951; Munch, Peterson and Armstrong,1958) have provided direct evidence that the bacterial population in modified. No significant changes in the microbial population were demonstrated (Bartley et al ,1951; Loosli et al,1951; Rusoff et al,1952).

These varying results of antibiotic feeding in mature ruminants where rumen development was complete, were interpreted due to change or modification in the microbial population and in their activity.

Since the chief end product of microbial digestion is short chain volatile fatty acids, the present study was undertaken to see the effects of chlortetracycline and oxytetracycline each at two different levels on the 'in vitro' fermentation of goats' rumen content with regards to total fatty acid production and pattern of fatty acid production. Efforts were also made to see the comparative action of these antibiotics on protein and carbohydrate rich substrates.

In addition studies were also undertaken to ascertain if the effect of the antibiotics on the microbial activity would in any way affect the extent of carotene destruction by them.

C H A P T E R - II

REVIEW OF LITERATURES

USE OF ANTIBIOTICS IN GROWTH

From the many published report,it is now evident that in addition to chlortetracycline (aureomycin),oxytetracycline (terramycin) and penicillin are susually effective in stimulating growth of young animals,but there are specie differences in responses. Bacitracin and several other antibiotics are less effective or have no activity at all depending upon other circumstances. For calves chlortetracycline and oxytetracycline have proved of greatest value.

Aureomycin

Increased growth rate and decreased incidence of scours in diary calves due to antibiotics feeding has been reported by many investigators (Bartley et al,1950; Loosli and Wallerman,1951; Rusoff,1951; Rusoff and Davis,1951; Volker and Cason,1951).

In general these calves in which antibiotics feeding produced growth stimulation were from 1 to 150 days of age or at an age when rumen development was far from complete.

Bartley et al (1950) reported that an animal protein factor concentrate containing aureomycin stimulated the growth rate of diary calves from birth to 42 days of age. These calves were fed the equivalent of about 15 mg. daily of aureomycin per 100 lb of body weight. The control calves gained 18.0 lb in 42 days and the 'animal protein factor' fed calves gained 30.8 lb, or 71.1 percent more. The incidence of calf scores was much lower in the aureomycin-fed calves and these calves also showed more thriftiness and improved over all condition. These data caused the authors to conclude that aureomycin enhanced the growth of the calves by preventing scores.

Loosli and Wallace (1950) found a growth response in young calves both from an animal protein factor concentrate containing aureomycin and from crystalline aureomycin. Both of the supplements were fed in a milk substitute at the rate of 10.0 g. per ton of crystalline antibiotics. The control calves gained 0.92 lb and the antibiotics-fed calves 1.11 lb daily for the first 8 weeks.

Perry et al (1954)also studied the effect of feeding aureomycin to growing and fattening beef animals in both suckling calves in which there was little or no rumen development and in older animals in which rumen development had progressed further. They observed that the administration of aureomycin to suckling calves at level of 24 mg. per 100 lb

body weight had decreased acouring and had given an apparent growth stimulation . Yearling steers receiving 75 mg. aureomycin per animal daily gained 43 lb more (P/0.05) than control animals on a ground corn cobs. Aureomycin fed steers required 18 percent less fed per unit gain. Aureomycin feeding had no effect on growth rate or feed efficiency of fattening steers on a controlled intake basis. Calves and yearling receiving 75 mg. aureomycin gained 27 lb more (stastistically significant) and required 20 percent less feed per unit of gain than control animals on a basal ration of ground corn cobs and simple minerals, free choice add per due cattle supplement A, 3.5 lb per day. In all cases the introduction of aureomycin to the ration caused a temporary depression in appetite for 2 to 4 days during the first week.

Bartley et al (1951) fed dairy calves and aureomycin supplement (Lederle) at levels of 3 and 9 g per 100 lb body weight (daily by capsule) from 1 to 22 weeks of age . They observed thatgains made by both the groups of supplemented calves were superior to gains made by controls, but the difference between the two supplemented groups was not significant. Calves were fed 3 g of supplement, the average weight at end of 22 weeks was 410 percent of the initial 1 week weight. Similarly calves fed 9 g averaged 400 percent of the initial weight and the control averaged 356 percent. The amount of T.D.N. and digestible protein used per lb of gain (from 1 to 12 weeks) was similar for control and supplemented groups. The average hay consumption (per unit of body weight) for the supplemented

and control groups was similar. However the supplemented calves averaged about 22 percent more grain consumption(per unit of weight)than the control averaged .

The effect of aureomycin upon the growth of dairy calves when administered orally, subcutaneously and intramuscularly were reported by Richardson et al (1953) the results indicated that oral administration at both levels used was effective in increasing body weight gain as compared to controls at the same age.

Hibbs and Conard (1953) used Jersey calves and observed the effect of adding 20 mg. of aureomycin in the form of Aurofac 2 A daily to the milk during the 7 weeks milk feeding period and 20 mg. of aureomycin per lb of dry matter consumed (hay+grain) after the milk feeding (8-12 weeks). Their result indicated that aureomycin feeding resulted in greater weight gains, higher feed intake and more efficient utilization of feed measured by T.D.N. per lb of gain.

Velu and Reed (1960) studied the effect of feeding aurofac on growth of Singhi X Jersey male calves reared in India to 6 months of age and found that supplementation with aurofac (containing aureomycin and vitamin B_{12}) caused significant improvements in gains in weight (by 18 percent) and height at withers (by 24 percent) of calves.

Terramycin

For the first time, Cason and Voelker (1951) fed two levels of a terramycin supplement to supply 15 and 30 mg of terramycin per 100 lb of body weight daily, for the first 8 weeks of calf's life. In this study a growth response was obtained, but it was not great enough to be statistically significant.

Voelker and Cason (1951) fed terramycin to calves at the rate of 30 mg. per 100 lb body weight, and average gain of 63 lb was noted for the supplemented calves as compared to 52 lb for the controls. The length of feeding period was not mentioned. At the rate of 100 mg. daily, 7 calves gained 28 percent over the control. No increase in feed utilization efficiency was observed.

Mackay et al (1953) studied the effect of feeding terramycin on the growth, efficiency of feed utilization, incidence of scours and general well being of dairy calves (i) from birth to 12 weeks of age and (ii) older ruminant animals and found that the feeding of terramycin supplement to young dairy calves receiving a liberal ration of milk, calf starter and good quality hay produced a significant increase in growth, stimulated the appetite and improved the general appearance as compared to the controls.

Feeding terramycin supplement to the older calves showed a lesser effect. After an initial increase in rate of growth for the first two weeks of feeding, the growth rate

gradually declined to the control level. Some improvement in general appearance in older calves was noted. No conclusion was drawn to the effect of terramycin in the control of scours due to its very slight incidence in these trials. According to these workers, terramycin appeared to be at least as effective as aureomycin in stimulating the growth of young dairy calves.

Kesler (1954) found that the addition of terramycin to a milk replacement diet for young calves stimulated growth significantly as measured by body weight gains and increase at height at withers. Supplemented calves consumed more concentrates and at an earlier age.

Mooddy et al (1954) reported that terramycin stimulated the growth rate of female calves more than that of male calves and found no evidence that small calves responded more to terramycin supplementation than did the larger calves.

No improvement in the growth of calves was observed when terramycin was included in milk replacement feed (Williams and Jenson,1954).

Use of antibiotics in fattening.

Jordan and Bell (1951) noted that fattening lambs receiving 6 to 12 mg. of aureomycin daily made faster and more efficient gain than the controls.

Jordan (1952) conducted four feeding experiments on 199 native and western lambs to study the effect of aureomycin in lamb fattening rations. Aureomycin in the form of Aurofac 2 A was incarporated in the soyabean meal and was fed at levels of 7.2, 10.8 and 14.4 mg. of aureomycin daily per lamb.

On the basis of their experiment, they recommended that aureomycin mightbe included in lamb fattening rations at levels from 7 to 14 mg. over a period of 100 days without causing the lambs to go off feed or increasing the incidence of scouring. Aureomycin fed at 14.4 mg. per lamb daily reduced the rate of gain and feed efficiency in both trials in which it was feed.

Bridges et al (1953) used aureomycin as feeding supplement and fed to lambs at levels of 1.1 to 15.0 mg. per lb of feed in rations composed of milo and alfalfa hay; and milo, cotton seed meal and alfalfa hay. increased in rate of gain resulting from feeding the aureomycin were relatively small and were not significant statistically. When aureomycin was fed at levels of 2.2 to 5.0 mg. per lb of total feed, an apparent improvent in feed efficiency was evident.

Jordan et al (1956) carried out experiments to find out the effect of chlortetracycline, stilbesterol and chlortetracycline, stilbesterol supplement on fattening lambs and reported that 21.6 mg. of chlortetracycline per lamb daily, 2 mg. of stilbesterol per lamb daily or a combination of chlortetracyclino

and stilbesterol at these two levels had no significant effect on the rate of gain, feed consumption or feed efficiency whether added to a ration with a concentrate-roughage ratio of 35-65 or 45-55. Carcass grade was slightly improved by the higher level of concentrate feeding and chlortetratycline addition. Carcass yeild was not effected by treatment or level of concentrate.

Hatfield and Garringus (1952) and Botkin and Paules (1954) found higher dressing percentages in lambs fed antibiotics .

Rusoff et al (1954) reported that chlortetracycline fed dairy calves were heavier and had larger carcasses and skeletal development than control calves.

In oxytetracycline fed yearling steers, Morrison et al , (1955) found a slightly higher dressing percentage and a lower carcass grade .

Landagora et al (1957) studied the effect of chlortetracycline on carcass yields including physical and chemical composition of dairy calves. They obtained in calves fed or injected with chlortetracycline, a significant increase in growth rate and efficiency of feed utilization; significantly heavier carcass weight and higher dressing percentage; increase in size and weight of the higher priced

cuts in the veal carcass; and no effect on tenderness as measured by the shearing strength of the 12th rib cut. Oral feeding appeared to increase carcass fat deposition whereas parenteral administration appeared to enhance protein synthesis. The growth promoting effect of chlortetracycline on young calves resulted in over-all body growth as indicated by muscle weights and measurements which according to these workers suggested that certain endocrine system were invloved or that had increased the efficiency in nutrient utilization.

Raun et al (1962) reported that supplementation of chlortetracycline to a high urea ration partly counteracted the effect of urea in lowering the carcass grading.

Use of antibiotics in older calves.

Bartley et al (1951) fed daily doses of 200-800 mg. of aureomycin per 100 lb of body weight to calves of 12-16 weeks old without any detrimental effects. One calf of 16 weeks old that had not previously received aureomycin was fed 2500 mg. of this antibiotic daily for 4 weeks without any harmful effects.

Fincham and Voelker (1953) divided 40 pairs of heifers into two groups and fed one group 80 mg. of aureomycin daily from birth to 200 days of age and 240 mg. daily to about 2½ years of age. They found that most of the growth stimulation

from the feeding aureomycin occured before the heifers were 6 months old, but the growth advantage obtained was maintained afterwards. At 18 months of age the control heifers weighed 755 lb and the supplemented heifers, 794 lb. The aureomycin fed heifers were bred 19 days earlier than the control heifers but required 1.6 services per conception as compared with 1.4 for the controls.

USE OF ANTIBIOTICS IN MATURE RUMINANTS

Warner (1952) and Loosli and Warner (1952) found that feeding of 700 mg. of aureomycin daily to cows had no effect on feed consumption but feeding of 1000 mg. daily caused feed refusals. The feeding of 100 mg. of aureomycin daily to milking cows produced neither harmful nor beneficial effects on milk production and feed consumption.

Chance et al (1953) showed that when steers were fed aureomycin (0.5 or 1.0 g. per day) there was an increase in the total bacterial count, a decrease in the numbers of streptococci and increase in the coliform organisms present.

Lassiter et al (1954) fed yearling dairy steers 500 mg. daily of crystalline aureomycin alone and in combination with a surfactant, Ethomid C/15 to study the effect on the digestion of feed nutrients. It was found that crystalline aureomycin decreased dry matter digestibility from 64.0 percent on the Basal ration to 60.5 percent.

This reduction was not statistically significant. Crude fiber digestibility was reduced from 35.5 to 22.7 percent and this reduction was significant. When aureomycin and Ethomid C/15 were fed in combination, the depressing effect of aureomycin on dry matter and crude fiber digestion appeared to be lessened. In this study aureomycin had no significant effect on the appetites, consistency of feces, overall condition of the steers, or digestion of dry matter, crude protein, ether extract, and nitrogen-free- extract.

Lassiter (1955) in his review concluded that no beneficial effects were derived from the feeding of antibiotics to mature cattle.

Experiments by Hungate et al (1955) indicated that chlortetracycline feeding altered the composition of the rumen microbial population of steers but did not alter the composition of rumen microbial activity.

Horn and associates (1955) found that 100 mg. of chlortetracycline per steerx daily depressed roughage digestion by microorganisms but 32 mg. of the antibiotic did not .

Evans and associates (1957) reported that feeding bhlortetracycline to sheep decreased the apparent crude fiber digestibility only if fed at a level greater than one mg. per lb of feed.

Lambert & Jacobson (1958) also fed chlortetracycline in a 13 mg. dose per 100 lb live weight daily to find out the effect of cellulose digestion in the bovine rumen. They reported that cellulose digestion (48 hours after the cellulose was introduced into the rumen in first experiment and 24,48,72 and 96 hours after cellulose introduction in the second) was not altered appreciably by chlortetracycline feeding.

Hougue et al (1956) used high nitrogen intakes with calves and found significantly greater apparent protein digestion, as well as greater nitrogen retention when chlortetracycline was fed than in its absence.

Klopfenstein et al (1964) found that antibiotic addition resulted in greater protozoal concentrations and increased nitrogen digestibility.

Effect of antibiotics on gas production.

Klopfenstein et al (1962) reported the effect of antibiotic and time on rumen activity. Manometric technique was employed to measure the effect of antibiotic upon rumen microbial activity. Whole rumen contents were diluted 1:1(V/V), with a buffer solution before transference to Warburg flasks. Antibiotics at two levels (high-400 mg. streptomycin and 40,000 units penicillin/flask; low-0.04 mg. streptomycin and 40 units penicillin/flask); and nutrient solution (urea,casein, glucose,starch,and cellulose) were added in various combinations at various time intervals.

Antibiotics added at the same time as the nutrient solution caused a greater decreases in gas production than when added 100 minutes after the addition of the neutrient solution. On the other hands nutrient added 100 minutes after the addition of antibiotic gave rise to significantly less gas than the simultaneous addition of both nutrients and antibiotics at this time. As the time of of incubation with antibiotic alone was extended to three hours, the inhibition of gas production, consequent upon nutrient addition, was increased. Incubation period greater than three hours did not further increased the inhibition. Gas production was increased for the first 100 minutes by both levels of antibiotic but the reverse was true at 250 minutes.

In all cases the effect of high level of antibiotic was greater than that of he low level. Gas production was significantly correlated with volatile fatty acid production and the final pH of the reaction medium.

Klopfenstein et al (1964) found that gas production 'in vitro' by rumen ogganisms taken from animals on the respective treatments showed that inhibition by the addition of antibiotic was significantly greater, when rumen organisms were taken from control lamb than when taken from the lambs fed 60 mg. aureomycin. Further gas production resulting from the addition of nutrients to prefeeding rumen samples was significantly greater with sample from animals receiving 20 mg. aureomycin, either before feeding or with the feed than with

samples from control animals or animals receiving 60 mg. aureomycin with the daily feed. Pooled data showed that gas production 'in vitro' rumen contents after feeding from animals receiving 20 mg. aureomycin in their ration, was significantly greater than by rumen contents from control animals.

Nešić and Ibrović (1963) maintained 20 sheep mostly on hay of moderate quality and studied the effect of some antibiotics on fermentative processes in the rumen on them. Samples of rumen contants were taken before the morning feed and individual sheep were given penicillin, streptomycin, chloramphenicol, terramycin, or aureomycin into rumen onform 3 to 5 successive days. Amounts given daily were 400,000 I.U., 0.5 to 1, 0.5 to 1, 0.25 to 0.5, 0.5 and 0.8 g. respectively.

Penicillin was the most harmful to ciliate population, terramycin and aureomycin had less effct on the protozoa and t the other antibiotics none. Bacterial activity was most affected by terramycin and aureomycin, although all the antibiotics to some extent inhibits fermentation of glucose and digestion of cellulose.

They suggested that these antibiotics could be given by mouth if it is disired to surpress batterial activity for example in bloat but rumen fluid from normal animals shouldthen be given to restore the flora. The antibiotics could be given by mouth therapeutically to young suckling ruminants before the flora was established.

STEAM VOLATILE FATTY ACID PRODUCTION

The significance of volatile fatty acid (VFA) production arising from fermentation within the rumen of ruminants has become an active area of research in ruminant nutrition and physiology. It has been established that the volatile fatty acids are the chief end product of microbial digestion and these acids are then absorbed from the reticulo-ruminal epithelium into the blood stream. The rate of absoption depends on the pH of the rumen and concentration of the VFA. With the isotope technique, Mukherjee (1960) worked out the rate of absorption with 'in vitro' experiment in fasting sheep to be 35.3 , 9.9. and 3.54 m.moles/hour for acetic, propionic and butyric acid respectively.

Unlike the simple stomached animals, these acids are extensively metabolised by the ruminant animals as source of energy and also for the synthesis of body fat. The concentration of total VFA in the artrial and venus blood in both the sheep and the cows varies with the concentration in the rumen. Fasting causes a steady depression of the volatile acid content of the rumen in the sheep and cow and the level of circulating VFA is equally depressed.

Propionic acid is khown to be a source of glucose and it enters the tricarboxylic acid cycle by carboxylation

to succinate (Pennington) and Sutherland,1956). It is thus an important product of digestion.

Butyric acid is rapidily metabolised by rumen epithelial and liver tissues in vitro. The in vitro experiments indicate that ketone bodies may be produced in considerable quanity from butyrate. Increases in the level of ketone bodies in the blood have been observed on injection of butyrate 'in vitro' (Shultz and Smith,1951;Jarrett and Potter,1950).

Acetic acid is quantitatively the most important acid produced in the rumen. It is metabolised by most body tissues and the contracting heart can utilize it as rapidily as glucose. Reid (1950) showed that itis metabolised by brain tissue. Phillipson (1947) regarded acetic acid as a glycogen former upto about 40 percent . The synthesis of milk from acetate is also established beyond doubt (Popjak et al 1951; Cowie et al,1951).

Armstrong et al (1957) demonstrated with help of indirect calorimetry that mixtures of acetic,propionic and butyric acids when used as the sole source of energy for fasting sheep, were utilized quite efficiently by the animals for their basal activities,as only about 15 percent of total metabolisable energy appeared as heat increment when 1117 Cal/24 hour of metabolisable energy was utilized by the animmals.

Stewart et al (1958) estimated that out of the 12.7 therms supplied by these VFA, 5.7 % was from valeric acid, 24.3% from butyric acid, 21.9% fron propionic acid and 48.1% from acetic acid.

Hence the amounts and proportions of these acids found in the rumen formed is important in supplying the ruminants with essential energy. Amounts and proportions of short chain volatile fatty acids produced are variable depending upon time after feeding, nature of diet, age of animals and inclusion of various additives in the feed. This variation is also due to type and number of microorganisms present in the rumen.

Protozoa and fatty acid production.

It has been demonstrated that rumen protozoa possess relatively large amounts of amylolytic, cellulolytic and proteolytic enzyme (Hungate, 1942; Williams et al, 1960).

According to Ferber(1928) protozoa may constitute 6to 10 percent of the weight of rumen contents and supply 20 to 25 percent of the total energy requirements of sheep and cattle.

Christiansen et al (1962) while describing a multiple -tube laboratory technique for studying factors influencing volatile fatty acid production by rumen protozoa in the

absence of bacteria, observed that protozoa perse se were capable of producing large quantities of VFA's since at the height of activity as much as 8 gm. of total volatile fatty acids (TVFA's) as acetic acid equivalents per hour per 100 gm. of organic matter were produced. They found that pH exerted an influence upon protozoal VFA production. The optimum pH for maximal VFA production was approximately 7.0. Below or above this pH range, the amount of VFA's produced was progressively less.

Time after feeding and fatty acid production.

Stewart et al (1958) observed that the concentration of VFA in the rumen increased after feeding from 4 to 6 hours. Rate of VFA production was greater than within the first two hours after feeding. The rates of production of propionic and valerric acid were proportionately greater than those of the other fatty acids for the first two hours after feeding. Volume of rumen contents and total quantities of VFA in the rumen were greater after the evening feeding.

Nature of diet and fatty acid production.

Stewart et al (1958) studied 'in vitro' VFA production from various feed by rumen microorganism. Their results were based on 12-hour production of VFA 'in vitro'. Urea was found to consistently increase VFA 'in vitro' regardless

of the substrates used although in subsequent 'in vivo' experiment, increase of VFA production was only slightly. Fresh haddclipped legume mixed grass caused a greater VFA production than did legume hay. The grass was found to markedly depress propionic acid production compared to hay. Molasses consistently decreased acetic acid production,beet pulp significantly increased acetic acid production and corn meal increased propionic acid production.

Mahapatro (1964) reported M % of ruminal acetate were lower ($P<.01$) and percent of propionate and butyrate were higher ($P<.01$, $P<.05$) respectively in lambs fed the hay or wilted silages than in those animals fed the wet silages. In another trial,they observed that M% acetate and butyrate did not vary ($P>0.05$) either with fineness or moisture content. Molar % propionate was higher ($P<.05$) in lambs which received the wet hays.

Ekern and Reid (1963) carried out experiments to find out the effect of that on the total and relative concentrations of VFA in ruminal ingesta. They fed three different diets viz. hay, silage and hay supplemented with lactic acid to three groups of young cattle. The results obtained showed the molar proportions of the following fatty acids as percentages ofthe total acids in the rumen ingesta of animals fed hay, silage and hay supplemented with lactic acid respectively as acetic acid 69.5, 58.3 and 57.4;

propionic acid 18.5, 20.7 and 28.7; butyric acid 9.8,15.4 and 11.0 and higher acids: 2.2, 5.6 and 2.8.

Thus the diet of hay alone was associated with a higher proportion of acetic acid and lower concentration of propionic acid and butyric acids in ruminal ingesta than were the diets of silage or of hay supplemented with lactic acid. Their observations indicated that the energetic efficiency of body gain in young cattle ingesting forage diets was favoured by diets resulting in higher proportion of propionic and butyric acid relative to acetic acid in the rumen.

Raun et al (1962) found that high concentrate (80%) rations fed group produced narrower acetate:propionate ratio, higher butyric acid levels and lower VFA and pH in the rumen than did a 50% concentrate fed group.

A series of experiments were undertaken in 1960 and 1961 by Parks et al (1964) to ascertain the effects of changes in apparent nutritive value and chemical composition of the diet on molar ratio of acetate, propionate and butyrate produced in the rumen fed gulf rye grass harvested at differentstages on maturity.

They reported that the intraruminal molar percentages of acetate tended to increase and those of

propionate and butyrate to decrease as the forage matured. Shifts in the intraruminal fatty acid ratios were shown to coincide with the changes in the apparent,nutritive value of rye grass especially in 1960. As the rye grass became less digestible over the growing season,the mixture of acids produced in the rumen of sheep became progressively higher in acetate and lower in propionate and butyrate.

The chemical component of rye grass which appeared to be most closely related to the intraruminal ratios were soluble sugar and protein in rye grass decreased and the level of lignin in the forage increased, the molar percent of propionate in the rumen content decreased and the intraruminal molar percent of acetate increased.

Effect of age on fatty acid production.

Omar et al (1964) found out the distribution and concentrations of intraruminal VFA in young ruminants of different ages and determined the relationship that such distributions might had to the extent and variability of ruminal development.

The maximum concentrations ofVFA were observed between 12 & 17 weeks. Acetic, propionic,butyric, isobutyric,valeric and iso-valeric acids were detected in

the ruminal content of all lambs with feed risidues in the rumen . The C_5 acids and butyric acid were not found in two lambs that received only milk.

The proportion of acetate was negatively related to level of total volatile fatty acids and to age, body size and stage of ruminal development. Displacement of acetate at higher concentrations of total VFA was by both propionate and butyrate but the displacement of acetate with growth and ruminal development was the result of a significant increase in the molar fraction of butyrate. Of the minor fatty acids, only iso-butyrate showed a significant relationship(negative) to age.

The simple product-moment, multiple and partial correlations ofacetate (negative) and of butyrate(positive) with ruminal characteristics were of such significance and magnitude that the workers indicated that the pattern of intraruminal fermentation was associated with factors conditioning both total ruminal development and papillary growth in creep-fed suckling lambs.

Species and fatty acid production.

'In vitro' studies of Negy et al (1962) on alfalfa substrate using deer,cow,sheep and cattle as rumen fluid donar,indicated that deer rumen fluid had resulted in lowest acetate and highest propionate percentages.

The following range was found in molar percentage of acetate, propionate and butyrate respectively; deer 42-49, 36-27, 17-18; cow 60-65, 18-16, 16-14; sheep 55-54, 23-27, 16-14; steer 55-54, 20-21, 19-20.

Heat stress and fatty acid production.

Weldy et al (1964) worked on the influence of heat stress on rumen acid levels and some blood constituents in cattle subjected to 90°F. Total VFA concentrations were lower at 90°F, with lower acetic acid concentrations being largely responsible changes in total VFA concentrations were consistently correlated to a significant degree with rectal tempature and rate of respiratory evaporative loss but the relationships of VFA level to dry matter and water intake, blood glucose and blood ketones were quite variable.

They concluded that the direct effect of the heat stress would bring about some changes in rumen acid concentrations and these changes in rumen acid levels as well as blood constituents (viz heamatocrit) might be involved in the adjustment to heat stress.

Effect of additives on fatty acid production.

Glucose was reported to increase 'in vitro' production of all three acids and total acids but agrozyme, an enzyme preparation, had no effect on 'in vitro' production

and a general increase in production of all the acids was found as the pH was raised from 4.5 to 6.5 but there was no significant change from 6.5 to 7.5 (Brown and Tucker,1962).

Raun et al (1962) added sodium bicarbonate and calcium carbonate to lamb rations high in concentrates and low in roughage and observed their effect on ruminal fermentation patterns.

Addition of sodium bicarbonate and calcium carbonate to lamb rations, high in concentrates and low in roughage had no appreciable effect on the acetate : propionate ratio or the total volatile fatty acids in the rumen fluids samples three hours ater the morning feed.

Miller et al (1964) also reported the effects of feeding of buffers to dairy cow on a high-concentrate ration. The four experimental treatments consisted of a control, 1.00 lb $KHCO_3$, 0.84 lb $NaHCO_3$ and 0.42 lb $MgCO_3$ per day.

Analysis of rumen samples showed that cows on the control ration had a significantly ($P<.05$) lower molar percent of acetic acid (49.2 vs 54.0) and significantly ($P<.05$) higher molar percents of propionic acid (37.4 vs 31.3), and n-valeric acid (2.8 vs 1.8) than while on the buffered rations. The molar percents of butyric and iso-valeric acids, although higher for the buffered rations, were non-significant.

Chalupa et al (1964) studied the influence of ethanol on rumen fermentation. Varying levels of ethanol(ml/100 g rumen DM) were added to a series of artificial rumina. Before feeding the distribution of individual fatty acids and total fatty acids were alomost identical but molecular concentration of acetic acid was 10 percent greater in rumen liquor obatined from ethanol-fed animals four hours after feeding.

Elam and Devis (1962) reported that microbial activity of ruminal samples was decreased by feeding the combination of sodium chloride and saliva salts but the total concentration of ruminal fatty acids and the proportions of these acids did not appear to be affected by treatments.

EFFECT OF ANTIBIOTICS ON VFA PRODUCTION

Reports that the feeding of aureomycin lowers the digestibility of various feed nutrients in ruminants suggests that the antibiotic could change VFA production in the rumen.

Hibbs and Conard (1953) made a study on the effect of feeding aureomycin supplement on the performance of calves raised on the high roughage system and did not find any difference by feeding aureomycin in the average total steam volatile fatty acids or acetic acid in rumen juice at 12 weeks of age. Propionic acid was slightly lower and butyric acid slightly higher in the aureomycin-fed group.

Hungate et al (1955à) concluded that the capacity of the rumen organisms to form fermentation products had not been changed by the antibiotic addition.

Leffel et al (1957) maintained nine lamb on alfalfa hay and barley diet and fed aureomycin at level of 0,7,12, or 24 mg per lamb per day. Their preliminary results indicated that antibiotic did not alter the amounts of ratios of VFA present in the rumen fluid and did not affect the pattern of dissimilation of c^{14}- labeled glucose by cell suspensions. Dissimilations of C^{14}- labeled glucose by washed cell suspensions from sheep and cows on normal rations consistently showed a high proportion of propionate production.

Preston et al (1959) studied the effect of low-level feeding of chlortetracycline on rumen fermentation in early weaned calves with regards to the mean pH and the mean concentration of SVFA in strained rumen liquor for the 10-week period. Both before and four hours after feeding, the rumen liquor from the calf which was fed concentrates supplemented with antibiotic had a higher pH and a lower concentration of steam volatile fatty acids, than the rumen liquor from its controlm mate.

Dinda (1960) while studying on some of the effects of chlortetracycline in the diet nutrition of the early weaned calf, found that antibiotic reduced the concentration of lower volatile fatty acids, ammonia and total reducing substances in the rumen and increased pH. The level of reducing sugar in the blood was found higher and that of urea lower when chlortetracycline was fed. Rate of passage of feed was not affected. The worker concluded that antibiotics increased growth of early weaned calves as consequence of increased feed intake and enhanced protein and energy metabolism and that these effects in turn, were secondary to changes in rumen fermentation.

Raun et al (1962) observed in lambs that in a low concentrate ration, chlortetracycline and urea did appear to exert and effect on ruminal fermentation as shown in table 2.1. While chlortetracycline and urea alone tended to widen

($P < .05$) the acetate- propionate ratio, a significant negative interaction was noted between the two. Urea and chlortetracycline alone tended to elevate acetate levels but together a negative interaction also occurred. Chlortetracycline also appeared to elevate ($P < .05$) butyric acid levels. Urea was added at zero and 0.7% levels and chlortetracycline at zero and 10 mg per lb of feed.

TABLE 2.1

Table showing the effect of chlortetracycline & urea additions in a low concentrate ration (50% concetrate-50% roughage).

Criteria	Basal	Chlor-tetracycline	+0.7% urea	+0.7% urea + Chlortetra-cycline	Average
VFAs molar%					
C_2	57.9	61.2	62.1	59.4	60.2
C_3	25.7	19.7	23.9	22.9	23.1
i - C_4	0.5	0.5	0.3	0.3	0.4
C_4	15.1	18.0	12.9	16.7	15.7
i - C_5's	0.3	0.2	0.2	0.2	0.2
C_5	0.5	0.3	6.5	0.4	0.4
Ratio C_2/C_3	2.3	3.1	2.6	2.6	2.6
Total VFA[a]	89	83	87	92	88
Buffering capacity[b]	2.2	2.1	2.1	2.2	2.2
pH	6.5	6.5	6.6	6.3	6.5
Av.daily gain	0.65	0.61	0.63	0.63	0.63
Feed/lb gain	5.86	6.46	6.35	6.00	6.17
Carcass grade score[c]	7.0	6.7	5.7	7.0	6.6
Dressing%	49.1	50.1	49.0	49.6	49.5
Seperable fat	26.1	24.8	27.5	25.7	26.0
Seperable lean	52.8	53.7	52.4	54.3	53.3
Seperable bone	21.1	21.5	20.1	20.0	20.7
% fat in logissimus dorsi	15.7	16.1	21.2	17.5	17.6

a Micromoles of volatile fatty acids per ml.

b Ml of N/10 KOH

c Federal carcass grade score: 5 -good,6-high good,7-low choice, and 8- choice.

Klopfenstein et al (1964) conducted experiments on cross bred whether lambs to find out the influenceof aureomycin on rurumen metabolism. Their each experimental period consisted of 12 days prefeeding, 8 days urine and fecal collection and one day of rumen sampling.

Treatments consisted of : A, control; B, the infusion of 20 mg of aureomycin (suspended in 5 ml of water)into the rumen via the fistula 90 min. prior to feeding; and C and D, 20 mg. and 60 mg., respectively.- of aureomycin fed in the ration.

They obtained that treatment B and C had tendency to produce more gas at one hour after feeding and treatment B also had tendency to produce greater VFA concentration'in vivo'. Significant differente in average daily VFA concentrations was not observed.

Purser, Klopefenstein and Cline (1964) in their 3X3 latin square design experiment, supplemented aureomycin(30 mg/day) and tylosine (15 mg/day) in two experimental groups of sheep while the third group was the control group. While studying on the effect of rumen liquor from antibiotic fed sheep on organism growth'in vitro', determined VFA ratio on samples taken four hours after fedding on the 14th day which showed :acetate/ propionate ratios of 4.37, 3.55 and 2.88; acetate /butyrate ratios of 6.32,6.01 and 5.70 and propionate/butyrate ratios of 14.7, 16.4 and 2.03 for the control,aureomycin and tylosin treatments respectively.

EFFECT OF MICROBIAL ACTION ON CAROTENE AND VITAMIN A.

Perry et al (1962) studied the synergism between an antibiotic and vitamin A utilization. They fed steers of one of their sub-groups, 80 mg. of chlortetracycline per head daily for this test. Control cattle receiving no supplemental vitamin A showed a growth depression from supplemental chlortetracycline.

Studies were conducted by King et al (1962)to determine whether appreciable losses of carotene and vitamin A occur in the rumino-reticulum. Destruction was observed when carotene and vitamin A were incubated 'in vitro' in rumen fluid.

Recovery of carotene from inoculated but non-incubated tubes averaged 97.5; whereas recovery from tubes incubated 9 hours averaged 65.6%. Similarly vitamin A recoveries (using U.S.P. Reference Standard solulation) were 81.1% in non-incubated versus 60.4% in incubated tubes. The difference in each case were significant ($P<.01$). Antioxidants(Santoquin and tocopherol) each reduced losses ($P<.01$) in a 10-hour incubation using a commercial, stablized vitamin A preparation.

A series of'in vitro' trials shwed recoveries varying from 11.5% to 100% when various commericial vitamin A feed preparations were compared. The destruction effect was comparable in rumen fluid from cattle and sheep and in fluid from cattle fed hay versus high grain rations.

Known amounts of carotene, vitamin A and Cr_2O_3 were fed to wethers and steers. Upon slaughter at 12, 24 or 48 hours following the meal, ingesta was collected quantitatively from the various segments of the digestive tract. Significant differences in the ratios carotene : Cr_2O_3 and vitamin A : Cr_2O_3 in feed versus injesta indicated losses from the rumino-reticulum (of approximately 40% in 12 hours) in addition to that resulting from passage into the lower tract.

Recent studies by Esplin et al (1963) suggested that carotene is poorly utilized on high-concentrate rations.

Devison and Seo (1963) reported the influence of nitrate upon carotene destruction during 'in vitro' fermentation with rumen liquor. Their results suggested that nitrate does not increase carotene destruction in the rumen, however rumen microorganisms were capable of destroying carotene (Table 4.2).

T A B L E 4.2

Tables showing carotene destruction during 'in vitro' fermentation with rumen liquor.

Nitrate added mg.	Animal	
	Control (%)	Nitrate fed (%)
0	25.2 ± 5.6	24.5 ± 8.2
3.25	19.3 ± 4.4	23.2 ± 6.3
7.5	20.8 ± 4.6	24.6 ± 5.5
15.0	20.6 ± 4.6	27.0 ± 8.1
Average	21.6 ± 2.6	24.5 ± 3.3

Keating et al (1964) designed their experiments to determine the 'in vitro' effect of rumen liquor from steers fed high -vs- low grain rations on the degradation of vitamin A and carotene and also to study the effects of ethoxycuin (santoquin), nitrate and nitrite.

Their studies with five fistulated steers on all roughage rations indicated that nitrite was effective in degrading both vitamin A and carotene provided the nitrite levels were sufficiently high. Nitrite was added 'in vitro' at levels corresponding to 0.25, 0.5 and 1% of the rumen liquor. A significant destruction of carotene with the 'in vitro' addition of 1% nitrite was indicated.

The ration ethoxyquin maintained the levels of vitamin A in both roughage and grain ration. The addition of 1% nitrate to the ration has little effect on the 'in vitro' destruction of vitamin A. Destruction of vitamin by rumen liquor from steer on a high roughage was significantly greater than with rumen liquor obtained from steers receiving a high grain ration. Destruction of carotene was found to be small or nil except with certain treatment notably nitrite and that only at equivalent levels that would never be encountered in usual feeding condition.

Shorland et al (1957) indicated that carotene was not affected by rumen fermentation.

Recently Klatte et al (1964) studied the stability of vitamin A acetate in ruminal and abomasal fluids. Ruminal and abomasal contents were obtained from dual-fistulated cattle and sheep which had been receiving low pigment diets without vitamin A. The contents were strained through cheese cloth and centrifuged at low speed. In each trial , six incubation tubes containing 10 ml. of either ruminal or abomasal fluid and approximately 20 I.U. of vitamin A acetate in oil dispersed in 20% tween 80 in water were prepared.

Three tubes were selected randomly and incubated under carbondioxide for 4 hours at 37°C. The other tubes were analysed for vitamin A immediately. Water, abomasal fulid and ruminal fluid were used in 11, 17 and 11 trials respectively. And additional trial of autoclaved rumen juice was used in 1 trial.

The average percentage of vitamin A recovered was 84.0% for water, 66.5% for abomasal juice and 63.9% for ruminal fluid and 87.8 for autoclaved rumen fluid. Destruction of vitamin A by ruminal and abomasal fluid was highly significant. Results obtained with autoclaved ruminal fluid suggested that destruction by ruminal fluid was dependent on microbial activity. Activity of fluids from steers and wethers were found similar.

Guerrant (1962) studied the efficiency of antibiotics in supplementing the growth effect of beta carotene in vitamin A -depleted rats.

In his experiment, weanling rats were depleted of vitamin A and then received for four weeks 1.0 microgram beta carotene in cotton seed oil daily, without or with 1 g. per kg. diet of chlortetracyline hydrochloride, chloromycetin, demethylchlortetracycline hydrochloride, procaine penicillin, sodium acrylate, streptomycin, terramycin hydrochloride, tetracycline hydrochloride or zinc bacitracin.

The additiona growth response in rats given antibiotic over that in rats given non ranged from only 4% for sodium acrylate or streptomycin to 44% for tetracycline hydrochloride; the other tetracylines also appeared to be effective.

C H A P T E R- III

MATERIALS AND METHODS

Rumen liquor used in incubation.

The rumen content used for incubation through out this experiment was brought from slaughtered goats from the butcher's shop very near to be college immediately after the slaughter. The rumen wall was slited by a sharp knife and a pound of rumen content was taken in a wide mouthed bottle which was previously cleaned and carbondioxide gas from the gas cylinder was filled in it by water displacement in the laboratory. The bottle filled with rumen liquor was immediately taken to the laboratory and carbondioxide gas was again passed and the bottle was immersed in warm water at about 39°C.

A 600 c.c. beaker was taken and carbondioxide was passed in it for a few minutes. The rumen contentxx was strained in the beaker through a double folded fine muslin cloth and 20 c.c. of rumen liquor was pipetted into each incubation flask.

Antibiotic treatment.

Aureomycin and terramycin at two levels i.e. 0.25 mg. per ml. and 1.0 mg. per ml. of rumen liquor were used as treatments thus 5 mg. and 20 mg. doses of aureomycin and

terramycin were added to the experimental flasks in duplication. The flasks were then gently shaken so that antibiotics might be well distributed.

While studying on the effects of these antibiotics in carbohydrate and protein rich substrate seperately, starch and casein were taken. One gm. of each was added in different experimental flasks.

'In vitro' incubation.

Incubation was carried out in 100 ml. conical flasks (Pyrex), fitted with rubber corks. Each rubber cork was provided with both inlet and outlet device, in which inlet tube was kept well below the outlet tube. This arrangement was made in order to fit all the experimental flasks in a series in the water bath.

To each of the flasks 20 ml. of the rumen liquor was pipetted and the experimental flasks were treated with respective treatments. 2 c.c. of 2 M phosphate buffer of pH 6.6 was added into the flasks. Two of the flasks were treated with 2 c.c. of 10 N sulphuric acid to stop the fermentation in these flasks and this constituted the unincubated sample.

All the other control and treated flasks were immersed in a water bath maintained at 39°C- 40°C and were then fitted tightly with corks connected in series. These were made air tight. Leakage was tested by placing drop of water at the junction of flask and cork. The incubation experiments were over in duplicate.

All the flasks were gassed with carbondioxide by gas cylinder through the inlet tubes for 15 minutes to maintain anaerobic condition which were tested by passing the outlet gas in Bariumchloride sodium and also by a lighted paper. After making sure that carbondioxide was arising from the outlet, via both the outlet and inlet were closed by the pinch cocks. The rumen content was thus incubated under anaerobic conditions. The flasks were gently shaken every fifteen minutes, throughout the incubation period.

After the specified incubation time all the flasks were acidified with 2 ml. of 10 N sulphuric acid. The whole process of starting incubation was carried out within 15 to 2o minutes from the time the sample was brough to the laboratory.

Estination of SVFA in rumen liquor.

The total SVFA in the rumen liquor was estimated by the method of Elsdon et al (1946a)

The acidified rumen liquor of each of the flasks was then taken to a measuring cylinder. Each flask was washed three times with 10 N suphuric acid and was poured in the measuring flask and the volume was made to 40 ml. In 20 ml. of the aliquots about 8 gm. of $MgSO_4$ was added . The addition of Magnessium sulphate served not only to increase the rate of distillation of the volatile fatty acids, but also to hold back HCl which otherwise might come over in appreciable quantities.

20 ml. of rumen liquor thus treated was taken in the "Markham steam distillation apparatus" and was steam distilled (Markham,1942).

Two successive distillates of 150 and 25 ml. respectively were collected and titrated against N/10 NaOH solution using 0.02 percent bromo - thymol blue as indicator. The second 25 ml. distillate always did not contained any acid. The duplicate distillation in the two stills that were regularly used, did not, as a rule, vary by more than 1 percent. Precautions were taken to see that steam distillation was not too fast to make the tip of the condensor hot.

CHROMATOGRAPHIC SEPARATION OF THE VFA

The chromatographic procedure followed was a modified procedure of Bueding and yale (1951). The whole process consists of the following steps which are described individually below:-

(1) Preparation of the VFA sample for chromatography

The first 150 ml. of Markham distillate was made distinctly alkaline after titration by the addition of a few drops of N NaOH and was then evaporated down to 20 ml. over an even slow flame of burner. The mall volume of distillate was then transfeered to a pyrex testus tube and was completely dried down in an oven.

The VFA was then extracted according to the method of Elsden (1946) into chloroform solution which is necessary

for chromatography. The dry powder was dissolved 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 tube. The powder was then extracted 6 times with small volume of 5% butanol in chloroform dried over Na_2SO_4 and then made upto volume.

(ii) 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 vigorously in concentrated hydrochloride acid for 30 minutes, to remome most of the iron in it. The supernatent HCl was decandet off and the celite was repeateadly washed with about 3 liter portions 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 washing, a fair amount of the finer particles of celite that failed to settle down within 15 minutes was also decandet 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 desiccator under continuous evacution.

8 gm. of this processed Celite was thoroughly ground in a mortar with 4.8 ml. of 2 M Phosphate buffer at pH 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 tampered again to remove the air bubbles 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 column. 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 roteted slowly and the edge of the pack was tampered 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. After the last addiion 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 detail so as to avoid the formation of channels and consequent poor resolution.

(iii) 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 just reached the surface of the Celite. From 3 - 5 ml. of the solution of the acids in the solvent containing 100 - 150 micromoles 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 (CB_0). The column was then filled with CB_0 and 5 or 10 ml. samples were collected for titration. Very often the first fraction of the elute was collected in bulk of 50 ml. to save time and error in the titration.

When the titration reading of the last sample of elute dropped down to blank value, the receiver was changed and after the remaining volume of CB_0 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 elute was carried out with N/200 alcoholic (ethanolic) KOH, using bromo thymol blue as the indicator. Before the titration of the CB_0 fractions 10 drops of ethanol was added to it so as to avoid the false end 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 CB_0 fractions as pointed out by Peterson and Johnson (1948).

The natural flow of the solvent through the column was sometimes found to be too slow. The efficiency of separation of the acids was found to be unaffected by variation in the rate of flow of the solvents.

Recovery of the solvents for repeated use.

The use solvents were pooled and vigorously shaken with about 5 ml. of strong ethanolic KOH to neutralize the free acids if any. The pooled sample was then washed with running tap water till the washing did not show any sign of alkalinity. The solvent was separated from the water with the separating funnel and was shaken with $CaCl_2$ granules for further drying. The dry solvent was distilled in a glass distillation apparatus. The distillate consisted of undetermined mixtures of chloroform and butanol which was used to prepare fresh CB_5 and CB_{20} by adding calculated amounts of pure chloroform or pure butanol to it based on specific gravity measurements with the hydrometer. The CB_0 was, however, always prepared from fresh chloroform. The same pooled solvent was used indefinitely without any apparent deleterious effect.

Reagents used

1. 2 M phosphate buffer, pH 6.5 - 104 g. K_2HPO_4 and 190 g. KH_2PO_4 per litre.
2. Butanol- n - butanol, reagent quality.
3. Chloroform - reagent quality, washed with distilled water for 2 - 3 hours to remove the alcohol added to it for stabilisation.
4. Ethanol - reagent quality.
5. Solvents - pure chloroform (CB_0), 5% butanol in chloroform (CB_5) and 20% butanol in chloroform(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.

ESTIMATION OF CAROTENE IN RUMEN CONTENT

The method suggested by Majumdar and Gupta (1960) after making a comparative study on the merits of the various adsorbents and solvent combinations, recommended by Moore and Ely (1941), Zcheile and Whitmore (1947), Bacharach(1950) and Bickoff et al (1952), was followed for the estimation

of carotene in rumen content. In stead of column chromatography, powder chromatrography was adopted in this case as suggested by Majumdar & Gupta (1960) in another paper.

Procedure applied

The whole process which was carried out consists of the following steps:-

Incubation of rumen samples:- The process for incubation was followed as in previous experiment except that in all the flasks 20 microgram per 2 ml of carotene was added.

Estimation of carotene:- 2 c.c. of rumen liquor was taken and was run in a Waring blendor and was homogenized for a minute with 25 c.c. mixture of 1:1 acetone and petroleum ether (b.p.40-60°C) as extractant and a pinch of quinol as antioxidant. For each sample four such extractions were made. The extract was then filtered through glass wool and taken in separating funnel. The glass wool was washed thrice with acetone : petroleum ether mixture and was collected in the separating funnel. The acetone present in the extract was then removedby washing with distilled water. Four such washings were made.

The residual petroleum ether extract was then taken in a 50 ml. measuring flask in which 10gm. of anahydrous sodium sulphate was kept. Thus the traces of water remained was removed. After that the extract was again taken in a beaker and was shaken with 6 gm. of bone meal.Xanthophylls and other non-carotenoid

pigments were thus adsorbed. The pure carotene extract thus obtained was then allowed to stand for half an hour in a dark place. This was then filtered through a Whatman No.40 filter paper and was then taking into a 50 c.c. measuring flask and the volume was made by the addition of petroleum ether. The carotene reading was then made in the photoelectric colourimeter using a blue filter No.42 having approximate spectral range 400 - 465 millimicrones. Before use the colourimeter was set at zero with blank.

Preparation of standard curve

A standard solution of carotene was prepared by taking 10 mg. of pure beta - carotene in 100 c.c. of petroleum ether with a boiling point of 40°C - 60°C . Thus 1 ml. of this solution contained 100 microgram. This solution in 0.1, 0.2, 0.3, 0.4, ~~25~~ 0.5, 0.6, 0.7, 0.8 c.c. were taken in duplicate in clean,dry pyrex test tubes and thus containing 10, 20, 30, 40, 50, 60, 70, 80, microgram respectively. In each tube, volume was made upto 10 c.c. with petroleum ether and the reading was directly taken in the colourimeter using blue filter No.42 having spectral range of 400 - 465 millimicrones. The standard graph was prepared and was attached.

S

STATISTICAL METHODS

1. Analysis of variance:- This analysis was done for total fatty acids produced and for the different fatty acids produced under various treatments.

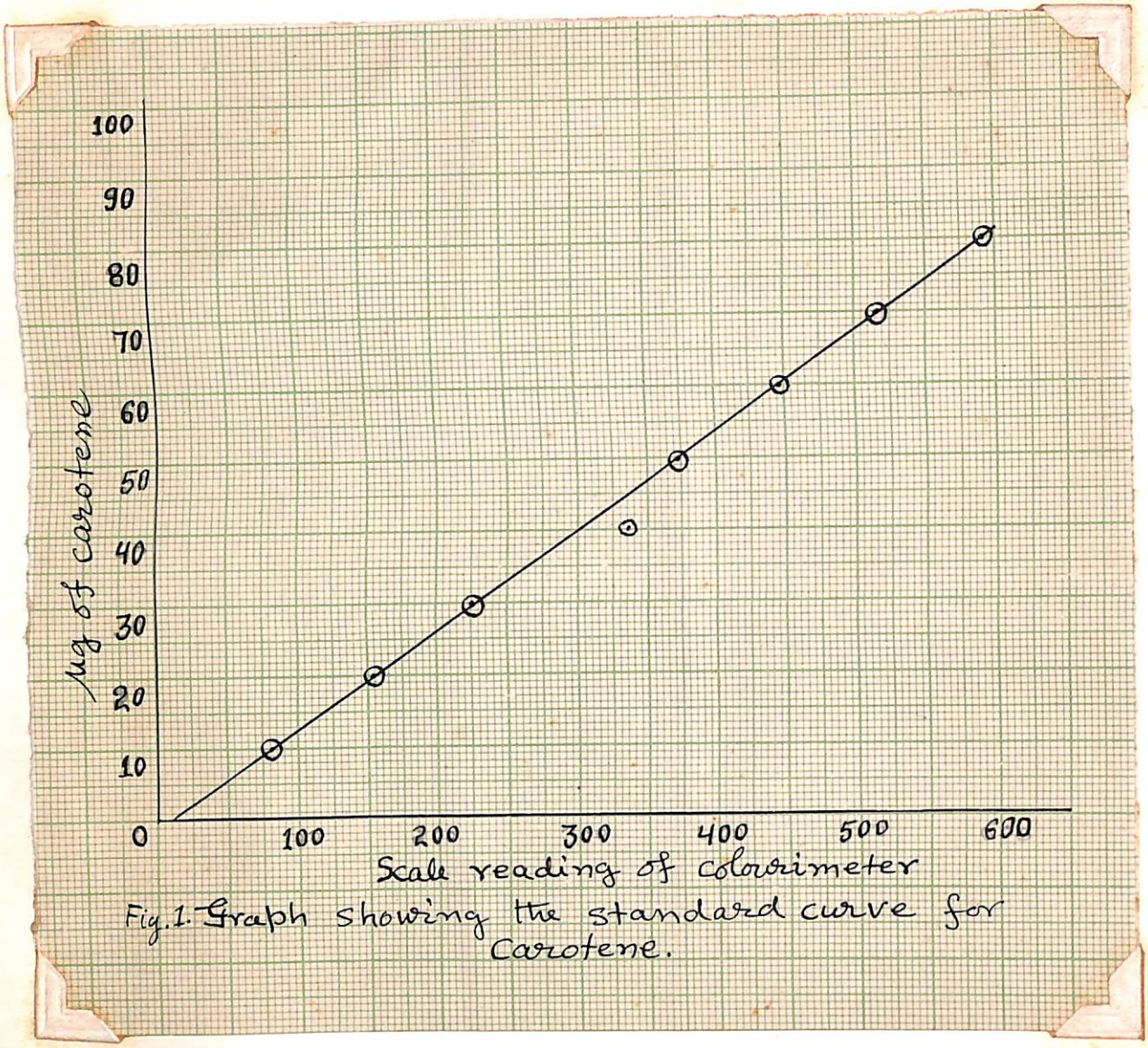

Fig. 1. Graph showing the standard curve for Carotene.

The following is the set up of the analysis:-

Sources of variation.	d. f.	Total S.S.	Mean Square	F.
Total	N-1	X	-	
Between treatments	Y-1	P	$\frac{P}{Y-1}$	$\frac{P}{Y-1}$
Within treatments. or error	N-Y	Z	$\frac{Z}{N-Y}$	$\frac{Z}{N-Y}$

where,

N- total number of observations.

Y- total number of treatments.

X- total corrected sum of squares.

P- between treatments corrected sum of square.

Z- error sum of squares.

The analysis of variance table provides a ready means of testing the significance of the difference between class means. A comparision of the mean square between treatments with the error mean square provides a test of significance of differences arising from treatments. The comparision is done by finding the ratio of the mean squrre concerned to the error mean square. This ratio is the variance ratio and is denoted by 'F'. This was seen in the F table at that particular degree of freedom for significance.

Critical difference:-

By this, the comparative effects ofdifferent treatments on VFA production from starch were studied. As significant differences between the treatments were obtained by F test in the analysis of variance, hence this test was done.

Critical difference was calculated both at 5% and 1% level by the following formula :-

$$\text{Critical difference} = t \text{ (with error d.f.)} \sqrt{s^2\left(\frac{1}{r_1} + \frac{1}{r_2}\right)}$$

t - table value with error d.f.

s^2 - error mean square.

r_1 and r_2 - No. of replicates.

The critical difference value was then compared with the difference of the averages between two treatments.

C H A P T E R - IV

ESTIMATION OF ERRORS IN VARIOUS EXPERIMENTAL PROCEDURES.

I Efficiency of Markham distillation:-

Markham(1942) designed a apparatus known as 'Markham distillation apparatus'. This apparatus is used for the distillation of known quantify of rumen liquor. Standard acetic acid solution was prepared and 10 c.c.of the standard acetic acid,solution was then added to the 'Markham Still' and was steam distilled. 50c.c.of distillate was collected and was titrated against N/10 sodium hydroxide. The next 15c.c.of distillate was also titrated and did not contain the acid.The result of the recovery is shown in table 4.1 .

T A B L E 4.1

Efficiency of distillation of Markham distillation apparatus.

Source	No.of observa-tions.	Amount of acid used (ml N/10 NaOH, mean value)	Amount of acid recovered (ml N/10 NaOH, mean value)	Percentage recovery.
N/10 Acetic acid	5	10 c.c.	10.06	100.6± .98

The table 4.1 indicates that recovery from the distillation apparatus was found to be about 100.6 persent.Too slow or too fast rate of distillation was found to produce errors.About 30 minutes of distillation was found to be optimum.

II. Efftiency of separation and recovery of acetic, propionic and butyric acids from mistures by chromatography on Celite.

A standard solution of known amounts of acetic, propionic and butyric acids in CB_5 was prepared seperately. A mixture of these three acids in the ratio of 3:2:1 was made which contained about 150 micromoles in 5 ml. Directtitration of the same amountof mixture with standared N/200 ethanolic KOH served as the standared.

5 ml. of the mixture was added to the chromatogram and the recovery of the three acids was tested. The result of the recovery is shown in table 4.2.

T A B L E - 4.2.

Percentage recovery of VFA by chromatography.

No. of columns used.	No. of observations	Eluents	Amount of acid used (ml.N/200 ethanolic KOH)	Amount of acid recovered (ml. N/200 ethanoloc KOH)	Percentage of recovery.
4	8	CB_0	4.02	4.06	100.95
4	8	CB_5	8.06	7.89	98.11
4	8	CB_{20}	11.80	11.70	99.15

The table 4.2 indicates that recovery for acetic propionic and butyric was 99.15, 98.11 and 100.95 percent respectively.

The elution curve foracetic, propionic and butyric acids in mixtures, based on the mean values of a number of runs in the same as well as in diferent columns, is represented in graph I in which abscissa represents successive aliquots of 10 cc and the ordinate the percentage of total acid in such aliqnt.

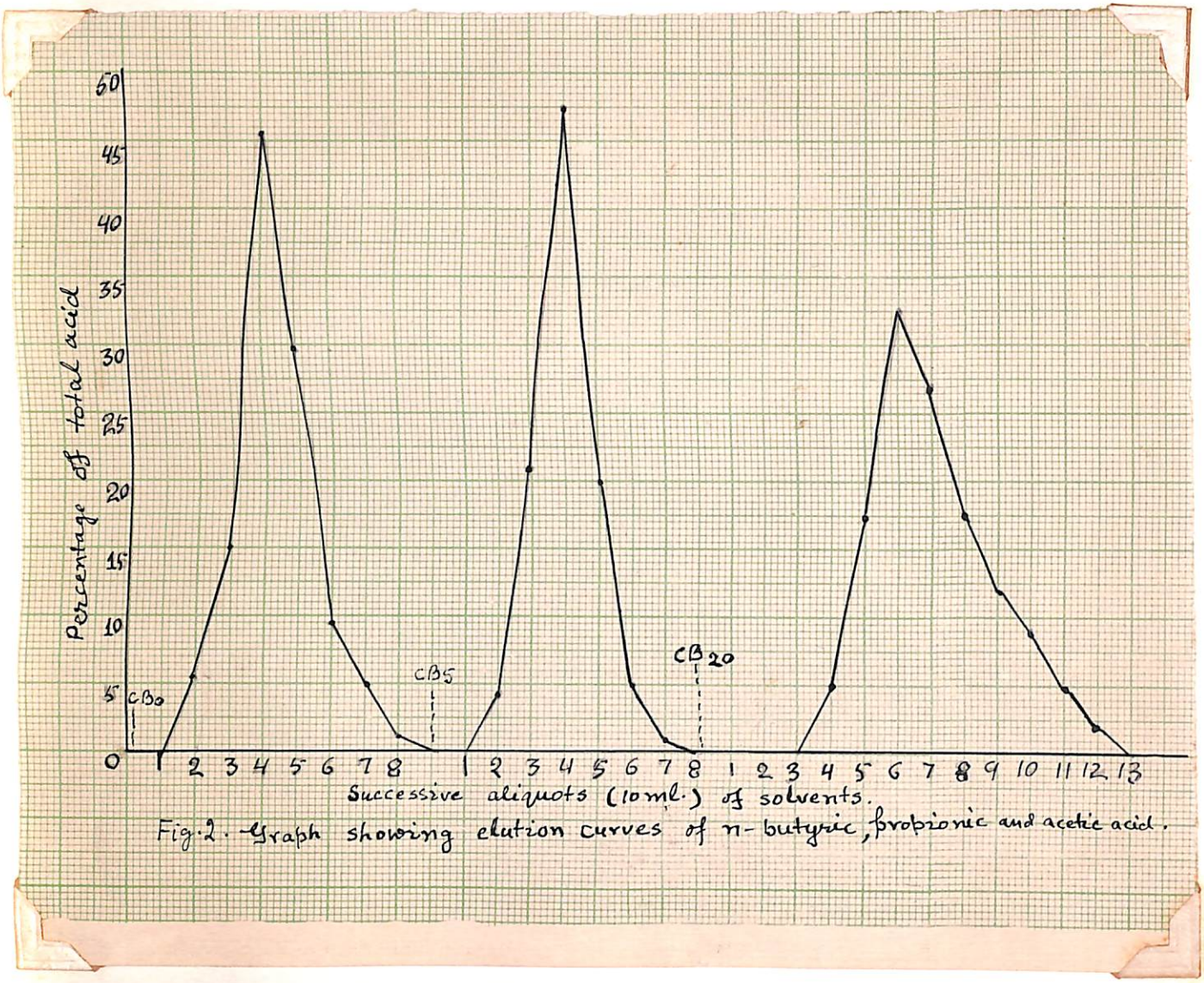

Fig. 2. Graph showing elution curves of n-butyric, propionic and acetic acid.

It is seen from the graph that on an average 80 ml of butyric, 70 ml of CB_5 and 120 ml of CB_{20} are required to completely elute butyric, proponic and acetic acid respectively. The interval between the complete elution of butyric and the appearance of propinonic is usually 10 ml of the eluent and that between propionic and acetic is 20 ml. The elution curve varied slightly between the columns and slight variation in the interval between the complete elution of the acids was also observed.

A standard solution of VFA was run successively in the same column and in the different columns, for chromatography to test the accurate reproducibility of the chromatographic readings in different runs of the same column and in the different columns. The result shown in table 4.2 was obtained by taking into consideration of the above mentioned factors.

It is seen from the above observations that chromatographic separation of these three acids by the procedure described in the Chapter three is invariably quentitative, accurate and highly reproducible between the columns and between the runs with the same column.

Slight variation in the speed of elution between the columns was observed but it was found that speed of elution did notaffect the accuracy of the process.

III. Efficiency of recovery of carotene.

In order to test the efficiency of carotene recovery, 20 microgram/ 2 ml. of rumen liquor of pure beta - carotene was added to the unicubated rumen liquor and carotene was estimated in the rumen liquor alone and in the added rumen liquor by the procedures described in detail in Chapter III. The results obtained is presented in table 4.3.

T A B L E 4.3

Percentage recovery of carotene in separate incubations.

Category	Carotene present in 2 ml. of rumen liquor (in µug.				Percentage recovery			
Sample No.	I	II	III	Average	I	II	III	Average
Rumen liquor (unincubated)	40.5 39.5	27.0 29.7	67.8 68.2	45.33	-	-	-	-
Do + 20 µug. beta carotene	57.6 58.4	47.5 46.5	85.5 86.5	63.66	96.66	97.91	97.72	97.43

The table 4.3 indicates that though there was variation in the carotene content of the rumen liquor from sample to sample as these were collected from the different animals under different feed regimes,,recovery of carotene was found to be ranging from 96.66 to 97.91%. The average percentage of recovery was found to be 97.43. The duplicates of the samples also did not vary much. Thus the method adopted was found to be quantitative and accurate.

C H A P T E R - V

RESULTS & DISCUSSION

As the end products of microbial digestion in the rumen are short chain fatty acids, this part of study was undertaken to find out the effects of antibiotics on the rate of fatty acid production and pattern of fatty acid production.

'In vitro' study :

In order to see the effects of antibiotics on the rates at which these different fatty acids are produced,'in vitro'study was adopted as the rates cannot be determined directly because of the following factors which stand on the way of their estimation 'in vivo' :-

(a) Fatty acids which are produced are being diluted by constant passage of saliva, intake of food and water.

(b) Because of appreciable interconversion of butyrate into acetate (Mukherjee, 1960).

(c) Due to metabolism of the acids, particularly butyric acid by the ruman wall itself (Pennington, 1952).

(d) Due to absorption of fatty acids through the rumen wall (Mc Anally & Phillipson, 1942; Barcroft et al, 1944 a).

(e) Determination of rate of production of VFA 'in vivo' is a complex one.

Carrol and Hungate (1954) and Halse and Vella (1956) reported an alternative approach to the problem of measuring ruminal VFA production which is based on the incubation of

ofhwhole rumen contents for short periods under conditions which simulate those of the rumen.

In the recent years, the artificial rumen technique has been found to be very useful in exploring the mysteries of rumen function. Hence for the sake of convenience and to get the preliminary information regarding the effect of antibiotics on rumen fermentation, the artificial rumen technique of 'in vitro' incubation was adopted.

Antibiotics selected for the study and their levels used

Chlortetracycline hydrochloride and oxytetracycline hydrochloride supplementations were reported to be the most beneficial for ruminants. These two antibiotics were, therefore, chosen for this study.

Different levels of antibiotics have been employed by different workers. Recently Klopfenstein et al (1964) reported on the effect of 5 mcg./ml and 500 mcg/ml of aureomycin on'in vitro' gas production. The low level of antibiotic did notproduce a measurable effect. Inhibition of gas production was reported by 500 mcg/ml dose of aureomycin.

As reported by Klopfenstein et al (1962) that gas production was significantly correlted with fatty acid production, it was thought to study the effect of the two levels of antibiotics i.e. one medium and another high to see the extent of effect of the antibiotics.

Bartley at al (1951) fed daily doses of 200-800 mg. of aureomycin per 100 lb body weight to calves 12 - 16 weeks old without any detrimental effects. One calf of 16 weeks old that had not received antibiotic was fed 2500 mg. of this antibiotic daily for 4 weeks without harmful effects.

Nešić & Ibrović (1963) used 0.25 to 0.5 and 0.8 gm. of terramycin & aureomycin respectively to the sheep in order to know the effect of antibiotics on fermentative processes in the rumen. Bacterial activity was found to be most affected by aureomycin & terramycin. They suggested that these antibiotics could be given by mouth if it is desired to suppress the bacterial activity for example in bloat but rumen fluid from normal animals to restore the flora, should be given.

As reported by Mukherjee (1960) the approximate capacity of rumen of sheep is four litres. Thus 250 mg. to 500 mg. terramycin & 800 mg. aureomycin has been used for 4000 cc capacity of rumen. Hence terramycin has been used in 0.06 to 0.12 mg. per cc and aureomycin in 0.2 mg. per cc of rumen content.

Therefore it was decided to take a medium dose of 0.25 mg/ml and a high dose of 1.0 mg/ml of both the antibiotics. The higher dose had been taken to see the extent of the effect of antibiotics on rumen fermentation.

Effect of chlortetracycline and oxytetracycline on 'in vitro' rate of production of VFA in rumen liquor in four hours of incubation.

Rumen samples were collected from the slaughtered goats with proper precautions as detailed in chapter 3. The goats were fasted forsseveral hours before slaughter and the period of fasting varied from animal to animal. Incubation was carried out (as described in chapter 3) for different samples separately and the total VFA was estimated in them. Results obtained has been presented in table 5.1.

TABLE 5.1

Total fatty acid production in 4 hours of incubation in individual incubation experiments.
(m.moles VFA/litre of rumen liquor)

Source	VFA in rumen liquor after 4 hours of incubation.					Amount of fresh VFA produced in 4 hours.				
	I	II	III	IV	V	I	II	III	IV	V
Rumen liquor (unincubated)	41	66	101	45	54	-	-	-	-	-
Rumen liquor (incubated)	51	85	151	57	81	10	19	50	12	27
do + Aureomycin 0.25 mg/ml	60	89	151	59.5	80	19	23	50	14.5	26
do + Aureomycin 1.0 mg/ml	50.5	80	150	57.5	81	9.5	14	49	12.5	27
do + Terramycin 0.25 mg/ml	63	83	153	57	81	22	17	52	12	27
do + Terramycin 1.0 mg/ml	54	83	150	58	80	13	17	49	13	26

It would appear from the above table thatthe initial VFA concentration in different experiments varied considerably and is due to the individual variation of the sources as also due to the nature of food material in the rumen and to the period of fasting preceeding collection of samples.

The rate of production during 4 hours of incubation was found to be correlated in most cases with the initial VFA present in the samples (as shown in the graph). Thus the sample showing the highest initial concentration of 101 m.moles/litre of rumen liquor produced as much as 50 m. moles as against the lowest is amount of 10 m. moles in the sample, also containing the lowest initial amount of 41 m. moles/litre of rumen liquor.

This trend was obtained with almost all the treatments and the addition of the antibiotics did not affect the rate of production ofthe acids irrespective of the initial amount and rate of fermentation of the rumen samples.

Effects on total fatty acid production and rate of production:

The summary of the result obtained from the average of five separate incubations is tabulated in table 8.2.

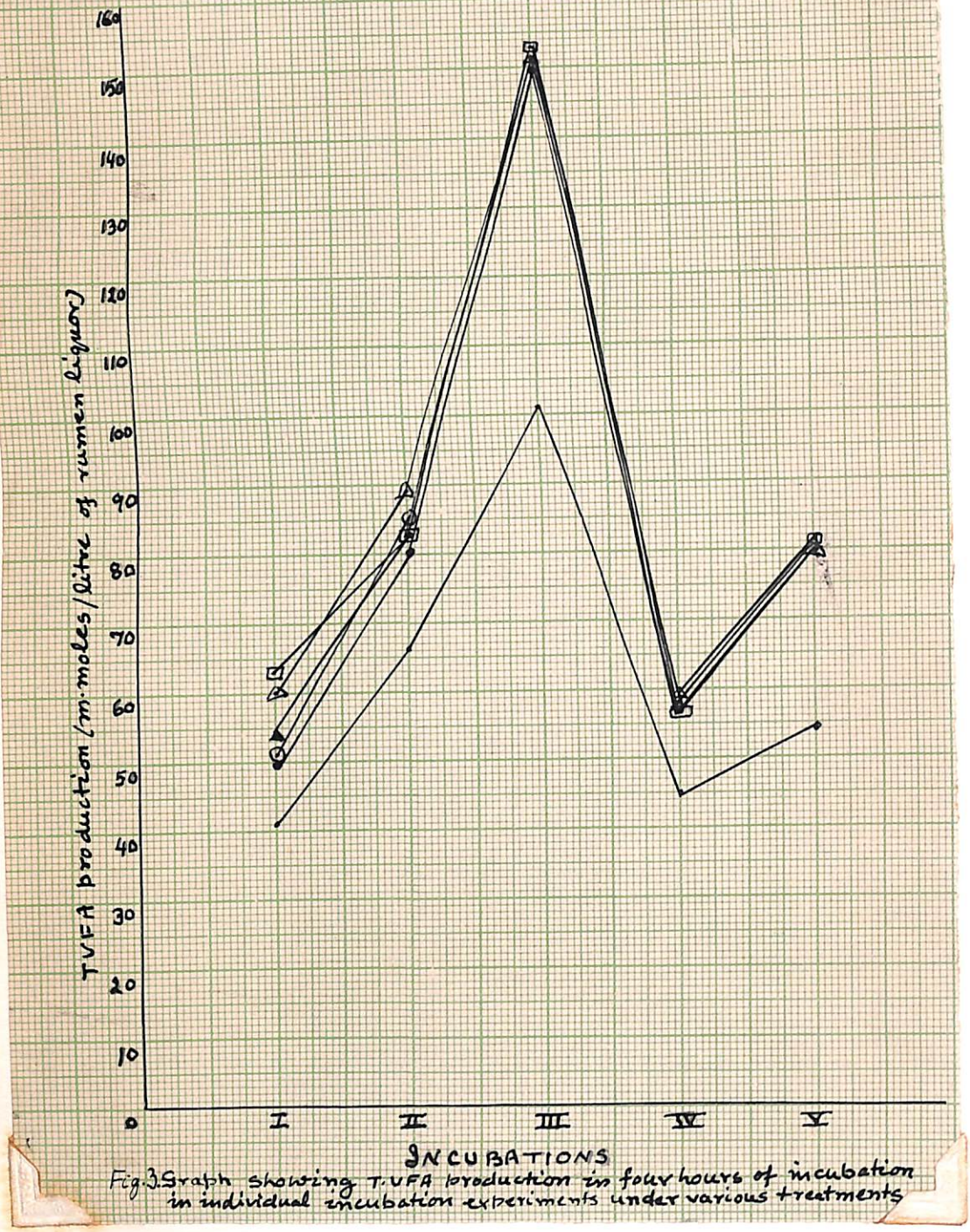

Fig. 3 Graph showing T.V.F.A production in four hours of incubation in individual incubation experiments under various treatments

T A B L E 5.2

Total fatty acid production in 4 hours of 'in vitro' incubation under various treatments. (m.moles of VFA/litre ofrumen liquor)

Source	No.of observations	Total VFA produced		Rate per hour	
		Average	Range	Average	Range
Rumen liquor	5	23.6 ± 7.2	10 to 50	5.8 ± 1.8	2.5-12.25
do + Aureomycin 0.25 mg/ml	5	26.5 ± 6.2	14.5-50	6.6 ± 1.5	3.62-14.7
do + Aureomycin 1.0 mg/ml	5	23.0 ± 6.8	9.5-49	5.75 ±1.7	2.5-17.25
do + Terramycin 0.25 mg/ml	5	25.4 ± 7.5	12 - 52	6.35 ± 1.8	3.25-13.2
do + Terramycin 1.0mg/ml	5	23.2 ±7.1	13 - 49	5.8 ± 1.8	3.25-12.2

The rumen liquor alone on 4 hours of incubation produced 23.6 m. moles of VFA per litre of the rumen liquor buthwhen treated with various doses of antibiotics, it seemed to remain almost the same.

After alysing the data statistically the variations between the treatments were found o be non significant. The result of the analysis of variance is shown in table 5.3.

TABLE 5.3

Analysis of variance of total fatty acids produced in 4 hours of incubation under various treatments

Sources of varition	d. f.	Corrected S. S.	Mean square	F Test
Total	24	4828.34		
Between treatments	4	59.24	14.81	$\frac{14.81}{238.45}$
Within treatments (error)	20	4769.10	238.45	

The rate of production of VFA of rumen liquor alone was found to be 5.8 m. moles/litre of rumen liquor per hour. The range as indicated in the table was 2.5 to 12.5 m. moles per hour per litre. This indicated large amount of variation from samples to samples. This wide variation may be due to some of the following reasons:

a) The samples were collected from different animals whose intakes were not similar;

b) the lack of optimum condition for 'in vitro' fermentation;

c) due to inharent variations in the factors controlling the rate of fermentation such as types and the no. of microflora present;

d) due to concentration and the nature of the substrate etc. in the rumen.

There was not much difference in the rates of average VFA production in untreated and treated samples and amongst the treatments.

Apparently observed slight fluctuations in the results with different treatments are within the range of experimental variation expected in a work of this nature.

Both the antibiotics i.e. aureomycin and terramycin behaved similarly with regards to total VFA production and rate of production. No significant difference was observed in behaviour of either ofthe two antibiotics as also between their two doses of administration.

Discussion

The above findings are in aggrement with Hungate at al (1955 a) that the capacity of rumen organisms to form fermentatio products hd not been changed by antibiotic addition. Raun at al (1962) found 89 micro moles/ml of rumen liquor in the basal ration while in chlortetracycline group, they had obtained 83 micro moles/ml.

Contrary to our finding, Preston et al (1959) and Dinda (1960) reported reduced concentration of lower volatile fatty acids while working on the early weaned calf whereas Hibbs and Connard (1953) did not find any difference in the average total VFA.

However our result obtained with goats maintained practically on roughage only are not comparable with the findings of Preston et al (1959) with early weaned calves whose ration of necessity is of a completely different nature.

The tendency of 0.25 mg./ml. of aureomycin and 0.25 mg. dose of terramycin to produce slightly more VFA though statistically not significant is in aggrement with Klophenstein et al (1964) whose treatment B i.e. infusion of 20 mg. of aureomycin (suspended in 5 ml. of water) into the rumen via the fistula 90 minutes prior to feeding, had a tendency to produce greater concentration of VFA 'in vivo'.

Effect of antibiotics on total VFA production in presence of carbohydrate and protein rich substrates.

Starch and casein were taken as the carbohydrate and protein rich substrates respectively. Excess amount of starch and casein were added to the control and experimental flasks. The main object was to study the comparative effects of antibiotics in carbohydrate rich and protein rich substrates. The total VFA production and rate of production in starch rich substrate is presented in table 5.4.

Carbohydrate rich substrate :-

T A B L E 5.4

Total fatty acid production in four hours of incubation by the addition of starch.
(m.moles of VFA/litre of rumen liquor)

Source	No.of obs.	Total VFA produced		Rate per hour	
		Average	Range	Average	Range
Rumen liquor	3	24.7± 12.2	10- 49	6.17± 2.03	2.5- 12.35
Rumen liquor+ starch	3	51.2± 12.8	29.5-74	12.8 ± 3.2	7.37-18.5
Do+Aureomycin 0.25 mg./ml.	3	44.5± 8.5	29.5-59	11.12± 2.1	7.37-14.7
Do+Aureomycin 1.0mg./ml.	3	35.0± 11.4	17-54	8.75± 2.8	4.25-13.5
Do+Terramycin 0.25mg./ml.	3	44.8± 11.3	28-66.5	11.2± 2.8	7.0 -16.62
Do+Terramycin 1.0mg./ml.	3	28.7± 10.8	12-49	7.17± 2.7	3.0 -12.25

The total VFA production by the addition of starch was raised. In rumen liquor alone it was found to be 24.7 m. moles/litre of rumen liquor while with starch,it gave 44.5 m.moles/litre of rumen liquor. But the production of VFA was found to be inhibited by all the treated levels of antibiotics.

In aureomycin 0.25 mg./ml. and 1.0 mg./ml. treatments, total VFA production was found to be 44.5 and 35.0 m.moles per litre of rumen liquor where as in terramycin 0.25mg/ml. & 1.0 mg./ml. doses it was 44.8 and 28.7 m.moles/litre of rumen liquor.

The rate of production also raised from 6.17 m.moles per litre of rumen liquor per hour in rumen liquor alone to 12.8 m.moles after addition of starch. The rate of production in 0.25 mg./ml.,1.0 mg./ml. aureomycin and terramycin treatments was found to be 11.12,8.75,11.2, and 7.11 m.moles/litre per hourz respectively.

Thus VFA production with higher doses of anitibiotics was found to be lower than VFA production with lower doses. Similar doses of both aureomycin and terramycin acted in a similar manner as there was no appreciable difference with reggrds to VFA production with equal levels of both the antibiotics.

Protein rich substrate.

Data of total fatty acid production and rate of production in protein rich substrate is presented in table 5.5.

T A B L E 5.5

Total fatty acid production in four hours of incubation by the addition of casein.
(m.moles of VFA/litre of rumen liquor)

Source	No. of obs.	Total VFA produced		Rate per hour	
		Average	Range	Average	Range
Rumen liquor	3	11.0±2.08	8 - 15	2.75± 0.52	2- 3.75
Rumen liquor + casein	3	42.3±14.7	14.5 - 64.5	10.57± 3.6	3.6- 16.1
do+Aur. .25 mg./ml.	3	19.17±6.9	8.5 - 32	4.79± 1.7	2.1- 8.0
do+Aur.1mg. /ml.	3	15.5 ±9.8	4.0 - 35	3.87± 2.4	1-8.75
do+Terr. .25mg./ml.	3	17.5 ±6.5	8.0 - 30	4.37± 1.6	2- 7.5
do+Terr. 1.0mg./ml.	3	13.17±4.3	7-23	3.29± 1.07	1.7 - 5.7

The data of table 5.5. indicates thatby casein addition also,VFA production raised from 11 m.moles to 42.3 m.moles per litre of rumen liquor while rate of production raised from 2.75 to 10.57 m.moles/litre of rumen liquor per hour.

The range of total fatty acid production by the addition of casein in four hours of incubation was found to be from 14.5 to 64.5 m.moles/litre of rumen liquor. A wide variation was observed. A probable reason for such a high value as 60.5 m.moles/litre in one sample may be due to prepondrance of deaminating organisms in the sample or as reflected by the high initial activity in this sample of rumen content.

In aureomycin 0.25 mg./-ml. and 1.0 mg./ml.sample,the total VFA production was found to be 39.17 and 15.5 m.moles per litre while in terramycin 0.25 mg. and 1.0 mg. per ml. sample, it was 17.5 and 13.17 m.moles/litre of rumen liquor respectively.

The rate of production in 0.25 mg./ml. and 1.0 mg./ml. aureomycin and terramycin treatments was found to be 4.79,3.87, 4.37 and 3.29 m.moles per litre of rumen liquor per hour respectively.

In samples treatdd by antibiotics,VFA production was reduced depending upon the levels of antibiotics used. In cases of both the antibiotics,reduction was slightly more with higher doses but both aureomycin & terramycin almost reacted similarly.

VFA production from starch and casein under various treatments.

The inhibition of total VFA production could not be found when rumen liquor without any nutrient was treated with various doses of antibiotics treatment as mentioned before but antibiotics at both the levels effected the VFA production in both carbohydrate and protein rich substrates. The VFA production from starch and casein under various treatment are presented in table 5.6 and 5.9.

VFA production from starch :-

TABLE 5.6

Total fatty acid production in four hours of incubation from starch under various treaments (m.moles of VFA/litre of rumen liquor)

Source	No.of obs.	Total VFA produced Average	Range	Rate per hour Average	Range
Starch	3	26.5	19.5- 35	6.62	4.87-8.75
do+Aureomycin 0.25mg./ml.	3	19.8	10 - 30	4.95	2.5 -7.5
do +Aureomycin 1.0 mg./ml.	3	10.3	5 - 19	2.57	1.25-4.75
do +Terramycin 0.25 mg./ml.	3	20.1	17.5- 25	5.02	4.37-6.25
do +Terramycin 1. mg./ml.	3	4.0	0- 10	1.0	0-2.5

The VFA production from starch was found to be 26.5 m.moles/litre in the rumen litre without treatment but there was significant inhibition in fatty acid production in all antibiotic treatments.

VFA production af from starch, in aureomy 0.25 mg./ml. and 1.0 mg./ml. treatments was 19.8 and 10.3 m.moles per litre respectively and in terramy cin 0.25 mg./ml. and 1.0 mg./ml. treaments 28.3 and 4.0 m.moles/litre respectively. ~~The rate~~

The rate of production of VFA from starch was found to be 6.62 m.moles/litre/hour which was reduced to 4.95, 2.57, 5.02 and 1.0 m.moles/litre/hour in 0.25 mg./ml. and 1.0 gm./ml. aureomycin and terramycin treatments respectively. The range of rate of production of the control as well as the treatments has also been indicated in table 5.6.

As indicated by the table, apparently in both the antibiotics, 1.0 mg./ml. dose was found to depress the production of VFA from starch more than 0.25mg./ml. dose. Treatment of both the antibiotics equally reduce the production but terramycin treatment in 1.0 mg./ml. dose was found apparently to depress the production slightly more than 1.0 mg./ml. treatment of aureomycin. A great variation in the production of VFA was noticed from starch when it was added in different samples as the range of production was found to be 19.5 to 35 m.moles per litre of rumen liquor.

VFA production fron starch under various treatments was statistically analysed and the result is presented in table 5.7 and 5.8.

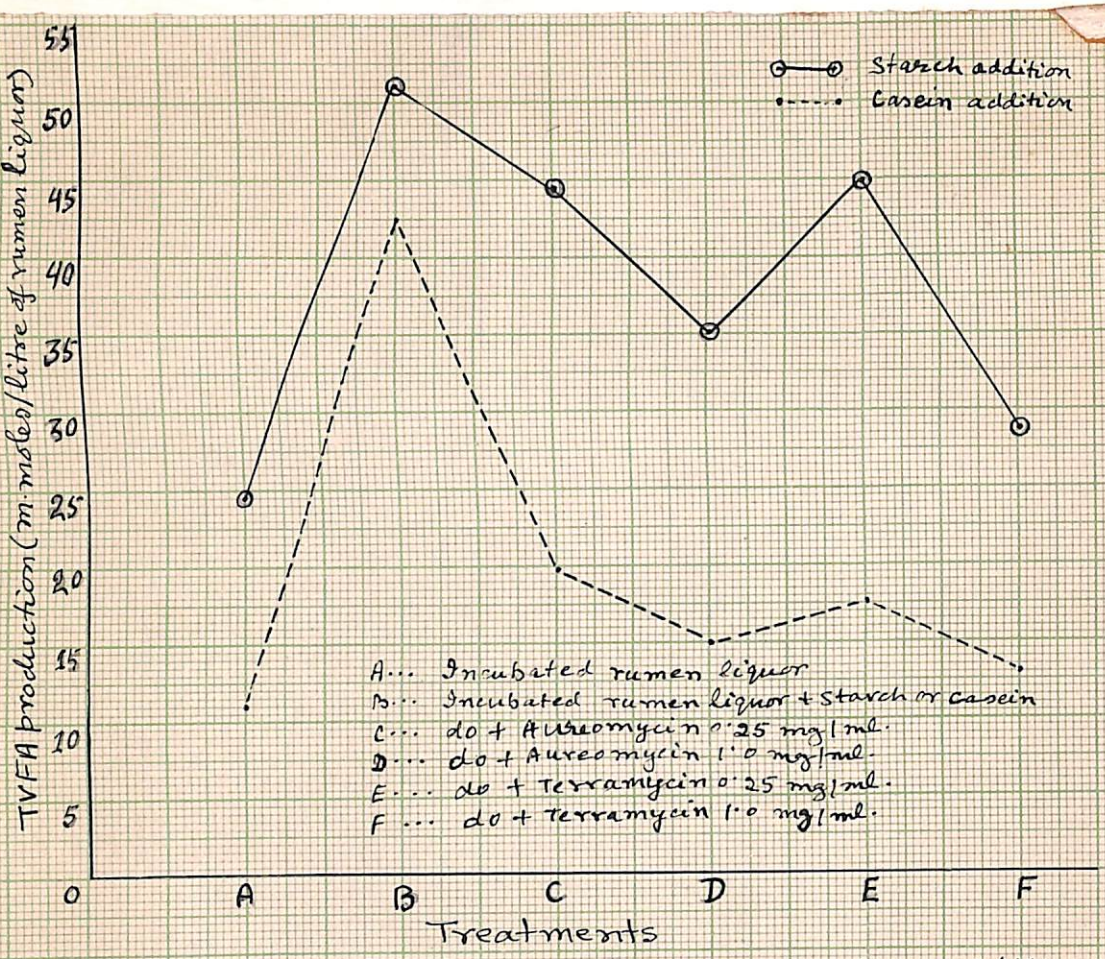

Fig.4. Graph showing TVFA production by the addition of starch or casein in four hours of incubation under various treatments.

T A B L E 5.7

Analysis of variance of VFA production from starch

Source of variance	d.f.	Corrected S.S.	Mean Square	F.Test
Total	14	1484.33		
Between treaments	4	954 .83	238.71	$\frac{238.71}{52.95}$ =4.58*
Within treaments (error)	10	529 .50	52.95	

*Significance at 5% level.

As significant difference between the treaments was obtained by F test, a comparative study ofdifferent treatments was made by applying critical difference test which is presented in table 5.8.

T A B L E 5.8

Comparision of fatty acid production from starch between various treatments by 'Critical difference test'

Treaments	Differences in averages	Calculatedvalue of critical difference at 5% level	at 1% level
		13.20	18.77
Starch vs Aureomycin 0.25 mg/ml.	6.7		
Starch vs Aureomycin 1.0 mg/ml.	16.2*		
Starch vs Terramycin 0.25 mg/ml.	6.4		
Starch vs Terramycin 1.0 mg/ml.	22.5**		
Aureomycin .25 mg/ml. vs Aureomycin 1.0 mg/ml.	9.5		
Aureomycin .25 mg/ml. vs Terramycin .25 mg/ml.	0.3		
Aureomycin .25 mg/ml. vs Terramycin 1.0 mg/ml.	15.8*		
Aureomycin 1.0 mg/ml. vs Terramycin .25 mg/ml.	9.8		
Aureomycin 1.0 mg/ml. vs Terramycin 1.0 mg/ml.	6.3		
Terramycin .25 mg/ml. vs Terramycin 1.0 mg/ml.	16.1*		

From the table 5.8, it is clear that higher doses of both the antibiotics significantly depressed the production of VFA from starch but the reduction is case of 1.0 mg./ml. level of terramycin treatment was found to be highly significant.

VFA production from casein :-

The VFA production from casein under various treatment is presented in table 5.9.

T A B L E 5.9

Total fatty acid production from casein under various treatments in four hours of incubation. (m.moles of VFA/litre of rumen liquor)

Soure	No.of obs.	Total VFA produced		Rate per hour	
		Average	Range	Average	Range
Casein	3	31.3	6.5- 54.5	7.82	1.62-13.
do +Aureomycin 0.25 mg/ml.	3	8.17	0.5- 17	2.04	0.12-4.2
do + Aureomycin 1.0 mg/ml.	3	4.5	-4.0- 20	1.12	-1.0- 5.0
do + Terramycin 0.25 mg/ml.	3	6.5	0-15	1.62	0- 3.75
do + Terramycin 1.0 mg/ml.	3	2.17	-1.0- 8	0.54	-0.25-2.0

The VFA production from casein was found to be of almost same order as starch. The average value of four three obervations was found to be 31.3 m.moles per litre of rumen liquor as compared to 26.5 m.moles in case of starch.

Fatty acid production from casein treated with aureomycin 0.25 mg./ml., aureomycin 1.0 mg./ml.,terramycin 0.25 mg./ml. and terramycin 1.0 mg./ml. was found to be 8.17,4.5,6.5 and 2.17 m.moles/litre respectively.

The rate of production of VFA per hour in the rumen liquor without casein was obtained as 2.75 m.moles/litre where as 10.57 m.moles/litre was found in the rumen liquor with casein. Rate of production from casein only worked out to be 7.82 m.moles of VFA per litre/hour.

The rate of production from casein i.e. 7.82 m.moles per litre per hour was reduced by the antibiotics treatments to 2.04,1.82, 1.62 and 0.54 in aureomycin 0.25 and 1.0 mg./ml. and in terramycin 0.25 and 1.0 mg./ml. respectively.

The effect of various doses of the antibiotics was found to inhibit the rate of fatty acid production. Apparently higher doses of both the antibiotics affected the production more than the lower doses. Apparently terramycin 0.25 mg./ml. and 1.0 mg./ml. treatments were found to reduced the production of VFA slightly more than aureomycin 0.25 mg and 1.0 mg. per ml. of rumen liquor dose.

The range of VFA production in 1.0 mg. treatment of aureomycin and terramycin was found to be -4.0 to 20 and -1.0 to 8 m.moles/litre of rumen liquor respectively.

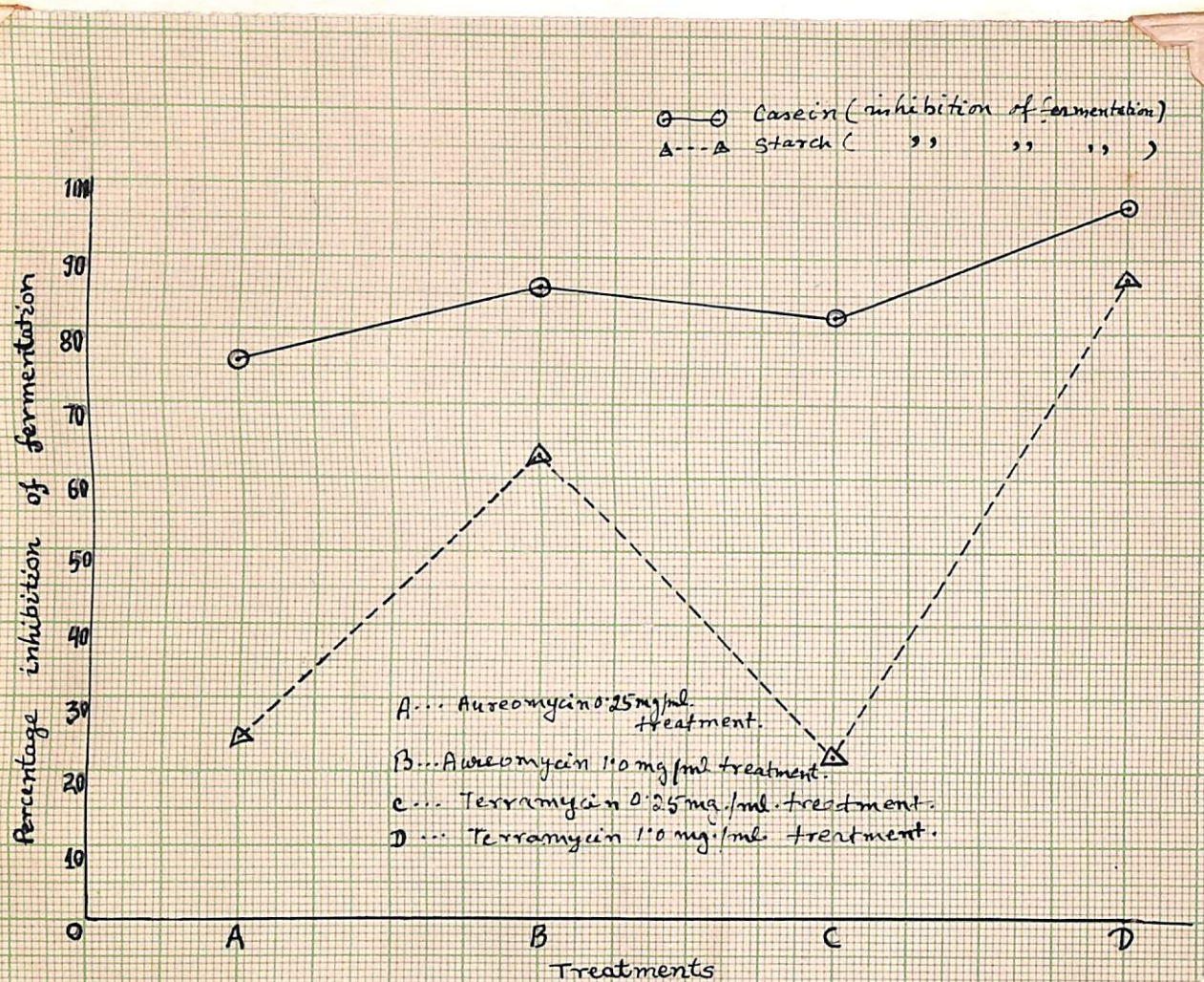

Fig. 6. Graph showing percentage inhibition of fermentation of casein & starch in four hours of incubation under various treatments.

Comparative effect of antibiotic treatment on VFA production from starch (carbohydrate rich) and casein (protein rich).

From the above tables and graphs, it is evident that all the antibiotic treatments have resulted in the inhibition of fatty acid production from both starch and casein. Percentage inhibition of fermentation of starch and casein in four hours of 'in vitro' incubation under various treatments was calculated by the following formula:-

$$\text{Percentage inhibition} = \left\{ \frac{\text{VFA produced from nutrient - VFA produced under treaments}}{\text{VFA produced from starch or casein}} \right\} \text{multiplied by 100.}$$

The percentage inhibition thus calculated is presented in table 5.10.

TABLE 5.10

Percentage inhibition of fermentation of starch and casein in four hours of incubation under various treatments.

Treatments	Percentage inhibition in starch fermentation	Percentage inhibition in casein fermentation
Aureomycin 0.25 mg./ml.	24.76	75.46
Aureomycin 1.0 mg./ml.	63.27	86.08
Terramycin 0.25 mg./ml.	22.09	82.09
Terramycin 1.0 mg./ml.	87.05	97.38

The perusal of the data of table 5.10 indicates that higher dose treatments of both aureomycin and terramycin affected the fermentation of both starch and casein.

The lesser dose 0.25 mg. doses of aureomycin and terramycin affected VFA production from casein more i.e 75.46 % and 82.09% than from starch i.e. 24.76% and 22.09% respectively. Higher doses of aureomycin and terramycin also inhibited VFA production from casein slightly more i.e. 86.03% and 97.38% than starch i.e. 63.27% & 87.05% respectively.

Discussion

Higher amount of starch and casein used in experiments resulted in higher production of fatty acids from both these nutrients in control groups. The higher amount i.e. one gm. per 200 c.c. of rumen liquor was added in order to visualize the effects of antibiotics more prominently on the production of fatty acid in carbohydrate and protein rich substrate.

Higher production of fatty acid after the addition of starch was due to its easily fermentable nature. The microbial population prefers these soluble sugars like starch and quickly ferments it to short chain fatty acids and lactic acid. Rao (1963) also reported higher fatty acid production by the addition of starch as substrate and also from the maize which is a starch rich substance.

The production of more VFA due to casein addition was quite in aggrement with el - Shazly (1952), Annison(1954) and Devis et al (1957). They studied the effect of protein rich

rations on the ruminal VFA and observed increased concentrations of total VFA's when high protein diets were fed. el- Shazly(1952) observed that the branched- chain acids arose chiefly as end-products of protein degradation, and also found a correlation between the concentrations ammonia and the C_4 and C_5 branched-chain acids in rumen after the injestion of protein - rich foods.

Through analysis of rumen contents McDonald(1954) and McDonald and Hall (1957) demonstrated that the rapid degradation of casein and the much slower degradation of zein was accompanied by the formation of ammonia and fatty acids.

Thus the higher m.moles VFA production due to 'in vitro' casein fermentation in rumen liquor has been found to be quite in aggrement with the findings of the above workers.

The wide variations in VFA production between samples from starch and casein were due to (i) samples obtained from the different animals under different feed regimes and (ii) variations in the activities of the microorganisms present.

Antibiotics treatments inhibited the production of VFA from starch. The reduction was more with 1.0 mg./ml. treatment of both the antibiotics than with 0.25 mg./ml.

treatment of both the antibiotics. Thus it seems that x activities of rapidly growing bacterias afe being affected by the antibiotic treatment. Aureomycin and terramycin affected in a similar manner.

Klopfenstein et al (1962) reported that gas production was significantly correlated with volatile fatty acid production. They observed further in course of their studies on effect of antibiotic and time on rumen activity that antibiotics added at the same time as the nutrient solution caused a greater decreasing in gass production than when added 100 minutes after the addition of nutrient solution. On the other hand, nutrient added 100 minutes after the addition of antibiotics gave rise to significantly less gas production than the simultaneous addition of both nutrients and antibiltics at the same time.

Thus from the above work,it is evident that nutrients had a tendency to produce more gas without x antibiotic but when antibiotic was given at the same time or before the nutrient addition,less gas production occured in spite of nutrient addition.

In our case also,nutrients had a tendency to produce more fermentative product like VFA in our case but when antibiotics were given at the same time,less VFA production from nutrients resulted. Hence our findings are in aggrement

with the above mentioned workers.

The beneficial effect of antibiotics in young ruminants might be due to their protein sparying action also as the young ones are generally given protein rich diets.

The influence of antibiotics on pattern of VFA produced on fermentation from rumen content.

This part of study was under taken to determine the effects of the antibiotics on the production of three principal products of cellulose digestion in the rumen i.e. acetic, propionic and butyric acids.

The percentage composition of these three acids in the rumen liquor before incubation and after four hours of incubation was determine with a view to get a clear picture of the chemical processes taking place during incubation. The results obtained have been shown in table 5.11.

TABLE 5.11

Comparative percentage composition of VFA in the rumen liquor before and after four hours of 'in vitro' incubation (average of three separate incubations).

Fatty acid	Percentage composition of VFA before incubation.	Percentage composition of VFA after incubation.
Butyric	18.7 ± 3.02	18.8 ± 1.3
Propionic	19.8 ± 0.39	20.3 ± 0.42
Acetic	61.5 ± 1.6	60.9 ± 1.4

The table 5.11 indicates that there was almost no change in the percentage composition of initial percentage of individual acids present in the rumen liquor which represented the 'in vivo' pattern and in the rumen liquor after four hours of 'in vitro' incubation represnting 'in vitro ' pattern. Thus it was found that percentage compositian of VFA on 'in vitro' incubation did not alter from the initial pattern of fatty acid in the rumen liquor of the slaughtered animal. Thus 'in vivo' pattern was to a great extent maintained in the 'in vitro' incubation.

Percentage composition of fatty acid in rumen liquor after incubation under various treatments.

The percentage composition of each fatty acid in rumen liquor under various treatments was calculated. The result has been presented in the table 5.12. After various levels of antibiotics treatment, the percentage composition of VFA in rumen liquor in four hours incubation, was found to be slightly altered.

TABLE 5.12

Percentage composition of fatty acid in rumen liquor in four hours of incubation under various treatments(average of three observations).

Treatment	No.of obs.	Butyric		Propionic		Acetic	
		Average	Range	Average	Range	Average	Range
Rumen liquor (unincubated)	3	18.7 ± 3.0	14.9 - 20.9	19.8 ± 0.39	19.1 - 20.4	61.5 ± 1.6	59.8 - 64.7
Rumen liquor (incubated)	3	18.8 ± 1.3	16.3 - 20.2	20.3 ± 0.42	20.0 - 20.4	60.9 ± 1.4	59.4 - 63.7
do+Aureomycin 0.25 mg/ml.	3	23.2 ± 1.4	20.2 - 24.7	16.6 ± 0.41	16.1 - 17.4	60.3 ± 1.8	58.0 - 63.7
do+Aureomycin 1.0 mg/ml.	3	23.4 ± 1.7	20.0 - 25.6	15.9 ± 0.43	15.4 - 16.8	60.7 ± 1.3	58.8 - 63.2
do+Terramycin 0.25 mg/ml.	3	21.8 ± 0.8.7	20.4 - 23.0	17.1 ± 0.69	16.8 - 17.5	61.1 ± 0.8	60.2 - 62.7
do+Terramycin 1.0 mg/ml.	3	22.8 ± 1.8	19.6 - 26.1	16.1 ± 0.53	15.3 - 17.0	61.1 ± 1.43	58.6 - 63.4

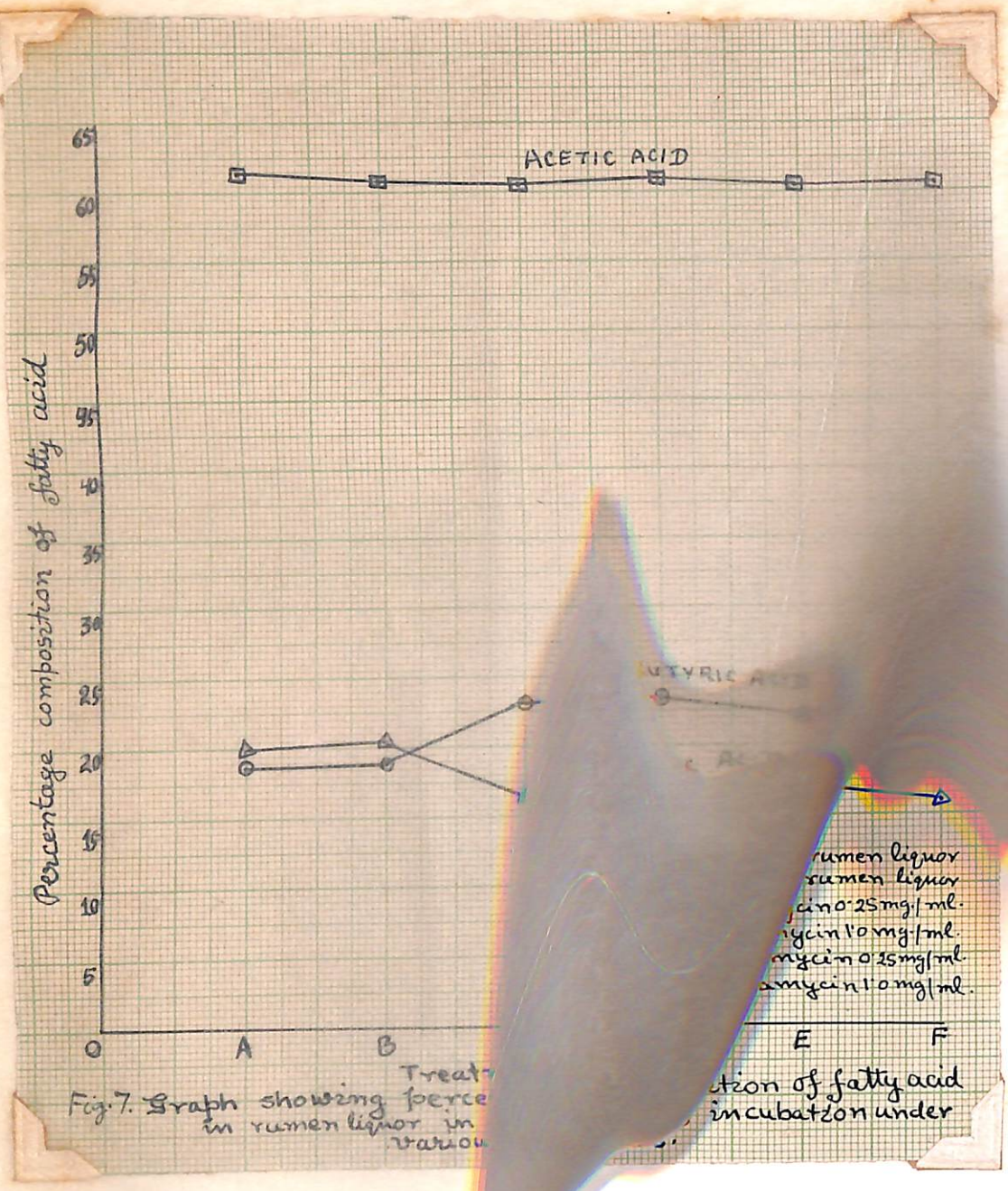

Fig. 7. Graph showing perce
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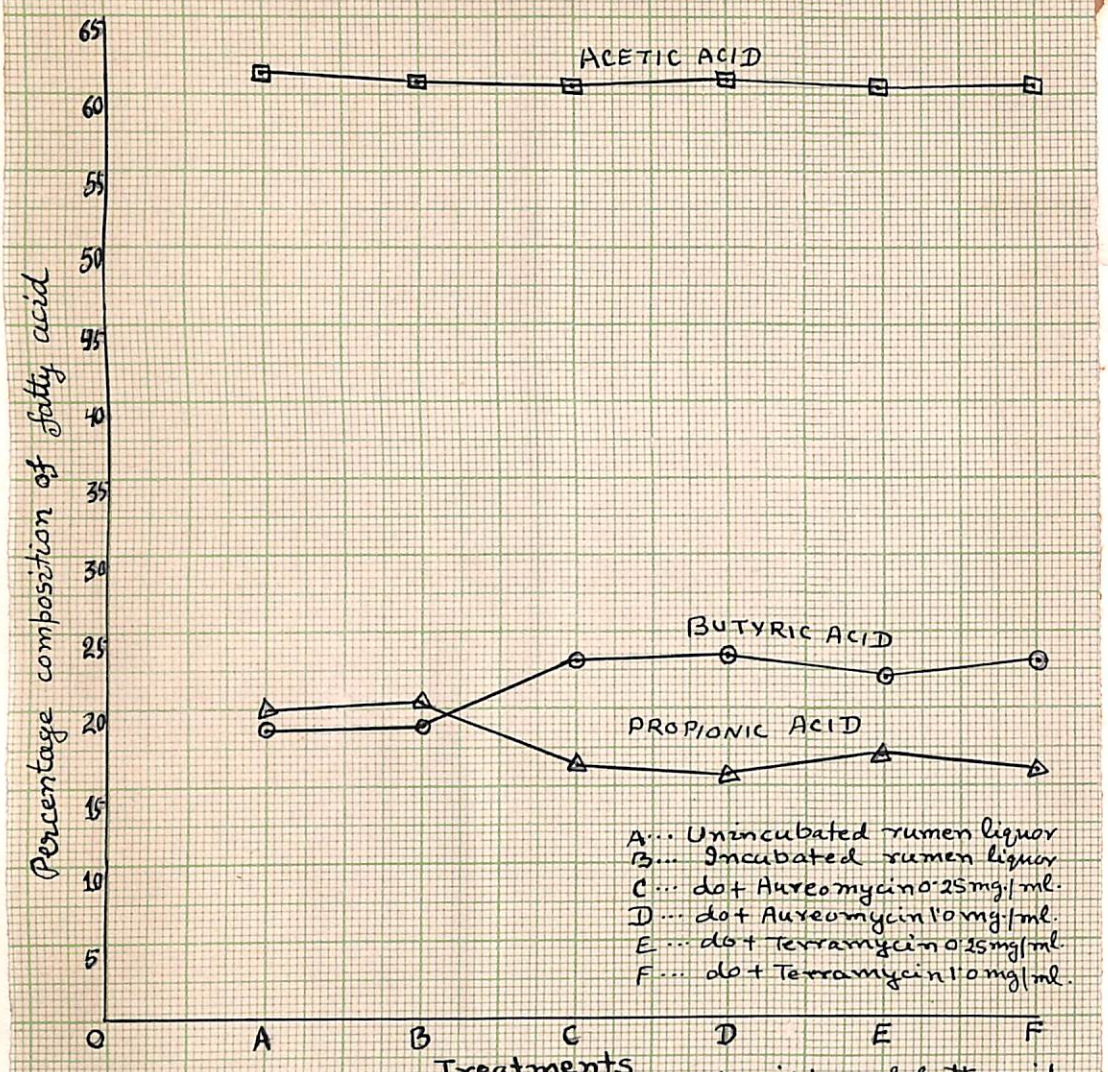

Fig. 7. Graph showing percentage composition of fatty acid in rumen liquor in four hours of incubation under various treatments.

It is evident from the table 5, ph that antibiotics in general apparently ra c acid percentage and decreased the percent c acid. No change in acetic acid percentage b biotic treatments was noticed.

1.0 mg. treatments of both aure amycin seemed to had a slightly greater teden the butyric acid percentage and to decreas ionic acid percentage, than the lower level of both the antibiotic. Aureomycin and terramyc affected similarly in the pattern of fatty acid

Composition of fresh VFA produced in fou incubation under various treatments.

With view to examine more critical ef ct of the antibiotics on the pattern of fermentatio am nt of each of the three acids produced during in io were calculated so that the masking effect of t it l acid present in the rumen content was eliminated he result is presented in table 5.13.

TABLE 5.13

Composition of fresh VFA produced in four hours of incubation under various treatments(m.moles/litre of rumen sample) .

Treatment	No.of obs.	Butyric		Propionic		Acetic	
		Average	Range	Average	Range	Average	Range
Rumen liquor	3	2.67±0.67	1.92-4.01	2.92±0.38	2.20-3.54	8.07±1.75	5.88-11.45
do + Aureomycin 0.25 mg/ml.	3	6.64±0.84	5.30-8.14	1.39±0.43	0.87-2.24	10.8 ±1.76	8.15-13.99
do + Aureomycin 1.0 mg/ml.	3	5.19±0.43	4.65-6.16	-0.10±0.17	-0.32-0.26	6.85±0.84	5.17- 7.88
do + Terramycin 0.25 mg/ml.	3	5.48±1.04	3.70-7.10	1.46±0.69	0.57-2.82	10.06±5.43	7.32-13.53
do + Terramycin 1.0 mg/ml.	3	5.31±0.82	3.70-6.43	0.51±0.67	0.09-0.76	8.51±0.81	7.10- 9.90

It is seen from the table 5.13 that a great variation had occurred in the pattern of fatty acid production in the treated group from the control. The average of each fatty acid produced in the control group was found to be : butyric 2.67, propionic 2.92 and acetic 5.88 m.moles per litre of rumen liquor.

In stead of that, in the xerox 0.25 mg. and 1.0 mg. aureomycin treatment and 0.25 and 1.0 mg./ml. terramycin treatment, butyric acid was found to be 6.64, 5.19, 5.48, 5.31 propionic acid 1.39, 0.10, 1.46, 0.51 and acetic acid 8.15, 5.17, 7.32, 7.10 m.moles/litre respectively.

The rate of production of individual fatty acid in four hours of incubation under various treatments was also calculated and has been presented in table 5.14.

TABLE 5.14

Rate of production of individual fatty acid in four hours of incubation under various treatments (average of three) (m.moles/litre of rumen liquor/hour).

Source	Butyric		Propionic		Acetic	
	Average	Range	Average	Range	Average	Range
Rumen liquor	0.67± 0.16	0.48- 1.00	0.73± 0.28	0.55- 0.88	2.02± 0.42	1.47- 2.86
do + Aureomycin 0.25 mg/ml.	1.66± 0.21	1.32- 2.03	0.35± 0.24	0.22- 0.56	2.70± 0.29	2.04- 3.49
do + Aureomycin 1.0 mg/ml.	1.30± 0.12	1.16- 1.54	-0.025± 0.04	-0.08- 0.06	1.71± 0.29	1.29- 1.97
do + Terramycin 0.25 mg/ml.	1.37± 0.24	0.92- 1.8	0.37± 0.17	0.14- 0.70	2.52± 0.46	1.83- 3.38
do + Terramycin 1.0 mg/ml.	1.33± 0.24	0.92- 1.6	0.13± 0.17	0.02- 0.019	2.13± 0.63	1.77- 2.47

Table 5.14 indicates that the rate of production in the rumen liquor alone was 0.67, 0.73, 2.02 m.moles/litre of rumen liquor per hour for butyric,propionic and acetic acid respectively.

The rate of production for butyric acid in the 0.25 mg. and 1.0 mg. treatment of aureomycin and 0.25 mg. and 1.0 mg. per ml. treatment of terramycin was found to be 1.66,1.30,1.37 and 1.33 m.moles per litre per hour respectively.

The rate of production for propionic acid in the 0.25 mg./ml., 1.0 mg./ml. aureomycin treatment and 0.25 mg/ml., 1.0 mg./ml. terramycin treatment was found to be 0.35, -0.025, 0.37 and 0.13 m.moles/litre/hour respectively while the rate of production for acetic acid in 0.25 mg./ml. and 1.0 mg./ml. treatments of aureomycin and terramycin was worked out to be 2.70, 1.71, 2.52 and 2.13 m.moles/litre of rumen liquor/hour respectively.

The difference in the composition of fresh fatty acids produced was statistitally analysed for each acid separately and the result has been presented in table 5.15.

T A B L E 5.15

Analysis of variance of fresh fatty acids produced under various treatments.

Fatty acid	Sources of variation	d.f.	corrected S.S.	Mean square	F test
Butyric	Total	14	43.4066		
	Between treatments	4	28.9750	7.2437	5.01*
	Within treatments (error)	10	14.4316	1.4432	
Propinic	Total	14	20.5062		
	Between treatments	4	15.1781	3.7945	7.06**
	Within treatments (error)	10	5.3281	.5328	
Acetic	Total	14	93.5157		
	Between treatments	4	30.0910	7.5227	1.18(N.S
	Within treatments (error)	10	63.4247	6.3425	

*Significant at 5% level, ** Highly significant at 1% level.
(N.S.- Non-significant.

It is evident from the above tables that antibiotics affected the butyric acid production positively and propionic acid production negatively. In the fresh fatty acidsproduced, there was not much difference in the acetic acid production taking the total fatty acids produced into consideration.

By statistical analysis also, it was found that there was significant difference between the treatments in the production of butyric acid ($P<.05$) and in propionic acid ($P<.01$) while in acetic acid production, difference between the treatments was found to be nonsignificant.

Percentate composition of the fresh fatty acids produced under various treatments.

Percentage composition of fresh fatty acids produced under various treatments was also calculated and has been presented in table 5.16 & 5.17.

Table 5.16 presents the comparative percentage composition in individual fatty acids found in the initial rumen liquor and in the fresh acids produced in four hours of incubation.

TABLE 5.16

Comparative percentage composition of fatty acid in the initial rumen sample and fresh fatty acid produced from it in four hours of 'in vitro' incubation without treatment.

Fatty acid	Fatty acid composition in initial rumen liquor		Percentage composition in fresh fatty acids produced	
	Average	Range	Average	Range
Butyric	18.8 ± 1.3	16.3-20.2	19.27± 1.35	17.5-21.1
Propinic	20.3 ± 0.42	20.0-20.4	21.96± 1.9	18.63-25.25
Acetic	60.9 ± 1.4	59.4-63.7	58.77± 1.2	57.25-60.27

T A B L E 5.17

Percentage composition of fresh fatty acids produced in four hours of incubation under various treatments(average of three).

Source	Butyric		Propionic		Acetic	
	Average	Range	Average	Range	Average	Range
Rumen liquor	19.27± 1.35	17.5- 21.1	21.96± 1.9	18.63-25.25	58.77± 1.2	57.25- 60.27
do + Aureomycin 0.25mg/ml.	34.99± 1.50	33.05-36.55	7.61± 2.3	3.78-11.8	57.04± 1.8	54.1 - 60.83
do + Aureomycin 1.0mg/ml.	43.63± 3.1	38.0 -48.9	-0.50± 1.1	-3.3 - 2.08	56.87± 1.0	54.4 - 59.92
do + Terramycin 0.25mg/ml.	32.76± 4.7	25.68-41.77	8.11± 2.7	3.35-12.81	59.13± 2.1	54.88- 61.5
do + Terramycin 1.0mg/ml.	36.99± 4.7	28.46-44.68	3.26± 1.3	0.69- 5.15	59.74± 3.5	54.6 - 66.39

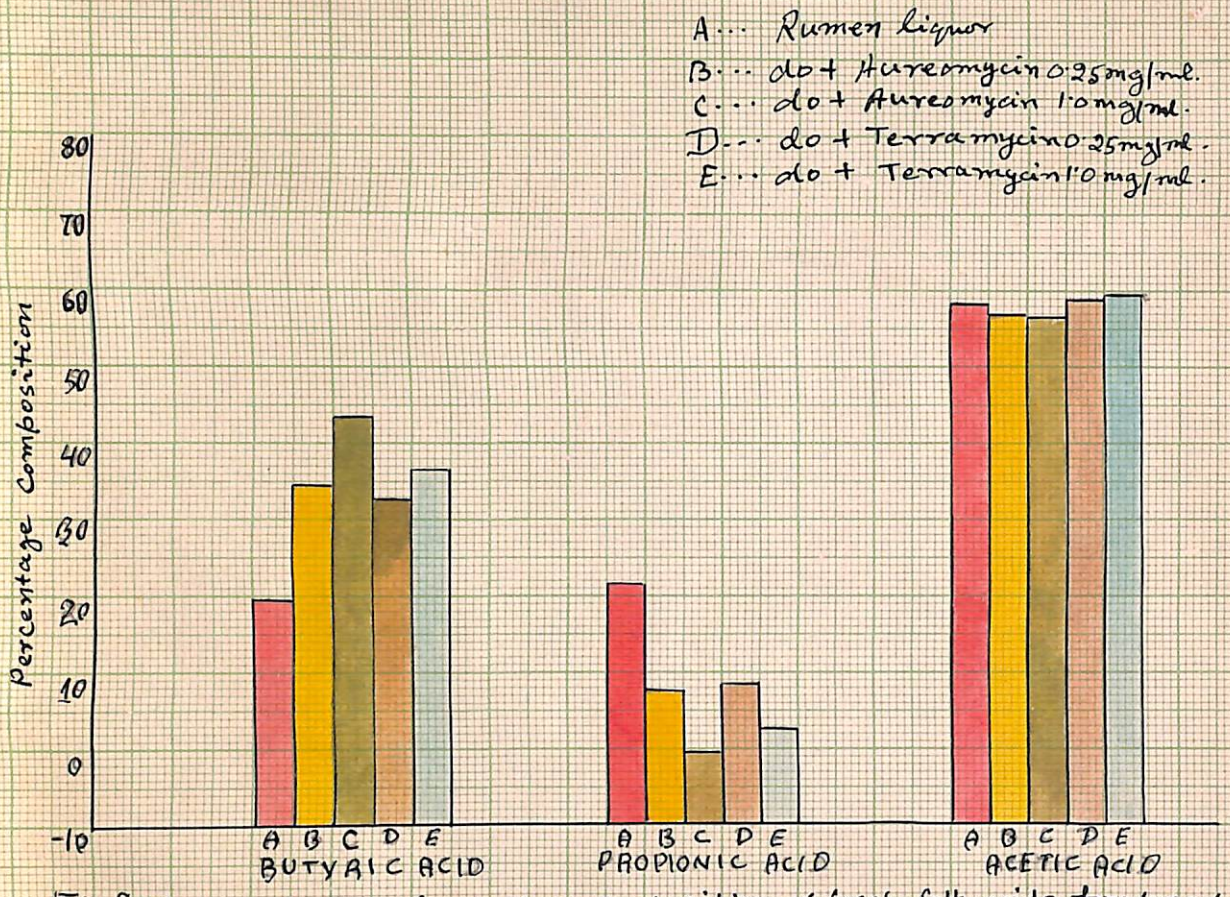

Fig. 8 Graph showing percentage composition of fresh fatty acids produced in four hours of incubation under various treatments.

It will be seen from the table 5.16 that much changes in composition were not found between the initial VFA and that produced during incubation. The composition in both the cases were - butyric 18-20%, propionic 20-22% and acetic 58-61%.

The antibiotic treatment markedly affected the percentage composition of fresh fatty acids produced as shown in the table 5.17 on page 96. Much changes were observed with regards to butyric acid and propionic acid by the antibiotic treatment while acetic acid percentage was not affected. The percentage of acetic acid in control as well as in 0.25 mg./ml. and 1.0 mg./ml. aureomycin treatment and in 0.25 mg./ml. and 1.0 mg./ml. terramycin treatment was found to be 58.77, 57.04, 56.87, 59.13 and 59.74 percents respecively. No marked difference was observed.

The percentage of butyric acid in control was 19.27% where as in 0.25 mg./ml. 1.0 mg./ml. aureomycin and terramycin treatments, it was 34.99, 43.63, 32.76 and 36.99% respectively. With higher levels of both the antibiotics, butyric acid percentage was apparently more than the lower levels.

The reverse was the case with propionic acid. Propionic acid percentage in control as well as in 0.25 mg./ml., 1.0 mg./ml. aureomycin and terramycin treatment was found to be 21.96, 7.61, -0.50, 8.11 and 3.26%/respectively.

Higher levels of aureomycin and terramycin had affected the propionic acid production markedly than the lower levels. The effect with aureomycin 1.0 mg./ml.treatment was so drastic that propionic acid percentage in fresh VFA was found to be in negative side, which is probably due to its matabolisation of the initial acid present in the rumen sample, by the microbial activity during incubation. 1.0 mg/ml. level of terramycin though affected the percentage of propionic acid in fresh VFA produced markedly but did not lower its amount to the negative side as was found with 1.0 mg/ml. treatment of aureomycin.

Ratio of the fatty acids after four hours of incubation ('in vitro ')under treatments.

Ratio of the fatty acids after four hours of incubation was also calculated for all the treatments and is presented in table 5.18.

T A B L E 5.18

Ratio of the fatty acids after four hours of incubation under various treatments.

Source	Acetic / Propionic	Acetic / Butyric	Propionic / Butyric
Rumen liquor	3.0	3.2	1.1
do + Aureomycin 0.25 mg/ml.	3.6	2.6	0.71
do + Aureomycin 1.0 mg/ml.	3.8	2.5	0.68
do + Terramycin 0.25 mg/ml.	3.5	2.8	0.78
do + Terramycin 1.0 mg/ml.	3.8	2.7	0.71

The data of table 5.18 indicates that with antibiotic treatment acetic: propionic ratio was wider than the control i.e. the ratio in the treated samples were obtained as 3.6, 3.8, 3.5 & 3.8 for 0.25 mg./ml., 1.0 mg./ml. aureomycin & terramycin treatments respectively, where as this ratio in control was 3.0 only. With higherlevels of both aureomycin and terramycin, this ratio was found to be wider than the low levels. Between the antibiotics, no difference was obtained with respect to their influence in this ratio.

Acetic : butyric and propionic : butyric ratios had a tendency to be narrower with the antibiotic treatment.

Discussion

The findings obtained by antibiotic treatment on pattern of VFA production was in aggrement with Hibbs & Conard (1953). They also reported no difference in the average total steam volatile fatty acids or acetic acid in rumen juice by antibiotic treatment. In their result also propionic acid wasslightly lower and butyric acid was slightly higher.

The work of Leffel et al (1957) indicated that antibiotic did not alter the amounts or ratios of VFA present in the rumen fluid. They might have obtained this result because of the lower doses they had used which was

not upto that extent to depress the propionic acid production and increase the butyric acid level as was observed in our case. The levels of aureomycin fed by these workers were 0,7,12, or 24 mg. per lamb per day. But in our 'in vitro' incubation, the level used was much higher i.e. 0.25 mg. & 1.0 mg. per ml. of rumen content.

Raun et al (1962) observed similar effects of antibiotics on rumen volatile fatty acid production. In a low concentrate ration, they observed that chlortetracycline did exert and effect on ruminal fermentation and tended to widen ($P<.05$) the acetate: propionate ratio. In their experiments also, chlortetracycbine hdd appeared to elevate ($P<.05$) butyric acid level. The comparative figures are presented in table 5.19.

T A B L E 5.19

Comparative effect of chlortetracycline treatment

VFA's molar %	Figures obtained by Raun et al (1962)		Present work	
	Basal	+ Chlor.	Rumen liquor	+0.25mg/ml. Chlor.
C_2	57.9	61.2	60.9	60.3
C_3	25.7	19.7	20.3	16.6
$i-C_4$	0.5	0.5		
C_4	15.1 (16.4	18.0 (19.0	18.8	23.2
$i-C_5$'s	0.3	0.2		
C_5	0.5	0.3		
Ratio C_2/C_3	2.3	3.1	3.0	3.6
Total* VFA	89	83	23.6	26.5

* Micromole/ml.

The perusal of the data on table 5.19 indicates that our results are almost similar to that of Raun et al(1962) except that total VFA production in our case was much lower. The reason for this was that the samples were collected in our experiments from those slaughtered goats which were kept fasting for 12 to 24 hours invariably before slaughter.

Pruser et al (1964) while studying on the effect of rumen liquor from aureomycin (30 mg/day) fed sheep on organism growth'in vitro', determined VFA ratios on samples taken four hours after feeding on the 14th day is presented in table 5.20 to compare with the VFA ratios obtained in the present work.

T A B L E 5.20

Comparative VFA ratios obtained in four hours of incubation.

Ratios	Pruser etal (1964)		Present work	
	Control	Aureomycin fed 30 mg/day	Rumen liquor	Aureomycin 0.25 mg/ml
Acetate / Propionate	4.37	3.55	3.0	3.6
Acetate / Butyrate	6.32	6.01	3.2	2.6
Propionate / Butyrate	14.7	16.4	1.1	0.71

The lack of aggrement apparent from the above table may be explained by the fact that in Pruser's experiment,the

antibiotic was not added directly to the nutrients at the time of incubation.

As regards the similarity in the percentage composition of fatty acid in the initial rumen sample and that produced during incubation, our results aggree with Mukherjee (1960) who also observed no appreciable changes in composition between the inital VFA and that produced during three hours of 'in vitro' incubation in fasting sheep maintained on hay and concentate. The composition in both cases were reported to be - butyric 10-12%, propionic 13 - 16% and acetic 72-75%. In our case also, it is evident from the table 5.18 that the composition of initial VFA and that produced during four hours of 'in vitro' incubation were similar. These samples were obtained from slaughtered goats who were fasted for several hours before slaughter.

Effect of antibiotics on carotene losses in the rumen liquor:-

It has been established now that carotene is less utilized by the ruminants thus they require greater amount of the provitamin than the actual requirement. One of the possible reasons is its destruction in the gastrointestinal tract. This experiment was conducted in order to determine whether appreciable losses occur in the rumen liquor and also to know the effects of antibiotics if any in preventing the possible losses occurring in the rumen.

The rumen sample was collected from the slaughtered goats and was incubated for four hours as detailed in Chapter-III. Carotene content was estimated in 2 mls.of rumen liquor under various treatments in three separate incubations. The results obtained is presented in table 5.21.

T A B L E 5.21

Carotene content of rumen liquor in 2 ml.after four hours of incubation under various treatments.

(in microgram)

Category	1st incubation.	2nd incubation.	3rd incubation.	Average
Rumen liquor(unincubated)	40	28	68	45.33
Rumen liquor(unicubated) +20 microgram carotene	86	45	86	69.66
Rumen liquor(incubated) +20 microgram carotene	34	22	44	33.33
do +Aureomycin 0.25 mg/ml +20 microgram carotene	43	34	47	41.33
do +Aureomycin 1.0mg/ml +20 microgram carotene	50	26	48	41.33
do +Terramycin 1.0mg/ml +20 microgram carotene	47	26	49	40.66

The perusal of data indicates wide variation in carotene content of rumen liquor of three incubations.This variation was due to the individual variation of the sources as also due to the nature of the food material present in the rumen.

In unincubated+20 microgram carotene per 2 ml. sample,the average carotene content was found to be 63.66 microgram where as inx the incubated sample with 20 microgram carotene was found to be 33.33 microgram only. With various antibiotics treatments,in which 20 microgram carotene was also added, carotene content in 2 ml. of rumen sample was found to be 41.33,41.33 and 40.66microgram. Thus it is seen thatwix with antibiotic treatment, less carotene was destroyed during incubation.

The percentage recovery of carotene after four hours of incubation under various treatments were also calculated and is presented in the table 5.22.

T A B L E 5.22

Percentage recovery of carotene after four hours of incubation under various treatments.

Category	Ist incubation.	2nd incubation.	3rd incubation.	Average
Rumen liquor(unincubated) +20 microgram carotene.	96.66	97.91	97.72	97.43
Rumen liquor(incubated) +20 microgram carotene.	56.67	45.83	50.0	50.83
do +Aureomycin 0.25mg/ml. +20 microgram carotene.	71.67	70.83	53.40	65.26
do +Aureomycin 1.0mg/ml. +20 microgram carotene.	83.33	54.16	54.54	64.01
do+ Terramycin 100mg/ml. +20 microgram carotene.	78.33	54.16	55.68	62.72

The percentage recovery in the unincubated rumen sample was found to be 97.43 percent where as recovery in the incubated sample was found to be only 50.83 percent. Hence in four hours of incubation the percentage loss of carotene was found to be 49.17 percent with rumen liquor.

With the antibiotic treatment, the percentage recovery was found to be 65.26,64.04,62.72 percent for the aureomycin 0.25 mg./ml. and 1.0 mg./ml. treatment and terramycin 1.0 mg/ml. treatment respectively.

Almost no difference was obtained in recovery percentage in the two levels of aureomycin and in the two tetracyclines used.

Discussion

Our findings are quite in aggrement with that of King et al (1962) who also observed the occurrence of destruction when carotene was incubated 'in vitro' in rumen fluid. In their case,the recovery of carotene from the tubes incubated for nine hours averaged about 65.6% against 97.5% recovery from inoculated but nonincubated tubes.

Devison & Seo (1963) also found while studying the influence of nitrate upon carotene destruction during 'in vitro' fermentation with rumen liquor that rumen microorganisms were capable of destroying carotene.

But Keating et al (1964) could not get any appreciable destruction of carotene in the rumen liquor from steers exeppt with certain treatment notably nitrite. Destruction in their case was found to be small or nil. Shorland et al (1957)while working on sheep indicated that carotene was not affected by rumen fermentation. These variations in results might be due to the conditions prevailing in the course of the experiments such as extent of anaerobic condition preventing spontaneous oxidation of carotene and perhaps amount of ether extract present in the rumen content.

Guerrant (1962) while working on the vitamin A depleted rats observed beneficial effect of antibiotics in supplementing the growth effect of beta- carotene.

The additional growth resposnse in rats given antibiotic over that in rats given none ranged from only 4% for sodium acrylate or streptomycin to 44% for tetracycline hydrochloride; the other tetracyclines also appeared to be effective.

It seems that antibiotics inhibited the destruction of carotene by microorganisms. Thus our findings are also in aggrement with these workers.

Therefore it seems that antibiotics especially tetracycline hydrochlorides together with beta -carotene or vitamin A will be more beneficial in the ruminants suffering

from vitamin A deficiency due to higher utilization of the vitamin in presence of antibiotics.

The necessity of more detailed 'in vivo' studies was felt in order to confirm this finding about the beneficial effect of antibiotics on carotene utilization.

C H A P T E R - VI

S U M M A R Y

1. A review on " antibiotics in ruminant nutrition " has been presented in Chapter-II.

2. Critical estimates of the probable experimental errors in the Chromatographic separation of VFA and also of estimation of carotene in rumen liquor were made and were found to be negligible.

3. Liquid - liquid Partition Chromatography with Celite'535' as the inert base, 2 M phosphate buffer of pH 6.5 as stationary phase and chloroform - butanol mixtures as the mobile phase was found to separate mixtures of acetic, propionic and butyric acids quantitatively, even when the acids were present in amounts of 5 - 10 micro moles each.

4. Two levels of two commonly used antibiotics were used. Chlortetracycline hydrochloride and oxytetracycline hydrochloride at 0.25 mg/ml. and 1.0 mg/ml. levels were tried to see their influence on fatty acid production.

5. Artificial rumen technique was employed for this study. The rumen samples were obtained from the slaughtered goat which were usually kept fasting for 12 to 24 hours preceding to slaughter.

6. Wide variation were observed in the rate of VFA production from sample to sample.of the rumen content. The rate of production during four hours of incubation was found to be apparently correlated in most ~~cases~~ with the initial VFA present in the samples.

7. Aureomycin and terramycin at both levels were found to had no significant influence on the rate of fatty acid production.

8. In presence of carbohydrate - rich substrate (starch in this case) antibiotics significantly inhibited the fermentation. The inhibition was found to be highly significant with higher doses of both the antibiotics where as low levels of both were found to inhibit only significantly.

9. Inp presence of protein rich substrate (casein in this case), both the levels of both the antibiotics also inhibited the fermentation.

10. Percentage inhibition of starch and casein fermentation under various treatments were calculated to be 24.76,75.46 for 0.25 mg/ml.aureomycin; 63.27,86.03 for 1.0 mg/ml. aureomycin; 22.09,82.09 for 0.25 mg/ml. terramycin and 87.05, 97.38% for 1.0 mg/ml. terramycin respectively.

11. No perceptible change in composition of VFA in rumen liquor was found during four hours of 'in vitro' incubation. The percentage in rumen liquor was- acetic 60-62%, propionic 19-21% and butyric 18-19%.

12. Treatments with antibiotics increased the butyric acid production in fresh fatty acids significantly ($P<.05$) while they decreased the propionic acid production significantly ($P<.01$) from the rumen liquor. No significant difference was found in the acetic acid amount by antibiotic treatment.

13. The composition of VFA in initial and that produced after four hours of incubation was found to be almost similar. The coposition in both the casss were butyric 18-20%, propionic 20-22% and acetic 58-61%.

14. Treatment with antibiotic markedly affected the percentage composition of fresh fatty acids produced from rumen liquor during four hours of incubation.

The percentage of butyric acid in control was 19.27% where as in 0.25 mg and 1.0 mg/ml. levels of aureomycin and terramycin treatments, it was 34.99,43.63,32.76 and 36.99% respectively and the propionic acid percentage in control as well as in 0.25 mg and 1.0 mg/ml. levels of aureomycin and terramycin treatments,it was found to be 21.96,7.61,-0.50,8.11 and 3.26% respectively. No marked difference was observed in the acetic acid production as it was found to be 58.77,57.04,56.87,59.13 and 59.74% in control as well as 0.25 mg and 1.0 mg/ml. levels of aureomycin and terramycin treatments respectively.

15. Treatments with antibiotics also affected the fatty acid ratios during incubation. Acetic:propionic ratio was found to be wider and other ratios were found to be lower.

Acetate : propionate ratio in control was found to be 3.0 were as in the antibiotic treated samples, it was found to be 3.6, 3.8, 3.5 and 3.8 ; acetate: butyrate ratio was 3.2 incontrol and 2.6 ,2.5,2.8 and 2.7 in antibotic treated samples and propionate: butyrate ratios were found to be 1.1 in control and 0.71,0.68 ,0.78,and 0.71 in the antibiotic treated samples.

16. Destruction of carotene was observed when carotene was incubated 'in vitro ' in the rumen fluid for four hours. The percentage recovery in incubated was found to be 50.83 percent as against 97.43 percent in unincubated rumen sample.

17. Antibiotics decreased the loss of carotene by microbial activity.No difference was found between the antibiotics used and between the levels of aureomycin.

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REFERENCES.

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

Arias,C.,Burroughs,W.,Gerlaugh,P. and Berthke,R.M. (1951).J.Animal Sci.,10 :683.

Bacharach,A.L.(1950) Analyst, 75:568.

Barcroft,J.,McAnally,R.A and Phillipson,A.T. (1944a)J.Exptl.Biol.,20: 120.

Bartley,E.E.,Fountaine,F.C., and Atkeson,F.W. (1950).J.Animal Sci.,9 :646.

Bartley,E.E.,Fountaine,F.C., and Atkeson,F.W. (1953)J.Dairy Sci. 36:604.

Bell,M.C.,Whitehair,C.K. and Gallup,W.D (1951)Proc.Soc.Expt.Biol.Med.,76:284.

Bickoff,E.M.,Livingston,A.L and Van Atta,G.R. (1952):J.Assoc.Off.Agric.Chem.,25:826.

Blaxter,K.L. (1954):Physiology of farm animals,1,3.

Bridges,J.H.,Miller,J.C., Kammlade,W.G.Jr.and Kunkel,H.O.(1953):J.Animal Sci.,12:660.

Brown,W.H.and Tucker,H. (1962):J.Dairy Sci.45:1390.(Abstr)

Cason,J.L.,and Voelker,H.H. (1951):J.Dairy Sci.,34:501 .

Chalupa W.,Evans,J.L., and Stillions,M.C. (1964):J.Animal Sci.23:802.

Chance,C.M.,Huffman,C.F., and Duncum,C.W. (1953):J.Dairy Sci.,36:253.

Christiansen,Wm.C.,Quinn,L.Y. and Burroughs,Wise. (1962):J.Animal Sci.21:706.

Colby,R.W.,Rau,F.A., and Miller,J.C. (1950):J.Animal Sci.,9:652.

Corrol,E.J.,andHungate,R.E. (1954):Appl.Microbiol.2:205.

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

Davis,R.F.,Woodhouse,N.S., Keeney,M.and Beck,G.H. (1957):J.Dairy Sci.,40:75.

Devison,K.L.and Seo,J. (1963):J.Dairy Sci.,46:862 .

Dinda,P.K. (1960):Ph.D.thesis.Annual Report ofR.R.I. Vol.18 .

Ekern,A.,Reid,J.T. (1963):J.Dairy Sci.,46:522.

Elam,J.and Davis R.E. (1962):J.Animal Sci.,21:327.

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

El-Shazly,K. (1952):Biochem.J.51:640.

Esplin,Grant,Hale,W.H, Hubbert,Farris Jr.and Taylor,B.R.Jr. (1963):J.Animal Sci.,22:695.

Evans,J.L.,Gmainger,R.B.and Thompson, C.M. (1957): J.Animal Sci.,16: 110.

Ferber,K-S. (1928):Z.Tierz.Zuchtungsbiol.12:31.

Fincham,R.C.,and Voelker,H.H.(1953):J.Dairy Sci.,36:594.

Gray,F.V. (1947):J.Expt.Biol.24:15.

Guerrant,N.B. (1962)Proc. Soc.Exp.Biol.Med.111:34.

Hibbs,J.W.,and Conrad,H.R. (1953):J.Dairy Sci.,36:593.

Horn,L.H.,Snapp,R.R.and Gall, L.S. (1955):J.Animal Sci.,14:243.

Hougue,D.E.,Warner,R.G.,Grippin,C.H. and Loosli,J.K. (1956): J.Animal Sci.,15:788.

Hungate,R.E. (1943):Biol.Bul. 84:157.

Hungate,R.E.,Fletcher,D.W. and Dyer,I.A. (1955a):J.Animal Sci.,14:997.

Jordan R.M.,Bell,T.D. (1951): J.Animal Sci.,10:1051.

Jordan,R.M. (1952)J.Animal Sci.,11:566.

Jordan,P.S.,Jordan,R.M. and Croom,H.G. (1956): J.Animal Sci.,15: 188.

Keating,E.K.,Hale,W.H., and Hubbert,F.Jr., (1964):J.Animal Sci.,23:111.

Kesler (1954):J.Animal Sci.,13:10.

King,T.B.,Lohman,T.G.and Smith,S.S. (1962): J.AnimalSci.,21:1002.(Abstr.)

Khatte,F.J.,Mitchell,G.E.Jr., and Little,C.O. (1964): J.Animal Sci.,23: (Abstr.)

Klopfenstein,T.J.,Purser,D.B. and Tyznik,W.J. (1962):J.Animal Sci.,21:1002.(Abstr.)

Klopfenstein,T.J.,Purser,D.B. and Tyznik,W.J. (1964):J.Animal Sci.,23:490.

Lembert,M.R and Jacobson,N.L.(1954):J.Dairy Sci.,40:633.

Landagora,F.T.,Rusoff,L.L,Harris, B(Jr)and Crown,R.M.and Bertrand,J.S.(1957):J.Animal Sci.,16:163.

Lassiter,C.A. (1955)J.Dairy Sci.,38: 1102.

Lassiter,C.A,Denton,T.W.,Davis, C.L.,and Taylor,J.A. (1954):J.Animal Sci.,13:991.

Leffel,E.C.,Brown,W.H.and Lakshmanan,S.(1957):J.Dairy Sci.,40: (Abst

Lewis. (1951): Biochemical J.48:175.

Loosli,J.K.,andWallace,H.D. (1950):Proc.Soc.Exptl.Biol.Med.75:531.

Loosli,J.K.,and Warner,R.G. (1952):Farm Research,18:3.

Loosli,J.K.,Wasserman,R.H.,and Gall,L.S. (1951): J.Dairy Sci.,34:500.

Mackay,A.M.,Ridell,W.H., and Fitzsimmons,R. (1953):J.Animal Sci.,12:19.

Mahapatro ,Bipin B.,and Leffel, E.C. (1964):J.Animal Sci.,23:883.(Abstr.)

Markham,R. (1942):Biochem.J.36:790.

Maynard,A.L.and Loosli,K.J. (1956):Animal Nutrition,4th edition.

Mazumdar,B.N.and Gupta,B.N. (1960): Annals of Biochamistry and Expt. Medicine,20:195.

Mazumdar,B.N.and Gupta,B.N. (1960):Current Science,29:434.

McAnally,R.A.,and Phillipson, A.T. (1942):J.Physiol. 101: 13.

McDonald,I.W. (1948a):Biochem.J.42:584.

McDonald,I.W. (1954):Biochem.J.56:120.

McDonald,I.W.and Hall,R.J. (1957):Biochem.J.67:400.

Miller,R.W.,Waldo,D.R.,Moore, L.A.and Hemken,R.W. (1964):J.Animal Sci.,23:885.(Abstr.)

Moddy,E.G.,Cook,A.P.,and Crumbaker,T.W. (1954):Proc.Ann.Meet.West.Div.A.D.S.A,35:141

Moore,L.A.& Ely,R. (1941):Ind.and Eng.Chem.Anal.Ed.13:600.

Mukherjee,D.B. (1960):PhD.thesis(Aberdeen).

Munch-Peterson,E.and Armstrong,J(1958):Australian J.Expt.Biol.36:77.

Nagy,G.,Vidacs,G.,and Ward,G.M.(1962):J.Dairy Sci.,45:691(Abstr.)

Nesic,P.and Ibrovic,M. (1963):Veterinaria,Sargevo,12:39.(Abstr.)

Neumann,A.L,Snapp,R.R.and Gall,L.S(1951):J.Animal Sci.,10:1058(Abstr.)

Omar,E.M.,Reagor,J.C.and Kunkal,H.O. (1964):J.Animal Sci.,23:729.

Parks,P.F.,Riewe,M.E.,Lyman, C.M.and Kunkel,H.O. (1964):J.Animal Sci.,23

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

Pennigton,R.J.and Sutherland,T.M.(1956):Biocham.J.63:618.

Perry,T.W.,Beeson,W.M.,Hornback, E.C.and Mohler,M.T. (1954):J.Animal Sci.,13:3.

Perry,T.W.,Beeson,W.M.,Mohler, M.T.and Smith,W.H. (1962):J.Animal Sci.,21:333.

Phillipson,A.T. (1947):J.Exptl.Bio.23:246.

Phillipson A.T.and Cuthbertson,D.P.(1956):Modern concepts of rumen digestion and metobolism.

Popjak,G.,French,T.H.,Hunter,G.D. and Martin,A.J.P. (1951): Biochem.J.48:612.

Preston,T.R.,Dinda,P.K., Macleod,N.A. (1959):Nutr.Soc. 18

Purser,D.B.,Klopfenstein,T.J. and Cline,J.H. (1964): J.Animal Sci.,23:888.(Abstr.)

Rao,S . (1963): M.Sc.Thesis submitted to Magadh Un.

Raun,N.S.,Burroughs,W and Woods,W.(1962):J.Animal Sci.,21:838.

Ray,S.N. (1942):Ind.J.Vet.Sci.,12:204.

Ray,S.N. (1958):Presidential address,Ind.Sci. Congr.Assoc.

Ray,S.N.,Chand,K.,and Rau,S.G (1941):J.Dairy Res.,12:109.

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

Richardson,C.W.,Ronning,M., Berousek,E.R.and Norton,C.L. (1953):J.Dairy Sci.,36:593.

Rusoff,L.L.and (1951):J.Dairy Sci,34:625.

Rusoff,L.L.,Haq,M.U.,Branton,C., Patrick,T.E.,and Arfenebourg,G.D.(1952):J.Animal Sci.,11:774.

Shaw,J.C.,Robinson,R.R.,Senger, M.E.,Leffel,E.C.,Doetsch,R.N., Lewis,T.R.and Brown,W.H. (1957):Maryland,Agric.Expt.Stat.Misc. Publication No.291:16.

Shorland,F.B.,Weenink,R.O., Johns,A.T.and McDonald,T.R.C. (1957):Biocham.J. 67:328.

Shultz,L.H.and Smith,V.R. (1951):J.Dairy Sci., 34:1191.

Sirotnak,F.M,Doetsch,R.N. Brown,R.E.and Shaw,J.C. (1953):J.Dairy Sci.,36:1117.

Stewart,W.E.and Schultz,L.H. (1958):J.Animal Sci.,17:737.

Stewart,W.E.,Stewart,G.D.and Shultz,L.H. (1958):J.Animal Sci.,17:723.

Sym,E.A. (1938):Cited in Biol.Rev.(1944),19:41.

Velu,J.G.and Reed,A. (1960):J.Dairy Sci.,43:1094.

Volkar H.H.and Cason,J.L. (1951):J.Animal Sci.,10:1065.

Warner,R.J. (1952):Proc.Cornell Nutr.Conf; p.126.

Williams,P.P.,Gutierrez,J.and Doetsh,R.N. (1960):Ophyroscolex Caudatus Ebertein. Bact.Proc.32.

Williams,J.B.and Jenson,C. (1954):J.Animal Sci.,13:1004.

Weldy,J.R.,McDowell,R.E., Van Soetst,P.J.and Bond,J. (1964):J.Animal Sci.,23: (Abstr.)

Zcheile,F.P.and Whitmore,R.A.(1947):Anal.Chem. 19:170.