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Dietary factors affecting volatile fatty acid production in the rumen

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DIETARY FACTORS AFFECTING VOLATILE FATTY ACID PRODUCTION IN THE RUMEN

by

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INTRODUCTION

Volatile fatty acids produced as a consequence of ruminal microbial degradation of ingested carbonaceous and proteinaceous materials meet a large portion of the ruminants' total energy requirements. These volatile fatty acids are absorbed directly into the portal circulation via the rumen epithelium. In that the ruminant host does not secrete any digestive enzymes or acids into the rumen, microbial attack of ingested food is solely responsible for these metabolic end products. Thus, these facts accentuate the importance of factors affecting production and absorption of volatile fatty acids.

The physiological and biochemical relationships between ruminal fermentation patterns and animal growth rate, feed efficiency and fattening are very poorly elucidated. However available evidence indicates acetate is the primary precursor of butyrate as well as milk fat whereas propionate is glucogenic. Although the exact relationship existing between these acids and animal biochemical systems is not clearly defined, it appears increased propionate production at the expense of acetate should enhance general performance of growing animals. Likewise increased ruminal propionate levels are usually associated with finishing fattening type rations. Therefore increased propionate production effecting improved utilization of acetate would not only increase growth rate and feed efficiency but would accelerate the fattening

process.

In many instances, the physical form of the ration ingredients influences the levels and ratios of ruminal volatile fatty acids. Similarly, specific carbohydrate and protein fractions appear to alter ruminal fermentation. Some lipids have been shown to affect ratios of ruminal volatile fatty acids. A comprehensive review of the literature indicates that acidity, buffering agents, select minerals and antibiotics might have an effect on ruminal fermentation patterns.

Current market conditions, grain surpluses and labor shortages have stimulated interest in increased ration concentrate level. However high concentrate-low roughage feeding regimes can precipitate digestive disturbances characterized by low ruminal pH and high lactic acid levels.

In cognizance of these physiological and biological phenomena, this study was initiated to appraise the effects of these dietary factors on ruminal fermentation patterns and animal performance.

REVIEW OF LITERATURE

Production of Volatile Fatty Acids

Formic, acetic, propionic, butyric and valeric acids arise largely from the microbial fermentation of dietary carbohydrates. Iso-butyric, 2-methylbutyric and iso-valeric acids arise primarily through the microbial deamination and decarboxylation of valine, leucine and isoleucine respectively (Annison 1954). Total concentrations and relative ratios of volatile fatty acids present in the rumen are dependent upon the composition of the rations and the feeding regime.

Acetic acid predominates with somewhat lesser amounts of propionic and butyric acids. Minor proportions of iso-butyric, 2-methylbutyric and iso-valeric acids are usually present. Although formic acid has been found in the rumen under some conditions, it is usually not present (Jamieson 1959 and Johns 1955). Steam volatile fatty acids beyond valeric acid are usually present only in minute traces (Annison 1954).

Highest total volatile fatty acid concentrations generally occur three to six hours after feeding (Gray and Pilgrim 1951 and Stewart *et al*. 1958). In silage fed sheep, Williams and Christian (1959) observed peak ammonia and volatile fatty acid levels one hour after start of feeding. Whereas Stewart *et al*. (1958) and Shaw *et al*. (1959) found volatile fatty acid ratios to be fairly constant throughout

the day, Gray and Pilgrim (1951) found the acetate-propionate ratio to narrow shortly after feeding, and then to widen thereafter. In an *in vitro* study, Bladen and Doetsch (1959) observed fairly constant volatile fatty acid ratios. Whereas little variation in volatile fatty acid levels occurred throughout the day with animals on diets containing only hay, more concentrated type diets gave fermentation peaks two to six hours after feeding (Balch and Rowland 1957). Reid *et al.* (1957) and Balch and Rowland (1957) observed narrowed acetate-propionate ratios and elevated butyrate levels in high concentrate diets. Propionate levels increased somewhat for a short time after feeding.

Gray *et al.* (1960) measured the rates of production of acetic, propionic and butyric acids in the normal rumen of sheep by the decline in specific activity of these acids after the introduction of their carbon labelled sodium salts. Since static levels of volatile fatty acids, as well as rumen volume, were fairly constant, rate of production was assumed to be equal to absorption rate. They determined the production rates of acetate, propionate and butyrate respectively to be 99, 44 and 21 millimoles per hour. Using a similar isotope dilution technique, Sheppard *et al.* (1959) found acetate to be synthesized at the rate of 90 millimoles per hour.

Gray and Pilgrim (1952b) concluded that acetate was a major precursor of butyrate. Sheppard *et al.* (1959) detected

considerable conversion of acetate to butyrate.

While rations containing large amounts of cellulose are characterized by high numbers of gram-negative cocci, high grain diets result in elevated numbers of gram-positive cocci with S. bovis predominating and with lowered numbers of the gram-negative cellulose digesting cocci (Hungate et al. 1952). Also under a high grain feeding regime, protozoa were killed, pH was lowered, total volatile fatty acid levels diminished and rumen motility was inhibited (Hungate et al. 1952).

In a review article, Oxford (1958) concluded that acetic acid was a primary product of cellulose digestion and propionate, lactate and acetate primary products of starch and soluble sugar degradation. Utilizing $C^{14}O_2$ in an in vitro study of the mechanism of propionic acid formation by a ruminant anaerobic micrococcus Veillonella gazogenes, Johns (1951a) and (1951b) concluded that CO_2 is fixed in the carboxyl group of propionic acid and that the amount of propionate formed depends upon the CO_2 concentration of the media. The following pathway was suggested: lactate $\xrightarrow{CO_2}$ pyruvate $\longrightarrow$ malate $\longrightarrow$ fumarate $\longrightarrow$ succinate $\xrightarrow{CO_2}$ propionate. Herschberger and Bentley (1956) presented evidence indicating the major pathway of propionic acid formation in rumen bacteria involves succinate decarboxylation. In contrast to this pathway, Johns (1952) concluded that Clostridium pro-

pionicum appears to form propionate from lactate by a more direct mechanism without production of intermediary dicarboxylic acids and without involving carbon dioxide fixation followed by subsequent decarboxylation. Elsden et al. (1956) isolated an unidentified organism from the rumen of sheep that fermented lactate directly to propionate. Ladd (1959) confirmed Elsden's observations.

Ruminal propionate levels are influenced by physical, microbiological and chemical factors. Moist steam heat treatment appears to effect an elevation of propionate levels at the expense of acetate. Phillipson (1952), Eusebio (1959), Ensor et al. (1959), Balch and Rowland (1957) and Shaw (1959) found that substitution of flaked corn for non-heated corn narrowed the acetate-propionate ratio and lowered butyrate levels. Phillipson (1952) observed that addition of cobalt to the diet containing flaked maize effected a further narrowing of the acetate-propionate ratio. In these studies he found elevated ruminal lactic acid levels with a disappearance of protozoa in the flaked maize diets. Clostridium butyricum, which normally produces acetate and butyrate, appeared to produce lactate at lower pH's found where flaked maize was fed. Ensor et al. (1959) found that whereas the steam heated corn effected a marked narrowing of the acetate-propionate ratio and a corresponding decrease in milk fat percentage, dry heated corn had no effect on ruminal volatile fatty acids or

milk fat.

Ground hay and pelleting moderately narrowed the acetate-propionate ratio (Ensor et al. 1959). Using lactating dairy cows, Balch (1958) showed that relatively more propionic acid was produced from a diet containing finely ground hay than from the long hay diet. Addition of linseed meal to flaked corn elevated butyrate levels (Eusebio 1959).

Annison (1954) and Balch and Rowland (1957) demonstrated that raising the protein level of a flaked maize diet increased butyric and branched chain acids. Johns (1953) and Waldo and Schultz (1960) found that glycerol is a precursor of propionate.

Lactic acid is widely considered to be a precursor of propionate (Waldo and Schultz 1960, Shaw 1956 and Eliot et al. 1957). Only traces of lactic acid are normally found in the rumen except on flaked maize, high grain, wheaten starch and other select diets (Balch and Rowland 1957, Hungate et al. 1952 and Phillipson 1952). Waldo and Schultz (1956) found lactic acid levels rose to a peak one-half to one hour after feeding and dropped rapidly after that. Supporting the hypothesis that lactate is an intermediate product in the production of propionate, a high correlation was demonstrated between decrease of administered lactate and increased propionate levels (Waldo and Schultz 1956). However, the immediate effect of elevated lactic acid levels was lowered

ruminal pH and total volatile fatty acid levels with no appreciable change in acid ratios (Reid et al. 1957). In a comparison of corn and hay silage as the only sources of roughage, Eliot et al. (1957) observed that corn silage, which was higher in acetic and lactic acids but lower in butyric acid, protein and fibre content than hay crop silage, gave higher ruminal volatile fatty acid levels and a higher proportion of propionic acid. Hay crop silage did not enhance total rumen volatile fatty acids but increased butyric and higher acid levels and decreased propionic and acetic acid levels.

Known pathways in the fermentation of lactate to propionate suggest that single carbon units play a vital role in this process. In a series of in vitro studies with rumen epithelial tissue, Pennington and Sutherland (1956a) found that when carbon labelled sodium bicarbonate was added to a medium containing propionate, essentially all of the radioactivity was found in the carboxyl group of lactic acid confirming carbon dioxide fixation. When propionate -1-C^{14} was added to the medium, the greater part of the isotope utilized appeared as $C^{14}O_2$ and the lactate was only slightly labelled. In a subsequent in vitro study, Pennington and Sutherland (1956b) found a diminished conversion of lactate to ketone bodies where a sodium bicarbonate buffer was used.

In cognizance of the microbial metabolism of lactate to propionate via carbon dioxide fixation and decarboxylation and

the influence of carbon dioxide on the metabolism of propionate by rumen epithelial tissue, the investigations of Matrone *et al*. (1957) and (1959) are of special interest. These authors found that quite satisfactory performance was obtained through the addition of either the salts of acetate and propionate or sodium and potassium bicarbonate to purified diets which contained casein, glucose, hydrogenated vegetable oil, vitamins and minerals. Where neither the salts of the volatile fatty acids nor the bicarbonates were added, very poor performance was obtained. Utilizing two sheep which had been fed on the foregoing purified diets with and without sodium and potassium bicarbonate additions, Van Campen and Matrone (1960) found that bicarbonate supplementation effected a narrowing of the acetate-propionate ratio. With *in vivo* and *in vitro* additions of carbon labelled sodium bicarbonate, most of the labelled carbon was found in propionate and the specific activity of valerate exceeded that of either acetate or butyrate in all cases. This indicates carbon dioxide is a precursor of propionate, and acetate and propionate are precursors of valerate.

In growth studies with calves and rabbits fed diets deficient in sodium, potassium and calcium, Thacker (1959) found that when these minerals were added in the form of acetates, bicarbonates and carbonates, mineral deficiency symptoms were alleviated. However, addition of these minerals as

chloride and sulphate salts did not effect any alleviation of mineral deficiency symptoms. He suggested the need of an anion capable of being oxidized to carbon dioxide and water with a cation of an opposite charge to achieve a cation-anion balance. This thus illustrates another vital role a single carbon unit can assume in ruminant metabolism. Wooley and Mickelsen (1954) observed a similar response with sodium and potassium bicarbonates and calcium carbonate. Black et al. (1952) found a greater amount of bicarbonate and propionate than acetate and butyrate incorporated in essential amino acids.

Factors affecting silage fermentations might also influence ruminal microbial fermentations. Klosterman et al. (1960) observed that an addition of one per cent non-dolomitic limestone or one-half per cent non-dolomitic limestone and one-half per cent urea to chopped whole plant corn silage at the time of ensiling increased the lactic acid content of the silage. Similarly, addition of one per cent non-dolomitic limestone to ear corn silage increased lactic acid levels. Cattle fed the treated silages gained faster and required less feed per pound of gain than those fed untreated silage. Where one per cent dolomitic limestone was added to the silage, lactic acid levels were elevated but no improvement in animal performance was noted (Klosterman et al. 1959).

Dietary nitrogen intake can have a pronounced effect on

ruminal fermentation. Jamieson (1959) observed that nitrate administration narrowed acetate-propionate ratios. Of interest was the fact that he associated high propionate levels with periods of poor growth. Formate generally appeared only where acetate levels fell. Whereas the only effect of a single dose of potassium nitrate was to narrow the acetate-propionate ratio, repeated doses also lowered total volatile fatty acid levels and elevated ruminal pH's. This author postulated that a lowered molecular percentage of acetic acid occurring as a consequence of nitrate administration may be due to a lack of acetic acid precursors usually present in the rumen, these probably being soluble carbohydrates or plant organic acids. Such a deficiency might be absolute, or brought about by competitive removal of these substrates in their function as hydrogen donors required to bring about the reduction of nitrate to ammonia. Such conditions can apparently occur during periods of high protein levels in the pasture. Annison et al. (1959a) and (1959b) observed elevated total volatile fatty acid, ruminal ammonia, amino nitrogen and lactic acid levels as well as narrowed acetate-propionate and acetate-butyrate ratios when sheep were transferred from dry roughage to lush green pasture. With elapsed time, rumen volatile fatty acid and ammonia levels were largely sustained. Observed lowering of pH and bicarbonate levels of the blood immediately after placement on pasture was

greatest where the animals had been previously fed high concentrate diets and least with high roughage. The acidosis noted occurred simultaneously with an increase in ruminal lactic acid concentration.

Stewart and Schultz (1958) observed that urea consistently increased volatile fatty acid production and narrowed the acetate-propionate ratio in vitro. However, these phenomena were not observed in subsequent in vivo trials (Stewart et al. 1958). Briggs et al. (1957) found that excessive ammonia concentration such as encountered in urea toxicity causes elevation of ruminal pH's above 8.0. Normal pH-volatile fatty acid relationships were modified to a considerable degree by high ammonia levels. High protein rations produced elevated ammonia and butyric acid levels (Orth and Kaufmann 1959). Barnett and Reid (1957) noted that whereas addition of a water extract of grass to an in vitro medium caused a widening of the acetate-propionate ratio, addition of the extracted grass markedly narrowed this ratio. Sirotonak et al. (1954) observed that l-aspartate and succinate were precursors of propionate.

Origin of the higher volatile fatty acids in the rumen was studied by Gray and Pilgrim (1952a) and Van Campen and Matrone (1960). Using labelled acetate, radioactive carbon appeared in the butyric and valeric acid fractions. With carboxyl labelled propionate, the valeric acid but not the

butyric acid became labelled. These data indicate a condensation of acetate to form butyrate and a condensation of acetate and propionate to form valerate. James et al. (1956) made the same conclusions. Using the organism LC, Ladd (1959) concluded synthesis of valerate represents a condensation of acetate and propionate. Shazly (1952) and Jamieson (1959) found that ruminal ammonia nitrogen values showed a highly significant correlation with concentrations of iso-butyric, 2-methylbutyric and iso-valeric acids. Shazly (1952) concluded that the power of microbes to decompose amino acids to ammonia and volatile fatty acids increased with elevated soluble protein diet levels.

Attempts to elucidate the physiological mode of action of antibacterial agents have not been too enlightening. Visek et al. (1959) proposed that select antibiotics suppressed bacterial urease activity. Young male rats fed a semi-purified basal diet supplemented with penicillin, chlortetracycline or arsanilic acid metabolized significantly less carbon labelled urea than unsupplemented controls. Chlortetracycline was most effective in suppressing urea metabolism. Penicillin and chlortetracycline significantly decreased ureolytic activity in the small intestine while chlortetracycline and arsanilic acid decreased ureolytic activity of the large intestine. Mangan et al. (1959) in bloat studies, observed that penicillin inhibited volatile fatty acid and

ruminal ammonia production, as well as protein breakdown. Reducing sugars and lactic acid accumulated while soluble protein concentrations rose rapidly during penicillin treatment. Using *in vitro* techniques, Brown (1959) concluded that penicillin apparently did not suppress general bacterial metabolism. Lambert and Jacobson (1955) observed that while chlortetracycline depressed *in vitro* cellulose digestion, no depression occurred *in vivo*. Horn *et al*. (1955) observed that whereas 100 mg. chlortetracycline per steer daily depressed roughage digestion by microorganisms, 32 mg. of the chlortetracycline or 100 mg. of penicillin did not. Hungate *et al*. (1955) concluded that although chlortetracycline feeding altered the composition of the rumen microbial population of steers, the potential for microbial activity was not appreciably affected. Although chlortetracycline feeding stimulated early growth rate of calves, this early weight advantage was lost when the calves reached one year of age.

Absorption of Volatile Fatty Acids

Much contradictory evidence exists in early literature concerning the absorption of volatile fatty acids. Due to poor experimental techniques and conditions, much of this early work must be viewed with considerable reservation. Much greater unanimity exists in more recent investigations reported herein.

Anatomically the rumen wall consists of a layer of

stratified epithelium backed by a smooth muscle coat which can be readily stripped from the epithelium. Volatile fatty acids produced in the rumen as a consequence of microbial fermentation are absorbed directly into the portal circulation primarily via the ruminal epithelium (Phillipson and McAnally 1942 and Gray et al. 1954). Forty to 70 per cent of the total energy requirements of the animal are met by these volatile fatty acids (Carroll and Hungate 1954 and McCarthy 1959).

Most recent investigations indicate absorption of volatile fatty acids from the rumen into the portal blood via the rumen epithelium is strictly a passive process (Gray et al. 1960, McCarthy 1959 and Stewart et al. 1958). Sander et al. (1959) found no preferential absorption of volatile fatty acids on a molar basis. Therefore the relative quantities of the various volatile fatty acids absorbed from the rumen are in general a reflection of the production or concentration of the individual acids in the rumen.

Many factors can affect the absorption process. Danielli et al. (1945) showed that absorption rates increased as pH decreased from 7.5 to 5.8. Stewart et al. (1958) made a similar observation. In further confirmation of the passive role played by the rumen epithelium, Danielli et al. (1945) observed that the permeability of the rumen epithelium is such that the pH of the ingesta tends to move towards

neutrality, independent of the neutralizing action of saliva. This suggests osmotic movement of buffering substances into the rumen by the same mechanism whereby rumen volatile fatty acids move into the portal blood. Masson and Phillipson (1951) presented a similar hypothesis.

In an early *in vitro* study, Pennington (1952) noted that the presence of carbon dioxide or bicarbonate in the rumen epithelium incubation media increased the uptake of propionate but had little effect on acetate and butyrate uptake. He also observed the *in vitro* conversion of butyric acid to ketone bodies by rumen epithelial tissue. This indicated ketone body production was not confined to the liver. In the following paper, Pennington (1954) again noted the effect of carbon dioxide in favoring absorption of propionate *in vitro* by sheep and ox rumen epithelial tissue. Carbon dioxide had no effect on rate of metabolism of propionate by sheep liver or kidney slices. Ammonium chloride inhibited the uptake of propionate by rumen epithelial tissue. In a later *in vitro* study, Pennington and Pfander (1957), utilizing rumen epithelial tissue demonstrated that butyrate lowered the uptake of propionate, acetate lowered the uptake of butyrate, and ketone body production from acetate was suppressed by the presence of propionate.

In studies on the portal blood of sheep, Annison et al. (1957) observed higher butyrate levels in the rumen than in portal blood. This difference was reflected in elevated portal blood ketone levels, which indicated metabolism of butyrate by the rumen epithelium to β-hydroxybutyric acid. Conrad et al. (1958) also observed proportionately lower butyrate levels in the portal blood than in the rumen. These in vivo observations lend credence to Pennington's in vitro data.

Using rumen perfusion techniques, Davis et al. (1958) and McCarthy (1959) did not detect any conversion of butyric acid to β-hydroxybutyric acid by the rumen epithelial tissue. In contradiction to the previously cited work, this indicates rumen butyrate was not a precursor of blood β-hydroxybutyrate.

Metabolism of Volatile Fatty Acids

In goat liver perfusion studies, McCarthy (1959) observed that propionic, butyric and valeric acids were completely removed from the portal blood and metabolized by the liver. Acetic acid was not removed by the liver. Consequently it appears that acetate absorbed from the rumen is made available as such to the extra hepatic tissues of the body. Formic acid and acetone bodies were formed by the liver. This author also demonstrated that introduced formate, propionate and

butyrate were recovered primarily in liver glycogen, blood glucose and lactate. These acids thus contribute much more to carbohydrate metabolism than to lipid metabolism. Very little acetate was recovered in these components, most of the label remaining in blood acetate. Kleiber et al. (1954) found that in the intact dairy cow there was appreciable labelling of milk lactose and casein from injected carbon labelled butyrate. Butyrate per se apparently contributed little to the formation of milk fat.

Using rat liver homogenates and soluble liver preparations, Pennington and Appleton (1958) found that the production of labelled carbon dioxide from carboxyl labelled acetate was almost completely abolished by propionate in only one-tenth the concentration of acetate. Butyrate was a less powerful inhibitor of acetate oxidation. This same inhibition occurred in sheep liver homogenate. Felts et al. (1958) concluded that butyrate and propionate caused a reduction in labelled carbon dioxide and lipid-C^{14} production from labelled glucose and lactate in rat liver slices.

Reid (1950) showed that acetic acid will not relieve insulin coma, whereas propionic acid is as effective as glucose. Schmidt and Schultz (1958) observed that in normal cows, sodium propionate feeding elevated ruminal propionate levels, but had no effect on blood sugar and ketones and milk production. Butterfat test was lowered slightly. However,

administration of sodium propionate for a six week period starting at calving increased blood sugar levels and milk production and decreased blood ketone levels. Administration of sodium propionate and sodium butyrate caused marked development of the rumen mucosa whereas sodium acetate did not.

Black and Kleiber (1958) indicated propionate is as important a precursor of aspartic acid and serine as it is of carbohydrate.

Using rat liver homogenates taken from rats on vitamin B_{12} deficient diets, Smith and Monty (1959) noted a severe depression in activity of the liver enzyme catalyzing isomerization of methylmalonyl-CoA and succinyl-CoA.

In a study of the heat increments of individually administered volatile fatty acids in fasting sheep, Armstrong and Blaxter (1957) determined the following heat increments: acetic acid 41 Cal., propionic acid 13 Cal. and butyric acid 16 Cal. per 100 Cal. of metabolizable energy. However, a mixture of the acids gave heat increment of 17 Cal. When heat increments of individual acids were measured in sheep receiving rations at or above maintenance level, considerably higher values were obtained, i.e. acetic acid 67 Cal., propionic acid 44 Cal. and butyric acid 38 Cal. (Armstrong et al. 1958). Thus whereas a mixture of acetate, propionate and butyrate administered to fasting sheep indicated these acids were essentially equivalent to each other in efficiency of utiliza-

tion, propionate and butyrate were much more efficiently utilized in growing and fattening rations than was acetate. However, Davis et al. (1960) observed that the simultaneous administration of propionate with acetate to an intact animal as compared to acetate alone did not diminish acetate oxidation.

Ensor et al. (1959) and Shaw et al. (1959) observed that dairy cow rations containing cooked high starch feeds narrowed ruminal acetate-propionate ratios and decreased the fat content of milk as compared to rations not containing cooked or steam heated starch. Decreased fat content of the milk was highly correlated with narrowed acetate-propionate ratios. Van Soest and Allen (1959) observed that high concentrate-low roughage type diets narrowed the acetate-propionate ratios and produced significant declines in milk fat percentage. Balch and Rowland (1959) found that the administration of sodium acetate to cows in which the milk fat percentage had been reduced by diets low in hay and high in concentrates usually brought about an appreciable improvement in fat percentage.

Feeding cod liver oil to milking cows effected a marked decline in milk fat percentage (Peterson 1932 and Shaw 1959).

EXPERIMENTAL AND RESULTS

Ruminal Volatile Fatty Acid Studies Using Fistulated Lambs

Experimental procedures common to all fistulated lamb trials

Procedures were developed by the author for collection of rumen fluid, measurement of rumen fluid buffering capacity and gas phase chromatographic separation of volatile fatty acids present in rumen fluid. Techniques developed by other workers were used for the determination of lactic acid and the steam distillation of volatile fatty acids in rumen fluid.

Collection of rumen fluid Twenty milliliters of rumen fluid was obtained via a small weighted suction strainer developed by the author coupled to a 50 milliliter plastic syringe. Fluid so collected was held in the syringe until discharged into a beaker for a pH determination using a Beckman pH meter, Model H-2. This technique facilitated pH measurements which were truly indicative of ruminal pH's without introduction of the electrodes directly into the rumen. Following pH measurement, one milliliter of saturated mercuric chloride was added to kill the microorganisms.

Steam distillation of ruminal volatile fatty acids Collected rumen fluid was brought to the laboratory where microorganisms and solid feed particles were removed by centrifugation. Following precipitation and filtration of pro-

tein, volatile fatty acids were steam distilled from the pH adjusted rumen fluid (McAnally 1944). A Markham steam distillation apparatus was used (Markham 1942). The resultant aqueous solutions of the volatile fatty acids were then titrated with potassium hydroxide, dried and stored for later gas phase chromatographic analysis. Details of the collection and processing of rumen fluid prior to gas phase chromatographic analysis are given in the Appendix.

Buffering capacity Centrifuged rumen fluid was taken down to pH 3.5 and base then added until a pH of 7.0 was reached. The amount of base taken to elevate the pH from 3.5 to 7.0 in 2 milliliters of rumen fluid was the measure of buffering capacity. Although the author was primarily interested in buffering capacity between pH 4.5 and 7.0, titration was commenced from pH 3.5 in that with the initial addition of acid, a sharp end point could be obtained at 3.5 whereas considerable difficulty was experienced in obtaining a good end point at pH 4.5. Also comparison of titration curves for centrifuged rumen fluid revealed great similarity of curves from pH 3.5 to 4.5. Thus the employed technique achieved precision and essentially measured differences in buffering capacity between pH 4.5 and 7.0. Details of this procedure are given in the Appendix.

Lactic acid determination Lactic acid levels of centrifuged rumen fluid were determined by the oxidation-steam

distillation technique outlined by Elsden and Gibson (1954). Details of this procedure are given in the Appendix.

Gas phase chromatographic analysis The gas phase chromatographic technique employed represents a refinement of a procedure outlined by Ralls (1960). He did not fully investigate either the method's potentials or limitations. The basis of this technique is a transesterification reaction of a properly heated dry mixture of potassium salts of the volatile fatty acids and the diester, potassium ethyl sulphate. The following reaction takes place:

$$R\overset{O}{\overset{\|}{C}}OK + C_2H_5O\overset{O}{\overset{\|}{S}}OK \xrightarrow[8\text{-}10 \text{ sec}]{325^{\circ} C} R\overset{O}{\overset{\|}{C}}OC_2H_5 + KO\overset{O}{\overset{\|}{S}}OK$$

In this technique, the dried potassium salts of the volatile fatty acids are manually mixed with potassium ethyl sulphate, a small portion of the mixed material added to a bent capillary tube, the tube inserted in the injection port of the gas phase chromatograph and the capillary tube heated with a tube containing 325° C oil. After approximately eight seconds, a spontaneous transesterification reaction takes place where the ethyl esters of the volatile fatty acids are volatilized directly into the chromatograph leaving potassium sulphate residually deposited in the capillary tube. This method has proven to be very reliable and does not entail any detailed or intricate manipulations. Examination of other workers' recordings using different techniques reveals that many experi-

enced extreme trailing in their peak patterns making accurate separation of components difficult, if not impossible. Using this dry distillation technique, formate, acetate, propionate, iso-butyrate, n-butyrate, iso-C_5 acids and n-valerate are separated. Only formate trails into acetate. Separation is complete between the straight chain and isomeric forms of the volatile fatty acids. However, 2-methylbutyric and iso-valeric acids cannot be separated due to their close boiling points, and as a consequence come through as one peak (designated as i-$C_{5's}$) between n-butyrate and n-valerate. The details of this procedure are given in the Appendix.

Fermentation pattern after feeding

Methods and results

Fistulated Lamb Trial 1 Description of the fermentation patterns present in the rumen with elapsed time after feeding vary considerably. This lack of unanimity is no doubt primarily due to differences in feed intake, ration components and method of feeding. Since the author was interested primarily in physiological and biochemical aspects of high concentrate diets, it was of primary importance that the fermentation patterns of these type diets be characterized.

Two fistulated wether lambs were selected for this study. The percentage composition of the 70 per cent concentrate-

30 per cent roughage ration which contained approximately 12.5 per cent protein is given in Table 1. Feed intake was constant during this trial and was limited to 1.3 pounds per animal twice daily. Rumen fluid collections were taken at

Table 1. Composition of rations fed in Fistulated Lamb Trials 1 and 2

Ingredient	Percentage
Ground alfalfa	30.0
Soybean oil meal	6.2
Rolled corn	57.0
Salt	.4
Limestone	.2
Molasses	6.2
Quadrex (gm./100 lb.)[a]	5

[a]Contains 10,000 I.U. of Vitamin A and 1250 I.U. of Vitamin D_2 per gram.

2, 4, 6, 8 and 10 hours after feeding. These animals were well adjusted to this diet before collections were made. Determinations of total volatile fatty acid levels and buffering capacity of centrifuged rumen fluid were made. Due to accidental contamination of the alkaline potassium salts of the volatile fatty acids with acetic acid vapors in the drying oven, gas phase chromatographic analysis of the samples was

not performed.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 2. A highly

Table 2. Fistulated Lamb Trial 1 - ruminal fermentation patterns with elapsed time after feeding[a]

Time after feeding	Total volatile fatty acids[b]	Buffering capacity[c]
2	125	3.2
4	125	3.0
6	115	3.0
8	96	2.6
10	97	2.5

[a]All values are based on the mean of two animals.

[b]Micromoles per milliliter.

[c]Milliliters of N/10 KOH.

significant decrease (linear regression $P < 0.005$) in total volatile fatty acid levels and buffering capacity of centrifuged rumen fluid occurred with elapsed time after feeding.

Fistulated Lamb Trial 2 In that the experimental data collected from the first trial was incomplete, this trial was repeated. Experimental procedures were the

same except that two different fistulated wether lambs were selected. Determinations of total volatile fatty acid levels, buffering capacity, pH and relative ratios of volatile fatty acids were made.

The results of this trial are given in Table 3. As in

Table 3. Fistulated Lamb Trial 2 - ruminal fermentation patterns with elapsed time after feeding[a]

Time after feeding	Percentage				Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	C_2/C_3			
2	56.0	24.4	.9	18.8	2.3	70	2.6	5.9
4	54.8	22.1	.8	20.8	2.5	89	2.5	5.8
6	57.2	19.5	1.4	20.3	2.9	70	2.1	5.9
8	58.7	19.6	1.1	18.6	3.0	71	2.0	6.0
10	57.1	17.7	1.7	20.8	3.2	65	2.0	6.0

[a]All values are based on the mean of two animals.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

the first trial, a decline in volatile fatty acid level and buffering capacity occurred with increased time after feeding, but to a lesser and non-significant degree (linear regression $P < 0.10$). However, a significant widening (linear regression $P < 0.01$) of the acetate-propionate ratio occurred with elapsed

time after feeding. The slight increase in pH was not significant.

Discussion and summary Results of Trials 1 and 2 indicated that peak ruminal fermentation values were achieved two to four hours after feeding on this high concentrate ration. As shown by Gray and Pilgrim (1951), these data demonstrated that after the fermentation peak is reached two to four hours after feeding, thereafter total volatile fatty acid and propionate levels decline, acetate-propionate ratios narrow and pH increases. Buffering capacity also declined in both trials after the fermentation peak was passed. All subsequent routine collections from fistulated lambs were made four hours after feeding since noted changes in the various measurement criteria were slight from two to four hours after feeding.

Effects of sodium bicarbonate, calcium carbonate, magnesium carbonate, cobalt sulphate and autoclaved corn

Methods and results

Fistulated Lamb Trial 3 An attempt was made in this trial to demonstrate that the addition of an alkalizing agent(s) to a high concentrate ration when shifting from a less concentrated to a more concentrated ration might prevent possible lactic acid accumulations and thereby shunt this "potential" lactic acid into propionic acid. Shaw (1956) has

shown that sodium lactate feeding enhances ruminal propionate levels. Van Campen and Matrone (1960) indicated sodium and potassium bicarbonate supplementation of sheep purified diets increased propionic acid levels. The composition of the 90 per cent concentrate-10 per cent roughage ration which contained approximately 11.7 per cent protein is given in Table 4. Ground alfalfa was blended with this ration to achieve lower concentrate levels. Three animals were started on a ration containing 75 per cent concentrate and 25 per cent roughage. The concentrate-roughage ratio was gradually increased until a 90 per cent concentrate-10 per cent roughage ration was attained for all animals. Then to this final high

Table 4. Composition of basic ration fed in Fistulated Lamb Trial 3

Ingredient	Percentage
Ground alfalfa	10.0
Soybean oil meal	8.0
Rolled corn	73.1
Molasses	8.0
Salt	.5
Limestone	.4
Quadrex (gm./100 lb.)[a]	6

[a]Contains 10,000 I.U. of Vitamin A and 1250 I.U. of Vitamin D_2 per gram.

concentrate ration, 1.25 per cent and 5.00 per cent of a sodium bicarbonate-potassium bicarbonate mixture[1] were added to the rations of animals 5 and 1 respectively whereas 4 received no supplementary bicarbonate. Determinations of volatile fatty acid levels and percentages, lactic acid levels, buffering capacity, pH and acetate-propionate ratios were made. Feed intake was constant during this trial and was limited to 1.3 pounds per animal twice daily. The animals were on the pretreatment ration for five days and the treatment rations for four days. While confounding of time-bicarbonate-concentrate effects occurs in this design, none the less comparison of responses of each animal before and during treatment provides a measure of the physiological role of buffering or alkalizing agents under this kind of a feeding regime.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 5. Where no bicarbonates were added to the 90 per cent concentrate-10 per cent roughage ration, a 38 per cent increase ($P < 0.01$) in total volatile fatty acid level, 12 per cent lowering in pH ($P < 0.01$) and a 62 per cent increase ($P < 0.01$) in buffering capacity occurred when the animals were shifted from the less concentrated to the more concentrated ration. Only slight

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[1] 66.6% $NaHCO_3$ and 33.3% $KHCO_3$.

Table 5. Fistulated Lamb Trial 3 - effect of dietary sodium and potassium bicarbonates on ruminal fermentation patterns

Animal and treatment[a]	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-$C_{5's}$	C_5	C_2/C_3			
#4										
Pretreatment a	59.9	22.9	.9	14.6	.9	.8	2.6	70	1.9	6.0
Trt (0% $^-HCO_3$)	55.7	23.4	.9	18.0	1.0	.9	2.4	96	3.0	5.3
#5										
Pretreatment b	55.1	29.9	.7	13.5	.3	.6	1.8	105	2.8	5.8
Trt (1.25% $^-HCO_3$)	47.9	34.5	.6	15.9	.3	.9	1.4	103	3.4	5.4
#1										
Pretreatment c	60.4	13.5	1.1	22.8	1.2	.9	4.5	75	2.2	6.2
Trt (5.00% $^-HCO_3$)	56.0	16.9	1.0	23.7	1.4	1.0	3.3	88	2.8	5.9

[a]All pretreatment values based on the mean of two samples taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

non-significant increases in volatile fatty acid levels, acidity and buffering capacity occurred in the bicarbonate supplemented animals.

Concurrent shift to the high concentrate ration with addition of the bicarbonates effected a 25.2 per cent ($P < 10$) narrowing of acetate propionate ratios whereas only a 9.2 per cent narrowing occurred in the ration not supplemented with bicarbonate. Lactic acid was undetectable in all cases.

Fistulated Lamb Trial 4 Klosterman et al. (1960) found that the addition of one per cent calcium carbonate to whole plant and ear corn silages at the time of ensiling approximately doubled the amount of lactic acid produced. A similar addition of high magnesium, dolomitic limestone increased lactic acid formation only 40 per cent. Whereas no improvements in growth performance were noted with the cattle fed the silages treated with dolomitic limestone, improvements in daily gain and feed conversion were noted in the cattle fed the limestone treated silages. In consideration of these observations, the glucogenicity of lactic acid and the neutralizing and buffering action of calcium carbonate, it was of interest to observe the effects of calcium carbonate additions to a high concentrate fattening type ration on ruminal fermentation patterns.

Other than the designated carbonate additions, the experimental procedure and rations used in Fistulated Lamb Trial

3 were also used in this trial. Four fistulated lambs were started on a 80 per cent concentrate-20 per cent roughage ration and the concentrate was gradually increased until a 90 per cent concentrate-10 per cent roughage ration was reached. At this time, no supplementary calcium carbonate was supplied to animal 4 whereas 0.625, 1.250 and 2.500 per cent calcium carbonate were added to the rations of animals 5, 6 and 3 respectively. The calculated calcium percentages of these final rations are given in Table 6. Measurements of volatile fatty

Table 6. Calculated amounts of calcium in diets used in Fistulated Trial 4

Animal	Per cent $CaCO_3$ addition	Percentage Ca
4	0.000	0.41
5	0.625	0.65
6	1.250	0.89
3	2.500	1.36

acid levels and percentages, lactic acid levels, acetate-propionate ratios, buffering capacity and pH were made. Feed intake was constant during this trial and was limited to 1.3 pounds per animal twice daily. The animals were on the pre-treatment ration for ten days and the treatment rations for four days.

Statistical analyses of the data appear in the Appendix

and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 7. As was true with sodium and potassium bicarbonate supplementation, shifting from the less concentrated to the more concentrated ration effected an increase ($P < 0.005$) in buffering capacity. Additions of calcium carbonate concurrently with the placement of animals on the 90 per cent concentrate-10 per cent roughage ration resulted in no change in buffering capacity from that observed when these animals were on the less concentrated ration. Also, as was true with bicarbonate supplementation, a marked reduction ($P < 0.05$) in pH occurred in the transition from the less to the more concentrated ration where no calcium carbonate supplementation was made whereas only a slight drop in pH occurred during this transition with calcium carbonate supplementation. Increasing levels of calcium carbonate appeared to lessen the narrowing of the acetate-propionate ratio when the lambs were switched from the lesser to the more concentrated ration. A highly significant narrowing ($P < 0.005$) of the acetate-propionate ratio occurred when the non-supplemented lamb was switched from the lesser to the more concentrated diet. An increase in n-valerate at the expense of the branched chain five carbon acids accompanied the narrowed acetate-propionate ratios. No significant effects on volatile fatty acid levels were noted either as a conse-

Table 7. Fistulated Lamb Trial 4 - effect of dietary calcium carbonate on ruminal fermentation patterns

Animal and treatment[a]	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-$C_{5's}$	C_5	C_2/C_3			
#4										
Pretreatment a	58.9	16.7	.9	21.9	1.1	.6	3.5	87	2.3	6.5
Trt (0% $CaCO_3$)	51.9	22.6	1.1	22.1	1.3	1.1	2.3	99	3.0	6.0
#5										
Pretreatment b	54.4	20.5	1.0	22.9	.7	.6	2.7	102	2.9	6.0
Trt (.625% $CaCO_3$)	50.6	26.2	1.0	20.6	.4	1.1	1.9	106	2.9	5.9
#6										
Pretreatment c	50.7	28.3	.6	19.2	.1	1.1	1.8	97	2.7	5.9
Trt (1.250% $CaCO_3$)	41.8	34.8	.6	19.5	.5	2.9	1.2	96	2.7	5.8
#3										
Pretreatment d	55.4	21.3	1.2	20.2	1.5	.4	2.6	87	2.5	6.1
Trt (2.500% $CaCO_3$)	52.5	18.5	1.0	25.6	1.8	.6	2.8	78	2.6	6.0

[a]All pretreatment values based on the mean of three samples taken on separate days. All treatment values based on the mean of four samples taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

quence of calcium carbonate supplementation or increasing concentrate level. Lactic acid was undetectable in all cases.

Fistulated Lamb Trial 5 In Fistulated Lamb Trials 3 and 4, the sheep were shifted gradually from a ration of lesser concentrate-roughage ratio to a 90 per cent concentrate-10 per cent roughage ratio ration. In this trial, the sheep received the 90 per cent concentrate-10 per cent roughage ration both before and during bicarbonate treatment. Also in contrast to Trial 3 where a combination of sodium and potassium bicarbonate was added, only sodium bicarbonate was added in this trial. After a very brief pretreatment stabilization period during which each animal established his own control values, one lamb was continued on the 90 per cent concentrate-10 per cent roughage ration without any bicarbonate supplementation, one received 1.25 per cent and two received 5.00 per cent sodium bicarbonate in their diets. The composition of the ration used is the same as used in Trial 3 with the exception of designated sodium bicarbonate additions. Feed intake was constant during this trial and was limited to 1.35 pounds per animal twice daily. Both the preliminary control and the subsequent treatment periods were four days in length, which was much too short for an accurate appraisal of control and treatment response. As a consequence any information gained from this trial is to be viewed with considerable reservation.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 8. Using each animal as his own control, statistical analysis of the data revealed only that sodium bicarbonate supplementation widened ($P < 0.025$) the acetate-propionate ratio whereas combined sodium and potassium bicarbonate supplementation in Trial 3 narrowed this ratio. An increase of iso-valeric and 2-methyl-butyric acids at the expense of valeric acid accompanied the widened acetate-propionate ratio. Sodium bicarbonate supplementation was without effect in altering total volatile fatty acid levels, buffering capacity and pH of centrifuged rumen fluid.

Fistulated Lamb Trial 6 Pretreatment and treatment periods were of insufficient length to provide an accurate measure of ruminal fermentation measurement criteria in previous trials where each animal served as his own control. Also in that no lactic acid accumulation resulted in Fistulated Lamb Trials 3 and 4 when animals were shifted from lesser to more concentrated rations, this experimental technique was abandoned in favor of a constant concentrate-roughage ratio ration during pretreatment and treatment periods. This design avoids confounding of concentrate-treatment effects. Again each animal served as his own con-

Table 8. Fistulated Lamb Trial 5 - effect of dietary sodium bicarbonate on ruminal fermentation patterns

Animal and treatment[a]	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-C_5's	C_5	C_2/C_3			
#1										
Pretreatment a	59.5	14.3	1.4	20.5	2.9	1.4	4.2	76	2.5	6.5
Trt (0% $NaHCO_3$)	60.1	16.3	1.3	19.4	2.0	1.0	3.7	89	2.7	6.1
#3										
Pretreatment b	42.2	30.8	1.3	21.5	2.1	2.1	1.4	95	2.9	6.1
Trt (1.25% $NaHCO_3$)	45.5	23.9	1.1	26.2	1.8	1.4	1.9	90	2.6	5.9
#5										
Pretreatment c	41.8	33.1	1.1	22.1	.4	1.5	1.3	118	3.1	6.1
Trt (5.00% $NaHCO_3$)	51.4	24.1	.9	22.0	.7	.8	2.1	118	3.0	6.3
#4										
Pretreatment c	55.4	20.2	1.3	19.7	2.1	1.3	2.7	91	2.6	6.7
Trt (5.00% $NaHCO_3$)	58.7	17.7	1.0	20.5	1.3	.8	3.3	79	2.2	6.6

[a]All pretreatment values based on one observation. All treatment values based on the mean of three samples taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

trol. The composition of the 80 per cent concentrate-20 per cent roughage ration which contained approximately 11.6 per cent protein is given in Table 9. Two animals were on the pretreatment ration for ten days and on treatment for 24 days. During this treatment period, one animal received 1.25 per cent sodium bicarbonate whereas the other received 5.00 per cent sodium bicarbonate. Feed intake was constant at 1.45 pounds per feed twice daily during the entire period.

Table 9. Composition of basic ration fed in Fistulated Lamb Trial 6

Ingredient	Percentage
Ground alfalfa	20.0
Soybean oil meal	6.0
Rolled corn	65.5
Molasses	8.0
Salt	.5
Quadrex (gm./100 lb.)[a]	6

[a]Contains 10,000 I.U. of Vitamin A and 1250 I.U. of Vitamin D_2 per gram.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 10. Both levels

Table 10. Fistulated Lamb Trial 6 - effect of dietary sodium bicarbonate on ruminal fermentation patterns

Animal and treatment[a]	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-$C_{5's}$	C_5	C_2/C_3			
#1										
Pretreatment a	58.1	18.8	.9	20.0	1.3	.9	3.1	90	2.6	6.5
Trt (1.25% $NaHCO_3$)	54.9	23.1	1.1	18.8	1.2	1.0	2.4	100	2.8	6.3
#6										
Pretreatment b	61.7	21.1	1.0	13.4	1.8	.9	2.9	94	2.4	6.4
Trt (5.00% $NaHCO_3$)	54.3	27.2	1.5	13.8	2.1	1.1	2.0	97	2.4	6.5

[a]All pretreatment values based on the mean of four samples taken on separate days. All treatment values based on the mean of 12 samples taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

of sodium bicarbonate supplementation effected a decrease in the acetate-propionate ratio; 1.25 per cent sodium bicarbonate narrowed this ratio 23 per cent ($P < 0.01$) and 5.0 per cent sodium bicarbonate produced a 31.5 per cent narrowing ($P < 0.005$). Total volatile fatty acid levels, levels of acids other than acetate and propionate, buffering capacity and pH were not affected.

Fistulated Lamb Trial 7 Since Phillipson (1952) observed that cobalt supplementation of a diet high in flaked maize narrowed the acetate-propionate ratio and lowered butyric acid levels, it was of interest to determine the effects of cobalt supplementation in a high concentrate fattening type diet. No supplemental cobalt was included in all previous fistulated lamb rations. These were calculated to contain approximately 0.16 ppm cobalt. Two lambs were selected to determine the effect of supplemental cobalt on volatile fatty acid ratios and levels, as well as buffering capacity and pH. One lamb went off feed, leaving only one lamb for this study. As in past trials, each lamb served as his own control. The composition of the 75 per cent concentrate-25 per cent roughage ration which contained approximately 11.6 per cent protein is given in Table 11. At the end of a 14 day pretreatment period 138.1 milligrams of cobalt sulphate ($CoSO_4 \cdot 7\ H_2O$) was added per 100 pounds of complete mix ration. This elevated the cobalt level to approximately 0.8 ppm. This cobalt fortified ration was fed during a 32 day treatment

Table 11. Composition of basic ration fed in Fistulated Lamb Trial 7

Ingredient	Percentage
Ground alfalfa	25.0
Soybean oil meal	5.0
Rolled corn	61.5
Molasses	8.0
Salt	.5
Quadrex (gm./100 lb.)[a]	6

[a]Contains 10,000 I.U. of Vitamin A and 1250 I.U. of Vitamin D_2 per gram.

period. Feed intake was constant and was limited to 1.3 pounds per animal twice daily.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 12. Butyric acid levels were reduced 26.0 per cent ($P < 0.01$) and acetate-propionate ratios were narrowed 13.5 per cent as a consequence of cobalt supplementation. These observations were in agreement with those of Phillipson (1952). Contrary to previous observations where an increase of valeric acid occurred at the expense of the branched chain five carbon acids when the acetate-propionate ratio was narrowed, in this instance the

Table 12. Fistulated Lamb Trial 7 - effect of dietary cobalt sulphate on ruminal fermentation patterns

Animal and treatment[a]	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-$C_{5's}$	C_5	C_2/C_3			
#5										
Pretreatment	50.3	20.0	.9	25.9	.9	1.9	2.5	121	3.1	5.9
0.64 ppm cobalt[d]	53.4	24.1	1.0	19.0	1.2	1.2	2.2	119	2.9	5.9

[a]All pretreatment values based on the mean of three samples taken on separate days. All treatment values based on the mean of 11 samples taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

[d]Supplied as $CoSO_4 \cdot 7H_2O$.

opposite relationship was observed. That is, the branched chain five carbon acids increased from pretreatment levels while valeric acid decreased. Total volatile fatty acid levels, buffering capacity and pH of centrifuged rumen fluid were not affected.

Fistulated Lamb Trial 8 Klosterman et al. (1959) and (1960) observed that the addition of one per cent non-dolomitic limestone to corn silage enhanced the lactic acid level and the feeding value of the silage. Addition of one per cent dolomitic limestone similarly increased lactic acid levels but did not effect any increase in average daily gain or feed efficiency. In that lactate feeding has been shown to enhance ruminal propionate levels (Hueter et al. 1956) and since the presence of excess dietary magnesium might interfere with normal ruminal fermentation or utilization of nutrients, it was of interest to investigate the possible effect of supplementary magnesium carbonate on ruminal volatile fatty acid levels, ratios and associated factors.

Although three animals were originally selected for this trial, one was discarded in that he repeatedly lost his rumen fistula plug, throwing him off feed and producing an abnormal ruminal fermentation. Each animal served as his own control as in past fistulated lamb trials. The basic 80 per cent concentrate-20 per cent roughage ration used in Fistulated Lamb Trial 6 was fed during the 25 day pretreatment period.

At the conclusion of the pretreatment period, 0.5 and 1.0 per cent magnesium carbonate were added to the foregoing basic ration to formulate the two treatment rations. The basic ration was calculated to contain approximately 0.216 per cent magnesium; the 0.5 and 1.0 per cent magnesium carbonate supplemented rations to contain approximately 0.359 and 0.503 per cent magnesium respectively. The treatment period lasted for 43 days. Feed intake was constant and was limited to 1.45 pounds per animal twice daily.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 13. Supplemental magnesium carbonate (0.5 per cent) effected an increase of 14.4 per cent ($P < 0.05$) in total volatile fatty acid levels while 1.0 per cent magnesium carbonate gave an increase of 8.9 per cent. Observed increases in buffering capacity, widening of the acetate-propionate ratios and elevation of pH as a consequence of magnesium carbonate treatment were not significant.

Fistulated Lamb Trial 9 Shaw (1959) and Phillipson (1952) and others have observed that the substitution of flaked corn for non-heat treated corn effected a narrowing in acetate-propionate ratios. Newland et al. (1960) observed that substitution of flaked corn for ground corn improved feed

Table 13. Fistulated Lamb Trial 8 - effect of dietary magnesium carbonate on ruminal fermentation patterns

Animal and treatment[a]	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-$C_{5's}$	C_5	C_2/C_3			
#1										
Pretreatment a	48.6	30.7	1.1	17.2	1.6	.8	1.6	102	2.8	5.8
Trt (0.50% $MgCO_3$)	52.6	26.2	.9	17.6	1.6	1.0	2.0	117	3.0	5.9
#6										
Pretreatment b	52.1	27.8	1.0	16.6	1.4	1.2	1.9	104	2.7	5.9
Trt (1.00% $MgCO_3$)	52.3	26.5	1.1	17.6	1.4	1.1	2.0	114	2.9	6.0

[a]All pretreatment values based on the mean of eight samples taken on separate days. All treatment values based on the mean of 12 samples taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

efficiency but slowed daily rates of gain. Salsbury *et al.* (1960) found that autoclaving with added water greatly increased the rate of *in vitro* starch digestion whereas ordinary dry steam autoclave treatment had little effect on starch digestibility. Their studies indicated that in contrast to the more slowly digested starches, the more rapidly digested starches had been subjected to considerable hydration. With this information, it was of interest to investigate the effect of dry steam autoclaved rolled corn on ruminal volatile fatty acid ratios, levels and associated factors. Two wether lambs were selected for this trial, with each animal serving as his own control. The basic 80 per cent concentrate-20 per cent roughage ration used in Fistulated Lamb Trial 6 was fed during the 14 day pretreatment period. Rolled shelled corn containing 12 per cent moisture that was autoclaved for 60 minutes at 15 pounds pressure per square inch was substituted for unheated rolled corn during the 36 day treatment period. Feed intake was constant and was limited to 1.3 pounds per animal twice daily.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 14. Autoclaving the corn effected a 46.2 per cent widening ($P < 0.005$) of the acetate-propionate ratio. Buffering capacity and total

Table 14. Fistulated Lamb Trial 9 - effect of autoclaved rolled corn on ruminal fermentation patterns

Animal and treatment[a]	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-$C_{5's}$	C_5	C_2/C_3			
Pretreatment	50.3	29.0	.9	17.5	1.2	1.1	1.7	116	3.0	5.8
Autoclaved corn	56.2	22.2	.9	17.8	2.3	.6	2.5	104	2.7	5.9

[a]All pretreatment values based on the mean of two animals, four samples per animal taken on separate days. All treatment values based on the mean of two animals, 10 samples per animal taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

volatile fatty acids levels were lowered, but not significantly as a consequence of autoclaving the corn. pH was not affected. Consistent with previous observations, the widened acetate-propionate ratio was accompanied by an increase of branched chain five carbon acids at the expense of valeric acid.

Discussion The bases for investigating the roles of sodium bicarbonate, calcium carbonate, magnesium carbonate, cobalt sulphate and autoclaving of corn have been presented. Due to convenience of sampling, fistulated lambs were employed. The only practical experimental design was one where each animal served as his own control because of limited numbers of animals and the high variability of response between animals to a given treatment.

Although the data reported represent a total of 175 separate samples, all information reported must be viewed with reservation. Constant difficulty was encountered in keeping these fistulated wether lambs on feed during the course of these seven trials. Minor stress conditions which would have little or no effect on an intact animal caused the lambs to go off feed, giving very abnormal fermentation patterns. Accidental removal of the rumen fistula plugs resulting in nearly complete evacuation of rumen contents generally produced anorexia, abnormally high propionate and abnormally low acetate levels. Usually a period of ten days would have to

elapse before the lamb returned to a normal microbiological and physiological state. The vulnerability of these lambs to these stress conditions provided a potential source of error and caused considerable delay in completion of many of the trials. Although the literature abounds with volatile fatty acid studies where fistulated lambs have been used as the experimental units, the extreme sensitivity of these individuals to stress factors encountered in these investigations very strongly suggests that they are unreliable experimental animals.

Nonetheless, some information is to be gleaned from these trials. Attempts to produce ruminal lactic acid accumulation when sheep were switched from less concentrated to more concentrated diets were unsuccessful. A combination of sodium and potassium bicarbonate supplementation, as well as sodium bicarbonate and calcium carbonate alone, concurrently with the shift from the less concentrated to the more concentrated diet dampened the drop in pH, had no effect on total volatile fatty acid levels and appeared to lower buffering capacity of centrifuged rumen fluid. This stability of buffering capacity is apparently caused by diminished movement of buffering substances from the blood into the rumen when a dietary buffering agent, such as sodium bicarbonate, is fed. Whereas combined sodium and potassium bicarbonate supplementation, and in one case sodium bicarbonate supplementation alone, narrowed

the acetate-propionate ratios, in Trial 5 sodium bicarbonate widened the ratio. Trial 5 however was of insufficient length. Although Van Campen and Matrone (1960) reported that sodium and potassium bicarbonate supplementation narrowed the acetate-propionate ratio, these fistulated lamb trials suggest that sodium bicarbonate has little effect on acetate-propionate ratios. However as reported by Pennington (1954), increased carbon dioxide tension favors absorption of propionate by the rumen epithelial tissue. No absorption studies were conducted in these trials.

Available evidence strongly indicates a single carbon unit such as a carbonate or bicarbonate could well affect the conversion of lactate to propionate. In that lactic acid accumulations were not produced in Trials 3, 4 and 5, no conclusions can be drawn as to bicarbonate or carbonate facilitating the shunting of lactate to propionate.

Interest in the role of calcium and magnesium carbonates on ruminal fermentation stemmed primarily from the reports of Klosterman et al. (1959) and (1960). They showed that addition of calcium carbonate alone to corn silages effected a greater increase in lactic acid levels than did dolomitic limestone additions. The limestone treated silage improved animal performance whereas dolomitic limestone treated silage did not. This of course suggested the possible enhancing role of calcium carbonate and the deleterious nature of excess

magnesium. In these investigations, calcium and magnesium carbonates were essentially without effect on ruminal fermentation as gauged by the outlined measurement criteria.

In Trials 3 and 4 increasing the amount of concentrate in the diet narrowed the acetate-propionate ratio as has been widely reported. Also, elevated valeric acid levels were correlated with narrowed acetate-propionate ratios.

Supplementary cobat narrowed the acetate-propionate ratio and significantly lowered butyric acid levels as reported by Phillipson (1952). Contrary to the relationship observed in other trials, here the narrowed acetate-propionate ratio was accompanied by an increase in the branched chain five carbon acids at the expense of valeric acid. As Gray and Pilgrim (1952a) consider propionate to be a precursor of valeric acid, the observed effect of cobalt sulphate supplementation in lowering valeric acid levels defies explanation in that cobalt supplementation elevated propionate levels.

While corn that has been subjected to wet steam treatment appears to narrow the acetate-propionate ratio (Eusebio 1959), in Trial 9 dry steam autoclaved corn effected a significant widening of the acetate-propionate ratio. Investigations of Salsbury et al. (1960) suggest little hydration of starch occurs with dry steam autoclaving whereas considerable hydration occurs under wet steam treatment. Total volatile

fatty acid levels, buffering capacity and pH were not affected as a consequence of dry steam autoclaving of corn.

Summary While recognizing the limitations of the experimental data obtained from these fistulated lamb trials, several conclusions can be made.

Sodium bicarbonate, potassium bicarbonate, calcium carbonate and magnesium carbonate have little effect on the absolute or relative amounts of acetate and propionate. Of these compounds, the bicarbonates tend to narrow acetate-propionate ratios. Also the bicarbonates tend to elevate ruminal pH's.

Supplementary cobalt tends to narrow acetate-propionate ratios and to lower butyric acid levels.

Narrowed acetate-propionate ratios or elevated propionate levels appear to presage elevated valeric acid levels at the expense of 2-methylbutyric and iso-valeric acids. This provides further evidence that acetate and propionate are the building blocks of higher molecular weight fatty acids. However the opposite relationship between acetate-propionate ratios and valeric acid was observed as a consequence of cobalt supplementation.

Dry steam autoclaving heat treatment appears to increase acetate at the expense of propionate.

Increasing dietary concentrate levels tends to narrow acetate-propionate ratios and lower pH.

Ruminal Volatile Fatty Acid, Growth and Carcass Studies Using Intact Lambs

Collection of rumen fluid was performed as described in the section devoted to comparison of fistulated and intact lambs. Samples were taken three hours after feeding in all intact lamb trials as contrasted to sampling four hours after feeding in all fistulated lamb trials.

All other laboratory procedures employed in these two intact lamb trials were identical to those used in the fistulated lamb trials.

Effects of calcium carbonate and sodium bicarbonate supplementation in a high concentrate lamb ration; effects of urea and chlortetracycline additions to a high roughage fattening ration

Methods and results

Intact Lamb Trial 1 Fifteen wether lambs weighing approximately 95 pounds were selected from lambs previously used in a group feeding trial. Three lambs were selected from each of five treatments. The lambs were individually fed for 23 days on the same rations as fed during the group feeding trial. The rations were: 1) shelled corn, supplement and long alfalfa hay, 2) basal complete mix, 3) basal plus 0.5 per cent calcium carbonate, 4) basal plus 1.5 per cent sodium bicarbonate and 5) basal plus 3.0 per cent sodium bicarbonate. Rations 2, 3, 4 and 5, which contained 80 per cent concen-

trates, were full-fed whereas intake of Ration 1[1] was limited to 1.60 pounds per animal twice daily. All rations contained approximately 12.5 per cent protein. The percentage composition of the supplement for Ration 1 and the complete composition of Rations 2, 3, 4 and 5 are given in Table 15.

Table 15. Composition of supplement used in Ration 1 and complete composition of Rations 2, 3, 4 and 5 used in Intact Lamb Trial 1

Ingredient	Percentage				
	1[a]	2	3	4	5
Corn		60.2	59.7	58.7	57.2
Cobs		20.0	20.0	20.0	20.0
Soybean oil meal	71.8	10.0	10.0	10.0	10.0
Dicalcium phosphate		.7	.7	.7	.7
Salt	10.0	.5	.5	.5	.5
Urea		.5	.5	.5	.5
Aurofac 10[b]	1.0	.1	.1	.1	.1
Trace mineral mix[c]		.2	.2	.2	.2
Calcium carbonate			.5		
Sodium bicarbonate				1.5	3.0
Vitamin A and D[d]	16.2	.8	.8	.8	.8
Molasses		7.0	7.0	7.0	7.0

[a]Supplement only.

[b]10 grams chlortetracycline per pound.

[c]Trace minerals added per pound of ration, in mg., were: Fe, 15; Mn, 8; Zn, 2; Cu, 1; and Co, 0.3.

[d]74,000 I.U. Vitamin A and 9,250 I.U. Vitamin D_2 per pound.

[1]0.86 lb. shelled corn, 0.08 lb. supplement, 0.66 lb. alfalfa hay.

Chemical composition of Rations 2, 3, 4 and 5 is given in Table 16.

Rumen fluid collections were taken three times weekly. One replication of each treatment was sampled each time

Table 16. Average chemical composition of the complete mix rations fed in Intact Lamb Trial 1[a]

Ration	Percentage	
	Protein	Calcium
2	12.5	.28
3	12.4	.50
4	12.1	.28
5	12.5	.30

[a]Determinations based on an air dry basis.

giving one sample per week per animal. A total of four rumen fluid collections were taken from each animal during the 23 day treatment period. Determinations of volatile fatty acid levels and percentages, acetate-propionate ratios and pH were made.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 17. Much wider ($P < 0.01$) acetate-propionate ratios were found in the long

Table 17. Intact Lamb Trial 1 - effect of ration form and concentrate-roughage ratio, dietary calcium carbonate and sodium bicarbonate on ruminal fermentation patterns and growth performance

Treatment[a]	Percentage						Ratio	Total VFA ml^{-1}[b]	pH	ADG	Feed conversion
	C_2	C_3	i-C_4	C_4	i-C_5's	C_5	C_2/C_3				
Long hay Shelled corn	50.4	24.4	1.0	21.4	1.1	1.6	2.1	91	6.6	.45	6.73
Basal	41.4	32.8	.5	21.2	.5	3.4	1.3	82	6.0	.32	8.60
+ 0.5% $CaCO_3$	42.8	37.0	.6	17.5	.5	1.7	1.2	81	6.2	.42	7.37
+ 1.5% $NaHCO_3$	41.5	36.5	.6	18.4	.5	2.5	1.1	101	6.1	.55	6.29
+ 3.0% $NaHCO_3$	49.8	26.3	.9	20.5	1.3	1.2	1.9	96	6.2	.39	7.76

[a]All treatment values based on the mean of three animals, four rumen fluid samples per animal taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

hay-supplement-shelled corn and the 3 per cent sodium bicarbonate rations than in the other rations. Higher pH ($P < 0.01$) was noted in the long hay ration than in the more concentrated complete mix rations. While not significant, sodium bicarbonate supplementation tended to elevate ruminal pH. No significant differences in total volatile fatty acid levels were noted. Interpretation of growth data differences is highly speculative in consideration of the method of selection of the experimental animals as well as the brevity of this trial.

Intact Lamb Trial 2 Critique of the first intact lamb trial affirmed the efficacy of using the intact lamb as an experimental unit in volatile fatty acid studies. The technique employed in sampling these lambs proved to be both rapid and simple to perform. Other than the differences noted between the long-hay (less concentrated) ration and the complete mix (more concentrated) rations, the possible effects of calcium carbonate and sodium bicarbonate supplementation were inconclusive. Also in the first trial, it was not possible to correlate growth performance with ruminal fermentation patterns in that the trial was too short and secondly, the lambs had attained market weight and finish prior to being used in ruminal fermentation studies.

It therefore seemed desirable to repeat this first intact lamb trial with the exception of the long-hay, shelled corn, supplement ration. Ration 1 had served only to provide a com-

parison between a complete mix high concentrate ration and a conventional lamb fattening ration.

Reports in the literature suggest that urea and antibiotics could have a dynamic effect on ruminal fermentation. Also, Visek et al. (1959) reported that a possible mode of action of an antibiotic was to inhibit bacterial urease activity. Jamieson (1959) reported that excessive ruminal ammonia levels were of course toxic to ruminants and produced narrowed acetate-propionate ratios. Burroughs et al. (1960) concluded that antibiotic supplementation most often shows a response for ruminants in a high roughage type ration. Urea toxicity occurs most readily in high roughage diets (Sapiro et al. 1949). Mindful of these facts and supporting evidence in the literature, it was deemed advisable to make a preliminary investigation of the effects of urea and chlortetracycline in a higher roughage type fattening ration than that used in the carbonate-bicarbonate trials.

Twenty-four western crossbred wether lambs weighing approximately 70 pounds were randomly allotted to eight treatment groups of three animals each. Prior to beginning the trial, they were vaccinated for sore mouth and enterotoxemia and drenched with phenothiazine. Whereas Rations 1, 2, 3 and 4 contained 80 per cent concentrate and 20 per cent roughage, Rations 5, 6, 7 and 8 contained 50 per cent concentrate and 50 per cent roughage. The composition of Rations 1, 2, 3 and

4 is identical to Rations 2, 3, 4 and 5 used in Intact Lamb Trial 1. Rations 5, 6, 7 and 8 were: (5) basal, (6) basal plus 10 milligrams chlortetracycline per pound, (7) 0.7 per cent urea and (8) urea basal plus 10 milligrams chlortetracycline per pound. These rations were calculated to contain 11 per cent protein. The percentage composition of Rations 5, 6, 7 and 8 is given in Table 18. Chemical composition of all rations is given in Table 19. The lambs were placed in individual feeding stalls twice a day for a 2 hour feeding period. Feed was kept before the lambs at all times during

Table 18. Composition of Rations 5, 6, 7 and 8 used in Intact Lamb Trial 2

Ingredient	Percentage			
	5	6	7	8
Soybean oil meal	7.25	7.25	2.25	2.25
Rolled corn	36.25	36.15	40.55	40.45
Molasses	5.00	5.00	5.00	5.00
Ground cobs	25.00	25.00	25.00	25.00
Ground alfalfa	25.00	25.00	25.00	25.00
Iodized salt	.50	.50	.50	.50
Mineral mix[a]	1.00	1.00	1.00	1.00
Urea			.70	.70
Aurofac 10[b]		.10		.10
Quadrex (gm./100 lb.)[c]	6	6	6	6

[a]Minerals added per pound of ration, in mg., were: Ca, 300; P, 231; Mn, 16; Co, 0.3.

[b]10 gm. chlortetracycline lb^{-1}.

[c]10,000 I.U. Vitamin A and 1,250 I.U. Vitamin D_2 gm^{-1}.

Table 19. Average chemical composition of the complete mix rations fed in Intact Lamb Trial 2[a]

Ration	Percentage	
	Protein	Calcium
1	11.8	.33
2	11.8	.50
3	12.1	.25
4	11.7	.30
5	11.4	.47
6	10.9	.39
7	10.5	.42
8	11.2	.37

[a]Determinations based on an air dry basis.

these periods. During the rest of the day, the lambs had free access to water and exercise lots. The initial, final and two week weights for the lambs were taken after 12 hour shrinks during which the animals did not have access to feed or water.

Rumen fluid collections were made during the last four weeks of the 62 day trial. Collections were taken three times weekly. One replication of each treatment was sampled each time giving one sample per week per animal. A total of four rumen fluid collections was taken from each animal. Determination of volatile fatty acid levels and percentages,

acetate-propionate ratios, buffering capacity and pH were made.

The lambs were slaughtered at a local plant where carcass weights and U. S. Government grades were obtained. Chilled carcass weight was obtained by shrinking the warm carcass weight by 2 3/4 per cent. Dressing per cent was based on the chilled carcass weight and the shrunk weight at the end of the experiment. Per cent separable fat and lean were determined from "hotel" rack sections.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance and correlation as described by Snedecor (1956).

Results of this trial are given in Tables 20 and 21. Total volatile fatty acid levels and pH were higher in the 50 per cent concentrate-50 per cent roughage rations than in the 80 per cent concentrate-20 per cent roughage rations ($P < 0.005$). Acetate levels were higher in the less concentrated rations ($P < 0.01$) whereas butyric acid levels were higher in the more concentrated rations ($P < 0.025$). Acetate-propionate ratios were narrower in the more concentrated rations ($P < 0.10$). Somewhat greater buffering capacity of centrifuged rumen fluid was noted in the less concentrated rations.

Calcium carbonate and sodium bicarbonate supplementation were without effect in altering levels and ratios of volatile

Table 20. Intact Lamb Trial 2 - effect of concentrate-roughage ratio, calcium carbonate, sodium bicarbonate, urea and chlortetracycline on ruminal fermentation patterns

Treatment[a]	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-C_5's	C_5	C_2/C_3			
	80 per cent concentrate-20 per cent roughage									
Basal	52.7	26.2	.4	18.7	.1	1.9	2.0	70	2.0	6.3
+ 0.5% $CaCO_3$	51.8	24.3	.3	22.0	.4	1.1	2.1	73	2.2	6.2
+ 1.5% $NaHCO_3$	52.2	24.4	.5	21.6	.4	.9	2.1	72	2.0	6.4
+ 3.0% $NaHCO_3$	55.4	24.4	.7	17.3	1.1	1.1	2.3	79	2.1	6.5
	50 per cent concentrate-50 per cent roughage									
Basal	57.9	25.7	.5	15.1	.3	.5	2.3	89	2.2	6.5
+ 10 mg. chlor. lb^{-1}	61.2	19.7	.5	18.0	.2	.3	3.1	83	2.1	6.5
0.7% urea basal	62.1	23.9	.3	12.9	.2	.5	2.6	87	2.1	6.6
+ 10 mg. chlor. lb^{-1}	59.4	22.9	.3	16.7	.2	.4	2.6	92	2.2	6.3

[a]All treatment values based on the mean of three animals, four rumen fluid samples per animal taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

Table 21. Intact Lamb Trial 2 - effect of concentrate-roughage ratio, calcium carbonate, sodium bicarbonate, urea and chlortetracycline on growth performance and carcass traits

Treatment[a]	Average daily gain	Feed conversion	Carcass grade[b]	Dressing per cent	Separable fat	Separable lean
80 per cent concentrate-20 per cent roughage						
Basal	.63	5.97	8.0	50.8	29.5	51.5
+ 0.5% $CaCO_3$	.71	5.13	7.7	52.2	33.3	51.2
+ 1.5% $NaHCO_3$	.62	5.95	8.0	51.1	32.1	50.5
+ 3.0% $NaHCO_3$	.64	5.80	7.7	52.5	32.6	51.2
50 per cent concentrate-50 per cent roughage						
Basal	.65	5.86	7.0	49.1	26.1	52.8
+ 10 mg. chloro lb^{-1}	.61	6.46	6.7	50.1	24.8	53.7
0.7% urea basal	.63	6.35	5.7	49.0	27.5	52.4
+ 10 mg. chloro lb^{-1}	.63	6.00	7.0	49.6	25.7	54.3

[a]All treatment values based on the mean of three animals.

[b]U. S. Government grade value: 5 equals good, 6 equals high good, 7 equals low choice, 8 equals choice.

fatty acids and buffering capacity in the high concentrate rations. Sodium bicarbonate tended to elevate ruminal pH.

In the low concentrate rations, chlortetracycline and urea did appear to exert an effect on ruminal fermentation. While both chlortetracycline ($P < 0.005$) and urea alone tended to widen the acetate-propionate ratio, a significant negative interaction ($P < 0.005$) was noted between the two. Similarly urea and chlortetracycline alone tended to elevate acetate levels, but again a significant negative interaction ($P < 0.005$) occurred between urea and aureomycin. Aureomycin also appeared to elevate butyric acid levels ($P < 0.005$).

Analysis of growth and carcass data reveal that carcass grade, dressing per cent and separable fat were higher in the 80 per cent concentrate-20 per cent roughage rations than in the 50 per cent concentrate-50 per cent roughage rations ($P < 0.005$). Also, more efficient feed conversion occurred in the more concentrated rations ($P < 0.05$). However, separable lean percentages were higher in the less concentrated rations ($P < 0.05$). The various treatments were without effect on average daily gain.

In the low concentrate rations, urea tended to have a depressing effect on carcass grade. However the combination or urea and chlortetracycline overcame the depressing effects of urea ($P < 0.10$).

No significant growth and carcass differences were noted between the high concentrate ration treatments.

Several correlations between volatile fatty acid patterns and growth and carcass characteristics were determined. Calculated correlations are given in the Appendix. A correlation coefficient of 0.81 was obtained between total volatile fatty acid levels and rumen fluid buffering capacity ($P < 0.01$). A significant correlation existed between carcass grade and ruminal butyric acid levels. Using all animals, a correlation coefficient of 0.63 was obtained ($P < 0.01$). Analyzing the high concentrate and low concentrate diets separately, correlation coefficients of 0.51 ($P < 0.05$) and 0.66 ($P < 0.01$) respectively were obtained. Whereas a correlation coefficient of 0.58 ($P < 0.01$) was obtained between separable fat and butyric acid levels when all animals were pooled, non-significant correlations were obtained when the high and low concentrate data were analyzed separately.

Discussion Concurrent collection of ruminal fermentation, growth and carcass data from intact lambs proved to be a reliable and productive technique. Only ruminal fermentation data were obtained from the first trial.

The first intact lamb trial revealed only that acetate-propionate ratios were narrower and pH's lower in high concentrate complete mix rations than in a long hay-shelled corn-supplement lamb fattening ration. Possible effects of calcium carbonate and sodium bicarbonate supplementation were ill-defined. Other than the noted effect of concentrate level on

ruminal fermentation, the only indication was that addition of 3 per cent sodium bicarbonate to a high concentrate ration tended to widen acetate-propionate ratios whereas 1 1/2 per cent sodium bicarbonate did not.

In view of the previously outlined limitations of the first trial, initiation of the second trial provided an opportunity to collect growth and ruminal fermentation data simultaneously, and associated carcass data at the termination of the trial. To better assess the value of calcium carbonate and sodium bicarbonate supplementation of high concentrate rations, these treatments were repeated per se in the second trial. Explanation has been previously given for exploring the effects of urea and chlortetracycline in a high roughage type ration.

An overall assessment of the fistulated lamb trials indicated that neither calcium carbonate nor sodium bicarbonate exerts a pronounced effect on ruminal fermentation patterns. However, there was an indication that sodium bicarbonate supplementation tended to narrow acetate-propionate ratios and elevate pH. This is compatible with the observations of Van Campen and Matrone (1960). The results of the first intact lamb trial indicated only that 3 per cent sodium bicarbonate widened acetate-propionate ratios in a high concentrate ration. This is contrary to the hypothesis presented previously by the author that sodium bicarbon-

ate or a similar single carbon buffering substance might facilitate the shunting of lactate to propionate. Review of the results of the second intact lamb trial certainly do not lend any credence to this hypothesis. In retrospect, it appears that sodium bicarbonate and calcium carbonate have little, if any, effect on ruminal fermentation patterns, growth and carcass characteristics. It must be emphasized, however, that no absorption studies were conducted. Extensive in vitro studies conducted by Pennington (1952) and (1954) strongly suggest sodium bicarbonate favors absorption of propionate by the rumen epithelial tissue. If this were true, ruminal propionate levels would not reflect absorption rates. Ruminal fermentation patterns observed in fistulated lambs that were off feed as well as those of Jamieson (1959) suggest that elevated propionate levels are often indicative of animals that are suffering from some digestive disturbance or of inherently poor performing animals. This accentuates the complexity of assessing the efficacy of a given biological measurement criteria in evaluating a ration treatment.

Visek et al. (1959) observed that a possible mode of action of antibiotics was to inhibit bacterial urease activity in the gut of the monogastric animal. If this were true, then an orally administered antibiotic could well perform the same function in the rumen where pH and other environmental conditions are quite similar to those found in the small

intestine. Further examination of the chemical nature of urea reveals it could have pronounced effects on ruminal fermentation. Urease catalyzes the enzymatic breakdown of urea to ammonia and carbon dioxide. Urea can act as a buffer or alkalizer (Klosterman et al. 1960 and Jamieson 1959). However, as is well known, excessive intakes of urea can produce the urea toxicity syndrome (Hale and King 1955). A commonly assumed mode of action of an antibiotic is to selectively inhibit certain strains of microorganisms. Thus an antibiotic could well effect an alteration in ruminal fermentation via selective microorganism inhibition. Comprehensive review of these facts concerning antibiotics and urea suggested these agents might well play integral roles in rumen fermentation.

While recognizing there were insufficient replications in the chlortetracycline-urea treatments, critique of the data indicates both chlortetracycline and urea exert separate as well as combined effects, on ruminal fermentation patterns, growth performance and carcass characteristics. Addition of 10 milligrams of chlortetracycline per pound of the 50 per cent concentrate-50 per cent roughage ration lowered feed efficiency. Antibiotic supplementation also produced a widening of the acetate-propionate ratio and increased butyric acid levels. Substitution of 0.7 per cent urea for soybean oil meal and corn on an iso-nitrogenous basis depressed feed

efficiency and carcass grade, widened acetate-propionate ratios and lowered butyric acid levels.

The combined additions of chlortetracycline and urea tended to overcome the depressing effects of each on feed efficiency and of urea on carcass grade. Not to be overlooked is the fact that chlortetracycline supplementation coupled with urea feeding tended to overcome the widening effect of each on acetate-propionate ratios. Perhaps antibiotic supplementation of a high urea ration could improve ruminant performance via increased ruminal propionate production as compared to a urea ration not containing antibiotic. Also of particular interest was that per cent separable lean increased and separable fat decreased somewhat concurrently with an improvement in carcass grade as a consequence of combined urea and chlortetracycline supplementation. Urea supplementation alone lowered carcass grade markedly and increased per cent separable fat. Although no nitrogen retention or ruminal ammonia studies were conducted, these trends indicate that chlortetracycline could very well inhibit bacterial urease activity effecting more efficient use of urea nitrogen.

These foregoing facts emphasize that antibiotic supplementation of urea containing rations might effect more efficient use of urea nitrogen, enable incorporation of higher levels of urea in high roughage rations and finally, to serve as a means of simultaneously improving carcass grade and per

cent separable lean.

Calculated correlation coefficients revealed no correlation between acetate-propionate ratios and gain, whereas a trend towards improved feed efficiencies was noted as acetate-propionate ratios were narrowed. Of major interest was the fact that carcass grade was highly correlated with butyric acid levels. In the high concentrate rations, a correlation coefficient of 0.51 was obtained; in the low concentrate rations, 0.66. While a high correlation was noted between separable fat and butyric acid levels when all animals were pooled, no correlation was noted when the high and low concentrate data were analyzed separately.

Summary Calcium carbonate and sodium bicarbonate were without effect in altering volatile fatty acid levels and ratios and buffering capacity of rumen fluid in high concentrate rations. Sodium bicarbonate had a tendency to elevate ruminal pH. Neither exerted any effect on growth or carcass characteristics.

Chlortetracycline supplementation of a high roughage fattening ration tended to decrease feed efficiency but had little effect on carcass characteristics. Addition of chlortetracycline appeared to widen acetate-propionate ratios and to increase butyric acid levels. Urea supplementation in a high roughage ration decreased feed efficiency and carcass grade, widened acetate-propionate ratios and lowered butyric acid levels.

Chlortetracycline supplementation of a high roughage fattening ration containing urea tended to overcome the depressing effects of urea on carcass grade, separable lean and feed efficiency. Also this combination tended to narrow acetate-propionate ratios from those observed with chlortetracycline alone.

Higher ration concentrate levels resulted in increased feed efficiency, carcass grade, dressing per cent and separable fat and decreased separable lean. Narrower acetate-propionate ratios, higher butyric acid levels, lower total volatile fatty acid and pH levels were noted in the high than in the low concentrate rations.

Effects of Sodium Bicarbonate and Lactic Acid on Ruminal Fermentation

Under high concentrate-low fiber feeding regimes, elevated ruminal lactic acid levels are apt to occur. Matrone et al. (1959) found that sodium and potassium bicarbonate additions to roughage-free purified diets, where lactic acid accumulations were apt to occur, gave superior lamb performance to those not receiving the bicarbonates. Subsequent isotope studies indicated higher propionate levels where bicarbonate supplementation had been made (Van Campen and Matrone 1960). Pennington (1954) indicated the presence of sodium bicarbonate favored the in vitro uptake of propionate by rumen epithelial tissue. Extrapolation of these observa-

tions suggested that sodium bicarbonate coupled with lactic acid supplementation would not only alleviate acidity produced by lactic acid, but would also elevate propionate levels above those produced from lactic acid alone.

Methods and results

Lactic Acid-Sodium Bicarbonate Trial 1 Several rumen fluid samples were taken from a fistulated lamb that was well adjusted to a 80 per cent concentrate-20 per cent roughage ration (Ration 2, Table 15). Samples were taken one and three hours after feeding. After taking collections on five different days, 90 milliliters of lactic acid was administered in an aqueous solution via rumen fistula. As the animal was immediately placed in stress, 60 grams of sodium bicarbonate was administered from three to eight hours after the initial lactic acid dosing. A final rumen fluid collection was taken 24 hours after dosing with lactic acid.

The experimental plan and the results of this trial are given in Table 22. During the pretreatment period, no differences were noted in volatile fatty acid levels and ratios, buffering capacity, lactic acid levels and pH of rumen fluid samples taken one and three hours after feeding. In the three hours following the administration of 90 milliliters of lactic acid, no change occurred in the relative amounts of the volatile fatty acids, but there was a tremendous drop

Table 22. Lactic Acid-Sodium Bicarbonate Trial 1 - effect of lactic acid and sodium bicarbonate additions on ruminal fermentation patterns[a]

Time and dosage[b]	Percentage							Ratio	Total VFA ml^{-1}[c]	Buffering capacity[d]	Lactic acid[e]	pH
	C_1	C_2	C_3	i-C_4	C_4	i-$C_{5's}$	C_5	C_2/C_3				
Pretreatment[f]												
1 hr. --		58.3	21.5	.9	17.8	1.0	.5	2.7	97	2.5	.6	6.2
3 hr. --		58.6	20.7	.9	18.6	.7	.5	2.8	98	2.4	.4	6.2
Lactic Acid												
0 hr.- 90 ml.												
1 hr.	10.0	56.2	18.7	.3	14.2	.5	.0	3.0	53	3.4	>27.5	4.3
3 hr.	3.0	57.8	20.9	.3	16.5	1.3	.3	2.8	57	2.9	>27.5	5.1
Added sodium bicarbonate												
3-8 hr.- 60 gm.												
24 hr.	8.2	38.1	29.2	2.2	11.2	3.4	7.6	1.3	82	2.1	--	6.6

[a]All values based on one fistulated lamb.

[b]Milliliters of lactic acid and grams of sodium bicarbonate.

[c]Micromoles of volatile fatty acids per milliliter.

[d]Milliliters of N/KOH.

[e]Micromoles of lactic acid per milliliter.

[f]Collections made one and three hours after feeding.

in total volatile fatty acid level and pH. A marked increase in buffering capacity occurred. Due to an error in adding insufficient oxidant in the lactic acid determination, it is known only that the amount of lactic acid per milliliter of rumen fluid was in excess of 27.5 micromoles. Nonetheless this indicates the lactic acid level was very high, which of course was to be expected as a considerable quantity of lactic acid had been added. The addition of 60 grams of sodium bicarbonate from 3 to 8 hours after the lactic acid dosing had quite a dramatic effect. As measured by a rumen fluid collection made 24 hours after the administration of the lactic acid, subsequent sodium bicarbonate supplementation sharply decreased acetic acid and markedly increased propionic acid levels. Concurrent with the marked increase of propionate was a similar increase in valeric acid. Addition of sodium bicarbonate returned total volatile fatty acid levels to normal, elevated pH above normal and surprisingly lowered buffering capacity to subnormal levels. The noted large amounts of formic acid produced as a consequence of lactic acid and sodium bicarbonate supplementation are of special interest as formic acid is normally not found in significant amounts in rumen fluid. No lactic acid was detectable after sodium bicarbonate administration.

Lactic Acid-Sodium Bicarbonate Trial 2 In the first trial, too large a dose of lactic acid was administered placing the animal in stress, throwing him off feed even though

sodium bicarbonate was given to alleviate the acidic condition. Whereas the initial plan was to administer lactic acid for a few days prior to combined lactic acid and sodium bicarbonate treatment, the extreme response produced from the lactic acid dosing necessitated immediate sodium bicarbonate therapy to neutralize excess lactate present in the rumen.

Therefore it was desirable to repeat Trial 1 with two modifications. First, administer lactic acid more slowly over a two day period and then follow this with sodium bicarbonate and lactic acid additions. Second, two intact sheep were substituted for the fistulated animals in that fistulated animals have repeatedly demonstrated themselves to be more vulnerable to digestive disturbances.

After becoming well adjusted to the same ration used in Trial 1, four collections were made from each of two sheep over a ten day period. Following this pretreatment period, 40 to 60 milliliters of lactic acid was administered in 20 milliliter increments throughout the day for two days. Two collections were made three hours after feeding during this treatment. Following the second day's collection, concurrent sodium bicarbonate and lactic acid supplementation was commenced. Two collections were made during this treatment regime. One was made five hours and the other three after sodium bicarbonate additions. Both the sodium bicarbonate and lactic acid were administered via stomach tube.

Results of this trial are given in Table 23. Lactic acid administration increased propionate levels markedly, primarily at the expense of acetate. In that dosage levels were much lower than in Trial 1, total volatile fatty acid levels, buffering capacity and pH were not affected by lactic acid administration. However, as would be expected, ruminal lactic acid levels were increased. In contrast to the narrowing of the acetate-propionate ration in Trial 1 produced by sodium bicarbonate supplementation, here it had a widening effect. However as was true with the first trial, sodium bicarbonate supplementation increased pH, and decreased buffering capacity and lactic acid levels. No formic acid was noted where only lactic acid was administered. However lactate combined with sodium bicarbonate produced some formate. Lactic acid was still detectable after sodium bicarbonate dosing.

Discussion

Narrowed acetate-propionate ratios and lowered pH are usually associated with high concentrate-low fiber type rations. Elevated ruminal lactic acid levels also resulted where flaked corn was substituted for rolled corn in high concentrate fattening rations (Phillipson 1952).

In contrast to this pattern, high roughage rations are characterized by widened acetate-propionate ratios and higher pH's than those found in a high concentrate ration. These

Table 23. Lactic Acid-Sodium Bicarbonate Trial 2 - effect of lactic acid and sodium bicarbonate additions on ruminal fermentation patterns[a]

Time and dosage[b]	Percentage							Ratio	Total VFA ml^{-1}[c]	Buffering capacity[d]	Lactic acid[e]	pH
	C_1	C_2	C_3	i-C_4	C_4	i-C_5's	C_5	C_2/C_3				
					Pretreatment							
	--	53.5	23.8	.7	20.2	.7	1.0	2.3	65	2.0	--	6.2
					Lactic acid							
1st day												
1100-20ml												
1400-20ml												
1600-20ml												
2nd day												
0800-20ml												
1030	--	42.0	31.2	.4	24.8	.6	.9	1.4	66	2.0	24.0	6.1
1600-20ml												
3rd day												
0800-20ml												
1030	--	45.6	28.3	1.0	21.9	1.5	1.5	1.6	62	1.9	8.0	6.2

[a]All values based on mean of two intact sheep.

[b]Milliliters of lactic acid and grams of sodium bicarbonate.

[c]Micromoles of volatile fatty acids per milliliter.

[d]Milliliters of N/KOH.

[e]Micromoles of lactic acid per milliliter.

Table 23. (Continued)

Time and dosage	Percentage C_1	C_2	C_3	i-C_4	C_4	i-C_5's	C_5	Ratio C_2/C_3	Total VFA ml^{-1}	Buffering capacity	Lactic acid	pH
Lactic acid plus sodium bicarbonate												
3rd day												
1100-20ml -30gm												
1600	4.3	48.0	25.6	1.2	18.2	1.5	1.1	1.9	52	1.6	6.7	6.8
1630-20ml -30gm												
4th day												
0800-20ml -30gm												
1030	--	47.6	28.8	.8	20.2	1.3	1.2	1.7	63	1.8	13.4	6.5

observations indicated that an agent that lowered pH would probably narrow acetate-propionate ratios whereas an elevation of pH would result in widened acetate-propionate ratios. Reid et al. (1957) observed that administration of sodium carbonate to lactic acid producing diets either had no effect or lowered propionic acid levels. However investigations by Van Campen and Matrone (1960) indicated sodium and potassium bicarbonate supplementation of a potential lactate producing diet enhanced propionate levels at the expense of acetate. As they administered these bicarbonates at extremely high levels, pH's were no doubt elevated. Thus two somewhat contradictory observations have been presented. However comprehensive analysis of these observations suggests that propionate levels could possibly be increased concurrently with increased pH.

Results of the first lactic acid-sodium bicarbonate trial indicated that the immediate effect of sodium bicarbonate in alleviating excess lactate acidity was to dramatically narrow the acetate-propionate ratio. Although it is recognized that lactate is a precursor of propionate, the sharp increase in propionate produced after sodium bicarbonate supplementation suggested that this alkalizing or buffering agent would elevate propionate levels above those obtained from lactic acid alone.

This pattern was not repeated in the second trial. Here,

lactic acid alone produced higher propionate levels than resulted from the combination of lactic acid and sodium bicarbonate. Thus the exact role sodium bicarbonate might play in lactic acid producing diets remains unclear.

Of major physiological interest in both trials was the fact that buffering capacity of rumen fluid either increased or remained the same with lactic acid supplementation, whereas buffering capacity diminished when sodium bicarbonate was added. This indicates that coincidental with the elevated ruminal acidity caused by lactic acid, there occurred a simultaneous movement of buffering substances from the blood into the rumen contents to temper this acidic condition. Similarly, administration of sodium bicarbonate had no effect on buffering capacity of rumen fluid probably because there was a diminished movement of buffering substances from the blood into the rumen because of the dietary buffer, sodium bicarbonate. Masson and Phillipson (1951) postulated that substances can enter the rumen from the blood stream or peritoneal cavity in the same manner that they are absorbed through the rumen wall.

Also noteworthy was the increased formic acid levels produced either by excessive lactate administration or by sodium bicarbonate addition following lactate supplementation. Jamieson (1959) observed that when acetate levels were reduced as a result of nitrate administration, formic acid

generally appeared. He also noted that appreciable amounts of formic acid were often found in poorly growing sheep, while formic acid was normally not detectable in healthy animals. Again the observations made in these two trials coupled with those of Jamieson (1959) suggest that formic acid accumulations occur as a result of abnormal ruminal fermentations.

Summary

As has been widely observed by other workers, these trials indicate lactic acid is a precursor of propionate.

Simultaneous administration of lactic acid and sodium bicarbonate does not appear to alter ruminal propionate levels from those produced by lactic acid alone. Sodium bicarbonate combined with lactic acid lowers ruminal lactate levels and elevates pH from thos existing when lactic acid alone is administered.

The concurrent administration of lactic acid and sodium bicarbonate tends to produce formic acid accumulations whereas lactic acid alone in moderate dosages does not elevate formic acid levels. Apparently conditions that diminish the reducing capacity of the rumen tend to precipitate formic acid accumulations.

Administration of lactate tends to increase the buffering capacity of rumen fluid. This appears to be caused by an influx of blood buffering substances into the rumen to

temper the added acidity produced by lactic acid. Sodium bicarbonate supplementation did not increase the buffering capacity of rumen fluid. In this instance, supplying the exogenous buffer apparently lessens the need for endogenous buffer movement into the rumen.

Comparison of Intact and Fistulated Lambs as Experimental Units for Volatile Fatty Acid Studies

Procedures developed for obtaining rumen fluid samples from intact lambs

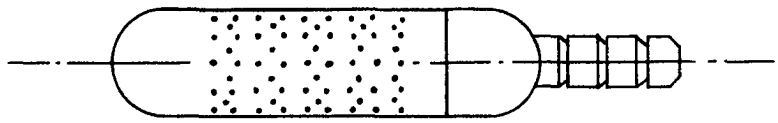
Fistulated lambs were selected initially for *in vivo* studies of ruminal fermentations due to the ease with which rumen fluid samples could be obtained. Critique of previously conducted trials utilizing fistulated lambs strongly indicated that they were very poor experimental units for assaying ruminal fermentation patterns under varying dietary regimes. These fistulated animals were prone to lose their ruminal fistula plugs resulting in semi-complete evacuation of rumen contents causing the animals to go off feed. Secondly, fistulated lambs appeared to be inherently much more vulnerable to digestive disturbances than were intact lambs. Both of these observed phenomena precipitate abnormal and distorted ruminal fermentation patterns. Thirdly, considerable leakage generally occurred around the rumen fistula. As a consequence, during summer months the immediate area surrounding the fistula was extremely vulnerable to screw-worm infestation.

While Drori and Loosli (1959) found similar digestibility of nutrients in intact and fistulated animals, it is certainly possible that as a consequence of leakage of material out of the rumen, entrance of atmospheric oxygen into the rumen around the fistula and also possible ruminal microorganism differences between intact and fistulated animals, that the ruminal fermentation patterns observed in fistulated animals could very well not reflect those in the intact animal. Fourthly, due to the expense, time and labor involved in fistulating animals, it is generally impractical and too costly to have sufficient numbers of fistulated animals to correlate growth performance with ruminal fermentation patterns.

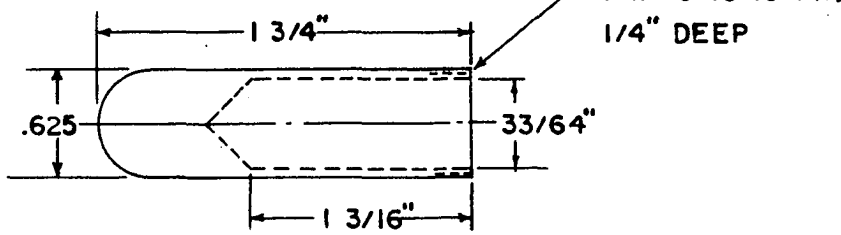
Suction strainer design It appeared advisable to explore the feasibility of developing a procedure whereby representative rumen fluid samples could be easily and rapidly taken from intact animals. To achieve this end, a suction strainer for passage via the oral cavity was designed using some of the basic principles employed in the suction strainer used in fistulated animals. The final design and dimensions of the suction strainer are illustrated in Figure 1. Fabrication of the suction strainer was done in the Physics Department Instrument Shop of Iowa State University. Stainless steel rod having a diameter of 0.625 inches was used as the starting material. One hundred and eighty 0.050 inch

Figure 1. Dimensional drawing of rumen fluid suction strainer

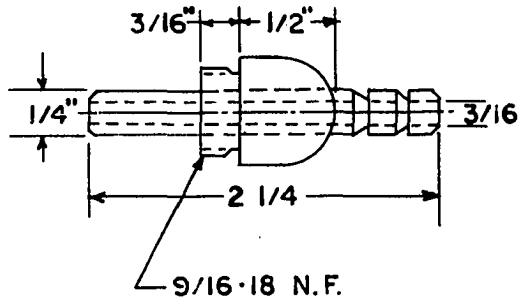
A.



B.



C.



A. COMPLETE SUCTION STRAINER

B. STRAINER

.0 50" HOLES, LONGITUDINAL ARRANGEMENT EACH 15°
EVEN 15° INCREMENTS HAVE 10 HOLES PER INCH
ODD 15° INCREMENTS HAVE 5 HOLES PER INCH

C. BASE

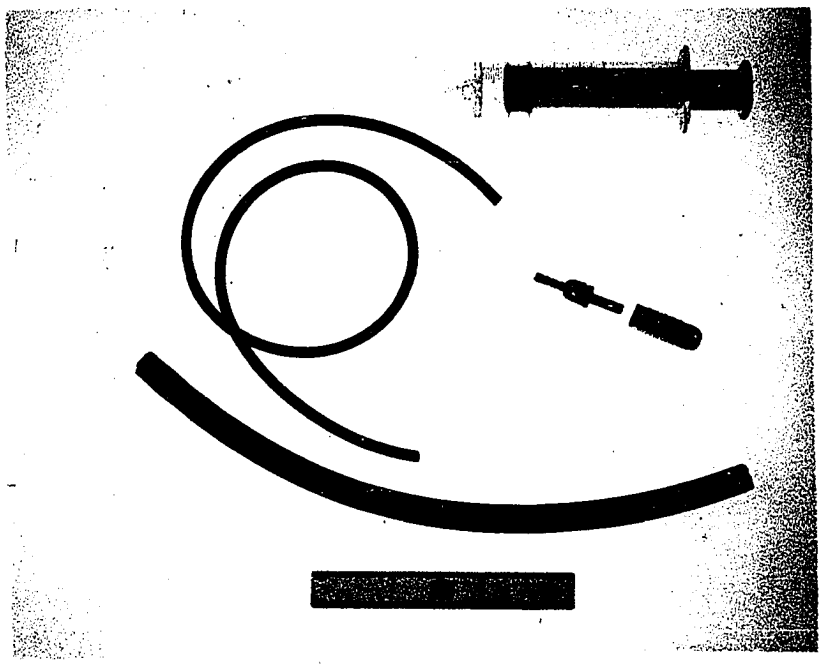
holes were drilled into the strainer. As illustrated in Figures 2 and 3, the strainer is coupled to a 3 foot length of 1/4 inch outside diameter rubber tubing to which a 50 milliliter plastic hypodermic syringe is connected.

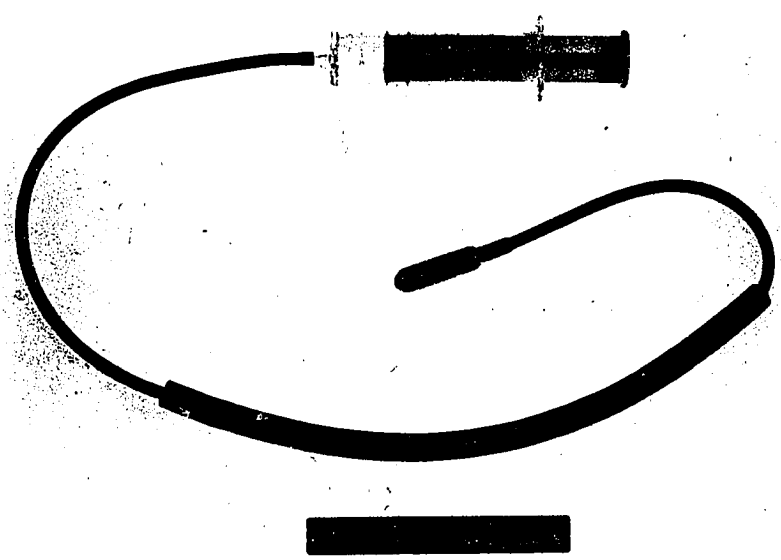
This overall design gave a strainer which was very durable and sturdy, easily passed into the rumen, sufficiently heavy to sink through the upper layers of the rumen contents into the lower fluid layers and one which permitted free entry of fluid without undue admittance of solid material.

Method used for passing suction strainer and collection of rumen fluid A 36 inch length of 1/4 inch rubber tubing to which the suction strainer was attached was passed inside a 3/4 x 18 inch section of rubber tubing (Figure 3). Leaving the sheep's mouth closed, the rubber "guide tube" with the strainer projecting out the end was passed between the molars and lower incisors, over the tongue and past the epiglottis. Holding the rubber guide tube, the strainer with the connected rubber tubing was allowed to pass down the esophagus into the rumen. After a few seconds, the strainer gravitated into the fluid layer. When in the fluid layer, blowing through the suction line gave a bubbling sensation. The suction line was then connected to the 50 milliliter hypodermic syringe and 20-30 milliliters of fluid were drawn. This fluid was then discharged back into the rumen, syringe disconnected and the line and strainer blown out. This was merely a "flushing

Figure 2. Components of rumen fluid sampling apparatus

Figure 3. Assembled rumen fluid sampling apparatus





action" which effectively eliminated any saliva contamination which might have occurred during the drawing of the initial sample. Contamination with saliva was of minor proportions for these reasons: 1) saliva cannot readily enter the strainer, 2) blowing into the line after the tube was passed to determine whether or not the strainer was in the liquid layer also effectively dispersed any saliva surrounding the strainer, 3) the saliva should be quite effectively stripped off as the strainer sinks into the rumen ingestum. After this "flushing", the syringe was again connected to the line, 20 milliliters of rumen fluid drawn (Figure 5) and the syringe then disconnected. The line and strainer were then blown out to remove feed clinging to the surface of the strainer. Holding the guide tube secure, tension was placed on the suction line, drawing the strainer out of the rumen, up the esophagus until it butted up against the guide tube, at which time the complete assembly was withdrawn from the animal. Rumen fluid was discharged from the syringe into a beaker for pH determination (Figure 4). This entire operation, including rinsing of syringe, suction line and strainer from a previous animal sampling took no more than 3-5 minutes.

Methods and results

Comparison of rumen fluid samples taken via rumen fistula and stomach tube from fistulated animals It was only

Figure 4. Discharging rumen fluid into beaker for pH determination

Figure 5. Drawing rumen fluid sample

remotely possible that different volatile acid ratios would exist in samples collected via stomach tube as compared to those taken via the rumen fistula. Nonetheless the possibility did exist that since stomach tube samples would be taken in the anterior section of the reticulo-rumen whereas rumen fistula samples would be taken more posteriorly that the constant inflow of saliva in the anterior ruminal area could produce a localized differential fermentation pattern. It was felt however that this influx of saliva could be reflected in total volatile fatty acid level, buffering capacity and pH differences.

To investigate these possibilities and in order to establish the validity of the stomach tube collection technique, eight samples were taken simultaneously via stomach tube and rumen fistula. These samples were taken over a six month period, from various fistulated animals that were on diets ranging from 50 to 80 per cent concentrates. Measurements of volatile fatty acid levels and ratios, buffering capacity and pH were made as previously outlined.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 24. No differences in volatile fatty acid percentages and ratios were noted. As would be expected due to salivary dilution in the

Table 24. Analytical comparison of rumen fluid samples taken by stomach tube and by rumen fistula in fistulated animals[a]

Collection route	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-$C_{5's}$	C_5	C_2/C_3			
Stomach tube	59.1	20.7	.8	17.6	1.2	.7	2.85	73.3	2.02	6.4
Rumen fistula	58.2	20.7	.8	18.3	1.4	.7	2.81	85.8	2.29	6.2

[a]Mean of eight animals.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

cardial area of the rumen, pH was elevated ($P < 0.005$). Although not significant, total volatile fatty acid levels and buffering capacity were lower in rumen fluid samples taken via stomach tube than those taken via rumen fistula.

Comparison of response to change in ration treatment between fistulated and intact lambs Although rigorous comparisons had not been previously made between the ruminal fermentations of intact and fistulated lambs, available data indicated that the ruminal fermentation patterns in fistulated lambs changed little when ration treatments were changed drastically while similar ration changes in intact lambs produced marked differences in ruminal volatile acid percentages.

Having made these preliminary observations, two intact and two fistulated wether lambs were selected to compare ruminal responses with change in ration treatment. All animals were placed on the 50 per cent concentrate-50 per cent roughage Ration 5 used in Intact Lamb Trial 2. The composition of this ration is given in Table 18. After a 14 day ration adjustment period, a series of four rumen fluid samples were taken over a ten day period. All animals were then slowly switched to an 80 per cent concentrate-20 per cent roughage ration (Ration 2, Table 15) over a ten day period. Another ten day adjustment period elapsed before any rumen fluid collections were taken. Again, a series of four samples were collected over a ten day period. All samples were taken

3 hours after feeding.

Intact lambs were sampled via stomach tube and fistulated lambs via the rumen fistula.

Statistical analyses of the data appear in the Appendix and were conducted by the method of analysis of variance as described by Snedecor (1956).

Results of this trial are given in Table 25. Ruminal fermentation patterns were very similar between intact and fistulated lambs when all were on the 50 per cent concentrate-50 per cent roughage ration. The only difference was that pH of rumen fluid was higher in the intact animals than in the fistulated lambs ($P < 0.005$).

However when all animals were switched to 80 per cent concentrate-20 per cent roughage rations, this similarity did not persist. While acetate-propionate ratios were narrowed considerably in the intact lambs, the acetate-propionate ratio did not change in the fistulated lambs when switched from the less to the more concentrated ration. Statistical analysis of the data revealed that both the variation due to type of animal and that due to treatment x type approached significance ($P < 0.10$). As was true when all animals were on the less concentrated diets, pH of centrifuged rumen fluid from intact lambs was higher than that from fistulated lambs ($P < 0.005$). Total volatile fatty acid levels and buffering capacity patterns were similar between intact and fistulated lambs.

Table 25. Comparative ruminal response of fistulated and intact wether lambs to change in ration treatments[a]

Ration	Percentage						Ratio	Total VFA ml^{-1}[b]	Buffering capacity[c]	pH
	C_2	C_3	i-C_4	C_4	i-C_5's	C_5	C_2/C_3			
Intact lambs										
50-50	60.3	21.1	.9	16.2	.8	.7	2.9	83	2.0	6.5
80-20	53.5	23.8	.7	20.2	.7	1.0	2.3	65	2.0	6.2
Fistulated lambs										
50-50	60.9	20.8	.8	16.2	.8	.5	2.9	86	2.1	6.3
80-20	58.4	20.0	.8	18.2	1.7	1.0	2.9	64	2.0	6.0

[a]All values based on mean of two animals, four rumen fluid samples per animal taken on separate days.

[b]Micromoles of volatile fatty acids per milliliter.

[c]Milliliters of N/10 KOH.

Acetate-propionate ratios tended to be narrower on the more concentrated rations ($P < 0.25$), butyric acid levels were higher ($P < 0.01$), and pH was lower ($P < 0.005$) than with the less concentrated rations. These observations were of secondary interest, but did confirm previous experimental data.

Discussion

Previously accumulated data very strongly suggested that fistulated and intact lambs oftentimes did not react similarly to change in ration treatment. Therefore it was of prime importance to first test the validity of the stomach tube collection technique in obtaining representative rumen fluid samples and second, to ascertain any differences in treatment response between fistulated and intact animals.

Although total volatile fatty acid levels were lower and pH was higher in samples taken via stomach tube as compared via the rumen fistula, these differences were consistent and of small proportions. These disparities are probably caused primarily by salivary dilution in the anterior section of the reticulo-rumen. No differences were noted in volatile fatty acid percentages in samples taken by the two methods simultaneously from a fistulated animal. This established the validity of the stomach tube collection technique.

As has been suspected, the fistulated animal is not a

reliable experimental unit for ruminal fermentation studies. The fistulated animal represents one that has been surgically altered, one where ruminal environmental atmosphere and pressure are often different than in the intact animal due to leakage around the rumen fistula plug, one where various skin irritations around the rumen fistula might alter animal behavior and appetite and one where semi-complete evacuation of rumen contents resulting from accidental removal of rumen fistula plug produces an extremely distorted fermentation pattern. Acetate-propionate ratios were not narrowed in fistulated lambs when they were switched from less concentrated to more concentrated rations while considerable narrowing occurred in intact lambs. Although the other ruminal measurement criteria were similar between intact and fistulated animals, the observed disparity in acetate-propionate ratios is a reflection of the outlined physical and physiological differences between these two types of animals.

Summary

The stomach tube collection technique is a valid and efficient method of obtaining rumen fluid samples. Although pH tends to be somewhat high and total volatile fatty acid level somewhat low in samples taken via stomach tube as compared via rumen fistula, these differences are small and consistent and therefore do not lend any error to comparative

measurements.

The intact lamb is far superior to the fistulated lamb as an experimental unit in ruminal fermentation investigations. Also the intact lamb can be used for combined growth, carcass and ruminal fermentation studies.

While total volatile fatty acid levels, buffering capacity and pH trends tend to be similar between fistulated and intact lambs, marked differences in relative percentages of acetate and propionate are often found. Ruminal acetate-propionate ratios narrowed considerably in intact lambs when switched from less concentrated to more concentrated diets. Little change occurred in fistulated lambs.

Effects of Calcium Carbonate, Sodium Bicarbonate and Protein Level in a High Concentrate Heifer Fattening Ration

Methods and results

Currently there is widespread interest in the feasibility of employing so-called "high" concentrate rations in cattle and lamb feeding. This interest has been spurred by the decrease in the cost of corn, sorghum and other feed grains relative to the cost of roughage materials. Mechanical feeding, labor and convenience considerations have similarly heightened interest in these "high" concentrate fattening type rations. In relationship to nutritional aspects of these type diets, it seemed desirable to investigate 1) the

role of an alkalizer such as sodium bicarbonate in preventing associated digestive disturbances, 2) the calcium requirement of such diets and 3) the effect of protein level on animal performance and carcass characteristics.

Fifty-seven yearling heifers weighing approximately 645 pounds were utilized in a completely randomized design. The percentage composition of the complete mix rations fed is given in Table 26. The average chemical composition of these rations is given in Table 27. Rations 1 through 5 contained approximately 12 per cent protein while Rations 6 through 10 contained 15 per cent protein. Calcium carbonate (0.5 per cent) was added to Rations 4 and 9 while 1.0 per cent calcium carbonate was added to Rations 5 and 10; 1.5 per cent sodium bicarbonate was added to Rations 2 and 7 and 3.0 per cent sodium bicarbonate to Rations 3 and 8. Rations in which no calcium carbonate additions were made contained approximately 0.3 per cent calcium; 0.5 per cent added calcium carbonate, 0.5 per cent calcium and 1.0 per cent added calcium carbonate, 0.7 per cent calcium. The heifers were fed a mixed ration in bringing them on feed. When up to full feed, the heifers were full fed the complete mix rations twice daily and were given free access to salt and water.

The feeding period extended over 155 days. The heifers were weighed on three consecutive days at the beginning and end of the trial. Weights were taken at 28 day intervals

Table 26. Percentage composition of rations fed in heifer fattening trial

Ingredient	Low protein					High protein				
	Basal	1 1/2% $NaHCO_3$	3.0% $NaHCO_3$	0.5% Ca	0.7% Ca	Basal	1 1/2% $NaHCO_3$	3.0% $NaHCO_3$	0.5% Ca	0.7% Ca
Rolled corn	74.5	74.5	74.5	74.5	74.5	65.5	65.5	65.5	65.5	65.5
Corn cobs	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Molasses	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Urea	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5
Soybean oil meal	3.85	3.85	3.85	3.85	3.85	12.85	12.85	12.85	12.85	12.85
Stilbosol[a]	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05
Ground alfalfa	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix[b]	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
Mineral premix[c]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Calcium carbonate				.5	1.0				.5	1.0
Sodium bicarbonate		1.5	3.0				1.5	3.0		

[a] 1 gm. diethylstilbesterol per pound.

[b] Contains approximately 725,000 I.U. Vitamin A and 90,000 I.U. Vitamin D_2 per pound.

[c] Composition: soybean oil meal, 309 lbs.; calcium carbonate (oyster shells), 48 lbs.; dicalcium phosphate, 90 lbs.; iodized salt, 150 lbs.; $MnSO_4 \cdot H_2O$, 3.63 lbs. and $CoSO_4 \cdot 7H_2O$, 42.9 gm.

Table 27. Average percentage chemical analysis of rations fed in heifer fattening trial[a]

	Dry matter	Ash	Protein	Ca
	Low protein			
Basal	89.8	3.3	12.1	.36
+ 1 1/2% $NaHCO_3$	90.5	4.5	12.2	.34
+ 3.0% $NaHCO_3$	89.8	5.3	11.9	.34
+ 0.5% $CaCO_3$	90.4	3.7	12.2	.49
+ 1.0% $CaCO_3$	89.6	3.8	12.0	.66
	High protein			
Basal	90.8	4.0	16.1	.37
+ 1 1/2% $NaHCO_3$	90.5	4.4	15.4	.31
+ 3.0% $NaHCO_3$	90.1	5.8	15.7	.35
+ 0.5% $CaCO_3$	90.8	4.0	15.9	.55
+ 1.0% $CaCO_3$	90.6	4.6	15.6	.70

[a]Determinations based on an air dry basis.

throughout the trial. At the end of the feeding period, cattle were trucked to a local packing house where federal carcass grades and carcass weights were obtained. Dressing per cent was determined on the basis of final live weights in Ames and hot carcass weights shrunk 2 1/2 per cent.

Results of this experiment are given in Table 28. No apparent differences in average daily gain, carcass grade and dressing per cent were observed between the 0.3 per cent calcium, 0.5 per cent calcium, 1.5 per cent sodium bicarbonate and 3.0 per cent sodium bicarbonate nor between the 12 and 15 per cent protein rations. The 0.7 per cent calcium rations

Table 28. Effect of dietary sodium bicarbonate, calcium carbonate and ration protein level on feedlot performance and carcass traits of fattening heifers

	Low protein					High protein				
	Basal	1 1/2% $NaHCO_3$	3.0% $NaHCO_3$	0.5% Ca	0.7% Ca	Basal	1 1/2% $NaHCO_3$	3.0% $NaHCO_3$	0.5% Ca	0.7% Ca
Animals/lot	6	6	6	6	6	6	5	5	5	6
Average daily gain	2.78	2.71	2.82	2.82	2.58	2.83	2.80	2.85	2.94	2.66
Average daily feed	20.7	21.4	22.3	21.2	19.7	21.0	22.2	24.3	22.5	20.9
Feed/100# gain-lbs.	745	790	792	751	765	744	793	854	767	784
Dressing %	58.5	57.9	59.0	58.2	57.9	59.3	59.5	58.1	58.9	58.1
Carcass grade[a]	6.8	6.5	6.3	6.3	5.8	6.5	6.0	6.8	6.8	6.6

[a]Carcass grade was computed using the following numerical values: high good, 6.0; low choice, 7.0; average choice, 8.0.

appear to cause depression in average daily gains. Observed lowering of feed efficiency, dressing per cent and carcass grade in the 0.7 per cent calcium diets is of too small dimensions to be meaningful. No differences were noted between the 0.3 and 0.5 per cent calcium diets. Sodium bicarbonate supplementation lowered feed efficiency. Due to the higher relative cost of soybean oil meal as compared to corn, feed costs were higher in the 15 per cent than in the 12 per cent protein rations.

Discussion

Bases for investigating the effects of sodium bicarbonate and calcium carbonate in high concentrate fattening rations have been previously presented in the fistulated and intact lamb experiments. In addition to these considerations, the observations of Hubbert *et al.* (1960) suggested that increasing the protein level would improve weight gains and feed conversion.

It appears the optimum calcium level in a high concentrate fattening ration lies between 0.3 and 0.5 per cent calcium. A calcium level of 0.7 per cent depressed weight gains.

Consistent with results of the intact lamb trials, sodium bicarbonate supplementation did not improve weight gains or carcass characteristics. Although feed efficiencies were not affected in Intact Lamb Trial 2, feed efficiency was decreased

as a consequence of sodium bicarbonate supplementation in this heifer fattening trial.

Increasing the protein level from 12 to 15 per cent only slightly improved weight gains and had no effect on feed efficiency or carcass characteristics. In contrast to previously cited data, these results indicate no advantage in protein levels in excess of 11-12 per cent. This conclusion is consistent with that of Goodrich et al. (1961).

Summary

The optimum dietary calcium level appears to be between 0.3 and 0.5 per cent in a high concentrate-low roughage type cattle fattening ration. Calcium levels in excess of 0.5 per cent appear to depress weight gains.

Sodium bicarbonate supplementation of high concentrate cattle rations had no effect on weight gains or carcass characteristics. Feed conversion was poorer in the bicarbonate supplemented rations.

Increasing the protein level from 12 to 15 per cent did not improve feedlot performance or carcass characteristics of heifers fed high concentrate fattening rations.

GENERAL DISCUSSION

When research was initiated on this research problem interest centered primarily in dietary factors that would increase ruminal propionate at the expense of acetate. It is reasonable to assume that narrowing of the ruminal acetate-propionate ratio should improve animal performance as propionate is universally considered to be glucogenic whereas acetate is not and since the heat increment of acetate considerably exceeds that of propionate in fattening rations.

Wide ruminal acetate-propionate ratios are normally found in high roughage rations while narrower ratios are found in high grain rations. However the physical and chemical nature of these grains influences the degree of narrowing of the acetate-propionate ratio. Flaked corn and bread narrow acetate-propionate ratios from those found with rolled non-steam heated corn (Shaw 1959). Phillipson (1952) observed that wheaten starch increased propionate levels above those found where corn starch was fed to lambs. Now in association with the narrowing effect of acetate-propionate ratios of flaked corn and wheaten starch, sizeable amounts of lactic acid accumulated in the rumen during fermentation immediately following feeding. Lactic acid is normally not detectable in appreciable quantities in the rumen and is considered to be an intermediate in ruminal fermentation. Lactic acid is widely considered to be both glucogenic and a precursor of

propionate. Nominal dietary lactic acid intake as well as temporary lactic acid accumulation in the rumen are not harmful. However as lactic acid is a strong organic acid as compared to the weak volatile fatty acids, either excess intake or ruminal accumulation of lactate produces a rumen dysfunction typified by low ruminal pH (4-5), ablation of ruminal motility and increased blood lactate levels.

In recognition of these facts, it was possible that addition of a dietary alkalizer such as sodium bicarbonate to these high lactate producing diets would be beneficial. Not to be overlooked at this point is the fact that high grain-low fiber rations, in which there is now considerable interest, can often precipitate severe digestive disturbances which are often typified by low ruminal pH's caused by excess lactic acid. The investigations of Matrone et al. (1959) indicated that dietary alkalizers such as sodium and potassium bicarbonate exerted a beneficial effect in the all concentrate purified diets that they used. This suggests suppression of lactic acid formation by the bicarbonates. Subsequent isotope studies conducted by Van Campen and Matrone (1960) indicated sodium and potassium bicarbonate supplementation of these all-concentrate purified rations enhanced ruminal propionate levels.

The in vitro studies of Pennington (1952) and (1954) indicated that increased carbon dioxide tension as supplied by

sodium bicarbonate increased uptake of propionate by ruminal epithelial tissue whereas the presence of ammonium chloride suppressed propionate uptake.

Johns (1951a) using washed cells of Veillonella gazogenes found that lactate was fermented only in the presence of carbon dioxide and that the amount of propionate formed increased with carbon dioxide tension of the medium. A process of carbon dioxide fixation followed by decarboxylation was involved in the fermentation of lactate to propionate.

A composite of these foregoing observations and hypotheses strongly suggested that sodium and/or potassium bicarbonate additions to high concentrate-low roughage type rations would serve two purposes: first, to suppress excess acidity caused by lactate accumulation and second, to elevate propionate levels by shunting more lactate to propionate via carbon dioxide fixation and decarboxylation.

A summary of three trials conducted with fistulated lambs and two with intact lambs indicates that sodium bicarbonate supplementation of high concentrate-low roughage type diets has little if any effect on relative amounts of acetate and propionate, total volatile fatty acid levels and buffering capacity of centrifuged rumen fluid. As would be expected, bicarbonate supplementation tended to elevate ruminal pH. It is to be recognized that whereas percentage additions of sodium and potassium bicarbonates ranged from 1 1/2 to 5 per

cent in diets containing from 85 to 90 per cent concentrates, Matrone et al. (1959) incorporated a 11.7 per cent mixture of sodium and potassium bicarbonates in their purified diets that contained no fiber. However, in view of the fact that the concentrate levels were below the 100 per cent level of Matrone's, it would be expected that considerably lower levels of bicarbonates would have a similar effect in a ration containing a lesser percentage of concentrates. Sodium bicarbonate supplementation definitely did not improve feedlot performance or carcass characteristics in a high concentrate-low roughage heifer fattening ration.

As lactic acid accumulations were not detected on the most concentrated diets, it is possible that with natural ration ingredients, the 100 per cent concentrate level would have to be approached before lactic acid would accumulate. Then, if this did occur, addition of sodium and/or potassium bicarbonate could well aid in shunting lactate to propionate, while simultaneously elevating pH. Rations containing some roughage or fiber were chosen in that it was felt considerable difficulty would be experienced with a ration containing no fiber in getting the animals on feed and establishing valid control values.

Investigations of the effect of administering sodium bicarbonate concurrently with lactic acid to sheep receiving a moderately high concentrate ration revealed that propionate

levels were no higher than when lactic acid alone was administered. However in the first of these studies when an extremely large dose of lactic acid was administered, subsequent sodium bicarbonate therapy appeared to greatly increase propionate levels over those produced by lactate alone. Of secondary interest in these investigations was the observation that simultaneous sodium bicarbonate and lactic acid supplementation resulted in formic acid accumulations that were otherwise not evident. This observation tends to confirm the suggestion of Jamieson (1959) that formic acid accumulations result as a consequence of abnormal ruminal fermentations.

Dietary sodium bicarbonate did not increase the buffering capacity of centrifuged rumen fluid. But a large dose of lactic acid markedly increased ruminal buffering capacity. These phenomena suggest the movement of buffering substances from the blood into the rumen under severe ruminal acidic stress as indicated by Masson and Phillipson (1951) and Danielli et al. (1945). Administration of a dietary buffer such as sodium bicarbonate obviates the need of this influx of blood buffering substances. These observations further support the theory of reciprocal movement of soluble substances between the rumen and the portal circulation via the rumen wall.

Fermentations taking place in silage and in the rumen

are somewhat similar. Klosterman et al. (1959) observed that addition of one per cent dolomitic-limestone appreciably elevated corn silage lactate levels. However, the treated silage gave no better animal performance than untreated corn silage. In a subsequent study, Klosterman et al. (1960) observed that the addition of one per cent non-dolomitic-limestone instead of dolomitic-limestone elevated silage lactic acid levels above those produced by dolomitic-limestone. Also this non-dolomitic-limestone treated corn silage improved animal performance over that obtained from non-treated silage. A combination of 0.5 per cent non-dolomitic-limestone and 0.5 per cent urea similarly increased lactic acid levels. The higher lactic acid levels in all instances were accompanied by a higher final silage pH when the fermentation reached a stable point. Obviously limestone and urea acted as buffering or alkalizing substances, permitting a greater accumulation of volatile fatty acids and lactic acid before a limiting pH was attained. Even though total organic acid levels were higher in the silages treated with the buffering substances, the final limiting pH's were higher in the treated than in the non-treated silages.

These highly interesting observations suggested that first, a buffering substance such as limestone might indirectly affect propionate production via the ruminal metabolic intermediate, lactic acid and secondly, that excess

magnesium might deleteriously influence ruminal fermentation.

In fistulated lamb trials, calcium carbonate and magnesium carbonate additions were without effect in influencing ruminal fermentation patterns. As previously emphasized this is not particularly meaningful since the author considers the fistulated lamb to be a notoriously poor experimental unit for volatile fatty acid studies. However the addition of 0.5 per cent limestone in two intact lamb trials had no effect on ruminal fermentation, growth, performance or carcass data. Neither did a 0.5 per cent limestone addition in a heifer fattening trial have any effect on growth performance or carcass characteristics. A 1.0 per cent addition of limestone appeared to depress rate of gain and feed efficiency.

Phillipson (1952) observed that cobalt supplementation of a cobalt deficient diet narrowed acetate-propionate ratios and lowered butyric acid levels. A similar ruminal fermentation pattern was observed in a single fistulated lamb trial.

Shaw (1959) and others observed that flaked corn and bread effected considerable narrowing of acetate-propionate ratios. Wet steam heating has been implicated as being responsible for this fermentation pattern. Salsbury et al. (1960) suggested wet steam heating increased starch hydration which in turn favored its more rapid degradation by rumen microorganisms. Fermentations that proceed at faster initial rates are generally associated with narrowed acetate-propion-

ate ratios than found in slower initial fermentation rates. They also noted that dry steam autoclaving apparently did not effect appreciable starch hydration as reflected by no increase in the rate of *in vitro* microbial degradation of starch. In a single fistulated lamb trial, where dry steam autoclaved corn was substituted for non-heated corn in a high concentrate ration, acetate-propionate ratios were widened. These combined observations indicate the type of heat is highly instrumental in determining the ruminal fermentation pattern. While moist heat treatment of high starch foods tends to narrow ruminal acetate-propionate ratios, dry steam heating does not have this effect.

As would be expected, increasing the concentrate percentage from 50 to 80 per cent increased feed efficiency and carcass grade. Narrowed acetate-propionate ratios, elevated butyrate and lowered total volatile fatty acid and pH levels resulted in the higher as compared to the lower concentrate rations. With the exception of the lowered total volatile fatty acid levels in the higher concentrate rations, the other observations are consistent with those widely reported in the literature. Of particular interest in Intact Lamb Trial 2 was the observed high correlation between carcass grade and butyric acid levels. There also tended to be considerable correlation between separable fat and ruminal butyric acid levels. Thus, one might postulate butyrate is a major pre-

cursor of body fat.

Review of the literature revealed that urea and antibiotics separately could likely exert dynamic effects on ruminal fermentation. Klosterman et al. (1960) observed that urea apparently acted similarly to limestone in its buffering or alkalizing action in favoring lactic acid accumulations. Also excessive intakes of urea cause marked elevation of ruminal pH (Briggs et al. 1957).

Several modes of action have been assigned antibiotics. Hungate et al. (1955) concluded that although chlortetracycline feeding did not alter appreciably the total potential for microbial activity, the composition of the rumen microbial population was altered. The observations of Visek et al. (1959) are of paramount interest. They suggested that select antibiotics suppressed bacterial urease activity. Therefore in ruminal fermentation, an antibiotic-urea interaction could certainly be possible. The observation of Mangan et al. (1959) that penicillin inhibited ruminal ammonia production supports this hypothesis.

Recognition of these possibilities was the basis for the 2^2 chlortetracycline-urea factorial portion of Intact Lamb Trial 2. Analysis of ruminal fermentation, growth and carcass data indicated chlortetracycline alone had little effect while urea tended to lower carcass grade and dressing per cent. However, chlortetracycline-urea interactions on

acetate-propionate ratios, feed efficiency, carcass grade, separable fat and separable lean were indicated. Although nitrogen balance, ruminal urease and ruminal ammonia levels were not determined, the apparent ability of chlortetracycline to overcome the depressing effects of urea on feed efficiency and carcass grade supports the theory of antibiotic suppression of bacterial urease activity. This could result in more efficient use of urea nitrogen. Also to be noted is the simultaneous increase in per cent separable lean and carcass grade when chlortetracycline and urea are fed concurrently as compared to urea alone. In other instances increased amounts of separable fat tend to be correlated with improved carcass grade. The possible action of an antibiotic in suppressing bacterial urease activity deserves further study.

Concluding comments made in this discussion will be devoted to some of the experimental techniques employed.

First, a gas chromatographic technique originally proposed by Ralls (1960) was refined and developed for separation of volatile fatty acids from formic through valeric acids. This method has proven to be not only reliable, but one which does not require highly trained technicians nor complex manipulations. Communications with other researchers in this field indicate many have experienced considerable difficulty in achieving separation of steam volatile fatty acids.

Secondly, this author is convinced and has presented

arguments and documentary evidence indicating that the fistulated lamb is a very poor experimental unit for ruminal fermentation investigations. Although Drori and Loosli (1959) found no difference in digestibility of nutrients between intact and fistulated steers, many of the shortcomings of fistulated lambs no doubt also apply to fistulated bovine.

Intact lambs can be used for concurrent ruminal fermentation, growth and carcass studies while fistulated animals can be used only in ruminal fermentation studies. However in many instances, intact animals have not been used due to the inconvenience of sampling, as well as inability to make accurate laboratory measurements on the collected rumen fluid. The stomach tube collection technique developed facilitated easy and rapid collection of rumen fluid samples that are representative of ruminal fermentation patterns and conditions.

Although comparative treatment data obtained from the fistulated lamb trials appears to be legitimate, this author seriously questions much of its validity. This apprehension is caused by personal knowledge of distorted ruminal fermentation patterns caused by stress factors that would have no effect on an intact animal. The real value of the fistulated lamb trials conducted was to demonstrate their shortcomings in assaying the effects of dietary factors on ruminal fermentation patterns.

SUMMARY

Trials have been reported that include: 1) ruminal fermentation studies utilizing fistulated and intact lambs, 2) combined ruminal fermentation, growth and carcass studies using intact lambs and 3) growth and carcass studies in a heifer fattening trial.

In rations containing approximately 85 per cent concentrates and 15 per cent roughage, sodium and potassium bicarbonate, calcium carbonate and magnesium carbonate additions were without effect in altering acetate-propionate ratios and total volatile fatty acid levels. Supplementary bicarbonates rather consistently elevated ruminal pH. Concurrent sodium bicarbonate and lactic acid supplementation produced ruminal propionate levels no higher than produced by lactic acid alone.

Using similar high concentrate rations, sodium bicarbonate and calcium carbonate additions were without effect on growth performance and carcass traits of fattening heifers and wether lambs.

Addition of cobalt sulphate to a high concentrate lamb ration tended to narrow acetate-propionate ratios and lowered butyric acid levels. Substitution of dry steam autoclaved corn for non-autoclaved corn effected a widening of acetate-propionate ratios.

Chlortetracycline supplementation of a high urea-high

roughage type lamb fattening ration tended to overcome the depressing effects of urea on carcass grade and feed efficiency. Where no urea was included in these rations, chlortetracycline additions tended to widen acetate-propionate ratios and to elevate butyric acid levels.

Narrowed acetate-propionate ratios, higher butyric acid levels, lower total volatile fatty acid levels and lower pH's were produced in the 80 per cent concentrate-20 per cent roughage rations than in those containing only 50 per cent concentrates. While overall weight gains were similar between the two types of rations, improved feed conversion, higher dressing percentage, higher per cent separable fat and lower per cent separable lean resulted in the higher concentrate rations.

In the second intact lamb trial, a high correlation was demonstrated between ruminal butyric acid levels and carcass grade in both high and low concentrate rations.

Rumen fluid buffering capacity measurements support the hypothesis that substances can enter the rumen from the blood stream or peritoneal cavity in the same manner that they are absorbed through the rumen wall.

An efficient gas phase chromatographic technique was perfected to determine percentages of volatile fatty acids from formic through valeric acid.

The fistulated lamb was shown to be a poor experimental

unit for ruminal fermentation studies. Thus to obviate the need of using fistulated lambs, a stomach tube sampling technique was developed whereby representative rumen fluid samples could be easily and rapidly taken from intact animals.

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APPENDIX

A. Collection and Steam Distillation of Ruminal VFA's

1. Withdraw 20 ml. of rumen fluid using a suction strainer coupled to a 50 ml. syringe.

2. Add 1 ml. of saturated $HgCl_2$ to kill the microorganisms to prevent any alteration of *in vivo* ruminal VFA ratios.

3. Centrifuge raw rumen juice in a high speed angle centrifuge for 40' to remove microorganisms and solid feed particles.

4. Decant or pipette off clear amber colored supernatant.

5. Place 5 ml. of this centrifuged rumen juice in a 100 ml. beaker.

6. Add 5 ml. of H_2O.

7. Add 10 ml. of McAnally reagent; i.e. solution saturated with $MgSO_4$ and containing 2 1/2% H_2SO_4.

8. Filter through Whatman #40 into a 50 ml. volumetric.

9. Adjust pH to 2.3 with 2N NaOH and 10N H_2SO_4 using bromphenol blue indicator; then dilute to volume (50 ml.)

10. Place 10-20 ml. aliquot (i.e. VFA's from 1-2 ml. of rumen juice) into Markham steam distillation apparatus.

11. Collect 150 ml. of distillate.

12. Using phenol red as indicator, titrate with N/10 KOH while maintaining CO_2 free atmosphere (CO_2free air bubbled through distillate). Use a 5 ml. buret, preferably one modified as an alkali and a filling buret in order that the buret need not be cleaned after each day's use and in order to facilitate easy filling.

13. After end point is reached, add 10% extra KOH.

14. Place aqueous potassium salts in 100°C oven, take to dryness or near dryness.

15. If necessary, dissolve in H_2O. Transfer to 5 dram snap cap opticlear vials or any other type of wide mouth vial of similar size. Take to dryness in 100°C drying oven. Store in dessicator. Do not store potassium salts of VFA's for extended length of time in 100°C oven.

CAUTION: It is imperative that an acid free atmosphere be maintained in the drying oven. If any acid is present (for example acetic acid), the extra base present in the dried sample will absorb the acid, resulting in completely distorted VFA ratios. Thus to insure complete protection against contamination by extraneous acids present in the oven atmosphere, it is advisable to <u>place only</u> titrated VFA samples in a particular oven.

B. Determination of Buffering Capacity

1. Place 2 ml. of centrifuged rumen fluid in beaker; add 5 ml. of H_2O; add bromphenol blue indicator.

2. Titrate to pH 3.5 using 0.1N HCl. Make final adjustment of pH with aid of pH meter.

3. Add phenol red indicator.

4. Titrate to pH 7.0 using 0.1N KOH. Make final adjustment of pH with aid of pH meter.

5. ml. of 0.1N KOH needed to titrate from pH 3.5 to 7.0 is the measure of buffering capacity.

C. Determination of Lactic Acid

1. Place 5 ml. of centrifuged rumen fluid in a 50 ml. beaker.

2. Add 1/2 ml. of 20% w/v $CuSO_4 \cdot 5H_2O$ and spatula full of solid $Ca(OH)_2$; let stand 30 minutes.

3. Filter through Whatman #40.

4. Add 10N H_2SO_4 to make concentration of H_2SO_4 1.0N in reaction flask.

5. Place 10 ml. of 0.5% w/v $NaHSO_3$ in receiving flask with tip just below the surface.

6. Heat the reaction mixture with the microburner until it just boils.

7. Turn steam on, discontinue flame, distill rapidly for 1-3 minutes.

8. Add 0.05N ceric sulphate dropwise until the yellow color becomes permanent; then add 3 ml. more.

9. Raise receiver to collect last 5 ml. (total collection approximately 25 ml.).

10. Cool in refrigerator.

11. Add 1 ml. of starch indicator.

12. Remove the excess bisulphite with strong iodine solution; back titrate carefully with 0.1N $Na_2S_2O_3$.

13. Add 15 ml. saturated $NaHCO_3$.

14. Titrate rapidly with dilute (0.01N) standardized iodine solution.

15. Before the end point is reached, add 1 ml. of 10% Na_2CO_3.

16. Titrate to end point.

17. Each ml. of 0.01 N iodine solution required equals 5 micromoles (um) of lactic acid.

D. Gas Phase Chromatographic Technique

1. Instrument

   a. Aerograph 110 C connected to a Brown-Honeywell 1 mv recorder.
   b. Column: stainless steel, 10 foot, 1/4 inch diameter packed with silicone stearate (Non-polar).

2. Materials

   a. 3.1 mm. x 11.5 cm. pyrex capillary tubes; 120° bend 1 1/8" from end.
   b. High boiling point oil
   c. $C_2H_5SO_4K$
   d. Filling funnels (medicine dropper with a rubber tube splicing unit).

3. Preparation of $C_2H_5SO_4K$

a. Mix 27.1 cc. of absolute alcohol with 25.0 cc. of reagent grade H_2SO_4. Maintain at 20°C during addition of acid to the alcohol. Allow reaction to proceed for 3 hours at room temperature.
b. At end of three hour period, neutralize the reaction mixture with an aqueous suspension of $CaCO_3$ (35-40 gm. of $CaCO_3$ required). As the equilibrium in the reaction of C_2H_5OH and H_2SO_4 is very far to the right, no danger exists in adding the aqueous $CaCO_3$. It must be added in the aqueous form in order that the precipitated $CaSO_4$ may be filtered out. Continue adding the aqueous $CaCO_3$ until effervescence discontinues. Filter out the $CaSO_4$ and wash with H_2O.
c. Next add slowly a K_2CO_3 solution until precipitation of $CaCO_3$ is complete. Digestion of the reaction mixture will aid in the precipitation of the $CaCO_3$ and will facilitate detection of the end point. Filter off the $CaCO_3$. Evaporate the filtrate ($C_2H_5SO_4K$ solution) to dryness. A yield of approximately 25 gm. should be obtained.

4. Preparation of Samples

CAUTION: With exception of weighing the $C_2H_5SO_4K$, perform all scraping, mixing and capillary tube filling in front of the oven as the potassium salts of the VFA's are extremely hygroscopic. Wearing of cotton gloves will facilitate handling of hot vials, spatulas, funnel and capillary tubes which must be kept in the oven to insure their complete dryness.

a. Place dried samples (K salts of VFA's) in 100°C drying oven.
b. Using a spatula, scrape the dried salts away from the bottom of the container. Place scraped sample back in oven.
c. Using the titration data and assuming an average molecular weight of 110 for the potassium salts of the VFA's, add an <u>equal weight</u> of $C_2H_5SO_4K$ to the potassium salts.
d. Thoroughly mix the potassium salts and $C_2H_5SO_4K$ with a small spatula. Place mixed sample back in oven.
e. While standing in front of oven, connect filling funnel and capillary tube. Hold this unit and the wide mouthed vial in one hand and then place

approximately 10 mg. of the reaction mixture in the capillary tube. It is not necessary to accurately weigh out a prescribed amount. A visual gauging of the amount in the capillary tube is sufficiently accurate. Gently tap the capillary tube to vibrate the mixture to the bottom of the tube. If filling is done rapidly and if the reaction mixture was perfectly dry prior to filling, very little of the reaction mixture will cling to the sides of the capillary tube. Store capillary tube in oven until ready to be inserted in injection port of gas phase chromatograph. Mixed samples should be analyzed a few hours after initial mixing of the salts and $C_2H_5SO_4K$.

f. Do not store $C_2H_5SO_4K$ in 100°C drying oven. Extended storage at this temperature degrades the $C_2H_5SO_4K$. Store in a closed container or dessicator. Any moisture present in the $C_2H_5SO_4K$ will be removed when the mixture of potassium salts of VFA's and $C_2H_5SO_4K$ are placed back in the oven after mixing.

5. Qualitative analysis in gas phase chromatograph

a. Operating conditions
   1) Oven temperature - 85-90°C
   2) Gas flow (helium) - 35-40 ml. per minute
   3) Injector and collector temperatures - 210-225°C
   4) Filament - 250 ma.
   5) Sensitivity - 64X

b. Place a silicone collector gasket in the injection port compression nut.

c. Allow machine to stabilize and sufficiently warm up (several hours) with an empty capillary tube inserted into the injection port and with the compression nut tightened only tight enough to prevent gas leakage.

d. When the machine has stabilized, remove charged capillary tube from oven, cover end with dry tissue, loosen injector compression nut slightly, remove old capillary tube and immediately insert new one. Retighten compression nut.

e. As some air is introduced into the chromatograph, allow 5-10 minutes for the machine to restabilize. In our case, this is indicated by the recording needle being on the base line without the benefit of any manual fine adjustment.

f. Heat a pyrex tube containing Dow-Corning 550, or any other high boiling point oil, till the oil reaches 325°C. Amount of oil in the tube should

be adjusted so that the tube is nearly full at 325°C.

g. Through use of this pyrex tube containing the 325°C oil, immerse the capillary tube as completely as possible in the oil. The following reaction takes place spontaneously 6-8 sec. after heating is commenced:

$$R\overset{\overset{O}{\|}}{C}OK + C_2H_5SO_4K \xrightarrow{325^\circ C} K_2SO_4 + R\overset{\overset{O}{\|}}{C}OC_2H_5$$

As the temperature of the oil and the injection port is far above the boiling point of these ethyl esters, they are volatilized into the chromatograph while K_2SO_4 is left residually deposited in the capillary tube. Continue immersion of the capillary tube for 30 sec., then remove the tube of hot oil.

h. After 25-30', all VFA's through n-valeric will have passed through the column.

i. The chromatograph is now ready for recharging with a new sample.

CAUTION: It is absolutely mandatory that the capillary tube and contained reaction mixture be <u>completely</u> free of any traces of H_2O to minimize trailing of peaks and to achieve optimal separation and sharpness of peak patterns. Thus, the foregoing procedures and precautions must be observed in order to achieve this end.

j. As the area under each peak is proportional to the molar amount of each acid, the relative ratios of the VFA's can therefore be obtained. Through reference to the total VFA level determined by titration, the absolute amount of each acid can be ascertained if desired.

If each peak reaches the base line before the next peak commences, the disc integrator recording will give directly the area under each peak. If not, some extrapolation will have to be performed and the adjusted peak area for each component may be measured through use of a planimeter.

Table 29. Fistulated Lamb Trial 1 - analysis of variance of the effects of time after feeding on ruminal fermentation patterns

Source	D.F.	Mean square: Total VFA ml^{-1}	Mean square: Buffering capacity
Total	9		
Animal	1	3803	2.294
Time	4	428	.213
Linear regression	1	1514	.772
Deviations from regression	3	67	.026
Remainder	4	4	.014

Table 30. Fistulated Lamb Trial 2 - analysis of variance of the effects of time after feeding on ruminal fermentation patterns

Source	D.F.	Mean square: Total VFA ml^{-1}	C_2/C_3	Buffering capacity	pH
Total	9				
Animal	1	392	.037	.296	.650
Time	4	276	.292	.173	.016
Linear regression	1	936	1.109	.545	.050
Deviations from regression	3	56	.019	.049	.004
Remainder	4	204	.038	.107	.023

Table 31. Fistulated Lamb Trial 3 - analysis of variance of the effect of dietary sodium and potassium bicarbonate on ruminal fermentation patterns

Source	D.F.	Mean square			
		Total VFA ml^{-1}	C_2/C_3	Buffering capacity	pH
Total	14				
Treatment	5	475	2.873	.723	.274
Pretrt vs trt	1	544	1.424	2.178	.560
Pretrt vs $^{-}HCO_3$	1	67	1.571	.838	.193
Pretrt a vs 0% $^{-}HCO_3$	1	830	.086	1.592	.456
Pretrt b vs 1.25% $^{-}HCO_3$	1	5	.327	.374	.147
Pretrt c vs 5.00% $^{-}HCO_3$	1	193	1.44	.466	.056
Remainder	9	65	.347	.148	.037

Table 32. Fistulated Lamb Trial 4 - analysis of variance of the effect of dietary calcium carbonate on ruminal fermentation patterns

Source	D.F.	Mean square			
		Total VFA ml^{-1}	C_2/C_3	Buffering capacity	pH
Total	27				
Treatment	7	32	1.859	.180	.126
Pretrt vs trt	1	12	2.144	.194	.247
Pretrt vs $CaCO_3$	1	21	.602	.001	.041
Pretrt a vs 0% $CaCO_3$	1	222	2.510	.846	.414
Pretrt b vs .63% $CaCO_3$	1	24	.914	.008	.027
Pretrt c vs 1.25% $CaCO_3$	1	0	.597	.000	.010
Pretrt d vs 2.50% $CaCO_3$	1	150	.148	.002	.008
Remainder	20	72	.204	.079	.076

Table 33. Fistulated Lamb Trial 5 - analysis of variance of the effect of dietary sodium bicarbonate on ruminal fermentation patterns

Source	D.F.	Mean square			
		Total VFA ml^{-1}	C_2/C_3	Buffering capacity	pH
Total	15				
Treatment	7	485	1.812	.182	.16
Pretrt vs trt	1	1	.498	.032	.03
Pretrt vs $^{-}HCO_3$	1	66	1.092	.122	.00
Pretrt a vs 0% $^{-}HCO_3$	1	139	.159	.049	.12
Pretrt b vs 1.25% $^{-}HCO_3$	1	16	.255	.034	.02
Pretrt c vs 5.00% $^{-}HCO_3$	1	51	.851	.078	.01
Remainder	8	246	.117	.189	.05

Table 34. Fistulated Lamb Trial 6 - analysis of variance of the effect of dietary sodium bicarbonate on ruminal fermentation patterns

Source	D.F.	Mean square			
		Total VFA ml^{-1}	C_2/C_3	Buffering capacity[a]	pH[a]
Total	28				
Treatment	3	132	1.524	.258	.102
Pretrt vs $^{-}HCO_3$	1	293	3.686	.078	.023
Pretrt a vs 1.25% $^{-}HCO_3$	1	344	1.313	.137	.101
Pretrt b vs 5.00% $^{-}HCO_3$	1	32	2.462	.001	.010
Remainder	25	261	.152	.108	.077

[a]Only 27 degrees of freedom.

Table 35. Fistulated Lamb Trial 7 - analysis of variance of the effect of dietary cobalt sulphate on ruminal fermentation patterns

Source	D.F.	Mean square		
		C_2/C_3	C_4	Buffering capacity[a]
Total	13			
Treatment	1	.133	111.73	.086
Remainder	12	.265	9.52	.061

[a]Only 11 degrees of freedom.

Table 36. Fistulated Lamb Trial 8 - analysis of variance of the effect of dietary magnesium carbonate on ruminal fermentation patterns

Source	D.F.	Mean square			
		Total VFA ml^{-1}	C_2/C_3	Buffering capacity[a]	pH[b]
Total	36				
Treatment	3	486	.420	.213	.102
Pretrt vs trt	1	1373	.914	.512	.045
Pretrt a vs 0.5% $MgCO_3$	1	1033	1.133	.316	.009
Pretrt b vs 1.0% $MgCO_3$	1	411	.083	.203	.043
Remainder	33	214	.322	.153	.037

[a]Only 31 degrees of freedom.

[b]Only 35 degrees of freedom.

Table 37. Fistulated Lamb Trial 9 - analysis of variance of the effect of autoclaved corn on ruminal fermentation patterns

Source	D.F.	Mean square			
		Total VFA ml^{-1}	C_2/C_3	Buffering capacity[a]	pH
Total	27				
Treatment	1	998	4.192	.378	.108
Animal	1	1473	2.611	.358	.023
Animal x trt	1	705	.135	.173	.079
Remainder	24	60	.319	.034	.027

[a]Only 23 degrees of freedom.

Table 38. Intact Lamb Trial 1 - analysis of variance of the effects of concentrate-roughage ratio, dietary calcium carbonate and sodium bicarbonate on ruminal fermentation patterns

Source	D.F.	Mean square		
		Total VFA ml^{-1}	C_2/C_3	pH
Total	55			
Time	3	1132	.250	.23
Treatment	4	873	1.675	.49
Time x trt	12	410	.328	.07
Remainder	36	548	.478	.08

Shortest significant ranges:

C_2/C_3

p:	(2)	(3)	(4)	(5)	
R_p:	.78	.81	.83	.84	
Treatments:	1	5	2	3	4
Means:[a]	2.07	1.89	1.26	1.16	1.14

pH

p:	(2)	(3)	(4)	(5)	
R_p:	.32	.33	.34	.35	
Treatments:	1	3	5	4	2
Means:[a]	6.56	6.23	6.21	6.10	6.04

[a]Any two means not underscored by the same line are significantly different at the 0.01 level of probability.

Table 39. Intact Lamb Trial 2 - analysis of variance of the effects of dietary calcium carbonate, sodium bicarbonate, urea and aureomycin on ruminal fermentation patterns

Source	D.F.	Mean square					
		Total VFA ml^{-1}	C_2	C_4	C_2/C_3	Buffering capacity	pH
Total	95						
Time	3	127	7	8	.098	.051	.12
Treatment	7	860	206	113	1.349	.112	.21
80-20 vs 50-50	1	5016	1222	426	4.502	.300	.48
80-20	3	157	30	62	.111	.100	.23
None vs $^{-}HCO_3$	1						
Con vs 1 1/2% $^{-}HCO_3$	1						
Con vs 3 % $^{-}HCO_3$	1						
50-50	3	177	43	59	1.536	.061	.10
Aureomycin	1	5	1	137	2.284	.000	.22
Urea	1	133	17	36	.102	.000	.02
Aureo x urea	1	393	110	2	2.223	.181	.05
Time x trt	21	84	13	23	.399	.065	.01
Time x 80-20	9	107	17	39	.632	.082	.02
Time x 50-50	9	78	4	8	.065	.046	.01
Time x con ratio	3	33	29	18	.700	.073	.01
Remainder	64	173	17	18	.216	.083	.04

Table 40. Intact Lamb Trial 2 - analysis of variance of the effects of dietary calcium carbonate, sodium bicarbonate, urea and aureomycin on growth performance and carcass traits

Source	D.F.	Mean square					
		Average daily gain	Feed conversion	Carcass grade	Dressing per cent	Separable fat	Separable lean
Total	23						
Treatment	7	.0027	.494	1.90	5.41	34.34	5.42
80-20 vs 50-50	1	.0024	1.321	9.38	29.56	203.59	29.26
80-20	3	.0048	.437	.11	1.29	8.30	.61
50-50	3	.0012	.275	1.19	1.48	3.97	2.27
Aureomycin	1	.0011	.056	.75	1.88	7.52	6.02
Urea	1	.0002	.000	.75	.34	4.20	.00
Aureo x urea	1	.0009	.766	2.08	2.22	.19	.79
Remainder	16	.0028	.242	.67	2.08	10.06	6.36

Table 41. Intact Lamb Trial 2 - correlations between ruminal fermentation, growth and carcass data

X	Y	Ex^2	Ey^2	Exy	r
Average daily gain	C_2/C_3	.06	4.09	-.06	-.12
Average daily gain	C_4	.06	268.45	.06	.02
Average daily gain	Total VFA ml^{-1}	.06	2613.52	.00	.00
Average daily gain	pH	.06	.79	-.05	-.24
Feed conversion	C_2/C_3	7.33	4.09	1.60	.29
Feed conversion	C_4	7.33	268.45	-16.04	-.36
80-20		2.26	91.40	-5.41	-.37
50-50		3.75	69.94	1.27	.08
Total VFA ml^{-1}	Buffering capacity	2613.52	.75	35.79	.81[a]
Carcass grade	C_2/C_3	23.96	4.09	-3.72	-.38
80-20		7.67	.89	.77	.29
50-50		6.92	1.77	-.83	-.24
Carcass grade	C_4	23.96	268.45	50.4	.63[a]
80-20		7.67	91.40	4.3	.51[b]
50-50		6.92	69.94	14.4	.66[a]
Separable fat	C_4	401.42	268.45	190.88	.58[a]
80-20		113.57	91.40	48.02	.47
50-50		84.26	69.94	-4.80	-.06

[a] P 0.01.

[b] P 0.05.

Table 42. Analysis of variance of the effects of route of collection of rumen fluid samples on ruminal fermentation patterns

Source	D.F.	Mean square		
		Total VFA ml^{-1}	Buffering capacity[a]	pH
Total	15			
Treatment	1	619	.250	.330
Remainder	14	265	.134	.023

[a]Only 13 degrees of freedom.

Table 43. Analysis of variance of comparative ruminal response of fistulated and intact wether lambs to change in ration treatments

Source	D.F.	Mean square		
		C_4	C_2/C_3	pH
Total	31			
Treatment	1	72.00	.456	.78
Type animal	1	7.80	.898	.24
Trt x type	1	7.61	.800	.01
Remainder	28	9.21	.236	.014