EFFECT OF PARTICLE SIZE, HEATING AND PELLETING OF RATIONS ON VOLATILE FATTY ACIDS PRODUCTION . IN THE RUMEN OF LAMBS

by

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INTRODUCTION

Volatile fatty acids (VFA) formed in the rumen have an important role in ruminant nutrition. Thus, the formation and utilization of VFA by cattle and sheep are an important phase in the conversion of feed to meat, milk and fiber and merit our continued attention both researchwise and in the practical application of feeding recommendations.

Historically, the formation of VFA in the rumen was observed almost 60 years ago but it was not until the 1930's and early 40's that the VFA formation was regarded as an essential step in the utilization of carbohydrates, especially cellulose. During the period between 1890 and 1940, the concept of microbial digestion of cellulose in the rumen and its utilization for energy was established. Perhaps the work at the Rowett Research Institute in Scotland (Elsden and Phillipson, 1948), and the analytical studies of the fatty acids found in rumen juice carried out by Elsden and his colleagues in England provided the clinching evidence, but many other European and American scientists have made extensive contributions.

Large amounts of organic acids, principally VFA, are produced within the rumen by microbial action, and these probably account for more than half of the energy derived from feed by the ruminant. In the past, the causes of the large variations which occur in the total amounts and relative proportions of these acids in the rumen have been little understood. Indeed, it has been only recently that some clarification has been obtained on the relation of the relative concentration of acids in the rumen to their production, absorption, and relationship to animal productivity. The purpose of this experiment was to determine the effect of particle size, heating and pelleting of rations on VFA production in the rumen of lambs and to try to establish a relationship between production of VFA and animal production and performance.

REVIEW OF LITERATURE

The nutrition and physiology of the ruminant differ from other herbivorous animals and particularly from the monogastric animals because of the nature of their digestive tracts in which approximately 70 percent of the feed energy is absorbed anterior to the true stomach (Hogan and Phillipson, 1960, Kameoka and Morimoto, 1959). The ruminant stomach is divided into four distinct compartments, the largest of which is the rumen. The reticulo-rumen comprises the "fermentation vat." The third compartment, the omasum, having characteristic folds which increase the surface area of the walls, is believed to function in absorption and to exert an abrasive action upon the material passing through to the abomasum. The abomasum is the true stomach and is analogous to the stomach of non-ruminants. Anything swallowed in a normal manner goes to the rumen where it is held for some time, where it is acted upon by bacteria and protozoa present. Carbohydrates are converted to VFA which are absorbed by the lining of the rumen consisting of a papillated stratified epithelium. The ingesta, in semidigested form, then moves through the omasum to the abomasum and then into the small intestine for further digestion.

Presence of VFA in the Rumen

Gruby and Delafond (1843) were among the first to speculate on the digestion in the ruminant stomach. They suggested that in the digestive tract of the ruminants a large number of organisms are present that are responsible for the digestion of vegetable matter. They estimated that 15 to 20 species of microorganisms existed in the four stomachs of ruminants.

Tappeiner (1883) demonstrated that the fermentation of cellulose in the

rumen of the ox resulted in the formation of large amounts of VFA, which he concluded contained at least 50 percent acetic acid.

For many years, VFA found in the rumen were considered to be of little nutritional significance. It has been established, however, that the VFA found in the rumen arise largely from the formentation of dietary carbohydrate. VFA production through bacterial fermentation of cellulose has been shown by Pringsheim (1921), Woodman (1930), Mangold (1934), Pochon (1935), Baker and Martin (1938), Baker (1939), Stephenson (1939), Baker (1942), McAnally and Phillipson (1942), Norman and Fuller (1942), Gray (1947), Hungate (1947), Hoflund et al. (1948) and Hershberger et al. (1956).

It is now generally known that acetic, propionic and butyric acids are the predominant organic acids produced in the rumen and that n-valeric, isobutyric and isovaleric normally exist in small amounts (Annison, 1954); in addition considerable amounts of acids of chain length greater than G₅ have been found in the rumen fluid of cows on certain special rations (Eusebio et al., 1959). When rations rich in starch are fed, lactic acid is often found in the rumen (Fhillipson, 1952). Amino acids can be converted to fatty acids (El-shazly, 1952, Sirotnak et al., 1954, and Annison, 1954) having a carbon structure similar to the acid or by the loss of carbon dioxide, to an acid with one less carbon atom. How much of the ingested protein and microbial protein actually goes to the fatty acids in the rumen is not known but probably the amount is small in comparison to the amount of fatty acids formed from carbohydrates.

Cellulose and Starch Digestion

The advent in recent years of the rumen fistule and the artificial rumen technique has provided research tools which have contributed greatly to our knowledge of rumen function. Ruminants are basically forage consumers and digest large amounts of cellulose and other complex carbohydrates. Since no cellulase is secreted by mammalian tissues, the process of cellulose digestion in the ruminant is entirely dependent upon the cellulases secreted by rumen microorganisms. The microorganisms usually gain access or attach themselves to plant structures at points of fracture (Baker and Harris, 1947). In the initial phase, the shape of the enzymatic cavities conforms to the outline of the microorganisms. Later, fusion of the zones leads to the formation of extensive zones of erosion. In view of the manner in which the microorganisms attack forage particles, it could be expected that physical factors such as churning or grinding could increase the number of fractures and thus enhance the rate of cellulose digestion. In the same respect lignin, being nondigestible, may hinder cellulose digestion by protecting it from the action of bacterial enzymes (Baker and Harris, 1947) and (Kam stra, Moxon and Bentley, 1958). Delignification of cellulose-containing plants greatly increases the rate of cellulose digestion in vitro. In this manner the cellulose of materials such as jute, hemp or sawdust can be made available for digestion. That lignin is a physical factor affecting cellulose digestion is further supported by the observation that the cellulose components of sawdust are digestible in vitro, without delignification if the material is finely ground (Virtanen. 1946).

X-ray diffraction studies have revealed differences which appear to be correlated with the digestibility of various cellulose sources (Baker et al., 1959). The observed differences were believed to be due to the degree of packing perfection. The degree of polymerization on the other hand does not appear to have an effect on cellulose digestion. This has been interpreted to mean that the dimensions of the intermiceliar spaces are more important than the

degree of polymerization in determining the rate of diffusion of enzymes into the material. It is also of interest that the callulose, altered by acylation or by formation of mathyl esters, is not digested in vivo (Baker and Harris, 1947).

Starch granules are derived from feed grains or other plants and are digested by the combined action of plant enzymes and rumen microorganisms (McNally and Phillipson, 1944). The digestion of starch, as in the case of cellulose, appears to occur at the point of direct contact between the bacteria and the starch granules. Cellulose and starch are converted to glucose (Kitts and Underkofler, 1954), most of which is further degraded to VFA. Cellulose appears to go through tetrose and cellobiose intermediates (Hash and King, 1954, Woodman, 1927). Fermentation of pentosans by rumen microorganicas in vitro has also been demonstrated (Howard, 1955).

VFA Production in the Rumen

A considerable volume of information is available on the production of VFA. Although the earlier work contributed much, it is the later studies, enhanced by the use of radioisotopes, which have served to clarify the situation to the greatest extent.

Numerous attempts have been made to measure production of VFA. Gray and Pilgrim (1951) followed the changes in the relative proportions of the acids in the rumen and attempted to assess the data obtained in terms of production and absorption.

Work on sheep, by Annison (1954a), demonstrated that total VFA in the rumen varied with the type of ration fed and the length of time after feeding. Animals fed corn exhibited VFA levels of 77, 87, and 92 (M per liter) of rumen fluid at 2, 5 and 8 hours, respectively. After feeding animals on a ration

of hay they had VFA levels of 117, 113, and 120 (M per liter) at the above time in ervals.

Phillipson (1942) reported fluctuations in VFA levels and ruman pH at various times before and after feeding sheep a ration of oats and bran. VFA reached their peak concentration at 4:5 hours after feeding with the pH level also at its most acid value. In a similar work, Hale et al. (1939) found that ruman VFA reached their peak six hours after feeding with ruman acidity highest at that point, with a gradual decline in acidity until the next feeling.

Williams and Christian (1956), using wethers for experimental animals, found that on a dry grass ration, the level of VFA in rumen liquor increased as consumption increased.

Jacobson at al. (1958) reported VFA concentrations of the rumen of 800to 1007-pound steers and heifers on a hay-silage diet an about 110 mM per milliliter.

Ratio of concentrates to roughage is also important in VFA production.

The investigation of Balch (1958), Stewart et al. (1958), Sheppard (1959), and Gray et al. (1960) indicated that 50 to 60 percent of the organic matter digested in the ruman is converted to VFA. In the usual types of roughage rations, acetic acid accounts for 67 to 70 mole percent of the total, or almost half of the VFA calories. The residue is a mixture of propionic, butyric and branched chain acids of four and five carbon atoms, and little or no larger carbon chain acids.

Analyses for VFA in rumen juice have shown that a ratio of acetic: propionic: butyric of about 65, 20 and 15, respectively, frequently occurs. This ratio has been accepted as relatively normal and fairly constant for dairy cattle receiving a ration composed mainly of roughage with some concentrates (Tyznic and Allen, 1951). Changes in the proportion of hay to grain and the

level of pro' in in the ration influence the acid ratio (Annison, 1954).

Th. actual molar percentage depends on many factors. Variables shown to have an effect include the ratio of roughage to concentrate (Elliot and Loosli, 1959; Peters et al., 1959; Woods and Rhodes, 1962), pelleting (Thompson, 1958; Haught et al., 1960), particle size (Balch, 1951), heat treatment (Chaw and Ensor, 1959; Ensor et al., 1959; Hayer et al., 1961), various oils (Shaw and Ensor, 1959), protein concentration (E1-Shazly, 1952), environment (Peters et al., 1959), feeding behavior or frequency of feeding (Knox and Ward, 1960, and Rhodes and Woods, 1962), mineral adequacy of the diet (Brink, 1961), the nature of the concentrate (Orth and Kaufmann, 1960, and Pfander et al., 1961), and the physical form of the ration (Cullison, A. E., 1962; Woods and Luther, 1962). Cellulose digestion favors the production of acetic acid, and any factor depressing cellulose digestion will increase the relative percentages of the other VFA's. Highly significant correlations have been reported between mole percentages of acatic acid and fat yield (Hawkins, 1959), fiber in the diet (Elliot and Loosli, 1959), and length of hay (Thompson, 1958). Rhodes and Woods (1962) found that pelleting a high concentrate ration for lambs had a marked effect on the pattern of VFA produced in the rumen.

Ensor (1959) stated that pelleting and feeding rations, containing 40 percent or more concentrate feeds, lowered the proportions of acetic acid and raised the proportion of propionic acid produced in the rumen.

The proportions of VFA were not altered when high roughage rations or rations containing 100 percent roughage were pelleted and fed.

Leffel and Komarek (1961) found little difference in the ratio between long, ground or pelleted alfalfa hay.

Hayer et al. (1961) observed that heat treatment of corn may affect rumen VFA. Steers were fed all-barley rations and compared steam rolled with dry rolled. A reversal of the usually observed acetate's propionate ratio resulted.

Ward (1962) studied the effect of artificial drying on the feeding value of hydrid, yellow dent corn fed to cattle in a fattening ration. Corn dried at three different temperatures was given to three different lots of steers in the following manner:

Lot No. 1 Corn dried by unheated forced air for 394 hours.

Lot No. 2 Corn dried with air heated to 180° F.

Lot No. 3 Corn dried with air heated to 230° F.

Results of the analysis of the rumen samples showed that there were no significant differences in total mM/100 ml. of VFA in rumen fluid or in the proportions of acetic and butyric acids. The proportions of propionic acid increased at higher drying temperatures with levels of 23.2, 26.7 and 28.1 percent respectively for the control, 180° F. and 230° F. drying temperatures.

Bartley (1961) found that feeding pelleted rations affects the normal metabolic process of the rumen. Rations that lower the fat content of milk usually decrease the molar percentage of acetic acid in the rumen and increase molar percentage of propionic and butyric acid.

The following table No. 1 is a compilation of data obtained at Kansas State University, (Bartley 1961) and shows the shifts which occur in the proportions of these acids with varying feeding regimes. It is apparent that the ratios of rumen VFA's can be altered by certain rations.

Table 1. Effect of different rations on ratios of rumen VFA. (Bartley 1961)

		Mol	Br % 1	VEA	
Investigator	Ration	C2	C ₃	C4	Valeric
Bartley (1961)	Long alfalfa hay (no grain)-	70	17	11	2
	Dehyd. alf. hay, pelleted	66	22	9	3
	Dehyd. alf. hay + steamed corn	60	21	16	3

Table 1. Continued.

		ar % 1	VEA	
Ration	^C 2	C ₃	C ₄	Valeric
)Long alfalfa hay + grain	68	17	12	
Finely ground hay + grain	60	25	14	
Medium ground hay + grain	66	18	13	
Above pelleted + grain	65	18	14	
Medium ground hay, pelleted + grain	67	17	13	
Medium ground hay + grain, pelleted	64	22	11	
Long alfalfa hay + grain	69	17	12	3
Long alfalfa hay + grain, pelleted	68	18	11	3
Long alfalfa hay, no grain	68	13	11	3
Alfalfa pasture (succulent)	63		13	3 2
Alfalfa pasture + saliva	65	19	13	2
	Dong alfalfa hay + grain Finely ground hay + grain Medium ground hay + grain Above pelleted + grain Medium ground hay, pelleted + grain Medium ground hay + grain, pelleted Long alfalfa hay + grain, pelleted Long alfalfa hay + grain, pelleted Long alfalfa hay, no grain	Ration C2 (2) Long alfalfa hay + grain Finely ground hay + grain 60 Medium ground hay + grain 65 Medium ground hay, pelleted 67 + grain 64 medium ground hay + grain, 64 pelleted Long alfalfa hay + grain 69 Long alfalfa hay + grain, 68 pelleted Long alfalfa hay, no grain 68 Alfalfa pasture (succulent) 63	Ration C2 C3	1)Long alfalfa hay + grain 68 17 12 Finely ground hay + grain 60 25 14 1) Medium ground hay + grain 65 18 14 1) Medium ground hay, pelleted 67 17 13 + grain 64 22 11 medium ground hay + grain, 64 22 11 pelleted Long alfalfa hay + grain, 69 17 12 Long alfalfa hay + grain, 68 18 11 pelleted Long alfalfa hay, no grain 68 13 11 Alfalfa pasture (succulent) 63 21 13

Results of preliminary studies at the Kansas station (Bartley, 1961) indicate that adding to the rumen either synthetic saliva or one pound of sodium bicarbonate daily increases the proportion of rumen acetic acid and decreases propionic acid when cown are fed ground roughage and heated corn. However, at Beltsville, Maryland, no change occurred in the ratio of rumen VFA's when 0.5 pound of sodium bicarbonate was fed daily (Elam, personal communication, 1961).

Balch and Rowland (1959) had noted that the feeding of equal parts of a finely ground hay and a mixed concentrate decreased the molar proportion of rumen acetate to 50 percent and increased that of propionate to 30 percent. Shaw (1959) indicated that in his studies the most interesting diet proved to be a combination of a small amount of steam-heated corn plus finely ground and pelleted hay. This combination effected a remarkable change in the rumen VFA and a marked decrease (53 percent) in the fat content of milk within 18 days. A still greater change in VFA was effected when the ration was made up

of smaller amounts of ground and pelleted hay plus larger amounts of steamheated corn (Shaw, 1958). Ground hay by itself produced only slight changes in the ruman VFA.

Balch (1951) stated that most experimental work confirms that grinding has little effect on digestibility. The rise in VFA production following eating is, however, more rapid with ground roughages than with those not ground. This must be linked with possible changes in the rate of breakdown of cellulose in different regions of the rumen associated with changes in the dry matter content of the digesta in those regions. Perhaps the most significant result of grinding roughages is the effect upon the proportion of the different fatty acids produced.

Ensor et al. (1959) studied the relation of diet to fat content of milk and to rumen soluble fatty acids. The results show:

- Grinding in general lowers acatic acid and increases propionisand butyric acid.
- 2. Heating further decreases acetic and increases propionic acid.

 Bartley (1961b) found that pelleting of grain has a depressing effect on butterfat content, because it depresses acetic acid and increases propionic acid. Farticle size reduction is the moviming important in hay. Pelleting reduces the particle size further. Pelleting is not a problem when the particle size of the roughage is large. Steam heat has more effect than dry heat.

Newland et al. (1962) in their study on the effect of heat-processing and pelleting corn for steers and lambs found that this processing of corn significantly narrowed the ruminal acetate and propionate ratio in both species.

Raun, Burroughs and Woods (1962) determined the effects of dietary sodium

bicarbonate, calcium carbonate, urea and chlortetracycline on ruminal fermentation, growth and carcass characteristics in lambs. Sodium bicarbonate and calcium carbonate additions to high concentrate-low roughage type diets were without effect in altering acetate-propionate ratio and total VFA levels in lambs. Supplementary sodium bicarbonate tended to elevate ruminal pH.

Chlortetracycline supplementation of a urea-high roughage lamb-fattening ration tended to overcome the depressing effects of urea on carcass grade. Where no urea was included in these rations, chlortetracycline additions tended to widen acctate propionate ratios and to elevate butyric acid levels. There appears to be an interaction between chlortetracycline and urea on acctate-propionate ratios and ruminal acctate levels.

Narrowed acetate-propionate ratios, higher butyric acid levels, lower total VFA levels and lower pH's were produced in the 80 percent concentrate as compared to the 50 percent concentrate diets. In one of the intact lamitrials, correlation was shown between ruminal butyric acid levels and carcass grade.

Woods and Luther (1962) studied the effect of altering the ration by heating or pelleting either the concentrate or the roughage portion of the ration which resulted in the same pattern of rumen fermentation as pelleting the complete ration. Further studies were also conducted to determine the effect of the fineness of grinding of roughage on patterns of VFA produced.

Rumen fluid from lambs fed the pelleted or reground pelleted rations had lower pH and the rumen fluid from lambs fed either of the heat-treated rations had higher pH than the rumen fluid from the control lambs. The micro moles of VFA were higher for the lambs fed the control and pelleted rations as compared to those fed the heated rations. The relative percent of acetate was lower for lambs fed reground pellets as compared to the control and heat

treatment. There was no statistical difference in the levels of propionate, butyrate or valerate for any of the treatments.

Again the total micro moles of VFA were higher for the lambs fed the complete pelleted ration than for the ration containing long hay or the ground mixed ration. The relative percent of acetate was lows:, the relative percent of propionate was higher and the acetate to propionate ratio was narrower in the rumen fluid of lambs fed the complete pelleted roughage ration as compared to those fed the long hay or complete mixed ration.

The moist heating of the ration did not produce significant difference in the measurements taken. There appeared to be no influence on VFA production by the method of drying the moist heated feed. Heating (moist or dry) did not bring about the changes noted in fermentation due to pelleting the entire mation. It was suggested that the method of heating does not equal the conditions associated with pelleting.

In the study of particle size the following results were obtained. The level of total VFA in the rumen fluid was higher for the lambs on the &-inch hay grind as compared to the &-inch grind. The acetate to propionate ratio was narrowed by the adding of concentrate to the ration. There was a form x concentrate interaction in the acetate to propionate ratio.

The adding of concentrate reduced the relative percentage of acetate in the rumen fluid for the lambs fed the rations containing pelleted hay. The feeding of concentrates increased the relative percent of propionate. There was a grind x concentrate interaction in the level of propionate in the rumen fluid. The adding of concentrates to the ration increased the relative percent of butyrate in the rumen.

The Absorption of VFA

abomasum and suggested that absorption must occur from the rumen, reticulum and omasum. Barcroft et al. (1944) suggested similar levels and, by introducing equimolar solutions of the sodium salts of acetic, propionic and butyric acids into the empty rumen of anaesthetized sheep, these workers showed that the acid anions were absorbed at different rates. Analysis of blood draining the rumen indicated that the rate of absorption under these conditions was acetate>propionate>butyrate. Detailed studies have verified and extended these earlier results (Danielli et al., 1945; Kiddle et al., 1951; Masson and Phillipson, 1951; Pfander and Phillipson, 1953; and Tsuda, 1956). It is now argued (Stewart et al., 1958; Gray et al., 1960) that average absorption from the rumen must equal production; however, it is felt by Pfander (1961) that a number of factors may cause temporary shifts and changes and that these may be extremely important in causing or intensifying disorders in either metabolism or digestive processes.

Factors shown to affect absorption are pH, Co₂ tension, electrolyte load, adrenaline, and monoiodoacetic acid (Gray, 1948; Pfander and Phillipson, 1953).

Danielli et al. (1945-46) showed that at pH7.5, when the amount of free acid was small, the rate of absorption of individual acids was in the same order as that given by Barcroft (1944) but that at pH5.8 the order was reversed: butyric>propionic>acetic. It was considered that free acids were absorbed at a greater rate than the anions and that different mechanisms of absorption were probably involved.

The permeability of the rumen epithelium is such that the pH of the ingesta tends to move towards neutrality. They estimated that due to more acidic pH in the rumen of sheep on pasture possibly twice as much VFA absorption might occur as compared to the same animal on a diet of hay. Gray (1948)

reported work with sheer in agreement with the previous authors in which acetic acid was not absorbed from an isolated rumen at pH of 7.5. He concluded that while rapid absorption of VFA from the rumen takes place at acid reactions, there is no absorption at all from alkaline solutions.

Gray (1947) investigated the effect of pH on the rate of absorption of the individual acids, and he showed that at a pH of 6.5 propionic acid disappeared more rapidly from the rumen than did acetic acid. No change in the ratio could be observed when sodium salts of the acids were placed in the empty rumen. Johnson (1951), in studies on the absorption of VFA's from the rumen of fasting goats and sheep, also found that butyric acid was absorbed more rapidly than acetic acid. Pfander and Fhillipson (1953) compared the ratio of acids int duced into a sheep's washed, isolated rumen with the ratio in which the acids disappeared. They found that the propioniciacetic ratios were 1:2.9 and 1:2.0 for the rumen and for the rate of disappearance, respectively; the corresponding butyric:acetic ratios were 1:3.3 and 1:1.5. These results appear to indicate that higher acids disappear at rates disproportionate to their concentration when compared to acetic acid.

The concentration of VFA in the rumen also influences blood flow (Dobson and Phillipson, 1956). Perfusion techniques have given dirferent values.

Brown at al. (1960) reported that the concentration of acetic, propionic and butyric acids in the blood was similar to that present in the rumen. McCarthy et al. (1958) found little conversion of butyrate to betones. Isolated systems have been useful in determining some of the general metabolic pathways in specific tissues. Ruman epithelium absorbs the VFA's and, in the process, changes a considerable portion of the butyric acids to betones. This reaction can be shown either in in-vitro systems (Pennington and Pfander, 1957; Seto et al., 1955) or by contrasting the VFA concentration in portal blood and in

the rumen vein (Mason and Phillipson, 1951).

Fate of VFA Absorbed from the Rumen

Phillipson (1947), realizing that the fatty acids were important sources of energy, estimated that they might supply at least 60 percent of the fasting energy requirements of the ruminants, and since then, other workers, including Marston (1948), Carroll and Hungate (1954) and Schambye (1955), have confirmed the significance of this energy contribution. In this respect the publication of Armstrong and his co-workers (1957a, b) at the Hanna Dairy Research Institute are of great interest. In a series of papers they have examined the utilization of energy of the lower VFA, administered singly or in combination, by sheep on either a fasting or fattening plane of nutrition. They observed a great deal of difference in the nature of utilization of the acids. It was concluded that the differences may be due to the fact that feed stuffs of variable composition produce different patterns of VFA's, which in turn may give rise to different heat increments when the animal is on a fattening diet.

Below maintenance, variations in acid distribution appear to have little effect, and the acids are all used with equal efficiency.

Acetic acid, apart from supplying energy, may act as a possible precursor of carbohydrate. Lorber et al. (1945), by the use of labeled acetate, demonstrated the conversion of acetic acid to glucose, but the evidence was not conclusive. Kleiber et al. (1952) detected radioactive carbon in the lactose and casein of milk from cows given labeled acetate, but Johnson (1955) suggests that this was due to participation in the Krebs cycle rather than as a net synthesis. He could show no consistent influence upon blood glucose level when acetic acid was given orally or when sodium acetate was injected intravenously, thereby confirming the studies of Jarrett et al. (1952). Phillipson

and Cuthbertson (1956) concluded that if acetate does cause an increase in total body carbohydrate it is by an indirect route.

Acetic acid is, however, actively concerned in the synthesis of milk fat by the ruminant, and some 40 to 80 percent of the acetic acid absorbed into the blood may be removed by the mammary gland. The mechanism is postulated as a stepwise elongation of a shorter acid by the addition of a \mathbf{C}_2 compound derived from acetate.

This function of acetic acid was first suggested by in vivo studies performed by Graham et al. (1938) and Reineke et al. (1941), as well as by the in vitro work of Folley and French (1949). Popjak et al. (1950) use: labeled acetate to demonstrate that fat was synthesized from acetate in the udder of the goat, and they later showed that during the six hours after injection of the carboxyl-labeled acetate a goat transferred 10 percent of the injected C to butterfat (Popjak et al., 1951). Kleiber et al. (1950, 1952) reported similar results for the cow; these workers (Kleiber et al., 1952, 1953, 1954) have, by injection of labeled fatty acids into the jugular vein of a lactating cow, studied the distribution of active carbon atoms among milk constituents.

The function of propionic acid is mainly glucogenic. Reid (1950) stated that propionic acid was the principal source of blood glucose for sheep, and McClymont (1951) has suggested that all ruminants maintain their blood-sugar level by gluconeogeneses from propionate. Clark and Malan (1956) showed that the administration of propionate into the rumen of a sheep produced a marked rise in blood sugar and had a strong anti-ketogenic effect, ar observation confirming the results of Jarrett and Potter (1950). Lauryssens et al. (1956) considered that the utilization of propionic acid observed by Kleiber was due mainly to the conversion of propionate in the liver or other organs into

compounds which were transported to the udder and used there as substrates for the synthesis of milk constituents. The activity of labeled propionate incubated with ruminant liver-tissue slices was found to be recovered mainly in malate, aspartate and lactate (Shaw et al., 1958).

Whatever the site of conversion, it is now apparent that propionate may be metabolized to form dicarboxylic acids by a route not involving pyruvate (Lorber et al., 1950; Shrueve, 1952). The formation of succinate from propionic acid by direct fixation of Co₂ has been demonstrated by Pennington (1954). Black and Kleiber (1955) proposed a scheme of alternative pathways for propionate metabolism, and their findings are expressed as follows (Correct and Reid, 1961):

They concluded the C₃ unit was transferred over routes (1) and (2) with about equal isotops dilution but more slowly over pathway (3) to pyruvate. They also concluded the propionate is as important a precursor for serine and aspartic acid as it is for carbohydrate.

Not much data is available regarding the mutabolic function of butyric acid. It has been shown by Kiddle et al. (1951) and Pfander and Phillipson (1953) to have a high energy value, and Johnson (1953) calculated that molar percentages of 60 percent acetic, 20 percent propionic and 20 percent butyric acid in the rumen could supply 41, 24 and 35 percent, respectively, to energy production.

There is some evidence (Kleiber et al., 1952, 1953, 1954) that butyrate, in contrast to acetate, is more closely concerned with the synthesis of lactose than of fat in the udder--from which it may be concluded that butyrate

may be a precursor of glucose without passing through the acetate pool.

The highly ketogenic activity of butyrate in the liver and rumen epithelial tissues has been demonstrated by Pennington (1952) and several other workers, but the effect of the acid on blood sugar is less well established. Schultz and Smith (1951) noted a rapid fall in blood sugar after feeding butyrate to goats, while Jarrett et al. (1952) on the other hand, obtained a rise after intravenous injection of the butyrate. Clark and Malan (1956) observed that administration of but, rate into the rumen caused a rapid increase in the concentration of ketone bodies, and that this was accompanied by a drop in blood sugar.

EXPERIMENTAL PROCEDURE

Wide variations in the proportions of acetic, propionic and butyric acids have been reported by several workers, due to differences in ration composition, preparation and treatment of the individual feedstuffs. The following data were gathered to provide information relative to proportions and concentration of VFA in rumen fluid taken from lambs on six different rations.

Experimental Plan

This experiment utilized 12 pens of 9 lambs each. The pans were approximately 12 feet by 40 feet each in size. The lambs used in this experiment were fine-wool ewe and wether feeder lambs purchased on February 4, 1963. They were drenched with phenothiazine for internal parasitus and vaccinated for enterotoxemia. They were implanted with 3 mgs. of stilbasterol, weighed and divided into six lots of 18 each on February 18, 1963, on the basis of sex and weight. Each of these six lots was subdivided into two

sublots of 9 each, one self fed (SF) and the other hand fed (HF). The six lots were assigned to the following ration:

Lot 1	
	Ground alfalfa hay55%
	Ground sorghum grain35%
	Molasses
Lot 2	
	Ground alfa fa hay55"
	Ground so ghum grain35%
	Molassec10%
	(Alfalfa hay was heated to 180°.)
Lot 3	
	Ground alfalfa hay55%
	Ground sorghum grain35%
	Molasses10%
	(Ground sorghum grain was heated to 180°.
Lot 4	
	Ground alfalfa hay55%
	Ground sorghum grain35%
	Molasses10%
	(for late ration pelleted and reground)
Lot 5	
	Ground alfalfa hay55%
	Ground sorghum grain35%
	Molasses10%
	(Complete ration pelleted)

Lot 6

Fine ground alfalfa hay.......55%

Ground sorghum grain......35%

Molasses...........10%

(Same as No. 1 but finer perticle size)

In order to keep the particle size of all the rations the same, except the ration given to lot No. 6, the rations were ground in the following way: rations given to lot Nos. 1, 2 and 3 were ground through \(\frac{1}{2}\)-inch screen; rations given to log No. 4 were ground through \(\frac{1}{2}\)-inch screen made into 3/4-inch pellet and then reground to a mash form in such a way as to cause a minimum amount of particle size reduction (This regrinding was done on a corrugated pellet crumble roll); rations given to lot No. 6 was ground through 6/64-inch screen. The details of the particle size of the rations are given in table No. 11.

Particle size was essentially the same for all lots except number 6.

The lambs were weighed every two weeks and their feed consumption and weight gains were also recorded. They were on trial for 77 days.

After 5 weeks rumen samples were taken from the lambs in early afternoon. The samples were obtained by introducing a stomach tube that was forced downward through the esophagus into the area of the rumen and ingesta drawn into the tube by means of a hand vacuum pump. The samples were collected in smell polythene freezer bags and frozen as quickly as possible. The samples remained frozen until used for analysis.

Analytical Technique

The samples were brought to room temperature and centrifuged at 2500 r.p.m. for 25 minutes. Supernatant was taken for analysis.

The analytical laboratory procedure used to obtain the data reported in this thesis is a modification by Tsein (1960) of that reported by Wiseman and Irvin (1957).

Chromatographic VFA separation technique involves essentially four major steps which are (1) the preparation of the absorbent (celite), (2) developing of the solvents, (3) colum preparation, and (4) titration of the eluents. The materials and reagents used are shown below, and laboratory procedures are briefly described.

Material and reagents

Celite, Johns-Manville's analytical filter aid

Skelly-Solve B (Hexane)

Acetone (practical grade)

Creosol red indicator. Add 1.3 ml. of 0.1 N sodium hydroxide to 50 mg. of 0-creosol sulphthalein in 20 ml. of alcohol; add water to 50 ml.

Sugar, extra-fine, granulated.

Alphamine red-R indicator, 0.4 gm. in 100 ml. of water.

Sulphuric acid, 0.15N.

Sulphuric acid (50:50) conc. acid: water.

Sodium sulphate (anhydrous).

Barium hydroxide (saturated).

Experimental

Adsorbent. 28 gms. of celite were carefully weighed and added to Skelly-Solve B and acetone solvent, 1 to 1 by volume, contained in a Waring blender. To this mixture were added 18 mls. of sugar solution (200%), 200 gms. in 100 mls. of water) and .1 to .12 ml. of .15 N sulphuric acid plus 3.5 to 4 mls. of alphamine red-R indicator solution. This was blended vigorously for 4 minutes and stored in a refrigerator until used.

Developing solvents. Various percentages by volume of acetone in Skelly-Solve B were made as follows: 1, 5, 30 and 40 percent. These are referred to as EA, BA, etc. To prevent gradual removal of water from the column by dry eluents, concentrations above BA, were equilibrated against the static phase as follows: 500 mls. of the solvent were stirred vigorously in the blender with 12 mls. of 50% sugar solution with 0.25 ml. of saturated barium hydroxide solution and 2 to 3 drops of creosol red indicator to free the solvent of carbon dioxide and traces of acids. After settling, the solvent was freed to suspended droplets by passing through filter paper. Column preparation. Using a small funnel, the slurry of the absorbent was added to a clamped-off chromatographic tube. The slurry, having been previously warmed to room temperature by removing it from refrigerator at least two hours before it was used, was then poured into the tube to about 30 cm. A glass rod was used to remove air bubbles from the slurry and the column was packed by applying 10 lb./sq. in. pressure of N from cylinder. This compressed the column to about 12 to 15 cm. in height. In the same way more slurry was added in order to get the total height of the column of almost 20 to 22 cm. The uneven surface at the top of the column was leveled by light tamping. Care was taken not to allow all the solvent to pass through the material so that the column might be prevented from drying.

 BA_1 was added as a fine stream down the side of the tube to avoid disturbing the top 6f the column. About 100 ml. were so added and forced through the tube with 5 lb. of air (nitrogen) pressure. The column was then clamped off, leaving at best 4 to 5 cm. of BA_1 above the surface of the column.

A 3-ml. sample of centrifuged rumen fluid that had been acidified with 1 to 2 drops of sulphuric acid (50-50) cone sulphuric acid:water) added to a 50-ml. beaker containing 2 gm. of sodium sulphate and 12 gm. of celite. This was stirred with a glass rod, forming a damp powder which was transferred to the top of the column. Approximately 1 gm. of celite was used to remove any remaining sample still in the beaker and was applied to the top of the capping material already in the tube. BA₁ was added until the capping material was wet. A rod was then used to remove air bubbles. This capping material was then pressed a little bit to even the top.

Approximately 100 ml. of BA_1 , BA_5 and BA_{30} (equilibrated) were used successively on the column to remove the butyric, propionic and acetic acids respectively from rumen fluid samples. These solvents were put under 5 lb. of pressure to speed their flow through the column.

Samples were collected in 250-ml. erlenmeyer flasks and titrated with a solution of approximately 0.011 N KOH containing an indicator. The end point of the titration was obtained just after the sample passed through a yellow color and became green. The amount of KOH solution used, multiplied by the strength of the solution, gave the mM of acid per 3 ml. of sample.

Results and Discussion

The results of the rumen VFA determinations are presented in tables No. 3 through 8. Analysis of variance of the data in table No. 9 indicated that only butyric acid and total VFA mM/100 ml. in the rumen fluid were significantly affected by different rations used, whereas acetic acid and propionic acid were not affected. It was also observed that of the three different treatments, heating, pelleting and particle size, the influence due to heating was most significant P<.01 using nonorthogonal comparison (Steel and Torrie, 1961).

The analysis of variance also showed an interaction between method of feeding and treatment (P<.05) in total VFA mm/100 mll in rumen fluid. This indicated that either hand feeding or self feeding (SF) or both might have some influence on the treatment. Table No. 2 shows that in most cases hand feeding produced more total VFA mM/100 ml. than self feeding and the same was true in case of efficiency. Efficiency seems to be better in the hand feeding lots as compared to self feeding lots.

Table 2. Total VFA mM/100 ml. and efficiency of all the lots.

Lot No.	Total VFA	Efficiency, lbs. of feed per 100 lbs. of gain
ot No. 1 HF	11.24	741.0
SF	9.38	723.7
Lot No. 2 HF	8.91	796.8
SF	11.56	746.5
Lot No. 3 HF	8.62	744.1
SF	7.53	823.7
ot No. 4 HF	12.50	607.3
SF	8.29	692.2
ot No. 5 HF	14.55	662.8
SF	7.39	646.3
ot No. 6 HF	12.90	744.0
SF	9.00	751.6

This trend was true in the case of lot Nos. 4 and 5 where lambs received pelleted and reground, and pelleted rations respectively. The method of feeding and pelleting had interactions. It appeared that hand feeding of pellets would produce a higher concentration of total VFA than self feeding of pellets. It appeared from table No. 2 that there was a slight correlation between total VFA production and efficiency but this was not statistically significant.

The results were also in agreement with the findings of Woods and Luther (1962) that the VFA mM/100 ml. was higher for the lambs fed the control and the pelleted rations as compared to those fed heated rations. Total VFA mM/100

ml. for lot No. 1 (control) and lot No. 5 (pelieted) was 10.31 and 10.97 respectively, whereas the total VFA mW/100 ml. for lot No. 2 (hay heated) and lot No. 3 (grain heated) was 10.23 and 8.08 respectively.

It was also observed that either heating the roughage or concentrate protion does increase butyric acid level in the rumen fluid. Butyric acid level in the rumen fluid of lambs receiving the control diet was 14 percent whereas the level of butyric acid in the rumen fluid of lambs from lot No. 2 and lot No. 3 was 16.6 percent and 16.0 percent respectively.

The effect of particle size of VFA production was studied by making the particle size of the ration given to Lot 6, much finer than the particle size of the ration given to other lots. This was confirmed by wet sieve analysis according to the method of Pfost (1961). The result of the particle size analysis are presented in Table 11 and figure 1. The comparison of total VFA mM/100 ml. in Lot 1 with Lot 6 indicated that the total VFA mM/100 ml. was slightly higher in Lot 6, though this was not significant. The efficiency of the lambs and VFA ratios were not different in these two lots. Particle size did not seem to influence VFA production and efficiency of the animal.

It has been shown by several workers, (Thompson, 1958; Bartley, 1961; Shaw, 1959) that pelleting decreases the acetic acid level and increases the propionic acid level in the rumen fluid. This was not observed in this trial. The reason was not understood. Total VFA mM/100 ml. was found to be higher.

Performence data in table 10 shows that self-fed lambs consumed more feed and gained faster than hand fed lambs. Eand fed lambs in Lot No. 4 and 5 gained faster than lambs in other hand fed lots, and both hand fed and self-fed lambs in Lot No. 4 and Lot No. 5 made more efficient gains than lambs in other lots. Total VFA mM/100 ml. in rumen fluid from the lambs in these lots were slightly higher than the total VFA mM/100 ml. in rumen liquor from the

lambs in other lots. Pelleting caused an increase both in consumption and in rate of liv. weight gain.

Pelleting involves: (1) adding moisture, (2) increasing temperature, (3) compression, (4) extension, and (5) air cooling, (Hastings et al., 1962). A combination of these factors probably made the ingredients of the pelleted rations more digestible. This in turn might have improved the feed consumption and live reight gains. Higher consumption of pellets than of ground feeds might be due either to changes in the animal's "appreciation" of these feeds or to difference associated with gut fill, but there are many difficulties in deciding which factor is most important.

SUMMARY

The purpose of this study was to determine the effect of particle size, heating of ration ingredients and pelleting of rations on ruman VFA in lambs.

One hundred and eight lembs were allotted to 6 rations, 18 lembs per treatment. The treatments were: (1) basal-grind-mixed ration; (2) basal ration in which hay was heated to 180°F.; (4) basal ration pelleted and reground; (5) basal ration pelleted; (6) basal ration was finely ground. Particle size of all the rations were the same except the ration in lot 6 which was slightly finer. Rumen fluid was collected and analyzed for VFA ratios and total VFA mM/100 ml. Lamb weights were recorded periodically for the 77 day feeding period and feed consumption by lots was obtained.

It was observed that of the three different treatments, heating, heating pelleting and particle size, heating influenced the VFA most significantly. Heating either roughage or concentrate portion of the ration increased the butyric acid level in the rumen fluid. Farticle size did not seem to influence VFA production or efficiency of feed utilization. Felleting did not influence

the VFA ratio in this trial, but total VFA mM/100 ml. was elevated. Pelleting was found to ploduce better and faster gains. In general, a slight trend was observed between total VFA mM/100 ml. and feed efficiency. The lot that produced more VFA mM/100 ml. showed great efficiency. Hand-feeding of pellets to lambs induced better gains and efficiency.

Table 3. Proportions and concentrations of VFA in Rumen Samples of Lambs in lot No. 1.

	Lamb. No.		Proportion		Total VFA
		Acetic	Proplonic	Butyric	mM/100 ml
land fed (HFI)	E100	66.59	18.07	15.35	9.987
Control	E91	62.31	.23.56	14.13	9.206
	E41	97.59	21.07	13.48	9.143
	E68	65.04	20.62	14.32	11.235
	96M	66.52	20.02	13.45	11.13
	W57	63.63	20.24	16.13	9.578
	W107	62.99	21.10	12.90	10.353
	W72	65.26	20.63	14.1	22.399
Average (HFI)		65.04	20.62	14.32	11.235
Self fed (SF1)	E12	63.38	19,53	17.08	9.655
	240	67.59	19.93	12.67	8.704
	645	67.43	19.34	13.19	9.433
	E70	65.00	21.82	13.30	7.672
	E92	66.99	21.22	13.78	10.274
	11.7	61.85	22.02	11.13	10.256
	W14	64.82	18.19	17.00	10.973
	06M	66.12	19.68	14.19	9.223
	76M	65.16	20.21	14.02	8.264
Average(SF1)		66.16	20.197	14.106	9.383
Average lot No. 1		64.59	20-4	14.21	10.300

Table 4. Proportions and Concentrations of VFA in Rumen Samples of lambs in lot No. 11.

Butyxic 18.34 16.93 15.87 15.87 16.05 16.87 16.05 16.72 17.53 17.53 17.53 17.53 14.99 22.26 13.83 14.99 16.67 16.67	Description	Lamb No.		Proportion		Total VEA
## 66.2 18.46 18.34 E47			Acetic	Propionic	Butyric	mM/100 ml
E47 661.58 13.48 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.93 16.95 16.85 16.95 16.85 1	Hand fed (HF2)	W8.2	62.3	18,46	18,34	6.03
#895 666.1 i8.03 15.87 #84 65.62 i6.76 ELO4 68.82 15.81 #34 66.76 17.18 15.3 #35 65.52 16.85 16.85 E31 66.32 16.85 E31 66.32 16.85 E85 65.46 17.68 16.72 E85 66.48 15.99 E87 67.45 18.71 13.83 E84 66.48 15.99 E87 67.45 18.71 13.83 E84 66.98 15.99 E87 67.45 18.71 13.83 E84 66.98 15.59 E85 66.98 15.59 E86 66.98 15.59 E87 67.45 18.71 13.83 E87 65.20 18.33 16.47 E87 65.33 18.00 16.60		E47	61.58	2.48	16.93	00
### 65.62		E95	66.1	:8.03	15.87	8.53
E104 66.85 15.81 15.3 H3 66.76 17.18 16.05 E85 66.12 16.87 16.87 E85 65.33 17.80 16.65 E31 66.32 16.74 16.65 E88 66.46 17.68 16.72 E88 66.48 15.99 17.53 E89 66.48 15.99 17.53 H3 61.84 17.90 17.53 H3 61.84 17.50 15.63 H3 65.20 18.33 16.47		575	65.62	16,276	17.60	7.44
#13 66.76 11.18 16.05 E85 65.12 16.87 E85 65.52 17.80 16.65 E81 65.46 17.80 16.72 E85 65.46 17.69 16.72 E85 66.48 17.20 17.53 E85 66.48 17.20 17.53 E84 63.39 12.22 14.99 E85 67.41 17.50 17.53 E84 63.39 12.22 14.99 E85 66.48 17.59 17.53 E84 63.39 12.22 14.99 E85 66.48 17.59 17.53 E86 66.48 17.59 17.53 E87 65.48 17.59 17.59 E87 65.49 17.59 E88 66.48 17.59 17.59 E89 65.49 17.59 E89 17.59 17.59 E89 66.48 17.59 E89 17.59 17.59 E89		E104	68.85	15.81	15.3	8.75
#54 66.12 16.85 16.87 16.87 16.85 16.85 16.55 17.80 16.65 16.65 16.65 16.42 16.44 16.55 16.45 16		W1.3	92.99	17.18	16.05	9.25
E31 66.53 17.80 16.65 E31 66.32 16.74 16.65 E88 63.43 14.49 22.26 E87 67.45 18.71 13.83 E84 66.48 12.22 14.99 E87 67.45 18.71 13.83 E84 66.48 12.22 14.99 E87 67.45 18.71 13.83 E84 66.91 17.50 14.99 E87 65.20 18.33 16.47 E88 66.91 17.50 15.67 E88 66.91 17.59 14.99 E89 63.39 12.22 E89 12.22 E89 12.23 E89 12.23 E8.60 18.60 16.60 E89 12.23 E8.60 18.60 16.60 E89 12.23 E89 12.24 E89 12.25		W54	66.12	16.85	16.87	10.6
E18 65.46 17.68 16.72 16.72 16.95 16.72 16.95 16.74 16.95 16.72 16		E85	65.53	17.80	16.65	10.70
E18 63.23 14.49 12.26 E67 67.45 18.71 17.58 E88 66.48 15.99 17.53 E84 63.39 12.22 14.99 W75 67.41 17.59 15.17 W75 67.41 17.50 15.17 W76 65.91 17.53 18.46 W106 63.08 21.05 15.63 W112 66.98 15.13 15.67 W16 65.20 18.33 16.47		E31	66.32	16.74	16.95	12.14
ELS 63.23 14.49 22.26 EST 67.45 18.71 13.83 ESS 66.48 15.99 17.53 ESS 66.48 12.22 14.99 W3 61.84 12.22 14.99 W35 67.41 17.50 12.07 W106 65.30 18.46 14.63 W112 66.98 15.47 ESS 65.33 18.00 16.47	Average (HF2	0	65.46	17.68	16.72	8.91
EGT 67.45 18.71 13.83 ESB 66.48 15.99 17.33 ESB 66.48 15.99 14.99 W3 61.84 12.22 14.99 W35 67.41 17.50 15.07 W410 66.91 17.50 15.63 W112 66.98 15.10 15.63 W412 65.20 18.33 16.47 65.33 18.00 16.60	elf fed (SF2)	818	63.23	14.49	22.26	99 0
E88 66.48 15.99 17.53 E84 63.39 17.53 E84 63.39 12.22 14.99 17.53 E84 63.39 12.22 14.99 14.99 E84 65.41 15.90 15.07 E8.06 E8.08 12.05 15.07 E8.06 E8.08 12.05 15.63 E8.00 18.33 16.47 E8.33 16.47		867	67.45	18.71	13.83	98.86
K34 63.39 12.22 14.99 K34 61.84 21.98 21.17 W75 67.41 1.30 15.07 W86 66.91 18.46 15.63 W106 63.08 21.05 15.63 W112 66.98 15.13 15.87 65.20 18.33 16.47		E88	66.48	15.99	17.53	9.42
W3 61.84 21.98 21.17 W75 67.41 17.50 15.07 W86 66.91 18.46 14.63 W12 66.98 12.13 15.63 W12 65.20 18.33 16.47		E94	63.39	12.22	14.99	11.79
NYS 67.41 17.50 15.07 NYS 66.91 18.46 14.63 NY106 63.08 21.05 NY112 66.98 15.13 15.87 65.20 18.33 16.47		W3	61.84	21.98	21.17	7.09
MISG 66-91 18-46 14-63 MIOG 63-08 21.05 15-63 MI12 66-96 15-15 65-20 18-33 16-47 65-33 18-00 16-60		W75	67.41	17.50	15.07	10.35
M106 63.08 21.05 15.63 W112 66.98 15.13 15.87 65.20 18.33 16.47 65.33 18.00 16.60		M86	66.91	18.46	14.63	17.86
W112 66.98 15.13 15.87		W106	63.08	21.05	15.63	20.14
65.20 18.33 16.47 65.33 18.00 16.60		W112	86-99	15.13	15.87	9.92
65.33 18.00 16.60	Average (SF2		65.20	18.33	16.47	11.56
65.33 18.00 16.60						
	verage of lot No.	11	65.33	18.00	16.60	10.23

Table 5. Proportions of and concentrations of VFA in Rumen Samples of lambs in lot No. 111.

	Lemb No.		Proportions		Total VFA
		Acetic	Propionic	Butyric	n#/100 ml
Hand fed (HF3)	w16	64.26	17.04	18.71	8.10
	W63	64.01	13,53	17.31	8.97
	E61	63.19	19.56	17.25	8.36
	183	65.49	10.6	17.91	11.75
	484	62.94	15.31	77.12	11.23
	W34	64.99	20.37	14.65	7.78
	W102	68,16	16.86	14.97	11.09
	W11	68.83	16.51	14.66	11.61
	E58	64.72	17,16	18.1	8.90
Average (HF3)		65.18	17.55	17.26	8.62
Self fed (SF3)	E	76 88	90 00	6	8
	1 0		00.04	13.02	7901
	1633	4.89	17.09	14.51	5.2
	E56	63.84	21.34	14.81	7.25
	E65	06.19	17.82	15.27	9.88
	E83	59.13	25.04	15.82	9.76
	W51	64.36	20.24	15.39	9.62
	64M	69.34	17.3	15.39	4.86
	W105	72.5	13.2	14.3	6.92
	W111	69.3	16.2	14.52	6.64
Average (SF2)		67.23	17.58	14.78	7.53
Average of lot 3		66.23	17 56	16 00	00
100000000000000000000000000000000000000		00 • 173	17.36	16.02	8,08

Table 6. Proportions and Concentrations of VFA in Ruman Samples of lambs in lot No. IV.

Description	Lamb No.		Proportions		Total VFA
		Acetic	Propionic	Butyric	Im COL/Man
Hand fed (HF4)	E19	68.49	19.1	12.4	10.32
	E36	63.86	17	14.50	19, 37
	E38	70.29	1.4-29	15.41	15.25
	E73	67.90	17.62	14.47	14.37
	E78	69.03	18.31	12,64	10.53
	W59	58.83	26.43	14.77	8.34
	998	68.00	18.42	14.04	16.37
	MYZ	68.99	15.84	15.22	11.30
	664	67.12	19.50	13.38	7.21
Average (HF4)		66.95	19.00	14.09	12.56
Self fed (SF4)	E25	67-17	17.38	15.45	6.88
	E50	65.64	19.82	19.79	9.97
	E55	63.81	17.98	18.20	10,36
	E74	68,38	19.32	13.3	7.05
	145	67.31	18.08	14.59	17.16
	W17	66.18	2. 82	12.48	7.39
	W22	96.99	19.79	13.27	6.35
	142	68,20	18.91	13,38	8.15
	WIOI	90-99	18.40	15.54	7.27
Average (SF4)		66.63	18.83	15.11	8.29
Average of lot No. 4	4	62-99	18,66	14.6	10.42

Table 7. Proportions and concentrations of VFA in rumen samples of lambs in lot No. V.

Hand feed (HFS) E15 62.03 22.39 15.57 18.16 60.42 22.39 18.07 22.35 18.16 60.42 22.39 18.07 22.35 18.16 60.42 22.35 18.07 22.35 18.07 22.35 18.07 22.35 18.07 22.35 18.07 22.35 18.07 22.35 18.07 22.35 19.07 10.03 19.07 10.0	Description	Lamb No.		Proportions		Total VFA
E15 62.03 22.39 15.57 E46 66.42 (6.22 16.26 E46 66.03 16.90 18.07 E47 66.03 16.90 16.42 E48 66.03 16.90 16.90 E48 66.03 16.90 16.90 E48 66.04 16.90 16.90 E21 16.00 16.90 E22 13 16.90 E23 16.00 16.80 E24 66.19 19.07 14.33 E25 16.00 16.90 E25 16.00 16.90 E26 16.00 16.80 E27 66.49 22.08 11.45 E28 66.00 20.12 16.01 E29 65.00 20.12 16.01 E20 65.00 20.13 16.01			Acetic	Propionic	Butyric	mM/100 ml
E22 60.42 750 18.07 2 E46 67.52 16.26 16.26 E49 69.76 17.52 14.42 WZ3 66.03 16.90 14.02 14.42 WZ3 66.03 16.90 16.80 16.80 WZ4 66.04 16.80 10.89 11.80 E21 66.19 19.07 14.33 E21 66.59 19.07 14.33 E23 65.00 20.12 14.63 E24 64.49 20.12 14.63 E25 65.59 19.67 14.63 E27 66.49 20.12 14.63 E28 65.50 20.12 14.65 WZ4 66.40 20.20 14.65 WZ4 66.40 20.12 14.65 WZ4 66.40 10.80 WZ4 66.40	land fed (HF5)	E15	62.03	22.39	15.57	18.16
E46 67.52 16.26 16.26 16.26 14.42		E32	60,42	250	18.07	22.35
E49 66.76 (52 14,42 14,42 14,42 14,42 14,42 14,43 16,63 16,63 14,01 14,42 14,64 14,63 16,63		E46	67.52	:6.22	16.26	11,41
W23 66,03 19,01 15,05 10 W26 69,09 16,85 16,85 14,01 14,01 W43 62,04 22,18 15,81 1 W410 68,46 22,13 10,09 1 W110 66,19 19,41 14,33 1 E21 66,19 19,41 14,33 1 E21 66,49 22,08 13,41 1 E37 64,49 21,6 13,44 1 E39 65,00 21,45 13,43 1 E39 65,00 20,12 14,63 1 W4 65,00 23,03 14,63 1 W4 64,40 20,32 14,63 1 W4 64,40 23,29 10,87 14,64 W4 64,40 23,29 10,87 14,64 W4 64,40 23,29 10,87 14,64 W4 64,40 23,29 1		E49	92.69	52	14.42	15.36
W426 66.09 16.85 14,01 1		W23	66.03	19.01	13,93	13 oil 7
W48 65.04 22.13 15.81 11 W67 70.11 18.50 15.81 11 W110 68.168 20.29 10.72 1 W11 66.19 19.41 14.30 1 E21 66.59 19.07 14.33 1 E31 64.49 22.08 13.41 1 E37 64.57 21.6 13.41 1 E39 65.00 20.12 14.63 1 W4 65.59 20.32 14.63 1 W44 65.59 20.32 14.63 1 W76 65.59 23.29 10.82 1 W76 65.11 21.21 13.72 1 65.11 20.31 14.01 1		W26	60.69	16.85	14.01	8.53
W67 70.11 18.50 10.89 1 W110 68.16 19.41 10.89 1 E21 66.19 19.41 14.30 1 E21 66.49 19.07 14.33 1 E21 66.49 19.07 14.33 1 E21 64.49 21.6 13.41 1 E39 65.00 21.46 13.43 1 E39 65.00 21.46 13.44 1 W4 65.59 20.12 14.63 1 W44 65.59 23.22 14.63 1 W44 65.59 23.22 10.82 1 W76 65.89 23.22 10.82 1 C65.11 21.21 13.72 1 C65.51 20.31 14.01 1		W48	62.04	22.13	15.81	10.93
Milo 68.68 20.59 10.72 1		W67	70.11	18.50	10,89	14.83
E21 66.59 19.07 14.33 E71 66.59 19.07 14.33 E71 64.49 22.08 13.43 E89 65.00 21.45 13.43 E89 65.00 20.12 14.5 13.54 E89 65.00 20.12 14.5 13.54 E89 65.59 20.32 14.63 14.64 E89 65.59 20.32 14.63 14.64 E89 65.59 20.31 14.04 65.40 23.22 10.07 14.06 E82 E89 65.40 23.22 10.07 14.06 E82 E89		W110	68.68	20.59	10.72	18.73
E21 66.59 19.07 14.33 14.33 15.45 15.49 15.49 15.41 13	Average (HF5	2)	66.19	19.41	14.30	14.55
E71 64.49 22.08 13.41 E87 64.57 21.6 13.83 E89 65.00 21.45 13.84 E103 65.48 20.12 16.01 E104 65.59 20.12 16.01 E105 65.59 20.12 16.01 E106 65.14 20.12 16.01 E107 E108 17.27 E108 E20.11 21.27 E20.11 13.72	elf fed (SF5)	821	66.59	19.07	14.33	7.74
E87 64.57 21.6 13.83 E89 65.00 21.6 13.84 E89 65.00 21.45 13.54 E89 65.00 20.12 16.01 14.65 19.95 19.57 14.63 14.65 19.57 14.63 14.65 19.57 12.27 14.65 19.57 12.27 12.27 12.27 12.27 12.27 14.65 19.57 12.21 13.72 12.21 13.72		E71	64.49	22.08	13.41	8.47
E89 65.00 21.45 13.54 13.54 13.54 13.54 13.54 13.54 13.54 13.54 13.54 14.63 14.63 14.65 14		E87	64.57	21.6	13,83	9.54
E103 63.88 20.12 16.01 WHY 65.54 20.32 14.63 WHY 65.59 19.67 14.64 WISC 65.89 23.32 10.82 65.11 21.21 13.72		E89	65.00	21.45	13.54	7.15
149 65.54 22.32 14.63 14.64 19.67 14.64 14.64 19.67 12.27 12.27 12.27 12.27 12.27 12.27 12.27 12.27 12.27 12.27 12.27 12.27 12.27 12.27 12.27 13.72 65.45 20.31 14.01 1		E103	63.88	20.12	16.01	6.4.3
W44 66.59 19.67 14.64 W52 64.40 23.32 12.27 W76 65.11 21.21 13.72 65.11 21.21 13.72		M9	65.54	2.3.32	14.63	4.68
H52 64.40 23.32 12.27 H76 65.89 23.29 10.82 65.11 21.21 13.72		477M	65.59	19.67	14.64	5.77
476 <u>65.89</u> <u>23.29</u> <u>10.82</u>		W52	64.40	23.32	12.27	7.89
65.11 21.21 13.72 65.65 20.31 14.01 1		M76	65.89	23.29	10.82	8.88
65.65 20.31 14.01	Average (SF5	()	65.11	21.21	13.72	7.39
TO*67 TC*07 CO*CO		ı			17.01	100
	verage or rot no	٠. ٢	60.60	70.07	T0.02	12.04

Table 8. Proportions and concentrations of VFA in rusen samples of lambs in lot No. VI.

Description	Lemb No.		Proportions		Total VFA
		Acetic	Tropionic	Butsvic	aw/loc ml
Hand fed (HF6)	56	66.18	19.63	14.18	17.69
	E35	67.64	13.68	16.44	16.50
	E69	71.10	14.24	14.65	8.94
	E108	68.55	15.28	16.66	14.72
	E109	09.99	18.81	14.58	13.43
	824	68.25	17.61	14.12	8.50
	W27	65.20	20.34	14.41	11.35
	1129	62.86	16.1	20.80	16.45
	W53	65.57	13.71	15.56	9.76
Average(HF6)		66.88	17.4	15.71	12.93
Self fed (SF6)	510	68,33	17.02	14.6	8.90
	E37	60.39	17.51	22.1	8.56
	E43	64.03	22.14	13.83	11,51
	Sec. S.	62,26	21.83	15.91	13.36
	WZO	67.76	18.44	13.80	5.89
	WZA	62.99	10.90	16.3,	8.39
	09M	64.79	14.07	21.13	84.6
	WBO	66.27	15.75	17.98	7.92
	868	62.99	23.90	13.07	7.03
Average (SF6)		64.85	18.62	16.58	9.01
Average of lot No. 6	9	65.86	18.00	16.14	11.62

Table 9. Analysis of variance. (Snedecor 1956).

	Source of Variation	Degrees of D freedom	Sum of squares	Mean square	F
Acetic acid	Treatment	5	215.2071	43.04142	.7826227
	method of feeding	1	38.234	38.234	.9348942
	treat. block interaction	5	257.0799	51.41595	.9348942
	error	96	5279.6534	54.99638	
Propionic	Treatment	5	196.5515	39.3103	.636954
Acid	method of feeding	1	.0129	.0129	.000209
	treat. block interaction	5	19.0072	38.61448	.625679
	error	96	592.4739	61.716	
Butyric	Treatment	5	103.203	20.64061	5.1303*
acid	method of feeding	1	.1403	.1403	.03488
	treat. block interaction	5	39.9699	7.99399	1.9869
	error	96	386.2324	4.02324	
Total VFA	Treatment	5	68.0855	13.617112	1.5424
mM/100 ml.	method of feeding	1	218.36801	218.36801	24.73554
	treat. block interaction	5	245.48172	49.09634	5.5613
	error	96	847.4983	8.8281	

^{*} Probability .05 ** Probability .01

Table 10. Av. Weights and efficiency of gain of the lambs.

Lot No. 1 Lot No. 1 Lot No. 1 Lot No. 1 Av. Lot No. 2 Lot No. 2 Lot No. 3 Av. Lot No. 3 Av. Lot No. 4 Lot No. 4 Lot No. 5 Lot No. 5 Lot No. 5 Lot No. 6 Lot No. 6	Description	Initial av. vt.	Final av. wt.	Av. gain per lamb	Av. daily gain per lamb	Lbs. of feed per lamb per day	Lbs. of feed per 100 W gein
10 69-4 105-7 36-3 -471 3-49 11 67-7 112-3 44-6 -579 4-19 12 67-7 1102-3 -44-6 -579 4-19 12 66-8 1100-6 33-7 -568 4-24 13 66-1 1104-2 36-1 -469 3-49 13 66-1 1104-1 44-1 -573 4-72 14 66-8 110-9 44-1 -573 4-72 15 66-0 105-7 -586 4-72 15 66-0 105-7 -586 4-72 16 67-1 102-6 4-72 -586 4-72 16 67-1 102-6 35-5 -586 4-72 16 67-4 109-4 4-20 -586 4-72 16 67-4 109-4 4-20 -586 4-78 16 67-4 109-4 4-20 -586 4-78 16 67-4 109-4 4-20 -586 4-78 16 67-4 109-4 4-20 -586 4-78 16 67-4 109-4 4-20 -586 4-78 17 18 18 18 18 18 18 18							
		FI) 69.4	105.7	36.3	.471	3.49	741.0
2. 66.9 100.6 40.5 .525 3.84 2.0 66.9 100.6 33.7 .438 3.49 2.2 66.9 110.6 43.7 .568 4,24 2.3 66.1 105.8 38.7 .568 4,24 3. 66.1 106.2 36.1 .469 3.49 3. 5 66.0 110.1 44.1 .573 4,17 4. 66.8 110.9 44.1 .521 4,10 4. 66.8 110.9 44.7 .588 4,07 5. 66.0 105.7 39.7 .530 3.78 5. 66.0 105.7 39.7 .569 3.72 6. 67.1 109.9 44.8 .569 3.72 6. 67.4 100.0 42.2 .46 42.2 6. 67.4 100.0 38.7 .569 4.14 6. 67.4 106.0 38.7 .569 4.14	Self fed (S	FL) 67.7	112.3	44.6	.579	4-19	723.7
(2) 66.9 100.6 33.7 .438 3.49 (2) 67.3 111.0 43.7 .568 4.24 (3) 67.1 110.8 38.7 .568 4.24 (3) 68.1 104.2 36.1 .469 3.49 (3) 68.1 110.1 44.1 .573 4.72 (4) 66.8 110.9 44.1 .573 4.10 (4) 67.3 112.6 45.3 .588 4.07 (5) 66.0 105.7 39.7 .516 3.78 (5) 66.0 105.7 39.7 .516 3.72 (6) 67.1 102.6 35.5 4.16 (6) 67.4 109.4 42.2 .465 (6) 67.4 100.4 38.7 .569 (5) 67.4 100.4 38.7 .563	IV. Lot No. 1		109.0	40.5	.525	3.84	732.4
(2) (6.5) 100.6 33.7 .438 3.49 (2) (6.7) 111.0 43.7 .568 4.24 (3) (6.1) 110.1 44.1 .568 4.24 (3) (6.1) 110.1 44.1 .573 4.72 (4) (6.8) 110.1 44.1 .573 4.72 (4) (6.8) 110.1 44.7 .520 4.72 (4) (6.8) 110.1 44.7 .530 3.48 (5) (6.0) 110.7 44.7 .530 3.78 (5) (6.0) 110.7 44.7 .530 3.78 (5) (6.0) 110.7 44.7 .569 3.72 (6) (7.1) 102.4 42.0 .569 3.73 (6) (7.1) 102.4 42.0 .569 4.14 (6) (7.4) 106.0 38.7 .503 3.78	ot No. 2						
(a) 66.7 111.0 43.7 .568 4.24 19.64.1 104.2 36.1 .469 3.49 (a) 66.0 110.1 44.1 .573 4.72 (b) 67.1 105.7 39.7 .588 4.07 (c) 67.1 102.6 4.7 .598 4.07 (c) 67.1 102.6 4.8 .569 3.72 (c) 67.1 102.6 4.8 .569 3.72 (c) 67.1 102.6 35.5 .461 3.43 (c) 67.1 102.6 35.7 .461 3.43 (c) 67.1 102.6 35.7 .461 3.78	-	F2) 66.9	100.6	33.7	6.38	3.49	206 8
105.8 38.7 .503 3.86 3) 68.1 104.2 36.1 .469 3.49 3) 66.0 110.1 44.1 .573 4.72 4) 66.8 110.9 44.1 .573 4.72 4) 67.3 112.6 45.3 .588 4.07 5) 66.0 105.7 39.7 .516 3.42 6) 67.1 102.6 35.5 .461 3.43 6) 67.4 109.4 4.20 36.7 6) 67.4 109.6 38.7 .503 3.78	Self fed (Sl		111.0	43.7	.568	4-24	766.53
(3) 68.1 104.2 36.1 .469 3.49 (3) 66.0 110.1 44.1 .573 4.72 (4) 66.8 110.9 44.1 .573 4.72 (4) 67.3 110.9 44.1 .573 3.48 (4) 67.3 110.6 45.3 .588 4.07 (5) 66.0 105.7 39.7 .516 3.42 (5) 66.0 105.7 39.7 .522 4.02 (5) 67.1 109.9 44.8 2.569 3.72 (6) 67.4 109.4 42.0 3.56 4.14 (6) 67.4 106.0 38.7 .563 4.14	v. lot No. 2		105.8	38.7	.503	3.86	771.6
(3) 66.1 1104.2 36.1 469 3.49 (3) 66.0 110.1 44.1 .573 4.72 (4) 66.8 110.9 44.1 .573 4.72 (4) 66.8 110.9 44.1 .573 3.48 (4) 67.3 112.6 45.3 .586 4.07 (5) 66.0 105.7 39.7 .516 3.78 (5) 66.0 105.7 44.7 .520 4.02 (5) 67.1 102.6 45.5 .466 3.72 (6) 67.4 109.4 42.0 .569 3.78 (6) 67.4 100.4 42.0 .565 4.14 (6) 67.4 106.0 38.7 .563 4.14	ot No. 3						
(4) 66.8 110.9 44.1 .573 4.72 (4) 66.8 110.9 44.1 .573 4.07 (5) 66.0 105.7 39.7 .588 4.07 (6) 67.1 102.6 45.3 .589 4.07 (7) 66.0 105.7 39.7 .516 3.42 (8) 67.1 102.6 4.8 .569 3.72 (9) 67.1 102.6 35.5 .461 3.43 (9) 67.4 106.0 38.7 .503 3.78	-	F3) 68.1	104.2	36.1	697°	67.5	7.744.1
(4) 66.8 110.9 44.1 .573 3.48 (4) 66.8 110.9 44.1 .573 3.48 (5) 66.0 105.7 39.7 .516 3.78 (6) 67.1 102.6 35.5 .461 3.78 (6) 67.4 109.4 42.0 .569 3.78 (7) 67.1 102.6 35.5 .461 3.43 (8) 67.4 109.4 42.0 .565 4.14 (9) 67.4 109.4 38.7 .503 3.78	Self fed (Sl		110.1	44.1	.573	4.72	823.7
(4) 66.8 110.9 44.1 .573 3.48 (4) 67.3 112.6 45.3 .588 4.07 (5) 66.0 111.75 44.7 .530 3.78 (5) 66.0 105.7 39.7 .516 3.42 (5) 66.1 114.2 49.7 .522 4.02 (6) 67.1 109.6 42.5 .661 3.43 (6) 67.4 109.4 42.0 .565 4.14 (6) 67.4 106.0 38.7 .563 4.14	v. Lot No. 3		107.15	40.1	.521	4.10	783.9
4,9 66.8 110.9 44.1 .773 3.48 4,0 67.3 112.6 45.3 .588 4.07 111.75 44.7 .530 3.78 5 66.0 105.7 39.7 .516 3.42 5 66.0 105.7 49.9 .622 4.02 6 7 67.1 102.6 35.5 .461 3.43 6 67.4 106.0 38.7 .565 4.14 6 67.4 106.0 38.7 .563 4.14	ot No. 4						
(4) 67.3 112.6 45.3 .588 4.07 5) 66.0 105.7 39.7 .516 3.42 5) 66.3 114.2 49.9 .622 4.02 6) 67.1 102.6 35.5 .461 3.43 6) 67.4 109.4 42.0 .565 4.14 106.0 38.7 .503 3.78	Hand fed (HI		110.9	44.1	. 573	3.48	607.3
5) 66.0 105.7 44.7 .530 3.78 5) 66.0 105.7 39.7 .516 3.42 5) 66.3 114.2 49.9 .522 44.02 6) 67.1 102.6 35.5 .461 3.43 6) 67.4 109.4 42.0 .565 4.14 106.0 38.7 .503 3.78	Self fed (SI		112.6	45.3	588	4-07	697.2
5) 66.0 105.7 39.7 .516 3.42 5) 66.3 114.2 49.9 .522 4.02 109.95 44.8 .569 3.72 6) 67.1 102.6 35.5 .461 3.43 6) 67.4 109.4 42.0 .565 4.14 106.0 38.7 .503 3.78	. Lot No. 4		111.75	44.7	.530	3.78	649.75
(5) 66.0 105.7 39.7 .316 3.42 (5) 66.3 114.2 49.9 .622 4.02 (6) 67.1 102.6 35.5 .461 33.43 (6) 67.4 106.0 38.7 .503 3.78	t. No. 5						
(5) 66.3 114.2 49.9 .622 44.02 109.95 44.8 .569 3.72 (6) 67.1 102.6 35.5 .461 3.43 (7) 67.4 109.4 42.0 .545 4.14 106.0 38.7 .503 3.78	-	5) 66.0	105.7	39.7	.516	3-42	8,649
109.95 44.8 .569 3.72 6) 67.1 102.6 35.5 .461 3.43 6) 67.4 109.4 42.0 .545 4.14 106.0 38.7 .503 3.78	Self fed (Si		114.2	49.0	.622	4.02	6.200
6) 67-1 102-6 35-5 .461 3.43 6) 67-4 109-4 42-0 .545 4-14 106-0 38-7 .503 3.78	v. Lot No. 5		109.95	8.44	.569	3.72	654.35
(6) 67-1 102-6 35-5661 3.43 (6) 67-4 109-4 42-0565 4.114 106-0 38-7503 3.78	ot No. 6						
6) 67.4 109.4 42.0 .545 4.14 106.0 38.7 .503 3.78		16) 67.1	102.6	35.5	.461	3.43	744.0
106.0 38.7 .503 3.78	Self fed (SE	6) 67.4	109.4	42.0	.545	4-14	759.6
	7. lot No. 6		106.0	38.7	.503	3.78	751.8

Table No. 11. Wet sieve analysis of the ration.

60	Description	6	16	Sieves 1	Sieves in Tyler Mesh 32 60	150	>150
	1. 6/64" Grind, mesh- given to Lot No. 6 % retained on the skeve	0	_	51.9	1.91	10.5	14.5
	2. 1/2" Grind mash given to 1, 2, 3 lots % retained	8.2	23.2	34.8	12.5	8.7	12.5
	3. 1 1/2 Grind mash for 3/4" pellet given to Log No. 4 % retained	16.5	20.9	33.2	5.4	O 1/2h	19.0
	4. 3/4" pellet given to lot No. 5 % retained	7.8	22.4	31.7	8.7	en 	21.1

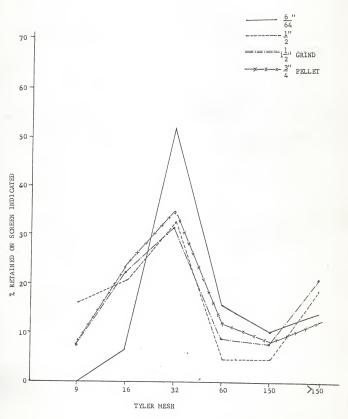


Figure 1

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EFFECT OF PARTICLE SIZE, HEATING AND PELLETING OF RATIONS ON VOLATILE FATTY ACIDS PRODUCTION IN THE RUMEN OF LAMBS

by

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Large amounts of volatile fatty acids are produced in the rumen by microbial fermentation. Several factors have been shown to alter the pattern of volatile fatty acid production. Objectives of this study were to determine the effect of particle size, heating and pelleting of rations on volatile fatty acid production in the rumen of lambs and to attempt to establish a relationship of production of VFA to enimal performance.

One hundred and eight feeder lambs, divided into 6 lots on the basis of weight and sex, were kept for 77 days on rations specifically designed for evaluating the effect of heating, pelleting and particle size on VFA production and feedlot performance. One-half the lambs in each lot were self-fed and one-half hand-fed.

Mixed rations of 55% alfalfa hay, 35% sorghum grain and 10% molasses were treated as follows and fed to the respective lots; (1) control-unheated; (2) hay heated to $180^{\circ}F$; (3) grain heated to $180^{\circ}F$; (4) pelleted and reground; (5) pelleted; (6) fine ground hay.

Rumen samples were collected by stomach tube 5 to 7 weeks after lambs were placed on the specific rations. The samples were frozen as soon as collected and later were analyzed for acetic, propionic and butyric acid by column chromatography.

Heating either the roughage or concentrate portion of the ration increased the butyric acid level in the rumen fluid. Particle size did not seem to influence VFA production, rate of gain, or efficiency of gain. Pelleting did not influence the VFA ratio in this trial, but total VFA mM/100 ml. was elevated. Pelleting produced more efficient and faster gains. In general a slight trend was observed between total VFA mM/100 ml. and efficiency of gain. The lots

that produced more VFA mM/100 ml. showed greater efficiency. Hand-feeding of pellets to lambs induced better efficiency. Hand-fed lambs gained less than self-fed lambs due to lowered feed consumption.