FURTHER STUDIES ON RUMINAL PARAKERATOSIS IN DAIRY CALVES

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INTRODUCTION

As with all other phases of agriculture, the animal industry has taken up the trend to increased efficiency of production. This trend has been stressed from two viewpoints. These are: (a) the push to greater total milk and meat output per animal; and (b) increased efficiency of this production. The field of animal nutrition has received much of this challenge due to the fact that the efficient modern high producer must be fed in such a way that maximum nutrient intake will occur along with maximum efficiency of utilization.

To help meet the challenge offered to the animal industry, several new methods of feed preparation for ruminants have been introduced. The more important of these methods include: (a) grinding to a greater degree of completeness; (b) pelleting part or all of the ration; (c) wafering the roughage portion of the ration; (d) steam treating certain of the grains; and (e) preparing all-in-one feeds containing a roughage material as well as concentrates in a predetermined ratio.

There have been several revisions in the feeding standards and feeding recommendations for dairy cattle during recent years. Whereas it was once felt that the protein level in the ration for ruminants was the limiting factor in performance, it is now widely agreed that the energy level is more often limiting. The revised feeding standards for ruminants largely pertain to the energy requirement of the animal. Due to the change in relative cost of many of the feedstuffs for ruminants,

it has become more practical, in many instances, to feed increased levels of the grains (concentrates) and lower levels of roughages. Prices for feedstuffs have been influential in the modification of feeding recommendations.

One of the major advances in the preparation of feed for animals has been the use of pelleted forms of concentrates, roughages, or both. Pelleted feeds have many advantages from the standpoint of mechanization of handling and storage. The storage space required for pellets is low due to the density of the pellet. Modern dairy operators can easily fit pelleted rations into their feeding program. Bulk feed handling in milking operations is made more efficient by the use of pelleted feeds. In addition to the added convenience in handling, pelleted feeds generally tend to promote a greater rate of intake by animals and increase palatibility in many cases.

There are some disadvantages in the use of pelleted rations for ruminants. Several investigators have observed a condition in the rumen called "ruminal parakeratosis" when pelleted feeds were used. This condition has been described as the incrustation and hardening of the papillae and the accumulation of excessive layers of keratinized, nucleated, squamous epithelial cells of the papillae. It has not been proven conclusively whether or not the condition has a consistent effect on milk production, rate of weight gain, or absorbtion of the end products of digestion from the rumen.

The specific objectives of this experiment were: (a) to determine the effect of urea vs. soybean meal as a protein supplement, on the incidence of ruminal parakeratosis and other rumen physiological conditions; and (b) to determine the effect of ruminal parakeratosis on

the absorption of volatile fatty acids (VFA) from the rumen.

LITERATURE REVIEW

Many of the topics reported upon by current researchers are related to ruminal parakeratosis. This review is meant to arrange some of these facts in such a way that their relationship to ruminal parakeratosis can be seen.

pH and VFA Production On Pelleted Feeds

Meyer et al. (29) have reported an increase in total VFA production in the rumina of sheep fed a pelleted form of roughage, and several workers have observed lower ruminal pH values on pelleted feeds. Cullison (9) suggested that a lowered rate of salivary secretion and, consequently, less neutralization of the rumen acids by the components of the saliva were important factors involved in bringing about the lower pH of the rumen. The fact that salivary secretion per pound of pelleted ration is less than that per pound of long roughage has been proven by Balch (3). Harris (15) observed that the pH of the rumen fluid from dairy calves fed pelleted grain and hay was significantly lower than that from calves fed hay in other forms. Hinders et al. (18) found that feeding dehydrated alfalfa pellets to dairy cows caused the ruminal pH to decrease from 6.9 to 6.0. The addition of Na_2HPO_4 raised the pH to 6.65 and decreased the titratable acidity of the rumen fluid. Salt and bone meal consumption by cows on pellets was noticeably greater. In further agreement with these workers, Rhodes and Woods (35) noted that

the pH in the rumina of lambs was decreased by feeding pelleted rations.

Harris (15) reported that the molar proportions of the VFA in the rumen did not differ greatly as a result of the feeding of hay in pelleted vs. long form. This is in contrast to work by King and Hemken (24) in which it was found that pelleting the ration did cause a difference in the molar proportions of the VFA in the rumen, however, it was suggested that lower hay consumption when pellets were fed may have been a factor. Harris (15) pointed out that when the ratio of hay to grain was changed from 1:1 to 1:4, the molar proportion of propionic acid increased while that of acetic acid decreased.

Characteristics of Rumen Ingesta and Rumination

Cole et al. (7) have reported that rumen ingesta of the frothy type contains a large amount of trapped gas. Harris (15) reported that animals fed pelleted rations had frothy ingesta which was subject to rapid expansion upon standing. Cole et al. (7) suggested that frothy ingesta results from incomplete expulsion of gas from the rumen by belching. Feeding long roughages causes an increase in the rate of belching by cattle. Meyer et al. (29) reported that CO_2 production was increased when pelleted rations were fed, and Rosen et al. (36) suggested that the CO_2 produced in the rumen by microbial decarboxylation of substrates is trapped in the frothy ingesta which has been stabilized by saponins and colloids. Thus, it appears that the incidence of frothy bloat might tend to be increased by the feeding of pelleted rations.

Garrett et al. (13) found that cattle fed pelleted rations spent less time ruminating than those fed long roughages. Similarly, Beardsley et al. (4) noted that steers fed finely ground, pelleted rations ruminated

very little, if any. Observations on rumination in dairy calves by Gilliland et al. (14) showed that calves on a ration including VFA salts ruminated slightly less than those on a control ration which did not include the VFA salts. It was suggested, however, that differences in intake level in favor of the control ration might have been responsible for these results.

With the rumen contents in a frothy, liquid condition when pelleted rations are fed, as noted above (7), it seems logical that the dry matter content of the ingesta would be lower than when long roughages are fed. However, Meyer et al. (29) found that although the ingesta was more fluid in appearance when pelleted feeds were fed, the percent dry matter was equal when compared to the injesta from cattle fed long hay.

Ruminal Parakeratosis - Incidence and Characteristics

Jensen et al. (21) observed parakeratosis in the rumina of 8.8% of fattened cattle in a random sample of 1535. He felt that the condition was related to rumenitis. In later work (22) he observed a high incidence of ruminal parakeratosis in lambs fed pelleted rations containing 40 to 50% alfalfa hay, but no parakeratosis in those trials where the lambs were fed chopped hay and unpelleted rations. In another trial which included 1569 animals, 39.2% of those fed pelleted rations had ruminal parakeratosis while only 7.7% of those fattened on pasture showed the condition.

In similar work, Hopkins et al. (19) reported that 38% of the lambs which were fed on various combinations of pelleted rations with 50% roughage developed ruminal parakeratosis. This compared to a 4% incidence in lambs fed unpelleted rations.

Thompson et al. (41) observed that feeding lambs a completely pelleted ration of 50% roughage resulted in changes in the appearance of the rumen mucous membranes which were characterized by increased length and width of the papillae, increased crusting of the tips and clumping of the papillae with the rumen contents, thickened corneum and increased accumulation of vesiculated, keratinized cells on the outer surface.

Beardsley et al. (4) observed tissue changes including ruminal parakeratosis in the rumens of steers fed a pelleted high-concentrate ration having a concentrate:roughage ratio of 70:30 and also in a control ration with long hay. The condition was not found in animals on a high-roughage unpelleted ration. In direct conflict to this work, McClure et al. (26) reported that pelleting rations with concentrate: roughage ratios of 75:25, 60:40, 45:55, and 30:70 had no effect on the occurance of ruminal parakeratosis.

Cullison (9) observed that steers on ground or pelleted rations which contained 30% hay had abnormal appearing rumen walls after 196-210 days on the ration. The appearance was characterized by the presence of abnormally long, dark colored papillae and by the sloughing off of areas of keratinized tissue from the rumen wall. Animals on a control ration which contained long hay did not develop the condition nor did animals fed straw plus the basic ground or pelleted ration. The length of time necessary for the development of ruminal parakeratosis was not determined, but one animal showed it after having been on the ration for 91 days. In a similar type of study, McCroskey et al. (28) observed a higher incidence of ruminal parakeratosis among animals in one trial fed a single ration containing 80% roughage than those fed the ration unpelleted. However, in other trials using the same rations no ruminal parakeratosis

was observed. Similar results were obtained by Haught et al. (16) in lambs fed pelleted or ground rations with 70% alfalfa.

A study was undertaken by Garrett et al. (13) to rate the severity of ruminal parakeratosis in relation to the method of preparation of alfalfa hay. The incidence of ruminal parakeratosis was greatest in steers fed either an all-alfalfa hay ration, or one with 50-60% concentrates, when the hay was ground and made into 1/4 or 5/8 inch pellets. The incidence was less when hay was fed which had been milled through a 1 inch screen or wafered after being ground through a 1.5 inch hammermill screen. When oat hay was added to the ration, an effective reduction in the severity of ruminal parakeratosis was observed, but the condition was not eliminated.

Harris (15) reported one trial with a 100% incidence of ruminal parakeratosis in 5-month-old dairy calves fed an all-pelleted ration which contained 50% alfalfa hay. A very slight amount of ruminal parakeratosis was observed in other calves fed similar hay in long or wafered form. In two subsequent trials in which the calves were fed alfalfa hay of higher fiber content in rations with 20% hay, either pelleted or long, no parakeratosis was observed.

Vidacs and Ward (44) observed changes in the rumen epithelial surface of fistulated cows similar to parakeratosis within 4-6 days after changing from hay to a beet pulp ration. Changing back to hay caused the epithelium to return to normal, but the recovery was slower than the development of the condition. When acetic acid was added to the beet pulp at a level found in the hay, the condition was arrested but not prevented. The addition of acetic acid to the hay ration slowed recovery. Hinders et al. (18) reported heavy ruminal parakeratosis among dairy cows

fed dehydrated alfalfa pellets as the only roughage. The addition of Na_2HPO_4 did not correct the condition. Palmquist and Ronning (31) observed that in cows fed pelleted alfalfa hay, the papillae of the rumen became very dark and hypertrophied. When the pelleted hay was replaced by long hay the condition was reversed. In related work, Gilliland et al. (14) found extensive ruminal parakeratosis in five out of eight calves fed a ration containing a 10% level of salts of propionic and butyric acids up to five weeks of age. In comparison to this, one calf out of eight fed a control ration with glucose in place of the VFA salts had the condition present in the rumen.

Related Conditions In Other Animals

Some workers have extended the study of ruminal parakeratosis to other animals besides ruminants. Vidacs et al. (43), in a study using white laboratory rats, found that when rumen fluid was fed from a cow with ruminal parakeratosis, nervousness and irritability occurred and was accompanied by keratinization of the squamous epithelium in the esophageal region of the stomachs of the rats. Another trial was conducted by the same workers (42) in which chickens were fed rations moistened with: (a) rumen juice from a cow with ruminal parakeratosis, (b) rumen juice from a cow with a normal rumen epithelium, (c) water, (d) water extract from dehydrated alfalfa pellets, and (e) water extract from dehydrated alfalfa hay. A condition similar to ruminal parakeratosis along with thickening of the epithelium was noted in the crops of chickens fed the ration which was moistened with rumen juice from the cow with ruminal parakeratosis.

Possible Causes of Ruminal Parakeratosis

Due to the wide variation in the incidence of ruminal parakeratosis reported in the literature, several theories have been asserted concerning the cause of the condition. Jensen et al. (21) believed that the condition was associated with rumenitis. In a later paper, Jensen et al. (22) stated that on the basis of the work which they had done with ground and pelleted rations, the condition appeared to be due to: (a) the finely ground feed having created a medium for bacteria to act and produce causative agents; (b) contaminants from the machinery used in the pelleting process; or (c) alterations of ingredients during processing. Cullison (9) reported that the low rumen pH on pelleted feeds, associated with ruminal parakeratosis, was possibly due to lack of salivation and subsequent neutralization of the VFA. Similar observations on ruminal pH were reported by other workers (15, 18, 35); however, ruminal parakeratosis has not always been associated with this situation (15). Garrett et al. (13) suggested that ruminal parakeratosis was related to the small feed particle size of ground and pelleted rations along with a higher rate of VFA production, less rumination, and a likely decrease in the buffering capacity of the rumen contents. On the basis of the results obtained with beet pulp rations Vidacs and Ward (44) suggested that a low acetate:propionate ratio in the rumen was the cause of the condition. In later work, Vidacs et al. (42) suggested that ruminal parakeratosis is a general reaction which occurs among ruminants fed pelleted diets and is caused by a chemical factor, a deficiency, or some special causative agent (possibly a heat-sensitive protein-bound factor). It is of interest to note that in the work by Harris (15) the animals which

were observed to have ruminal parakeratosis were fed pelleted rations which contained 50% roughage, while those which did not have the condition were fed rations with only 20% of a higher fiber hay. Similar results appear in the data of Garrett et al. (13). This data, along with the other theories presented above, tend to suggest that ruminal parakeratosis is caused by a combination of factors which act together to result in the observed condition. This might explain the discrepancy in the reported observations regarding the incidence of ruminal parakeratosis, since the lack of one of the factors might prevent the incidence of the condition.

VFA Absorption and Utilization

The subject of VFA absorption from the rumen is a topic of long-time interest. Danielli et al. (10) reported that at pH 7.5 the order of absorption is: acetic > propionic > butyric while at pH 5.8 the order is: butyric > propionic > acetic. It was suggested that more free acids would probably be absorbed at low pH since more would be present in proportion to the anion form. It was also suggested that these free acids would be absorbed at a faster rate and by different mechanisms than would the anions. At low pH, the fatty acids leave the rumen through lipid and water filled pores, while at high pH the anions leave the rumen by simple diffusion. Similarly, Sutton et al. (40) reported that absorption of total VFA is increased at a low rumen pH and acids of longer chain length are absorbed at a greater rate. These data agree with work by Annison et al. (1) in which it was found that as the rumen pH was lowered, the order of absorption of the VFA changed from one of near equality to: buteric > propionic > acetic.

However, Blaxter (5) suggests that the rate of absorption of the individual VFA is more closely related to the production of each. Bloomfield et al. (6) reported that total VFA absorption from the rumen increased at low pH while rumen ammonia absorption decreased. Kiddle et al. (23) observed that the proportion of fatty acids in the blood draining the rumen was similar to that of the acids in the rumen with the exception that less butyric acid was present. Pennington (32) reported that while each of the VFA is metabolized to a certain extent by the rumen epithelium, butyric acid is used in the greatest amount, its end product being ketone bodies. It was suggested that the energy obtained by the metabolism of butyric acid in the rumen epithelium is used in the "active absorption" of other metabolites. Shaw et al. (37) did not find butyrate to be metabolized to such an extent as previous work indicates.

Rhodes and Woods (35) observed a greater utilization of butyric acid by rumen epithelium among sheep on pelleted rations compared to those on long hay. Haught et al. (16) reported no difference in the amount of VFA absorbed among sheep on ground or pelleted rations. No ruminal parakeratosis was noted in the same animals. Vidacs et al. (45) suggested that VFA absorption is not uniform over the rumen surface and may be altered by lesions caused by the incidence of ruminal parakeratosis. Hinders and Owen (17) indicated that absorption of total VFA from the rumen decreases as ruminal parakeratosis develops. Similar work was done by Harris (15) in which a buffered VFA solution and polyethylene glycol were introduced into the emptied rumen. While VFA absorption from the rumen apparently increased during the early stages of the development of ruminal parakeratosis, absorption decreased as the condition became more severe.

Effect of Ruminal Parakeratosis On Performance

The proof of the significance of ruminal parakeratosis is in the effect produced on the performance of the animal, whether as a meat animal or as a milk producer. Jensen et al. (22) found that among the lambs in the lot in which ruminal parakeratosis was noted, those with normal rumina gained an average of 0.3889 lb./day as compared to 0.3213 lb./day for those which had ruminal parakeratosis. Garrett et al. (13) observed that steers fed long oat hay and finely ground pelleted rations made greater gains and had larger fat-corrected carcasses than did those fed the same rations without the oat hay in which severe ruminal parakeratosis was noted. Beardsley et al. (4) observed that gains decreased among steers fed various roughage:concentrate ratios in pelleted form as the proportion of concentrates increased. It was also noted that a high incidence of ruminal parakeratosis occured on the high-concentrate pelleted ration. A low feed intake on the high-concentrate ration might have been responsible for the lower gains. Cullison (9) found a lower average rate of gain among steers with ruminal parakeratosis on a ration which contained 30% roughage. No definite assumption could be made since feed intake was lower in the group with lower gains. It was also noted that the feeding of long oat straw increased the consumption of pelleted rations, increased the rate of gain and also reduced the incidence of ruminal parakeratosis.

Hopkins et al. (19) noted that pelleted rations containing 50% roughages caused an increase in feed efficiency when fed to lambs. A relatively high incidence of ruminal parakeratosis was noted among the lambs on the pelleted ration but no comparison was made within the group

to determine the effect of the parakeratosis on the rate of gain. McCroskey et al. (28) found that although considerably lower rates of gain were produced among steers in one group fed a pelleted ration with a 4:1 ratio of grain to hay, no ruminal parakeratosis was observed. In another trial in which ruminal parakeratosis was observed, there appeared to be little relationship between the condition of the rumen wall and the rate of weight gain by the steers (27).

Kunkel et al. (25) reported that lambs with mucosal damage in the rumen of a different type than ruminal parakeratosis had lower rates of weight gain, but there was no relationship between the incidence of mucosal desquamation and diet, treatment, the size of papillae, or the pigmentation of the rumen. Rhodes and Woods (35) observed increased rates of gain when pelleted rations were fed to lambs. It was also noted that the pelleted rations were associated with dark colored rumens. McClure et al. (26) reported that pelleting a ration containing 75% concentrates lowered rates of gain in lambs but that no difference in the incidence of ruminal parakeratosis was noted between lambs fed pelleted or unpelleted rations.

Although the effect of ruminal parakeratosis on milk production has not been accurately determined, some workers have suggested that there is a relationship. Hinders et al. (18) fed four pairs of identical twins (three lactating and one dry) on rations of alfalfa hay or dehydrated alfalfa pellets. The pelleted ration caused heavy ruminal parakeratosis and also a drastic reduction in milk yield and fat percent. When the figures are placed on an equal fat basis, the average fat-corrected-milk (FCM) production per day by the cows on alfalfa hay was 41.2 pounds compared to 27.6 pounds for the same cows fed dehydrated alfalfa pellets and

having ruminal parakeratosis. The results of work done by Garrett et al. (13) prompted the suggestion that a relationship exists between rations causing ruminal parakeratosis and those causing low-fat milk production. On the basis of the limited information available, it is felt that ruminal parakeratosis does have an effect on the milk production performance of dairy cattle. Whether the effect is due to a decrease in the absorption of the VFA or to other factors remains to be investigated.

EXPERIMENTAL PROCEDURES

A single trial, using 24 male Ayrshire calves, was conducted to test the effect of different pelleted rations on the incidence of ruminal parakeratosis and other chemical and physiological conditions in the rumen. A second trial, using a pair of male Ayrshire calves and a pair of male Holstein calves, was conducted to determine the effect of ruminal parakeratosis on the absorption of volatile fatty acids (VFA) from the rumen. Polyethylene glycol (PEG), a nonabsorbable substance, was used in this trial as a marker.

Pre-trial Period

The calves used in both of the trials were obtained from the Oklahoma State University dairy herd and three cooperating dairymen in the state. The calves were left with their dams for 72 hours after birth, after which they were put into individual metal calf pens, bedded with wood shavings, and fed whole milk at the daily rate of 10 percent of the 3-day weight. The calves continued on this feeding program until they were five weeks old, at which time they were weaned. In addition to the milk, the calves were offered a calf starter containing 80% grain and 20% alfalfa pellets. This starter was fed free choice along with access to fresh water at all times. After the calves were weaned, long alfalfa hay was offered free choice along with the grain. Weekly body weight determinations were made to check on growth progress and daily observations were made to insure that

prompt attention was given to any disorder. This management program was continued until the calves were 8 weeks of age at which time they were transferred to another barn and started on the experiment.

Experimental Period

Trial I: The 24 calves were assigned to the four pelleted experimental rations in a randomized block design to help correct for any deviations in performance due to season. Each animal was maintained on the same ration assigned to him at the start for the entire 12-week experimental period. The four pelleted rations were: (a) 1:1 ratio of pelleted grain to pelleted hay, grain with 1% urea; (b) 1:1 ratio of grain to hay, no urea; (c) 4:1 ratio of grain to hay, grain with 1% urea; (d) 4:1 ratio of grain to hay, no urea. The grain and hay pellets were weighed, mixed, and fed in amounts large enough to allow maximum intake with minimum refusal and still maintain the desired ratio of grain to hay. Feeding was done twice daily. The refused feed was weighed weekly and the amount recorded.

The calves were housed in individual stalls equipped with expanded metal screens to alleviate the need for bedding. Water was available at all times. Daily observations were made concerning appetite and possible physical disorders. The calves were weighed weekly and heart girth measurements were made to indicate growth rate and performance.

Rumen fluid samples were taken at 4-week intervals starting at 12 weeks of age. The sampling was done three hours after the morning feeding with a stomach tube equipped with a strainer as described by Raun and Burroughs (33). The pH of the rumen fluid was determined using a Beckman Model N portable pH meter with a single glass electrode. The sample was

TABLE I

COMPOSITION OF PELLETED GRAIN RATIONS

RATION I		RATION II	
Ingredient	%	Ingredient	%
Ground corn	38.0	Ground corn	33.0
Ground milo	20.0	Ground milo	20.0
Crimped oats	15.0	Crimped oats	15.0
Wheat bran	10.0	Wheat bran	10.0
Dried molasses	5.0	Dried molasses	5.0
Dicalcium phosphate	1.0	Dicalcium phosphate	1.0
Trace mineral salt	0.6	Trace mineral salt	0.6
Quadrex[a]	0.1	Quadrex[a]	0.1
Aurofac 10[b]	0.3	Aurofac 10[b]	0.3
Soybean meal	9.0	Soybean meal	15.0
Urea	1.0		
	100.0		100.0

[a]Contains vitamin A, 10,000 I.U. and vitamin D_2, 1,250 I.U./gram.

[b]Contains aureomycin (chlortetracycline hydrochloride), 10.0 grams/lb.

then divided into two equal parts for VFA and rumen NH_3 analysis, respectively. Saturated mercuric chloride solution (1 ml/100 ml rumen fluid) was added to the VFA sample to stop microbial activity. Both samples were centrifuged to remove small feed particles. The samples to be analyzed for rumen NH_3 were diluted 50:50 with 0.1N HCl to prevent loss of the NH_3. Approximately 15 ml of the rumen samples for VFA and NH_3

saved. The rumen samples taken upon slaughter were obtained directly through a small incision in the rumen wall as soon after death as possible and were strained through cheese cloth. Otherwise they were processed in the same manner as the other samples. All processed rumen fluid samples were frozen until the analyses could be made.

A six hour observation on rumination and the time spent eating, drinking, and lying down was made after each of the first 20 calves had been on the experimental ration for six weeks. The observation period was started two hours after the morning feeding.

In addition to the above mentioned procedure, the last four calves in the trial were maintained on the experimental rations an extra month using low fiber pelleted alfalfa hay. Samples were taken at two week intervals and at slaughter for the determination of total VFA and buffering capacity in addition to pH, VFA proportion, and rumen NH_3. The total VFA sample (100 ml) was strained through cheesecloth and 1 ml of saturated mercuric chloride was added to stop microbial activity. The sample was then frozen pending the laboratory analysis for total VFA. The buffering capacity of the rumen fluid was determined immediately using the method of Nicholson et al. (30), in which the buffering capacity is expressed as the number of milliequivalents of HCl required to lower the pH of 100 ml of strained rumen fluid to pH 4.5. A pH meter was used to indicate the pH in this study.

The calves were slaughtered at 20 weeks of age to observe any development of ruminal parakeratosis. This was done in the Anatomy Laboratory of the College of Veterinary Medicine. The rumen of each calf was removed and the weight of the contents and the washed reticulo-rumen was determined. The washing was done by careful dipping in water. The

rumen wall was examined for incrustation, clumping, or discoloration of the papillae. The liver of each animal was given a visual check for the presence of abscesses. A 2X2 inch square section was cut from the dorsal sac of the rumen and preserved for further examination.

Trial II: Two pairs of male calves, one Holstein and one Ayrshire, were raised up to 2 months of age using management practices similar to those described in Trial I. At approximately 2 months of age the calves were fitted with plastic rumen fistula plugs, 3 inches in diameter. It was decided that due to the rather large size of the fistula used in relation to the body size of the calf, some modifications in the method commonly used were needed. Therefore, a transverse incision was made in the body wall as high in the region of the paralumbar fossa as possible. The fistula plug was inserted and the skin was sutured together around the plug. The results were very desirable and almost no leakage occurred.

The fistulated calves were placed on expanded metal screens with no bedding. One member of each pair of calves was assigned to each of two experimental rations. These were: (a) 1:1 ratio of pelleted grain to pelleted alfalfa hay; (b) 1:1 ratio of pelleted grain to long alfalfa hay. The rations were fed in amounts large enough to allow maximum intake and minimum refusal, while keeping the desired ratio of grain to hay. Feed refusals were weighed back daily. The grain ration designated as Ration II in Table I was used for this trial.

Rumen samples were taken at two week intervals by dipping some of the contents out through the fistula. The samples were prepared for the determination of pH, VFA proportion, rumen NH_3, total VFA, and buffering capacity as mentioned above. In addition, samples were taken for the determination of the dry matter of the rumen contents.

Absorption Studies

Determination of the absorption of VFA from a buffered VFA solution introduced into the rumen was made at 4 and again at 5 months of age. The rumen was emptied by removing the fistula plug, removing the rumen contents, and washing the rumen about 6 times with a total of about 3 gallons of warm water. This insured that almost all of the material was removed from the rumen. The fistula plug was replaced and 3 liters of a buffered VFA solution (Table II), at pH 6.6, containing known amounts of acetic, propionic, butyric, and valeric acids as well as polyethylene glycol (PEG) was introduced. A portable pH meter was used to check the pH of the solution and 10N H_3PO_4 was used to maintain the pH at about 6.6. Fifteen milliliter samples of the solution were taken through the fistula for PEG and VFA analysis at 5 minute intervals for the first 30 minutes and at 30 minute intervals after that until 120 minutes had elapsed. At the end of 120 minutes, a concentrated solution of PEG was introduced into the VFA solution in the rumen and samples were taken at 5 minute intervals for 15 minutes to measure the final volume of the solution.

Preparation of Test Solutions Used in Absorption Study

The test solutions introduced into the washed rumen were similar to those used by Sutton et al. (39), the difference being the modification by Harris (15) which eliminated the use of calcium chloride in the buffer because of erroneous readings on the colorimeter in the determination of polyethylene glycol (PEG).

The solution of buffer and VFA was made up to 2.5 liters and the final .5 liter containing the PEG was made up and added just prior to the

addition of the solution to the rumen to avoid deterioration of the PEG. The pH of the test solution was adjusted to 6.6 with 5N NaOH during the preparation. A pH check was made prior to the mixing of the VFA and phosphate buffer solutions to insure that the pH of both solutions was 6.6. A final pH check was made just prior to the addition of the test solution to the rumen.

TABLE II

COMPOSITION OF TEST SOLUTION INTRODUCED INTO THE RUMEN

COMPONENT	g/3 liters
VFA, pH 6.6	25.5042
Acetic	13.3847
Propionic	6.6026
Butyric	4.5918
Valeric	0.9251
K Cl	1.120
KH_2PO_4	0.410
$MgSO_4 \cdot 7H_2O$	0.739
Phosphate buffer, pH 6.6	74.0428
KH_2PO_4	20.3583
$Na_2HPO_4 \cdot 12H_2O$	53.6845
Polyethylene glycol (60 mg/100 ml.)	1.80

The reason that the PEG (mol wt 4000) was included in the test solution was to serve as a nonabsorbable marker to permit absorption to be distinguished from dilution. The source of this dilution could be saliva or fluid moving across the rumen wall by osmosis. Losses can also

occur as some of the fluid moves down the digestive tract. In this study the analysis for PEG was carried out by the turbidimetric method of Hyden (20). The amount of VFA absorbed was determined by subtracting the VFA concentration at different time intervals, corrected for dilution, from the initial concentration.

Determination of Rumen Ammonia

The rumen fluid samples preserved for determination of rumen ammonia were analyzed using the method of Conway (8) as modified by Sherrod (38). Two milliliters of rumen fluid (diluted 50:50 with HCl) were incubated for 1 hour at 40° C with saturated K_2CO_3 in a Conway unit. The ammonia released from the rumen fluid was absorbed by a 1% boric acid-indicator solution in the center well of the unit. The ammonia in the center well was titrated with standardized HCl to give the concentration of ammonia in millimoles per 100 ml of rumen fluid.

Determination of Individual VFA

The relative proportion of the VFA in all of the rumen samples was determined using a modification of the gas chromatograph method of Erwin et al. (11). The samples were prepared for analysis by centrifuging 5 ml of the rumen fluid with 1 ml of 25% metaphosphoric acid. The supernatant was saved for analysis. A 10 µl sample of the prepared rumen fluid was injected into the injection block which was maintained at 300° C to quickly vaporize the sample. The instrument used was an Aerograph Model A-600-B "Hi-Fi" with a hydrogen flame ionization detector. A gold-plated detector shield was used to reduce corrosion problems. A range of sensitivity of 1 million was available on the instrument through the use of an 11-step

attenuator which reduced the response by half at each step from 1 to 1000, a high-low input impedence control (10^9 to 10^7 ohms) which reduced the signal by 100 times, and an output sensitivity control which increased the output signal by 10 times.

An Aerograph hydrogen generator model A-650 was used to supply filtered hydrogen (20 ml/min) and air (300 ml/min) which were necessary for the operation of the flame ionization detector. The hydrogen produced by the generator came from water by the process of electrolysis. A 36 inch column, 1/8 inch in diameter, packed with carbowax 4000 was used with an oven temperature of 125° C.

The fatty acids in the rumen samples and standard were eluted with nitrogen as a carrier gas. With a nitrogen flow rate of 30 ml/min, sharp symmetrical peaks were obtained in approximately 18 minutes, the order being acetic, propionic, butyric and valeric. The eluted fatty acid peaks were recorded by a Sargent Model SR recorder and were measured by the method of triangular approximation by which the area of the peak was determined as the product of the height of the peak and the width of the peak at half-height. The measurement of the peaks was done with a ruler graduated in millimeters.

In the latter part of the VFA work, oxygen (60 ml/min) was used in place of the air in the hydrogen flame ionization detector. This increased the sensitivity and stability of the machine by a large degree.

Preparation of VFA Standard

A standard solution of VFA which contained known amounts of acetic, propionic, butyric, and valeric acids was prepared for use in the quantitative determination of the individual fatty acids in the rumen

samples. The standard (Table III) contained a quantity of VFA closely corresponding to the average reported level (12 mM/100 ml) for rumen fluid and the individual acids were in the proportion of acetic, 60%; propionic, 25%; butyric, 13%; and valeric 2%. In order to compute the amount of each of the acids needed in the standard, the proportion of each was multiplied by 12 and then by the millimolar weight of the individual acid. This figure was multiplied by 10, with the result being the respective weight of each of the acids needed to prepare a liter of standard. These amounts were weighed out by difference into a small, stoppered volumetric flask on an analytical balance. The acids were then emptied into a one-liter flask and the weighing flask was rinsed several times with distilled water. After making the solution up to one liter, stoppering, and shaking to mix the solution, the flask was stored in the cooler until it was needed.

TABLE III

STANDARD VFA SOLUTION

Acids	g/liter	µg/µl	µM/10 µl
Acetic	4.3246	4.3246	0.7201
Propionic	2.2773	2.2773	0.3074
Butyric	1.3244	1.3244	0.1503
Valeric	0.2572	0.2572	0.0251

Calculation of VFA

The fatty acids in the rumen fluid were calculated from the peaks obtained after injecting 10 µl of the prepared sample into the gas chromatograph instrument. The amount of each individual fatty acid in the sample was determined by dividing the area of the peak obtained for the rumen fluid sample by that obtained for the same acid in the standard VFA solution and multiplying this by the calculated number of micromoles of VFA per 10 µl of standard. In doing this, it was assumed that the peaks obtained after injection of the standard represented the computed number of micromoles per 10 µl.

To minimize any sampling errors, several samples of the standard were used and the results averaged to get the respective factors. Also, the first one or two samples analyzed at the start of a series of samples were discarded to minimize any alterations in the equillibrium of the column as suggested by Erwin et al. (11). Samples of the standard were injected at the beginning and end of each series of samples.

Determination of Total VFA

The total VFA in the rumen fluid were determined by a modification of the method of Fenner and Elliot (12). Ten ml of centrifuged rumen fluid were adjusted to pH 2.0 with H_2SO_4, antifoam agent was added, and the sample was steam distilled for 20 minutes. The distillate was titrated to a phenolphthalein endpoint with standardized NaOH (0.0620 N) from which the milliequivalents of acid were determined. A water blank, adjusted to pH 2.0 with H_2SO_4, was distilled and titrated. The titre thus obtained was used to correct the titration values for the samples.

A VFA standard was used to standardize the procedure and a recovery of 99% was obtained on the standards.

Analysis of Feeds

A proximate analysis was determined on composite samples of all of the pelleted feeds used in both trials. In addition to this, a method was devised to measure the density of the pelleted feeds. The results of these analyses may be found in Table IV.

To determine the pellet density, a sample of the pellets was weighed and then placed in a graduated cylinder containing a known amount of water which, when the pellets were added, would not rise above the graduations of the cylinder but would completely cover the pellets. The final volume of the water was immediately noted and the density of the pellets was calculated as the number of grams of feed per cubic centimeter of volume of the pellet. The final volume of the water was noted immediately to avoid absorption of water by the pellets.

TABLE IV

PROXIMATE ANALYSIS AND PELLET DENSITY OF PELLETED FEEDS USED IN ALL TRIALS

Nutrients	Pelleted Grain (Ration I)	Pelleted Grain (Ration II)	Pelleted Hay (Early Cut)	Pelleted Hay (Regular Cut)
Crude protein (%)	14.80	14.20	21.55	17.55
Fat (%)	3.43	3.40	1.99	1.70
Carbohydrates				
NFE (%)	60.80	61.02	41.32	36.80
Crude fiber (%)	3.88	4.51	16.19	28.80
Moisture (%)	10.02	10.08	9.62	8.98
Ash (%)	7.07	6.79	9.33	6.17
Pellet density (gr/cc)	1.21	1.23	1.13	1.05

RESULTS AND DISCUSSION

Examination of Rumina Post-mortem

Upon slaughter of the calves in Trial I of this study, the rumina were removed and examined for the occurrance of ruminal parakeratosis and any other anatomical abnormalities associated with the papillary surface of the ruminal epithelium. While none of the animals in the trial developed the parakeratosis condition to any extent (Table V), some interesting observations were made. Only 23 of the original 24 calves in the trial were slaughtered, 1 being lost due to bloat.

All of the animals fed the 1:1 grain to hay ration with urea and four of the animals fed the 1:1 ration without urea exhibited slight amounts of ruminal parakeratosis in the form of capping of the papillae in scattered areas of the rumen. In direct contrast to this, none of the animals fed the 4:1 grain to hay rations showed capping of the ruminal papillae. While of a much lower degree of severity, these results are similar to those reported by Harris (15) who found that animals fed a grain to hay ratio of 1:1 developed severe ruminal parakeratosis and those fed a 4:1 grain to hay ratio did not develop the condition. Since the animals in the present trial were fed a pelleted hay of higher fiber content (16.2% vs. 28.8%) than were those fed by Harris (15), while all other feeding and management practices were similar, a difference in fiber content of the ration may have been a contributing factor in the failure of the animals to develop the condition to the

extent observed previously (15).

At 4 months of age, the last 4 animals on the trial were switched from the hay ration which they had been receiving and were fed early cut, low fiber alfalfa pellets along with the grain. The rations fed were all in the ratio of 4:1 grain to hay. Upon slaughter of these animals at 6 months of age, the rumina were free from parakeratosis but clumping of the papillae was observed. These results, along with the fact that the animals used in the absorption work in this study developed parakeratosis when fed the same grain and hay in a 1:1 ratio, suggest a relationship between the feed ratios used and the development of ruminal parakeratosis. These observations do not agree with most of the reported work in that most workers have observed ruminal parakeratosis in high concentrate feeding situations.

Another interesting observation made during the post-mortem examination of the rumina was the incidence of the clumping together of the papillae in those animals fed the 4:1 grain to hay rations. It was noted that clumping of the papillae occurred in all of the animals fed the 4:1 grain to hay diets, whereas clumping was only noted in 50% of those animals fed the 1:1 grain to hay diet. The clumping of the papillae was characterized by many groups of papillae being tightly packed together in firm clusters. The clusters found in this study were from 1-2 inches in diameter and could be felt by palpation of the rumen wall through the serosa. When the papillae in these clumps were separated, there appeared to be a large amount of extremely dry feed particles binding the papillae together. From the manner and tightness with which the clumps were held together, it seems very unlikely that any rumen fluid would penetrate these clumps after they were once formed even though the rumen contents of those animals

on the 4:1 diets were more liquid than that of the calves fed the 1:1 diets. The clumping of the papillae may be of importance in animal performance.

Areas of the rumen walls in 3 of the calves fed the 1:1 grain to hay ration without urea were found to be eroded and devoid of papillae. The majority of this denuded area was noted in the ventral areas of the rumen. Only one other incidence of this was noted, that being in a calf fed the 1:1 ration with urea. The reason for this occurrance cannot be explained at this time on the basis of the data obtained in this trial.

Several interesting conditions were noted which did not appear to be related to the diet of the animal. In 7 of the 23 rumina examined, scattered areas of red-tipped papillae were observed. The surface of these papillae was very soft and had the appearance of new tissue. The cause of these red-tipped papillae is not known, but it is possible that these papillae were previously capped and the caps rubbed off exposing the developing layers of the epithelium. Another possible cause might be the accumulation of blood in the capillaries of the papillae due to poor drainage at the time of sacrifice. In contrast to previously reported work (4, 25, 31, 35) in which pelleted feeds caused dark colored rumina, only 4 of the animals in this study were found to have this condition. Although no study was done to determine the reason for the variations in rumen color observed, the fact that the darker colored rumina appeared in those Ayrshire calves which had a predominance of red in the hair coat might suggest that work is needed to determine the relationship between skin and hair pigmentation and that of the rumen.

Since no real incidence of ruminal parakeratosis was observed in this trial, no further work with these rumina was conducted from the

standpoint of the histological and cytological study of the condition of ruminal parakeratosis.

TABLE V

OBSERVATIONS ON RUMINA OF CALVES SLAUGHTERED AT END OF EXPERIMENT

No. of calves per group[a]	Ratio of grain:hay	Degree of parakeratosis	Degree of clumping of papillae
5	1:1 (with urea)	Very slight	Slight
5	1:1	Very slight	Slight - moderate
6[b]	4:1 (with urea)	None	Severe
7	4:1	None	Severe

[a]No. of calves at end of experiment. Last 2 calves on 1:1 ratio of grain to hay changed to 4:1 ratio 1 month prior to slaughter. This is the reason why more calves are listed in 4:1 groups.

[b]One calf lost due to bloat.

Observations on Ruminal pH

In agreement with work reported by others (9, 15, 35), the pH of the rumen fluid taken from the calves fed the pelleted diets in this study was lower at 3 hours after feeding than is usually expected when long roughage is fed (Table VI). The normal pH value is close to 6.9 for cattle fed long roughage (18). There did not seem to be any pronounced effect on the ruminal pH when urea was fed as compared to when it was not fed; however, a slight increase in the ruminal pH at slaughter was noted

when the rations containing urea were given. This change could be due to the buffering action of ammonia.

The pH determinations made on the rumen fluid taken at the time of slaughter are considered to be more accurate than those made on rumen fluid obtained by the stomach tube. The use of the stomach tube and pump may cause high readings due to saliva which may collect in the anterior region of the rumen. Another source of error involved in the use of the stomach tube is the lack of consistancy with which the tube may be positioned in the rumen prior to the taking of the sample, thus getting unrepresentative samples. In several cases during the present study the samples obtained through the stomach tube had a pH value greater than 7.0 and appeared to have excessive amounts of saliva mixed with the rumen fluid in the sample. The presence of varying amounts of saliva in the stomach tube samples is thought to be the reason why the average pH value of these samples was higher than that of the samples taken at slaughter. It is very likely that the low ruminal pH values observed in this study were due to a lack of sufficient salivary secretion to neutralize the end products of the fermentation reactions occurring in the rumen. Balch (3) reported that when long, dry roughages were limited or removed from the diet, salivary secretion decreased.

The pH of the rumen fluid samples taken from the bottom of the rumina of the calves in Trial II was higher (6.2 vs. 5.8) than that of the samples from the center of the rumina.

Proportion of Ruminal VFA

The results obtained from the analysis of the rumen samples for the molar proportions of the VFA are reported in Table VII. A relatively

TABLE VI

pH OF RUMEN FLUID AT 1-MONTH INTERVALS

Ratio of grain:hay	No. of calves	Age at sampling (months) 3[a]	4[a]	5[b,c,d]
1:1 (with urea)	6	6.16	6.52	5.70
1:1	6	5.88	6.51	5.50
4:1 (with urea)	6	6.06	6.16[e]	5.62
4:1	6	5.89	6.54	5.54

[a]Samples taken by stomach tube approximately 3 hr. after feeding.

[b]Samples obtained from rumen immediately after slaughter.

[c]Average of 5 calves in 1:1 rations; 6 calves in 4:1 ration with urea; 7 calves in 4:1 ration without urea. Last calf on each 1:1 ration changed to 4:1 ration at 4 months of age; last 4 calves on 4:1 rations slaughtered at 6 months of age.

[d]Statistically significant ($P < 0.05$): Urea vs. non-urea rations.

[e]Average of 5 calves; one calf lost due to bloat.

large difference in the molar proportions of acetic and propionic acids was noted between the 4:1 and 1:1 grain to hay rations, with an increase in the propionate and decrease in the acetate proportion noted when the 4:1 grain to hay ratio was fed. These results agree with those reported by several workers (15, 34, 44) in which a 4:1 grain to hay ratio caused a narrowing of the acetate to propionate ratio as compared to a wider ratio when a higher level of roughages was fed. There was no difference between rations in the levels of butyrate and valerate in the rumen samples taken in this trial at 3 and 4 months of age but an increase in the molar percent of butyrate was noted at slaughter in the animals fed urea (11.6 vs. 8.8).

As can be seen from the data presented (Table VII), the molar proportions of acetate and propionate in the rumen can be changed to an appreciable extent by dietary means. In terms of the efficiency with which the produced VFA are used for energy under practical conditions of fattening and growth, the mixture of VFA in the rumen with a high proportion of acetate is not used with as great an efficiency above maintenance intake levels as when a mixture containing a greater proportion of propionate is present in the rumen (2, 5). The heat loss incurred by the high acetate ratio accounts for much of the difference in efficiency (2).

Observations on Ruminal Ammonia

The rations which included urea in Trial I tended to cause slightly higher ruminal ammonia levels (Table VIII). However, the only consistently higher values were obtained when the 1:1 grain to hay ration with urea was fed. These results, when compared to the pH values obtained (Table VI), do not show that the rate of ammonia absorption is greater at higher pH levels than at the lower values as reported by Bloomfield et al. (6), although the range of pH values obtained in this study was probably too limited to prove this statement. Also, with the presence of urea and since the actual amount of ammonia produced and absorbed was not measured, it cannot be stated that the values reported are indicative of the absorption of ammonia. At the lower pH values, the ammonia exists as NH_4^+ which is impermeable to the lipid layer of the ruminal epithelium. At higher pH levels, the ammonia is in the readily absorbable free ammonia form. On the basis of the limited data obtained in this trial, it can be generally stated that the presence of urea, a readily hydrolyzable

TABLE VII

MOLAR PROPORTIONS OF VOLATILE FATTY ACIDS IN RUMEN FLUID OF CALVES IN TRIAL I

Ratio of grain:hay	No. of calves		Age at sampling[a] (months) 3	4	5[b]
				(mole %)	
1:1 (with urea)	6	acetic	58.8	54.9	56.9
		propionic	31.2	32.9	29.4
		butyric	8.4	10.1	11.5
		valeric	1.6	2.1	2.2
1:1	6	acetic	57.6	51.5	58.0
		propionic	27.9	35.8	30.1
		butyric	11.6	9.7	9.1
		valeric	2.9	3.0	2.8
4:1 (with urea)	6	acetic	50.3	46.6[c]	45.7
		propionic	32.5	42.1[c]	40.0
		butyric	12.5	8.7[c]	11.7
		valeric	4.7	2.6[c]	2.6
4:1	6	acetic	46.5	51.8	50.4
		propionic	42.6	35.5	38.1
		butyric	9.6	9.8	8.6
		valeric	3.2	2.9	2.9

[a] 3 and 4 month samples taken by stomach tube; 5 month sample taken direct from rumen after slaughter.

[b] Average of 5 calves in 1:1 rations; 6 calves in 4:1 ration with urea; 7 calves in 4:1 ration without urea. Last calf on each 1:1 ration changed to 4:1 ration at 4 months of age; last 4 calves on 4:1 rations slaughtered at 6 months of age.

[c] Average of 5 calves; one calf lost due to bloat.

nitrogen source, causes the level of ammonia in the rumen to increase.

In Trial II, samples taken at the bottom of the ventral sac of the rumen had slightly lower concentrations of ammonia (.335 mM/100 ml) than did the samples taken in the center of the rumen (.404 mM/100 ml). It can also be noted that the pH at the bottom of the ventral sac was higher than it was in the center of the rumen. This might explain the difference in ammonia concentration based on the theory discussed above.

TABLE VIII

CONCENTRATION OF RUMINAL AMMONIA IN RUMEN FLUID SAMPLES OF CALVES IN TRIAL I

Ratio of grain:hay	No. of calves	Age at sampling[a] (months)		
		3	4	5[b]
		(mM/100 ml)[c]		
1:1 (with urea)	6	.6712	.6863	.8350
1:1	6	.5662	.5251	.6726
4:1 (with urea)	6	.4856	.5544[d]	.7413
4:1	6	.2964	.3963	.7100

[a]3 and 4 month samples taken by stomach tube; sample at 5 months taken direct from rumen.
[b]Avg. of 5 calves in 1:1, 6 in 4:1 with urea and 7 in 4:1 rations without urea. Last calf on each 1:1 ration changed to 4:1 ration at 4 months; last 4 calves on 4:1 rations slaughtered at 6 months.
[c]None of differences among treatment means were statistically significant at 5% level of probability.
[d]Average of 5 calves; one calf lost due to bloat.

Buffering Capacity of Rumen Fluid

The buffering capacity of the rumen fluid taken from the last four calves of Trial I and the four calves used in Trial II is shown in Table IX. The buffering capacity was defined as the number of milliequivalents

of HCl needed to lower the pH of 100 ml of rumen fluid to 4.5. The rumen fluid from those calves fed the rations with no urea showed a greater resistance to a pH change than did that from the calves fed rations with urea. The reason for this is not known.

A greater buffering capacity was found in the calves of Trial II fed long hay than in those fed pelleted hay. Also, when samples of the injesta were taken from the center and bottom of the rumina of these calves fed long hay, the buffering capacity of the rumen fluid from the bottom of the rumen was greater (6.1 vs. 5.6) than that of the fluid from the center of the rumen. Samples were not taken in more than one part of the rumina of the calves fed pelleted roughage due to the liquid condition of the injesta. The presence of more saliva in the bottom of the rumen is felt to be the cause of the greater buffering capacity.

Part of the difference in the buffering capacity of the rumen fluid from the calves fed long as compared to pelleted hay may be explained on the basis of salivary secretion. As was reported by Balch (3), the secretion of saliva was greater when long roughage was fed. Since the saliva contains fairly large amounts of bicarbonate and phosphate, as sodium and potassium salts, a decrease in the secretion of saliva could result in a decrease in the level of these important buffers in the rumen.

It has been suggested by Garrett et al. (13) that a decrease in the buffering capacity of the rumen contents is one of the predisposing factors leading to ruminal parakeratosis. The fact that the calves in Trial II fed pelled hay developed ruminal parakeratosis and also had a lower buffering capacity agrees with this idea, however, the absence of ruminal parakeratosis in the calves of Trial I which had an even lower buffering capacity cannot be explained on this basis.

TABLE IX

BUFFERING CAPACITY OF RUMEN FLUID FROM CALVES IN TRIALS I AND II

Ratio grain:hay	Form of hay	No. of Calves	Buffering capacity[a] (meq·HCl/100 ml)
Trial I:			
4:1 (urea)	Pelleted	2	2.62
4:1	Pelleted	2	4.21
Trial II:			
1:1	Long	2	5.85
1:1	Pelleted	2	4.30

[a]No. of milliequivalents of HCl to lower pH of 100 ml rumen fluid to 4.5.

Total Volatile Acidity

The total volatile acidity (total VFA), as determined by steam distillation, in the rumen samples taken from the last four calves in Trial I and the calves in Trial II is expressed as millimoles of VFA per 100 milliliters of rumen fluid (Table X). There appeared to be more variation between samples from the individuals within a ration than there was between the rations fed to the calves. It may be stated, however, that a figure of the magnitude of 12-16 millimoles of VFA per 100 milliliters of rumen fluid is a reasonable one to use as the level of VFA in the rumen. This agrees with the figure of 12 millimoles of VFA per 100 milliliters of rumen fluid that was used in the preparation of the VFA standard used in this study and in others (15) in the chromatographic

analysis for individual VFA.

TABLE X

TOTAL VOLATILE ACIDITY IN RUMEN FLUID FROM CALVES IN TRIALS I AND II

Ratio of grain:hay	Form of Hay	No. of calves	Total Volatile Acidity (mM/100ml rumen fluid)
Trial II:			
1:1	Long	2	16.34
1:1	Pelleted	2	14.12
Trial I:			
4:1 (urea)	Pelleted	2	14.21
4:1	Pelleted	2	15.29

Performance of Animals on Pelleted Rations

The data concerning the performance of the calves in Trial I is presented in Table XI. There appears to be little relationship between the grain to hay ratio of the ration and the estimated pounds of total digestible nutrients (TDN) per pound of gain, pounds of feed per pound of gain, daily rate of gain, total weight gain, pounds of feed consumed per day, or estimated pounds of TDN consumed per day. The difference noted between the 1:1 and 4:1 grain to hay rations and the pounds of feed per pound of gain can be explained on the basis of a higher TDN content in the 4:1 ration (70%) as compared to the 1:1 rations (63%).

The differences in nutrient intake (Table XI) are related to the body

size of the animal. Those animals that had the lowest rate of gain and feed intake were also the smallest at the start of the trial. Because none of the animals developed extensive ruminal parakeratosis, no comparison on the effect of the condition on the performance of the animals can be made.

The fact that the calves fed the 4:1 grain to hay rations had a slightly greater efficiency of gain than those fed the 1:1 grain to hay rations might be due to the increase in propionate and decrease in acetate noted in the rumen fluid of the calves fed the 4:1 rations (Table VII). The efficiency of utilization of feed energy for body gain is known to increase with an increase in the molar concentration of ruminal propionate (2). The differences observed in this trial (Table XI) were very slight.

Observations on Rumination

The 6-hour observations made on twenty of the animals in Trial I gave some interesting results. Although no characteristics peculiar to a specific ration were observed, all of the animals on the pelleted diets showed nervousness as exhibited by frequent lying down and getting up and constant moving about. In addition to this, very little rumination was observed in many of the animals and four animals showed no visible ruminal contractions during the 6-hour period (Table XII). The time spent ruminating ranged from 0-66 minutes during the 6 hours. Similar observations were reported by Beardsley et al. (4). Cud-chewing was also lacking in six of the animals, and those which did chew their cuds did so for very short periods of time (Table XII). The reason for this lack of cud-chewing is thought to be due to the fact that the hay and grain fed were finely ground and pelleted and thus were readily dispersed in the

TABLE XI

PERFORMANCE OF ANIMALS FED PELLETED RATIONS FOR A 3-MONTH EXPERIMENTAL PERIOD

Ratio of grain:hay	No. of[a] calves	Estimated Total Digestible Nutrients (TDN) (%)	Body Wt. Gain Total (lbs.)	Body Wt. Gain Daily (lbs.)	Feed Consumption Total (lbs.)	Feed Consumption Daily (lbs.)	TDN/day (lbs.)	Feed/lb. gain (lbs.)	TDN/lb. gain (lbs.)
1:1 (urea)	5	62.8	145	1.76	672	7.95	5.02	4.71	2.92
1:1	5	63.2	127	1.54	636	7.51	4.77	4.92	3.07
4:1 (urea)	4[b]	69.8	132	1.55	533	6.47	4.53	4.26	2.93
4:1	5	70.4	152	1.79	643	7.52	5.31	4.33	3.00

[a]First 5 calves of each block of 6. Last calf of each block maintained on experiment for 4 months.

[b]Lost one calf due to bloat.

fluid ingesta. The most obvious activity engaged in by the animals was the chewing on the concrete stalls and licking of the hair coat which all animals did for a considerable length of time. These actions, along with the general nervousness displayed by the animals, may have been due to the lack of any long roughage in the diet and the consequent craving for this roughage by the animals.

TABLE XII

OBSERVATIONS ON RUMINATION[a] AND CUD-CHEWING ON TWENTY ANIMALS IN TRIAL I

Ratio of grain:hay	No. of calves	Time spent ruminating		Time spent chewing cud	
		Range	Average	Range	Average
		(min)			
1:1 (with urea)	5	0-61	27	0-83	16
1:1	5	0-66	31	13-25	21
4:1 (with urea)	5	0-60	29	0-69	29
4:1	5	0-32	23	0-62	24

[a]Rumination defined as the visible contractions of body walls which were not respiration.

The Effect of Ruminal Parakeratosis on VFA Absorption

The four fistulated calves used in this study were paired on the basis of breed (Holstein and Ayrshire), age, and weight. One member of each pair was fed long hay and the other member was fed pelleted hay. Otherwise the calves were cared for and fed in identical fashion. The grain to hay ratio was 1:1, with no urea in the grain. At 4 mo. of age,

when the first absorption trial was conducted, both calves fed pelleted hay had developed moderate to heavy ruminal parakeratosis. The condition persisted through the second absorption trial conducted on the same calves at 5 mo. of age. In addition to this, both animals fed the long hay had also developed slight parakeratosis at 5 mo. of age. The reason for this occurrance cannot be explained; however, it is felt that this observation may lend support to the idea that the ratio of the grain to hay in the diet is as important as is the physical form of the diet in the consideration of the causes of ruminal parakeratosis.

The results obtained in the absorption trials conducted are presented in Tables XIII and XIV. A noticeable decrease in the amount of VFA absorbed from the rumina of the calves with ruminal parakeratosis was noted at 5 months of age (Table XIV) when compared to the calves which had little or no parakeratosis. However, in the trial conducted at 4 months of age, this was not found to be true (Table XIII). The reason for this cannot be explained at this time. Also, the level of acetate was quite variable in the rumen of the calf with severe parakeratosis (Table XIII), with no real trend in the concentration being observed. The cause of this occurrance may have been due to insufficient mixing of the solution prior to sampling or to diffusion of blood acetate across the rumen wall into the solution. These variations were not noted in the other acids to the extent found in the acetate. A definite decrease in the absorption of VFA from the rumina of the calves with more advanced development of ruminal parakeratosis was observed in the absorption work at 5 months of age (Table XIV). The concentrations of the VFA at the beginning and end of each absorption period are given in Tables XV and XVI. From these data it can be seen that the total change in VFA concentration

over the 2-hour experimental period was greater for those calves which did not have ruminal parakeratosis, meaning a greater amount of the VFA were absorbed from the rumen. In terms of the percentage change in the individual VFA concentrations, it appears that the longer chain fatty acids (C_4, C_5) were absorbed to a greater degree than were the shorter chain fatty acids (C_2, C_3) as can be seen by the increase in the molar concentrations of the shorter chain acids at the end of the absorption period (Table XVI). This would agree with the literature (10, 40) which states that at acid pH the longer chain fatty acids are more readily absorbed than are the shorter chain fatty acids; however, it is questionable to assume that the pH of 6.6 maintained in this trial was acidic enough to cause this change.

Problems Needing Further Research

During the course of this study several questions have arisen which need further study before any concrete statements may be made concerning the practical importance of ruminal parakeratosis. The first of these is the question of the importance of the clumping of the papillae of the rumen. If these clumps are impermeable to rumen fluid, the absorptive area of the rumen might be decreased more than when a moderate case of ruminal parakeratosis was observed.

The fact that no parakeratosis was noted in four animals fed rations with a 4:1 ratio of grain to hay in Trial I of this study, while moderate to severe incidences were observed when the same grain and hay rations were fed in a 1:1 ratio in Trial II, offers a distinct challenge to future research. It appears that the proportions of acids in the rumen may be a causative agent in conjunction with other factors, unknown at

TABLE XIII

EFFECT OF RUMINAL PARAKERATOSIS ON ABSORPTION OF VFA FROM THE RUMEN AT 4 MONTHS OF AGE

Pair	Calf no.	Ration[a]	Intervals after introduction of VFA (min.)	(Initial conc.) - (Conc. at specified intervals corrected for dilution) Acetic (mg/100 ml)	Propionic	Butyric	Valeric	Degree of Parakeratosis
I	48	Long hay / Grain II	15	42.0	17.7	39.2	2.0	None
			60	134.9	78.6	65.3	13.9	
			90	206.4	108.1	84.0	16.6	
			120	239.3	117.2	104.4	20.6	
	91	Pelleted hay / Grain II	15	155.5	54.4	77.2	-1.6	Severe
			60	219.1	120.8	54.6	16.9	
			90	150.1	112.7	40.9	18.4	
			120	307.6	183.1	112.4	23.0	
II	77	Long hay / Grain II	15	2.3	5.5	-11.1	-0.7	Very slight
			60	156.1	79.1	35.5	15.9	
			90	231.8	132.2	72.3	23.4	
			120	267.8	99.9	82.6	27.5	
	84	Pelleted hay / Grain II	15	11.7	42.8	19.5	4.6	Slight
			60	-9.6	46.3	35.3	16.1	
			90	48.0	71.0	44.9	22.6	
			120	134.2	110.7	76.6	24.7	

[a]Composition of rations shown in Table I; hay and grain fed in 1:1 ratio.

TABLE XIV

EFFECT OF RUMINAL PARAKERATOSIS ON ABSORPTION OF VFA FROM THE RUMEN AT 5 MONTHS OF AGE

Pair	Calf No.	Ration[a]	Intervals after introduction of VFA	(Initial conc.) - (Conc. at specified intervals corrected for dilution)				Degree of parakeratosis
				Acetic	Propionic	Butyric	Valeric	
			(min.)	(mg/100 ml.)				
I	48	Long hay / Grain II	15	2.5	26.9	15.4	-13.5	Slight
			60	143.1	80.8	58.3	15.7	
			90	266.5	155.3	139.6	21.4	
			120	316.1	196.2	143.2	26.8	
	91	Pelleted hay / Grain II	15	-32.0	13.7	0.2	6.6	Severe
			60	49.5	71.8	43.2	10.6	
			90	92.9	87.4	82.9	15.2	
			120	192.0	134.2	99.6	20.7	
II	77	Long hay / Grain II	15	33.8	18.7	9.2	2.5	Very slight
			60	136.9	69.1	58.5	19.4	
			90	166.7	101.2	67.1	22.4	
			120	240.7	128.7	95.9	26.7	
	84	Pelleted hay / Grain II	15	64.9	11.3	16.3	3.7	Moderate
			60	143.1	72.3	55.1	16.2	
			90	138.0	117.1	81.0	23.4	
			120	166.0	103.2	73.1	24.3	

[a]Composition of rations shown in Table I; hay and grain fed in 1:1 ratio.

TABLE XV

INITIAL vs. FINAL CONCENTRATION OF VOLATILE FATTY ACIDS IN THE RUMINA OF CALVES (TRIAL II)

Pair	Age (months)	Calf No.	Time[a]	Acetic	Propionic	Butyric	Valeric	Degree of ruminal parakeratosis
				(mg/100 ml.)				
		48	Initial	353	179	123	23	None
			Final	114	61	19	2	
I	4							
		91	Initial	449	231	154	27	Severe
			Final	141	48	42	4	
		48	Initial	371	194	168	29	Slight
			Final	132	34	24	2	
I	5							
		91	Initial	309	161	113	23	Severe
			Final	117	26	14	3	
		77	Initial	392	188	112	30	Very slight
			Final	124	88	29	2	
II	4							
		84	Initial	423	237	154	37	Slight
			Final	289	127	78	12	
		77	Initial	411	181	116	29	Very slight
			Final	170	52	21	3	
II	5	84						
			Initial	396	180	111	30	Moderate
			Final	230	76	38	5	

[a]Initial = 5 min. after introduction of VFA solution into rumen.
Final = 120 min. after introduction of VFA solution into rumen.

TABLE XVI

INITIAL vs. FINAL PROPORTIONS OF VOLATILE FATTY ACIDS IN THE RUMINA OF CALVES (TRIAL II)

Pair	Age (months)	Calf No.	Time[a]	Acetic	Propionic	Butyric	Valeric	Total VFA conc.	Degree of ruminal parakeratosis
				(mole %)				(mM/100 ml)	
		48	Initial	59	24	15	2	9.33	None
			Final	64	28	7	1	1.72	
I	4								
		91	Initial	59	25	13	3	12.51	Severe
			Final	67	19	13	1	2.23	
		48	Initial	58	25	15	2	8.50	Slight
			Final	74	17	8	1	1.31	
I	5								
		91	Initial	58	25	15	2	7.56	Severe
			Final	74	19	6	1	1.20	
		77	Initial	61	24	12	3	9.75	Very slight
			Final	57	33	9	1	2.22	
II	4								
		84	Initial	57	26	14	3	9.50	Slight
			Final	64	23	12	1	3.26	
		77	Initial	62	22	13	3	10.40	Very slight
			Final	74	19	6	1	2.36	
II	5								
		84	Initial	62	23	13	2	10.28	Moderate
			Final	72	19	8	1	3.91	

[a]Initial = 5 min. after introduction of VFA solution into rumen.
Final = 120 min. after introduction of VFA solution into rumen.

this time, but which may also be related to the actual fermentation processes in the rumen and the level of fiber in the diet.

In the dairy field, the ultimate problem concerned with ruminal parakeratosis is the possible effect on milk production. Therefore, work is needed to study the effect of the condition on milk production from the standpoint of the length of time needed for the development of ruminal parakeratosis in the adult animal and also the long-term effects on the animal. Included in this should be a study to determine whether the condition is permanent or will clear up without a change in the ration.

SUMMARY AND CONCLUSIONS

A single trial involving 24 male Ayrshire calves was conducted to determine the effect of rations on the development of ruminal parakeratosis and the subsequent effect on animal performance and certain biochemical conditions in the rumen. A second trial involving one pair of Ayrshire and one pair of Holstein male calves was conducted to determine the effect of ruminal parakeratosis on the absorption of VFA from the rumen, using polyethylene glycol as a nonabsorbable marker to measure dilution.

All of the calves in both trials were on experiment between the ages of 2-5 months and were maintained on expanded metal floors to alleviate the need for bedding. The calves in Trial I were fed four rations having a 1:1 or 4:1 ratio of pelleted grain to pelleted hay with urea as a protein supplement in one ration of each ratio. Rumen samples were taken at 1-month intervals, 3 hours after feeding, for the determination of pH, ruminal ammonia and volatile fatty acids. In addition, samples were taken from the last four calves in Trial I and the calves in Trial II for the determination of total volatile acidity and buffering capacity.

In Trial I, no cases of ruminal parakeratosis were observed; however, slight amounts of capping were observed in the rumina of calves fed the rations with a 1:1 grain to hay ratio. Also, extensive clumping of the ruminal papillae was observed in the calves fed the 4:1 grain to hay ratios.

The pH of the rumen fluid from calves fed urea in the ration was slightly higher than that in the calves fed rations without urea. There was little other difference in the pH between the rations. The molar proportion of acetate was lower and that of propionate was higher in the calves fed the rations with a 4:1 grain to hay ratio as compared to those fed 1:1 grain to hay ratios. A slight increase in the ruminal ammonia level was noted in the calves fed rations with urea. This is probably due to the fact that urea is readily hydrolyzable.

The buffering capacity of the rumen fluid from the calves in Trial II fed long hay and grain in a 1:1 ratio was greater than that of any other rations. This is thought to be due to a greater salivary secretion when long hay is fed. The total volatile acidity in the rumen fluid from four calves in Trial I and the calves in Trial II varied within the range of 11-22 mM/100 ml of rumen fluid. Rather large variations between feeding and sampling time (5-7 hrs.), due to the fact that samples were taken at the time when the rumen was emptied prior to the introduction of the VFA solution, are thought to be the reason for the wide range in values.

The performance of the animals in Trial I appeared to be due more to the initial weight of the animals than to any ration factors. A slight increase in efficiency of gain was noted in the animals fed urea in the ration.

The calves in Trial II were fistulated at two months of age and paired on the basis of breed, weight, and age. The rations fed were in a 1:1 ratio of grain to hay, with the hay long or pelleted. One member of each pair was fed each of the two rations for the entire 3 month period. Both calves fed the pelleted rations had developed ruminal parakeratosis at 4 months of age. The condition persisted through the observations at 5

months of age. A definite decrease in the amount of VFA absorption from the rumina of calves with extensive ruminal parakeratosis was observed.

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