

STUDIES ON RUMINAL PARAKERATOSIS IN DAIRY CALVES

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## INTRODUCTION

Advances occurring in animal nutrition and the rising cost of labor during recent years have resulted in an increase in automation in feeding livestock. As new techniques and labor devices are adopted in our feeding regimes, it is often necessary to vary the methods by which livestock feeds are processed.

Interest in pelleted rations has increased during recent years due to the advantages reported for the use of pelleted feeds. Some of these advantages center around (a) increased feed consumption and weight gains in cattle receiving pelleted feed, (b) less labor resulting from self-feeding a complete pelleted ration, (c) decreased storage space required by pellets, and (d) ease of conveying feed using mechanical equipment.

Even though the pelleting of feed appears promising, some investigators have observed an increase in the incidence of a condition in the rumen termed, "ruminal parakeratosis" when pelleted feeds were used. This condition involves an excessive incrustation and hardening of the papillae and the accumulation of excessive layers of keratinized, nucleated squamous epithelial cells of the papillae. Although ruminal parakeratosis has become a common observation in rumina removed from animals at the slaughter house, neither the basic cause for the development of this condition nor its effects on nutrient utilization and animal performance has been satisfactorily determined. Much of the

previous work has dealt with incidence of parakeratosis and descriptions of the effect of the condition on the physical appearance of the papillae.

Since most absorption from the rumen appears to occur in the ventral portion of the rumen where incrustation and clumping of the papillae develop, research is needed to determine the importance of this condition on absorption of volatile fatty acids (VFA) from the rumen.

The specific objectives of the present experiment were: (a) to find by means of pilot feeding trials a ration, describable as to content and form, which will consistently produce ruminal parakeratosis in the bovine, (b) to determine what relationship, if any, exists between the occurrence of parakeratosis and certain biochemical conditions within the rumen, particularly with respect to absorption of VFA.

## LITERATURE REVIEW

### Incidence of Ruminal Parakeratosis

While studying the rumenitis-liver abscess complex in beef cattle in 1954, Jensen et al. (39) observed parakeratosis in the rumina to the extent of 8.8% of a random sample of 1535 cattle. The parakeratosis was thought to be associated with rumenitis.

A high incidence of experimentally-produced ruminal parakeratosis was observed by Jensen et al. (40) in lambs fed pelleted rations containing 40 to 60% alfalfa hay, while none of the condition was present in two trials in which the lambs were fed unpelleted rations in which the hay was chopped. Ground cereal grains and molasses were fed as a source of concentrate. In another trial involving 1569 animals, 39.2% of the lambs fed a pelleted ration had ruminal parakeratosis, while only 7.7% of those fattened on pasture developed the condition. The condition was described as a noncontagious disease characterized, grossly, by hardening, enlargement, and clumping of mucosal papillae and, microscopically, by accumulation of excessive layers of keratinized, nucleated, squamous epithelial cells on the papillae.

The degree of parakeratosis was varied, being generalized and severe in many animals. The highest incidence was in the anterior-ventral sac. Although the specific cause of ruminal parakeratosis was not known, three possible etiological factors were suggested:

(a) finely ground feed which may have predisposed by creating a medium for bacteria to produce causative agents; (b) contaminants from the machinery which may have entered the feed at the time of pelleting; and (c) deficiencies which may have resulted from alterations of ingredients by processing.

Hopkins et al. (35) made observations on the incidence of ruminal parakeratosis in 90 lambs fed rations containing 50% roughage. It was found that 38% of the lambs fed pelleted feed and 4% of those fed unpelleted feed had ruminal parakeratosis.

The effect of different methods of processing alfalfa hay was studied by Garrett et al. (28) to determine its apparent association with incidence and severity of parakeratosis. The disease was most prevalent in animals fed the finely ground roughage prepared in 1/4 or 5/8 inch pellets, but minor incidence was also noted in the groups fed chopped hay (milled, 1 inch) or 4 inch wafers (ground through a 1.5 inch hammermill screen). Less parakeratosis was found when oat hay was added to the pelleted rations. It was suggested that an increased rate of fatty acid production on finely ground pelleted alfalfa compared to chopped alfalfa, coupled with less rumination and a likely decrease in the buffering capacity of the rumen, possibly leads to factors predisposing to parakeratosis.

Beardsley et al. (15) compared various concentrate:roughage (C:R) ratios (70:30, 55:45, and 40:60) fed either finely ground and pelleted or coarsely ground and unpelleted and noted that steers on the finely ground pelleted feed ruminated only occasionally or not at all. Examination of rumina following slaughter revealed marked

tissue changes, including parakeratosis, in steers on the high concentrate pelleted ration and the control ration but not in steers on a high roughage, unpelleted ration. No explanation was given for the occurrence of the condition in the control group. In contrast, McClure et al. (49) found that pelleting rations having several C:R ratios (75:25, 60:40, 45:55, and 30:70) had no effect on the incidence of ruminal parakeratosis. Also, Haught et al. (32) found no significant differences in the gross appearance of the rumina of sheep fed pelleted rations as compared to ground rations containing 70% alfalfa and 30% grain.

Thompson et al. (63) observed that the feeding of completely pelleted rations containing 50% roughages, compared with conventional forms of the same ration resulted in changes characterized by increased length and width of papillae, incrustation and clumping of the papillae with rumen contents; microscopic changes included a thickened corneum with increased accumulation of vesiculated keratinized cells on the outer surface layer, enlarged central core, enlarged papillary bodies extending farther into the epithelium, and increased folding of the malpighian layer. Cullison (22) observed varying degrees of an abnormal rumen wall condition at the time of slaughter among steers which had received either a ground or pelleted ration containing 30% costal bermuda-grass hay for approximately 7 months. The condition was characterized by the presence of excessively long, dark, unhealthy appearing papillae and the sloughing off of areas of keratinous tissue from the rumen wall. Animals on the control rations of concentrates and long hay exhibited a uniformly normal rumen wall

condition, as did steers which received straw in addition to the basic ration in the ground or pelleted form. Similarly, in one trial, McCroskey (51) observed a higher incidence of ruminal parakeratosis in steers fed a complete ration having 80% roughage as pellets or re-processed pellets than when the ration was unpelleted. However, in other trials no rumen parakeratosis was observed in steers fed the same type of rations.

Pelleted rations have not been fed in every case in which ruminal parakeratosis has been observed. Vidacs and Ward (67) observed rumen epithelial changes in fistulated cows within four to six days after changing from hay to a dried beet pulp ration. Upon changing the ration to hay again the rumen epithelium returned to normal but recovery was slower than the development of parakeratosis. Addition of acetic acid to the beet pulp ration at a concentration equal to that found on the hay diet arrested, but did not correct, the necrosis. Acetic acid added to the hay ration slowed recovery. It was suggested that the low acetate/propionate ratio associated with the beet pulp ration may be the causative agent of this condition and that, in turn, destruction of papillae may result in alteration of the VFA absorption. Gilliland et al. (29) found a high incidence of ruminal parakeratosis in dairy calves fed a ration containing a 10% level of salts of propionic and butyric acids to five weeks of age, whereas very little of this condition was observed in animals receiving a control ration in the same form. The cause of the condition was not determined. A similar condition was noted by Tamate et al. (62) while studying the effect of various dietaries on the calf's stomach. Extensive

sloughing and hemorrhage was observed in the rumen of one calf receiving butyric acid while a clumping condition of the papillae was noted in two calves receiving acetic and propionic acids. Prominent folds of the mucous membrane in the whole area of the rumen and atypical papillae were observed quite consistently in the calves receiving VFA or their salts.

Kunkel et al. (44) reported ruminal desquamation in 7-8 month old lambs fed a total mixed diet for 72 days. Of the 201 ruminal specimens examined at the close of experiment I, 26 showed varying degrees of desquamation. The depapillated and scarred or denuded areas ranged in size from about 1 cm. in diameter to almost all of the surface of the ventral sac. No apparent reason was offered for the differences noted in type of condition as compared to that observed by other workers.

#### Biochemical Changes in the Rumen as Related to Ruminal Parakeratosis

Rhodes and Woods (58) noted that pH was lower in the rumen for lambs receiving pelleted rations. Hinders et al. (34) reported that the use of a dehydrated alfalfa pellet ration decreased ruminal pH from 6.9 to 6.0 and caused heavy ruminal parakeratosis. Addition of phosphate decreased the titratable acidity of the rumen fluid, raised the pH to 6.65, but did not eliminate the parakeratosis. Salt and bonemeal consumption was considerably greater for animals receiving pelleted rations. In agreement with these data, Cullison et al. (22) observed a significantly lower rumen pH level in steers receiving a pelleted ration. An inadequate secretion of saliva was offered as

an explanation for this phenomenon. Balch (11) found that for every 10 lb. hay consumed 43-57 lb. saliva were added during eating, but with every 10 lb. concentrates only 12-15 lb. saliva were added. With concentrates the rate of secretion of saliva was faster than with hay, but the rapidity of ingestion was raised so that the dry-matter content of concentrate boluses was high. Less saliva was added to concentrates given as cubes, although by the time the cubes reached the reticulo-rumen they had been almost completely ruptured.

Danielli et al. (23) investigated the effect of pH on the rate of absorption of the individual VFA. At pH 7.5 the order of absorption was: acetic > propionic > and butyric; whereas at pH 5.8 the order was reversed and became: butyric > propionic > and acetic. It was considered that free acids (present at pH 5.8) were absorbed at a greater rate than the anions and that different mechanisms of absorption were probably involved. At a lower pH the proportion of free acid absorbed would probably be greater, as the relative proportion of the free acid in the rumen solution would be increased. It is now recognized that at pH 5.8 the fatty acids leave the rumen by way of lipid and water filled pores, whereas at pH 7.5 penetration of the wall is by simple diffusion. Kiddle et al. (43) indicated that the mixture of acids in blood draining the rumen was similar to those present in the rumen contents, except that it contained appreciably less butyric acid. Pennington (55) showed that fatty acids which disappear from the rumen are partially metabolized by the epithelium. The extent to which this happens is least for acetate, and greatest for butyrate. Rhodes and Woods (58) found that butyric acid utilization by rumen



epithelium from lambs on mixed or pelleted rations tended to be higher than from lambs on long hay rations. Observations by Vidacs et al. (68) on rumen epithelial changes occurring when cattle are fed different rations led to the conclusion that the absorption of VFA may not be uniform over the entire surface of the rumen, and may be altered by lesions caused by the development of ruminal parakeratosis. Recently Hinders and Owen (33) have indicated a decrease in the percent of total VFA absorbed from the rumen as ruminal parakeratosis continues to develop in the animal receiving a pelleted ration. These data are in agreement with work presented by Harris et al. (31) on the absorption of VFA from the rumen where known amounts of acetic, propionic, butyric and valeric acids and polyethylene glycol were introduced into the emptied rumen. Thus, it appears that during early development of parakeratosis, absorption of VFA are accelerated, but as the condition continues to develop VFA absorption from the rumen is decreased.

Albino rats were used by Vidacs et al. (65) to demonstrate their importance as a test animal to study the effect of various diets on the esophageal area of the stomach. The animals received a commercial rat feed, and different rumen fluids as a drink from ruminants receiving a variety of rations. Those receiving rumen fluid from ruminants on a dehydrated alfalfa pellet diet showed a very definite thickening of the mucosa, whereas those that obtained the rumen fluid from a ruminant fed alfalfa hay showed no epithelium changes. Autoclaving the rumen fluid obtained from the ruminant receiving the pelleted diet resulted in a condition closer to the control. The

authors suggested that a heat-sensitive or protein-bound factor caused the ruminal parakeratosis. Pure VFA added to the rats' drink did not cause any change in the thickness of the epithelium. Similar work by Vidacs et al. (66) using the chick as a test subject showed a thickening of the stratum corneum when the diet consisted of a standard feed mixed with rumen fluid from a cow fed pelleted dehydrated alfalfa hay showing heavy parakeratinization of the rumen.

#### Performance Noted in Animals Having Ruminal Parakeratosis

Although no significant difference was noted in rate of gain in body weight between lots of animals by Jensen et al. (40) a within-lot examination showed that lambs having normal rumina made a significantly higher rate of gain in body weight than lambs which had ruminal parakeratosis. No statistically significant difference was observed between the group of animals receiving pelleted feed as compared to the group receiving unpelleted feed. Hopkins et al. (35) noted that the pelleting of rations having a 1:1 ratio of hay to grain did not affect the rate of gain of lambs but did increase feed efficiency to some extent. The effect of the relatively high incidence of ruminal parakeratosis on the rate of gain of the lambs receiving pelleted feed is not clearly defined in this report, however, since no within-group comparisons were made.

Garrett et al. (28) observed that steers fed long oat hay with the finely ground pelleted rations (1/4 and 5/8 inches) made significantly greater gains and had larger fat-corrected carcass weights than animals receiving the pellets alone and showing a high incidence

of ruminal parakeratosis. Cullison (22) found a definite association between the incidence of ruminal parakeratosis and the average rate of gain of different groups of beef steers receiving complete rations having 30% hay; however, no causal relationship was necessarily indicated since the average daily feed consumption was lower for the groups having the lower rates of weight gain.

Beardsley (15) observed a relatively low rate of gain for beef steers fed a pelleted high-concentrate ration and having a high incidence of ruminal parakeratosis; however, other factors, including a lower feed intake, were involved in the lower rate of gain for the steers receiving the high-concentrate pelleted ration. McCroskey et al. (50) observed no incidence of parakeratosis in cattle fed a pelleted ration having a 4:1 ratio of grain to hay, although the animals gained less in one trial when the ration was fed in pelleted form than they did when it was fed unpelleted. Moreover, in the limited trial in which McCroskey (50) observed a relatively high incidence of parakeratosis in animals receiving feed which had been pelleted, there appeared to be no relationship between the condition of the rumen and rate of weight gain.

Kunkel et al. (44) reported that animals with mucosal damage had significantly lower rates of gain, but the incidence of ruminal desquamation was distributed generally and was without significant implication as to an effect of treatment or diet, the initial weight of the lamb, the size of papillae, or the pigmentation of the mucosa.

The Effect of Parakeratosis on Milk Production

The effect of ruminal parakeratosis on milk production has not been thoroughly investigated. Hinders *et al.* (34) divided 4 identical twins (3 in production and one pair fistulated and dry) into two groups (A and B) and divided the lactation into 3 periods (2, 7 and 2 weeks, respectively). The results obtained are presented in Table I. The average daily milk production is considerably less in the second period for the group receiving dehydrated alfalfa pellets. A drastic decrease may be noted in the butterfat per cent when the ration was changed from alfalfa hay to dehydrated alfalfa hay in both groups. Each fistulated animal developed heavy parakeratosis after changing from alfalfa hay to dehydrated alfalfa pellets.

TABLE I  
THE EFFECT OF TYPE OF RATION ON DAILY MILK  
PRODUCTION AND FAT PER CENT

Period	A		B			
	Ration	Daily milk prod.	Per-cent fat	Ration	Daily milk prod.	Per-cent fat
I	Alfalfa hay	48.1	3.02	Alfalfa hay	51.3	3.02
II	Dehydrated alf. pellet	37.5	2.32	Alfalfa hay	46.5	2.90
III	Dehydrated alf. pellet / Na <sub>2</sub> HPO <sub>4</sub>	34.0	1.64	Dehydrated alf. pellet / Na <sub>2</sub> HPO <sub>4</sub>	44.5	2.27

## EXPERIMENTAL PROCEDURE

Three trials, involving 10-14 male Ayrshire calves each, were conducted to determine the effect of different rations on ruminal parakeratosis and certain biochemical conditions in the rumen. A fourth trial involving two pairs of male Ayrshire calves was conducted to determine the effect of parakeratosis on volatile fatty acid (VFA) absorption from the rumen, using polyethylene glycol as a nonabsorbable marker.

### Management of Calves

All calves were selected from the Oklahoma State University dairy herd and two other Ayrshire herds in the State. The calves were removed from their dams at approximately 3 days of age and placed in individual stalls which contained shavings for bedding. Whole milk was fed twice daily at the rate of 10% of body weight for two weeks and at 8% of initial body weight for the remaining three weeks. In addition, a palatable calf starter was fed. The calf starter contained, in addition to the grain ration, 20% alfalfa pellets (3/16 inch diam) to assure consumption of both hay and grain. At two months of age each calf was assigned to an experimental trial as outlined below.

During the course of the trials all feed pellets which were fed were 5/16 inch in diameter. During the last week of the first

three trials rumen samples were collected by stomach tube at 4- and 8-hour intervals. After determining the pH, the samples were strained through cheese cloth, centrifuged and frozen for future determination of VFA.

Trial I. Twelve male Ayrshire calves were assigned to 3 treatment groups according to a randomized block design. The feeding regime for the three groups consisted of a pelleted grain mixture plus one of the following forms of alfalfa hay: (I) long; (II) wafered; and (III) ground and pelleted. The composition of the grain ration is given in Table I and is designated as Ration B.

TABLE II  
COMPOSITION OF PELLETTED GRAIN RATIONS

Ration A		Ration B	
<u>Ingredient</u>	<u>%</u>	<u>Ingredient</u>	<u>%</u>
Ground milo	50	Ground corn	33.15
Crimped barley	25	Ground milo	20
Wheat bran	10	Crimped oats	15
Liquid molasses	7	Wheat bran	10
Cottonseed meal	5	Soybean meal	15
Urea	1	Dried molasses	5
Dicalcium phosphate	1	Dicalcium phosphate	1
Salt	1	Salt	0.5
		Aurofac 10 <sup>a</sup>	0.25
		Quadrex <sup>b</sup>	0.1
	<u>100.0</u>		<u>100.0</u>

<sup>a</sup>Contains aureomycin (chlortetracycline hydrochloride), 10.0 grams/lb.

<sup>b</sup>Contains vitamin A, 10,000 I.U. and vitamin D<sub>2</sub>, 1,250 I.U./gram.

The rations were fed twice daily in amounts such that there was a minimum amount of feed refused and so that the hay and grain was consumed in approximately a 1:1 ratio. One month was allowed for the calves to become accustomed to the new feeding regime. Beginning at 3 months of age feed consumption was recorded for each calf, with any refused feed being weighed back at the end of each week. Measurement of body weight, circumference of heart girth, and height at withers was made weekly during the experimental period. Daily observations were made for any indication of sickness of the animals.

Trial II. The feeding regime and management practices in Trial II were similar to those in Trial I. Fourteen Ayrshire male calves were assigned at two months of age to two treatment groups. All calves received the same pelleted grain mixture plus one of the following forms of alfalfa hay: (I) long; and (II) pelleted. The composition of the grain mixture is presented in Table I and designated as Ration A. The hay and grain were fed in a 1:4 ratio with a minimum refusal. The animals were housed in tie stalls with shavings for bedding.

Trial III. This trial was conducted in similar manner as Trial II with the following changes. Ten Ayrshire male calves were divided into two groups according to age and housed under conditions similar to dry lot feeding conditions. The calves were maintained in individual 12' x 12' concrete indoor stalls. No shavings were offered for bedding.

Trial IV. Four Ayrshire male calves were paired on the basis of age and weight, fistulated at two months of age, and used on experiment

during the period from 3-5 months of age. The calves were maintained on expanded metal floors with no bedding.

The feed for Pair I consisted of a pelleted grain mixture (Ration A) and one of the following forms of alfalfa hay: (I) long; and (II) pelleted. Calves in Pair II were fed similarly except that Ration B (Table I) was used. The rations were fed twice daily so that there was a minimum amount of feed refused and the hay and grain were consumed in approximately a 1:1 ratio.

#### Slaughter of Calves

The calves in Trials I, II, and III were slaughtered at 5 months of age to observe the development of ruminal parakeratosis. All calves were sacrificed in the Anatomy Laboratory of the College of Veterinary Medicine. The reticulo-rumen was removed and emptied of its contents. Cleaning was accomplished by dipping the opened rumen into water several times until all feed particles were removed. The rumen was then studied in detail for detection of any parakeratosis. A 2 x 2 square inch section was taken from the dorsal cranial sac of the rumen to make microscopic sections for histological study. After careful examination for parakeratosis the reticulo-rumen was wrapped and frozen until the completion of all the trials. The rumina were then thawed and compared as to the development of parakeratosis and pigmentation. Photographs were taken for later group comparisons.

#### Studies on VFA Absorption

Absorption studies were made on each pair of calves in Trial IV at 4 and 5 months of age. The ruminal plugs were removed and the



rumina emptied and rinsed several times with warm water so that very little material remained in the rumen. Soft paper towels were used to remove the presence of any remaining pools of water. The plugs were then refitted and the buffered solution at pH 6.6 (Table III) containing known amounts of acetic, propionic, butyric and valeric acids and polyethylene glycol, were introduced into the emptied rumen. The pH of the solution in the rumen was determined using a portable pH meter, and was maintained at near 6.6 by the addition of 10N phosphoric acid as needed. Samples were withdrawn for analyses at 5-min intervals during the first 30 min and at 30-min intervals thereafter during a 2-hr experimental period.

Preparation of Test Solutions. The test solutions introduced into the emptied, washed rumen were a modification of the test solution as reported by Sutton et al. (61). The use of calcium chloride was omitted as a component of the buffer since it appeared to be causing an error in the Klett readings for the determination of polyethylene glycol (PEG).

A 5 N solution of NaOH was added to the VFA solution to adjust it to a pH of 6.6. Prior to mixing the VFA and phosphate buffer solution, a check was made to assure a reading of pH 6.6 for both solutions. Another pH reading was taken prior to introducing the buffered solution into the emptied rumen. The PEG was added to the solution a few minutes before introduction into the rumen to avoid deterioration of the PEG.

Criterion of Absorption. A decrease in the concentration of VFA in the rumen may be due to absorption of VFA from the rumen, dilution

of the rumen contents with saliva, movement of digesta through the digestive tract, or the movement of fluid across the rumen wall due to osmotic pressure differences. The PEG (mol wt 4000) was included in the test solution as a nonabsorbable marker to permit absorption to be distinguished from dilution. The absorption was determined by subtracting the concentration at specified intervals, corrected for dilution, from the initial concentration. Analysis for PEG was conducted according to the turbidimetric method of Hyden (37).

TABLE III  
COMPOSITION OF TEST SOLUTION INTRODUCED INTO THE RUMEN

Component	Molarity	mg/3 liters
VFA, pH 6.6	0.121	24.57
Acetic	0.072	13.0
Propionic	0.030	6.7
Butyric	0.016	4.12
Valeric	0.003	0.75
KCl	0.005	1.118
KH <sub>2</sub> PO <sub>4</sub>	0.001	0.408
MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.001	0.739
Phosphate buffer, pH 6.6	0.1	60.6252
KH <sub>2</sub> PO <sub>4</sub>	0.05	20.4136
Na <sub>2</sub> HPO <sub>4</sub> · 7H <sub>2</sub> O	0.05	40.2116
Polyethylene glycol (60mg/100 ml.)	0.2	2400.00

#### Determination of Rumen VFA

The rumen fluid samples from all trials were analyzed for VFA using a modification of the method reported by Erwin *et al.* (26). A 10  $\mu$ l sample of the prepared rumen fluid was used for injection into the stainless steel injector block maintained at 300°C to provide rapid vaporization of the sample. The instrument used was the Aerograph

Model A-600-B "Hi-Fi" with a hydrogen flame ionization detector (a gold-plated detector shield was used to avoid corrosion problems) and an 11-step precision attenuator that reduced the signal by half with each step from 1 to 1000. A high-low input switch ( $10^9$  to  $10^7$  ohms) further reduced the signal by 100 times and a third switch increased the output signal 10-X. With these three controls a total dynamic range of 1 million is available and linearity is excellent throughout this range.

The Aerograph hydrogen generator Model A-650 was used to supply filtered hydrogen and air (300 ml/min) necessary for the operation of the flame ionization detector. The hydrogen was produced by electrolysis of water.

The fatty acids were eluted with a nitrogen carrier gas flow rate of 30 ml/min to produce sharp symmetrical peaks in approximately 15 minutes. From 5-10 minutes were allowed following the elution of valeric acid before the next injection. The eluted fatty acid peaks were recorded by a Sargent Model SR and measured with a compensating polar planimeter.

Preparation of VFA Standard. A standard solution of VFA containing acetic, propionic, butyric, and valeric acids was prepared to use in determining the unknown quantity of the individual fatty acids in rumen fluid. The quantities of the VFA used in the standard corresponded closely to previously reported amounts (12 mM/100 ml) present in rumen fluid. The molar percentage generally accepted is acetic, 60%; propionic, 25%; butyric, 13%; and valeric, 2%. The molar proportion of each fatty acid was multiplied by 12 and then by the millimolar weight of each individual fatty acid. To prepare a liter of standard solution, approximately ten times these respective amounts of individual acids were

used. Approximately 4.12, 2.24, 1.44, and 0.26 ml of acetic, propionic, butyric and valeric acids, respectively, were measured into a small beaker containing distilled water and weighed individually with an analytical balance. The contents were then emptied into a one-liter flask. After rinsing the beaker several times and emptying into the flask the solution was made-up to one liter, shaken and placed in the cooler.

Calculation of VFA. The fatty acids in the rumen were calculated from the peaks obtained following the injection of 10  $\mu$ l of prepared rumen fluid in the gas chromatograph instrument. The area of the respective peaks eluted following the injection of an aliquot of the standard solution (Table IV) was assumed to represent the computed micromoles per 10  $\mu$ l. The amount of each individual fatty acid in the rumen fluid was then computed by dividing the area obtained for the rumen fluid sample by that obtained with the standard fatty acid solution and multiplying the result by the computed micromoles per 10  $\mu$ l in the standard solution.

TABLE IV  
STANDARD VFA SOLUTION

Acids	ml/liter	g/liter	$\mu$ g/ $\mu$ l	$\mu$ M/10 $\mu$ l
Acetic	4.12	4.4440	4.4440	0.7393
Propionic	2.24	2.2488	2.2488	0.3036
Butyric	1.44	1.4901	1.4901	0.1691
Valeric	0.26	0.3112	0.3112	0.0305

According to Erwin et al. (26) some fatty acids are eluted when distilled water is injected into the column following a fatty acid mixture. However, it was suggested that an equilibrium exists between the fatty acids retained and those moving through the column. For this reason the first one or two samples were ignored at the beginning of each series of samples analyzed. Each time a series of rumen samples were analyzed for VFA a standard containing a fatty acid concentration similar to the biological samples was analyzed.

#### Measurement of Particle Size of Ground Alfalfa Hay

A measurement of particle size was obtained for the ground alfalfa hay used in each of the three trials since there is some evidence that finely-ground or pelleted hay and roughage influence rumen function and lead to changes in the molar proportions of rumen volatile fatty acids. The alfalfa hay for the different trials was obtained from different fields, but hay from the same source was used within each trial.

The alfalfa hay was prepared for pelleting by grinding in a mobile unit owned and operated by Norton's Implement Company, Stillwater, Oklahoma. Samples of the ground hay were collected and the particle sizes were measured by using American Standard Testing Material (ASTM), Tyler Mesh. The ground hay was introduced in small quantities, and the nest of sieves was then shaken by hand for an appropriate length of time. The procedure was repeated several times until a sufficient amount of each size was collected. The particles collected on each mesh were removed and weighed. The distribution of particle size is presented in Table V.

TABLE V  
DISTRIBUTION OF GROUND ALFALFA HAY RETAINED BY VARIOUS SIZE MESH

ASTM, Tyler Mesh (openings/linear inch)	Per cent ground alfalfa hay retained by the mesh		
	Trial I	Trial II	Trial III
8	7.44	6.54	5.47
14	13.11	10.42	13.92
20	13.05	14.04	16.35
40	27.98	27.35	26.71
60	11.29	13.80	9.86
80	11.00	12.52	11.45
100	9.05	9.12	7.98
Less than 100	<u>7.08</u>	<u>6.21</u>	<u>8.26</u>
	100.00	100.00	100.00

Criteria used for Pellet Density Measurements

The term density or specific gravity has recently come into use in the description of feeds where volume is being used as a criteria. The term density is defined herein as a measurement of grams of alfalfa pellets displacing 1 milliliter of water. The determination was conducted at room temperature or approximately 34°C. At this temperature the density of water is 0.9944.

The density of the alfalfa pellet was determined by weighing into a 250 ml graduated cylinder 50 gms of alfalfa pellets. A measured amount of water was poured over the pellets until they were fully submerged. The pellets were stirred occasionally during a period of 30 minutes to assure expulsion of any air bubbles present. The graduated cylinder was then filled to the 250 ml mark and the density of the pellet calculated by dividing the grams of pellets by the amount of water displaced.

## RESULTS AND DISCUSSION

### Ruminal Parakeratosis

The results will be discussed in relation to previous research when appropriate. In order to acquaint the reader with the affected site of ruminal parakeratosis, attention will be given to both the physical and chemical nature of the rumen and factors affecting changes within the rumen.

#### Post-mortem Examination of the Rumina

It is generally known that the mucosal surface of the rumen is covered with papillae which vary in size and shape in different regions. In the present study the papillae were observed in some cases to be conical, but more often they tended to be tongue-like in shape. The longest of the latter type were about 2-4 mm. broad and 6-10 mm. long.

The presence of the papillae greatly increases the area of the rumen wall available for the absorption of metabolites. Dobson et al. (24) described the papillae as consisting of a central core of densely packed collagen fibers surrounded by stratified epithelium. The papillae covering the surface have a rich blood and lymphatic supply in intimate contact with the basal layer of the stratified squamous epithelium. The basal layer of the epithelium consists of columnar cells arranged around the capillaries with the long axis of the cells being perpendicular to the length of the capillaries.

Numerous mitochondria found in the columnar cells are often elongated and orientated in the long axis of the cells. In comparing the stratified epithelium covering the papillae with that of the skin, Barcroft et al. (14) noted that the layer of cornified cells was thin; the layer of granular cells (stratum granulosum), although distinct in many places, is by no means a continuous layer; and no sebaceous glands were present. Consequently, there was no greasy protection to assist in preventing the passage of water through the epithelium.

In Trial I, calves receiving an all pelleted ration developed severe ruminal parakeratosis during the duration of the experiment (Table VI), whereas calves receiving wafered or long hay showed only slight to moderate parakeratosis. The condition (Fig. 1-3) was very extensive and uniform throughout the rumina of calves receiving the all-pelleted ration. The affected papillae in the dorsal portion of the rumen were not distinct and appeared to have become attached and grown together with heavy dark incrustation covering them. The incrustations on many papillae appeared as rough and scaly and others appeared as though the papillae had been capped. The capping appearance of the papillae was more prevalent in the ventral sacs where the papillae were more numerous and greater in length. In this area, the affected papillae were firm, leathery, and enlarged. The removal of the crust or cap from the papillae revealed a hardening of the stratum corneum of the papillae, not just the formation of ingesta around the papillae. Although parakeratosis was observed throughout the anteroventral sac and along the transitional portion of the reticulum, it appeared less extensive in this region as compared to the



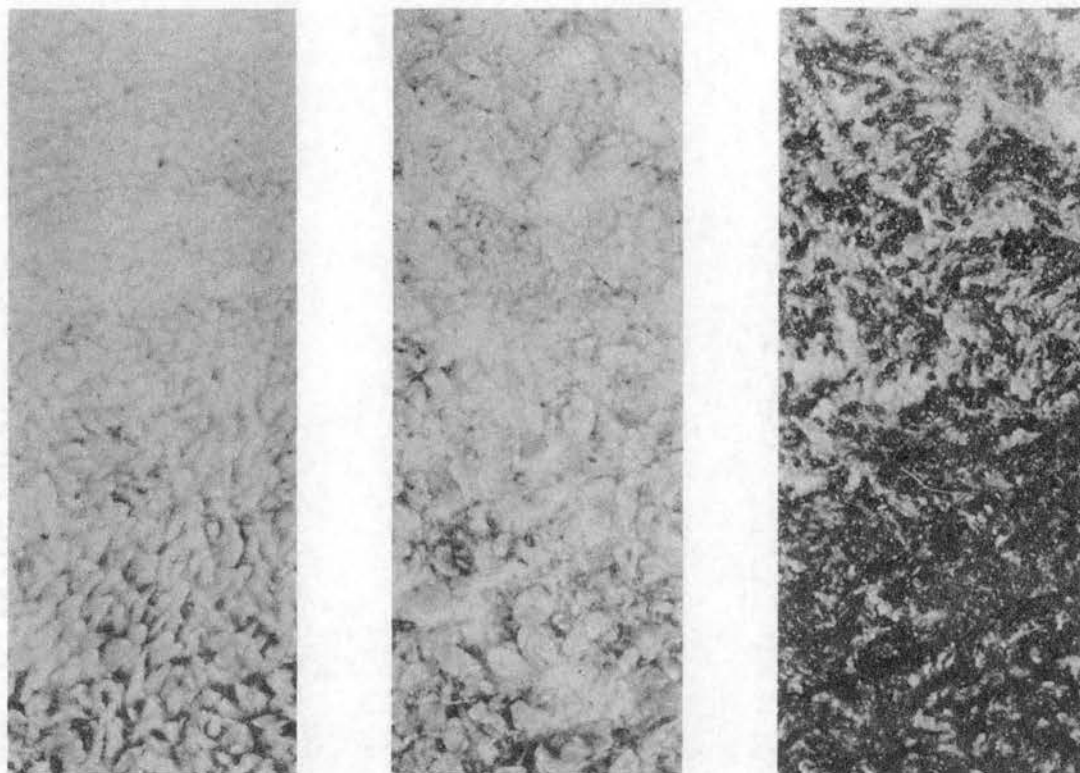


Figure 1. Sections showing papillae in dorsal area of rumen: a) no incrustation from calf on long hay; b) slight incrustation and eroded areas from calf on wafered hay; c) heavy incrustation from calf on pelleted hay.

TABLE VI  
 INCIDENCE OF RUMINAL PARAKERATOSIS IN CALVES SLAUGHTERED  
 AT FIVE MONTHS OF AGE

Trial	No. of calves per group	Ratio of grain:hay	Form of hay	Degree of parakeratosis	Remarks
I	4	1:1	Long	Slight	Calves maintained on metal screens without bedding
	4	1:1	Wafered	Slight-moderate	"
	4	1:1	Pelleted	Severe	"
II	7	4:1	Long	None	Wood shavings used for bedding
	7	4:1	Pelleted	None	"
III	5	4:1	Long	None	Dry lot conditions with no bedding
	5	4:1	Pelleted	None	"

remainder of the rumen. Many papillae on the floor of the ventral sacs and a few in the dorsal region were clumped together. Clumping of the papillae was observed to a greater degree in the calves receiving an all pelleted ration and to a slight degree in the other groups, but did not appear to be always associated with ruminal parakeratosis. The clumps of papillae ranged in diameter up to one inch in the ventral sacs. In several instances the clumps of affected papillae were visible and palpable from the serosal surface through the intact ruminal wall.

Eroded areas of papillae were observed in the ventral sacs of the rumina where the papillae were the greatest in length. It was not established whether or not this is a normal occurrence within the rumen since the treatments appeared to have no consistent effect on erosions of the papillae. The eroded areas appeared to result from the abrasive nature of the ingesta on the papillae. The size of the eroded areas was observed to be as large as 6 inches in diameter in some rumina.

Lesions were noted on the posterior pillar of two calves (No. 2 and 67) receiving a pelleted ration in Trial III. The cause of these lesions was not determined. Brownlee (17) noted that the rumen mucous membrane in calves fed concentrates without the addition of other suitable solid food was found to be easily torn, and also areas of superficial erosions of the mucous membrane covering the posterior pillar were observed.

Although ruminal parakeratosis, as denoted by incrustations of the papillae, was not observed in Trials II and III at the time of

slaughter, most of the rumina contained various degrees of clumping of the papillae. The clumping appeared to be a natural process apparently due to the form of ingesta. Even though clumping was more prevalent in some rumina than others no consistent pattern as to type of treatment was apparent. The rumina from calves of Trials II and III were mostly greyish in color with some rumina having a reddish appearance. Upon closer examination an eroded area was observed at the tips of some papillae. Most of these were observed on the floor of the anterior dorsal sac, while a few were observed in the posterior dorsal sac and all ventral sacs. On these papillae it appeared as though the mucosa was eroded from the tips. Although the red tipped papillae appeared to be more consistent with the groups receiving pelleted alfalfa hay, it was also observed to a lesser degree in the other groups. This condition was not observed in Trial I where the pelleted hay group showed severe parakeratosis.

#### Observations on Rumen Ingesta

The rumen ingesta (Fig. 4 and 5) removed from calves receiving an all pelleted ration was observed in all trials to be frothy and expanded rapidly on standing. Expansion of the ingesta probably was a result of gas trapped in the rumen contents. However, no bloat was observed in any of the calves receiving an all pelleted ration. Frothy type ingesta has been shown to contain large amounts of free gas (19). Quantitative studies by Washburn and Brody (69) established that the main constituents of rumen gas were carbon dioxide (70%) and methane (30%). Cole et al. (20) have reported that foamy ingesta results from



Figure 4. Rumen ingesta from calf receiving long hay.



Figure 5. Rumen ingesta from calf receiving pelleted hay.

inefficient expulsion of gas. Similarly, Rosen et al. (59) have suggested that carbon dioxide becomes trapped in frothy ingesta due to saponins and colloids as a result of microbial decarboxylation. Yadava (73) demonstrated a release of gas from frothing rumen contents by the addition of saliva.

Attention has been given to the salivation and the behavior of the parotid and submaxillary glands of cattle and sheep for many years. It is recognized that secretion from the parotid gland is alkaline and contains considerable quantities of bicarbonate and phosphate, as sodium and potassium salts. Further, the secretion is continuous, but is greatly enhanced during the mastication of food. In contrast, the submaxillary glands secrete during the feeding of the cow and produce a mucous-rich saliva, but are quiescent during rumination. The sublingual glands, however, are described as secreting continuously as do the superior molar and the glands on the roof of the mouth. Together, the salivary glands secrete large volumes of alkaline and well-buffered saliva which serves to stabilize the pH and water content of the fermenting ingesta contained in the rumen.

Balch (11) has demonstrated that less saliva is secreted when feed is given as cubes as compared to conventional forms. Salivary secretion rates were calculated to be 5-6, 7-12, and 3-5 lb per 10 min for hays, concentrates, and grass, respectively. Also, Bailey (9) observed that the amount of saliva added to a given weight of food varied greatly with the different foods. The variation in rate of salivary secretions was found to be mainly due to the differences in the rate at which the foods were eaten.

Bailey and Balch (10) reported that the addition to the ruminal contents of sufficient acetic acid to lower the mean pH from 6.8 to 5.4 and to increase the total VFA from 93 to 167 meq/liter had no obvious effect on the rate of secretion of mixed saliva. It was suggested that the effectiveness of tactile stimulation shortly after a meal was inhibited by some factors, possibly sensory nerve endings becoming fatigued. In contrast, Ash and Kay (8) noted that the introduction of VFA buffered at acid pH causes a transient increase in salivary secretion. Further, Ash (7) reported inhibition of rumen motility following the administration of solutions of VFA buffered at acid pH into the empty rumen.

The pillars and walls of the reticulo-rumen are in regular and almost constant movement. There is general agreement that the frequency of contractions is greatly increased during feeding. The length of a complete cycle may vary up to 100 sec. The cycle is initiated by a sharp, biphasic contraction of the reticulum and rumino-reticular fold. With the second phase of reticular activity, a contraction wave involving the anterior and dorsal rumen sac, anterior pillar, posterior and dorsal coronary pillars sweeps posteriorly, followed by sequential relaxation. Toward the end of this contraction wave, a simultaneous contraction of the anterior, posterior, and ventral coronary pillars, the ventral rumen sac and posterior dorsal blind sac, is initiated. The anterior pillar then contracts downward, where it remains until the subsequent cycle, while the remaining structures relax. This activity results in a revolving of ingesta in a roughly clock-wise direction as viewed from the left side of the animal. Coordinated with this



rumino-reticular activity is contraction of the omasal orifice and omasum, serving to control the passage of ingesta into the omasum (19).

#### Observations on Ruminal pH

In addition to foamy ingesta, the pH of the ruminal contents from calves receiving an all pelleted ration was lower as compared to the other treatment groups (Table VII). This difference was found to be statistically significant at 4 hr after feeding in Trials I and III. Cullison et al. (22) suggested an inadequate secretion of saliva as the reason for a lower ruminal pH in steers receiving a pelleted ration and showing incidence of parakeratosis.

Rate of salivary flow, as influenced by the dietary composition, may well be a contributing factor in the difference in ruminal pH observed between the groups receiving pelleted and long or wafered hay in the present investigation. It is common knowledge that scabrous materials will stimulate an increased flow of saliva from the salivary glands. The influence of scabrous materials, Weiss (70) suggested, is mediated by reflex salivary secretion which inhibits frothing. Dougherty et al. (25) postulated a stimulation of sensory fibers by the froth which inhibits the reflex relaxation of the esophageal sphincters.

It has been established for many years that increased production of carbon dioxide causes a lowering of the pH toward acidity. Cole et al. (20) have pointed out that saliva secreted at pH 8 that is in equilibrium with 6% CO<sub>2</sub> will tend to absorb CO<sub>2</sub> when exposed to 70% CO<sub>2</sub> in the rumen until its pH is reduced below about 6.9. Also, with the continual production of VFA in the rumen, any decline in the flow

TABLE VII  
pH OF RUMEN FLUID DURING LAST WEEK ON EXPERIMENT

	No. calves	<u>Hours after feeding</u>	
		4	8
Trial I			
Long hay	4	6.5 <sup>a</sup>	6.6
Wafered hay	4	6.6 <sup>a</sup>	6.7
Pelleted hay	4	5.7 <sup>b</sup>	5.9
Trial II			
Long hay	7	6.1	6.1
Pelleted hay	7	5.7	5.9
Trial III			
Long hay	5	6.2 <sup>c</sup>	6.3
Pelleted hay	5	5.4 <sup>c</sup>	5.4

<sup>a,b</sup>Values with unlike superscripts differ significantly ( $P < 0.01$ ).

<sup>c</sup>Difference between treatments statistically significant at 1% level.

of saliva would tend to reduce the buffering capacity of the rumen contents, thereby resulting in a lower rumen pH. This has been amply confirmed by Turner and Hodgetts (64) where it was shown that when no saliva was secreted the VFA produced in the rumen would lower the pH to a value below 3.0. Therefore, it appears feasible that any decline in salivary secretion coupled with an increased CO<sub>2</sub> production could have been responsible for the lowered rumen pH noted in the present experiment.

Since calves in Trials II and III on a pelleted ration had a rumen pH similar to calves in Trial I on the pelleted ration, no correlation was established between a lower rumen pH and incidence of parakeratosis. However, this does not rule out a lowered rumen pH as a possible predisposing factor in the development of parakeratosis since the pelleted grain ration and grain:hay ratio were different in Trial I and Trials II and III.

#### Molar Proportions of VFA

Results obtained on the molar proportions of the VFA in Trials I, II, and III are presented in Table VIII. A statistically significant difference ( $P < 0.05$ ) was obtained in Trial I in the molar proportion of acetic acid at 8 hr following feeding. In general, it appears that the molar proportions of the VFA in the rumen do not differ markedly as a result of feeding the hay in a pelleted vs. long form. However, it may be noted that when the ratio of hay to grain was changed from a 1:1 to a 1:4 ratio, the molar proportion of propionate increased with a corresponding decrease in acetic acid. These data are in

TABLE VIII  
 CONCENTRATION AND MOLAR PROPORTIONS OF VOLATILE FATTY ACIDS  
 IN RUMEN FLUID OF CALVES IN TRIAL I, II, AND III

	Long hay		Wafered hay		Pelleted hay	
	<u>Hours after feeding</u>		<u>Hours after feeding</u>		<u>Hours after feeding</u>	
	4	8	4	8	4	8
	(mole %)		(mole %)		(mole %)	
Acetic	59.1	54.3 <sup>a,b</sup>	58.8	60.4 <sup>a</sup>	55.4	50.9 <sup>b</sup>
Propionic	27.2	32.3	26.3	27.7	29.1	34.8
Butyric	10.6	11.3	12.3	9.5	12.2	10.4
Valeric	3.0	2.1	2.7	2.5	3.6	3.9
<u>TRIAL II</u>						
Acetic	49.9	49.7	---	---	46.8	47.5
Propionic	36.6	37.3	---	---	40.4	40.0
Butyric	10.4	9.9	---	---	9.8	9.4
Valeric	3.1	3.1	---	---	3.0	3.1
<u>TRIAL III</u>						
Acetic	51.0	56.5	---	---	52.5	44.2
Propionic	36.5	31.8	---	---	36.2	42.6
Butyric	8.5	8.2	---	---	7.8	10.3
Valeric	4.0	3.5	---	---	3.5	2.9

<sup>a,b</sup>Values with unlike superscripts differ significantly ( $P < 0.05$ ).

agreement with previous reports (12, 60) that the molar proportions of the ruminal VFA, especially acetate and propionate, can be controlled within wide limits to a remarkable degree by rather simple dietary means.

Shaw et al. (60) consider that the type of concentrate fed is relatively more important than the level of roughage included in the ration. The inclusion of ground hay and steam heated grains for lactating cows caused a significant depression in the production of milk fat, and this depression was shown to be associated with a decrease in acetate and an increase in propionate and higher acid fractions in ruminal fluid.

The importance of VFA as sources of energy to the ruminant animal is not challenged. Phillipson (57) estimated that they might supply at least 40% of the fasting energy requirements of the ruminant. Also, in a series of papers by Armstrong et al. (4, 5, 6) the importance of molecular proportions of the VFA have been considered. The VFA were administered singly or in combination to sheep on either a fasting or fattening plane of nutrition. When the acids were given individually to fasting sheep, to supply approximately 700 calories per day, they found that acetic, propionic and butyric acid produced heat increments corresponding to about 41%, 13%, and 16% of their respective caloric values. A mixture of the acids in the molecular proportions of 5:3:2 gave a heat increment of 17% instead of the expected 27%. Acetic acid could be varied greatly without increasing the heat increment appreciably when propionic acid was present. It is apparent that the animal has difficulty in utilizing acetic acid rapidly, but that, under more natural circumstances, the other acids have a pronounced synergistic effect with respect to heat increment. The utilization of mixtures of the acids, irrespective of their

composition, was highly efficient in the fasting animal. When the sheep were placed on a fattening ration it was found that the nature of utilization of the acids changed markedly. Administration of individual acids gave heat increments of 67%, 44%, and 38% for acetic, propionic, and butyric acids, respectively. Moreover, when the acids were given in combination, composition of the mixture appeared to have a decided effect on the magnitude of the heat increment. Johnson (41) estimated that if the molar percentages of the acids produced in the rumen are 60.0 acetic, 20.0 propionic, and 20.0 butyric, the relative contributions to energy production are 41%, 24%, and 35%.

#### The Effect of Parakeratosis on VFA Absorption

Four fistulated calves, paired on the basis of age and weight, were used for this aspect of the study. Neither calf of Pair I, as noted in Table IX, had developed ruminal parakeratosis at 5 mo. of age, whereas the calf of Pair II receiving an all pelleted ration had developed slight and severe parakeratosis at 4 and 5 mo. of age, respectively. Both pairs of calves were receiving the same form of feed, differing only in the composition of the pelleted grain ration (Table II). Calves in Pair I received grain Ration A, whereas Pair II received grain Ration B (Table IX). These data coincide with results obtained in the first three trials where ruminal parakeratosis was noted in Trial I with the use of grain Ration B, whereas the condition did not develop when grain Ration A was fed in Trials II and III. It appears that even though pelleted hays may be an important factor in causing the development of ruminal parakeratosis when fed as the only

source of roughage, consideration must also be given to the composition of the grain ration being included in the overall diet. At the present time it is a matter of conjecture as to what ingredient, if any, was present in grain Ration A preventing the development of ruminal parakeratosis.

During early development of parakeratosis, absorption of VFA was increased, but as the condition became more severe, VFA absorption from the rumen decreased (Table IX). Similarly, Hinders and Owen (33) have recently reported a decrease in the total percentage of VFA absorbed from the rumen following the development of parakeratosis.

The molar proportions of VFA initially introduced into the rumen and the molar proportions following a two-hour experimental test period are presented in Table X. A slight difference occurred in the molar proportions initially introduced and those present following the two-hour experimental test period. Apparently, acetic acid was absorbed to a lesser degree on a molar basis than were the other acids. The buffered VFA solution introduced into the rumen was maintained at pH 6.6 during the two-hour experimental test period. According to previous literature (23, 56, 61), one might expect that at an acid pH the specific absorption rates of the acids would be in the order butyric > propionic > acetic. A second theory would indicate some diffusion of acetic acid back through the rumen epithelium. In line with this reasoning, it has been demonstrated that when a solution, free of fatty acids, is placed in an isolated reticulo-rumen sac, small quantities of acetate accumulate (24). Under such conditions, the systemic plasma VFA fraction is composed largely of acetate.

TABLE IX  
 PRELIMINARY STUDIES ON THE EFFECT OF RUMINAL PARAKERATOSIS  
 ON ABSORPTION OF VFA FROM THE RUMEN

Pair	Age (mos.)	Calf No.	Ration <sup>a</sup>	Interval after introduction of VFA (min.)	(Initial - (Conc. at specified concentration) - intervals corrected for dilution)				Degree of Parakeratosis
					Acetic	Propionic	Butyric	Valeric	
					(mg/100 ml)				
I	5	16	Long Hay	15	167	60	61	9	None
			†	30	208	106	101	14	
			Grain A	60	291	186	148	22	
				120	461	285	165	27	
	5	86	Pelleted	15	83	11	37	5	None
			†	30	210	115	85	22	
			Grain A	60	301	167	111	28	
				120	352	201	126	32	
	5	25	Long Hay	15	54	10	27	3	None
			†	30	138	69	51	11	
			Grain B	60	159	102	85	17	
				120	293	173	117	28	
II	4	74	Pelleted	15	166	122	66	8	Slight
			†	30	327	180	113	18	
			Grain B	60	445	266	122	27	
				120	526	329	194	31	
	4	25	Long Hay	15	144	80	67	6	None
			†	30	311	199	147	20	
			Grain B	60	331	215	170	23	
				120	447	256	214	30	
5	74	Pelleted	15	74	41	3	5	Severe	
		†	30	167	92	55	14		
		Grain B	60	239	155	75	21		
			120	355	220	136	27		

<sup>a</sup>Composition of rations shown in Table II; hay and grain fed in a 1:1 ratio.



TABLE X  
 INITIAL VS. FINAL PROPORTION OF VOLATILE FATTY  
 ACIDS IN THE RUMEN OF CALVES

Pair	Age (mos.)	Calf No.	Time <sup>a</sup>	Molar %			
				Acetic	Propionic	Butyric	Valeric
I	5	16	Initial	51	30	17	3
			Final	70	22	8	0
		86	Initial	52	27	17	4
			Final	66	23	11	1
II	4	25	Initial	52	28	16	4
			Final	61	27	10	2
		74	Initial	51	29	17	3
			Final	62	23	13	2
II	5	25	Initial	49	26	22	3
			Final	63	20	21	0
		74	Initial	51	28	18	3
			Final	74	15	10	1

<sup>a</sup>Initial = introduction of VFA solution;  
 Final = Two hours following introduction.

The molar proportions of the rumen VFA also vary. This was demonstrated in the first three trials when the ration was changed from a 1:1 to a 4:1 ratio of grain to hay. Acetic acid always accounted for the majority of the fatty acids present, but decreased progressively as the concentrate portion of the ration increased. Moreover, Brown et al. (16) observed that the relative molar proportions of VFA in the rumen vein blood were nearly identical to those in the rumen fluid. The addition of either propionic or butyric acids, which drastically shifted the ratio of the acids in the rumen fluid, caused drastic changes in proportions of these acids in the rumen vein blood. Further, Annison et al. (2) demonstrated that changes in the total VFA content of the rumen ingesta after feeding were paralleled by similar changes in the portal blood.

#### Effect of Ruminal Parakeratosis on Body Weight Gain

The average daily gain for the groups of calves in each trial is presented in Table XI. No statistical significant difference in body weight gain was observed between the groups in any of the trials.

In some cases where calves were receiving an all pelleted ration, consumption of the ration was lower than for those receiving long hay. Some calves appeared to prefer an all pelleted ration, whereas others preferred long hay.

In Trial I the consumption of wafered hay by Group II was low during the early part of the one-month adjustment period. However, as calves became accustomed to the wafered hay no problem in consumption was noted. The average daily gain, as shown in Table XI, was highest

during the experimental period for the group receiving wafered hay in Trial I.

#### Effect of Particle Size on Ruminal Parakeratosis

The fineness of grind of the alfalfa hay prior to pelleting was measured through a series of test sieves and the distribution of particle sizes, as percentage of hay retained on sieves, is given in Table V. No apparent differences in particle size appeared to exist between the three sources of ground alfalfa hay used in the three trials. Although particle size may be an important critical factor in ruminant nutrition in some feeding experiments, the variation among trials in the present work suggests responsible factors causing parakeratosis other than particle size. In contrast, Garrett *et al.* (28) suggest an apparent association between ruminal parakeratosis and feed particle size.

In order to further evaluate the difference between pellets, density measurements were obtained on the pellets. Thus, 0.76, 0.83, and 0.72 g alfalfa hay were required to displace one milliliter of water used in Trials I, II, and III, respectively. There was no apparent association between density and incidence of parakeratosis.

#### Problems Arising Needing Further Investigation

Although the objectives of the present study have dealt with the development of ruminal parakeratosis and its effect on volatile fatty acid (VFA) absorption from the rumen, further investigations are needed to find the cause of this condition. Since one concentrate

ration was not effective in causing the condition when fed in combination with the same pelleted alfalfa hay, perhaps certain ingredients present in the ration were responsible.

The grain ration in Trial IV, appearing to prevent the development of ruminal parakeratosis, was noted to contain 1% urea. Urea may pass from the blood to the rumen contents either in the saliva or directly through the rumen epithelium. The organic constituents of saliva, principally mucus and urea, have been suggested to be of importance in bloat and are a source of nitrogen to the micro-organisms of the rumen (Kay, 42). Urea accounts for most of the nitrogen present in parotid saliva and its concentration is proportional to, but rather lower than, its concentration in plasma. Further studies are needed to test the effectiveness of low levels of dietary urea in preventing ruminal parakeratosis.

The pH of the rumen contents is usually between 5.5 and 7.3. The location within this range is usually dependent upon time of eating, type of diet, rate of passage through digestive tract, absorption of VFA and flow of saliva. In the present study animals receiving an all pelleted ration were generally noted to have a lower rumen pH than those receiving long hay. Also, during early development of parakeratosis absorption of VFA were increased, whereas a decrease in VFA absorption was noted as the condition became more severe. It would be interesting to determine whether VFA absorption was increased initially due to increased blood flow through the rumen epithelium, or if the increase resulted from a change in the histological nature of the rumen epithelium.

The effect of feeding buffers in the ration to partially control rumen pH has not been satisfactorily investigated. More work is needed to test their effectiveness as a preventative for parakeratosis over a long period of time.

## SUMMARY AND CONCLUSIONS

Three trials, involving 10-14 male Ayrshire calves each, were conducted to determine the effect of different rations on ruminant parakeratosis and certain biochemical conditions in the rumen. A fourth trial involving two pairs of male Ayrshire calves was conducted to determine the effect of parakeratosis on volatile fatty acid (VFA) absorption from the rumen, using polyethylene glycol as a nonabsorbable marker.

Calves in all trials were on experiment between the ages of 3-5 months. Volatile fatty acid and pH determinations were made on rumen samples taken from calves in the first three trials at 4 and 8 hr after feeding during the eighth week. In Trial I the calves were maintained on expanded metal floors. A ration having a 1:1 ratio of concentrates to roughage was fed with the hay baled, wafered, and pelleted for three respective treatment groups. In Trials II and III a 4:1 ratio was fed, with the hay being baled or pelleted. In Trial II, wood shavings were used for bedding, whereas calves in Trial III were maintained under dry lot conditions.

In Trial I, extensive ruminal parakeratosis was observed in calves fed pelleted hay, whereas calves fed wafered or long hay had only slight parakeratosis. No ruminal parakeratosis was observed in the rumina of calves on Trials II and III.

In all trials the pH of the rumen ingesta was lower in calves fed pelleted hay than in calves fed hay in other forms. This difference was found to be statistically significant ( $P < .05$ ) in Trial I and highly significant ( $P < .01$ ) in Trial III at 4 hr following feeding. Inadequate secretion of saliva and an increased amount of ruminal gases in the frothy ingesta are postulated as responsible factors causing a lower rumen pH and as predisposing factors in the development of ruminal parakeratosis. In the first 3 trials, when the grain to hay ratio was changed from a 1:1 to a 4:1 ratio, the molar proportion of acetate decreased and propionate increased in a similar manner regardless of the form of hay included in the diet.

Calves in Trial IV were paired on the basis of age and weight and fistulated at two months of age. The calves were maintained on expanded metal floors with no bedding. Both pairs received a 1:1 ratio of grain to hay, the rations differing only in the grain composition. Calves in Pair I were fed the same pelleted grain mixture as calves in Trials II and III, whereas calves in Pair II received a pelleted grain ration similar to calves in Trial I. One calf in each pair received either long or pelleted hay.

At the end of 4 and 5 months neither calf of Pair I had developed ruminal parakeratosis, whereas the calf of Pair II, receiving an all pelleted ration, showed slight and severe parakeratosis at 4 and 5 months of age, respectively. It appears that during early development of ruminal parakeratosis the absorption of VFA is increased, but as the condition becomes more severe, VFA absorption from the rumen decreases.

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A P P E N D I X

TABLE XI  
 AVERAGE DAILY GAIN FOR CALVES IN TRIALS I, II, AND III  
 DURING A TWO-MONTH EXPERIMENTAL PERIOD

Trial		Daily gain	TDN/lb. gain
I	Group I (long hay)	1.61	3.55
	Group II (wafered hay)	1.81	3.05
	Group III (pelleted hay)	1.64	3.42
II	Group I (long hay)	1.13	3.92
	Group II (pelleted hay)	1.38	2.97
III	Group I (long hay)	1.81	2.65
	Group II (pelleted hay)	1.59	2.73



TABLE XII  
 THE pH OF RUMEN INGESTA IN TRIAL I TAKEN AT 4 AND 8 HR  
 FOLLOWING FEEDING AND AT TIME OF SACRIFICE

Groups	4 hr	8 hr	<sup>a</sup> Time at sacrifice
(Calf No.)	(pH)	(pH)	(pH)
I (long hay)			
98	6.7	6.7	5.9
19	6.3	6.2	5.5
223	6.4	6.2	5.1
18	6.7	7.3	5.7
II (wafered hay)			
56	6.3	6.4	5.5
9	6.8	6.8	5.7
92	6.4	6.8	5.6
201	6.8	6.6	6.0
III (pelleted hay)			
91	6.0	6.2	5.9
220	5.4	5.9	5.4
40	5.3	5.1	5.3
63	6.1	6.5	5.3

<sup>a</sup>Rumen sample taken approximately 30 min following sacrifice.

TABLE XIII  
 THE pH OF RUMEN INGESTA IN TRIAL II TAKEN AT 4 AND 8 HR  
 FOLLOWING FEEDING AND AT TIME OF SACRIFICE

Groups	4 hr	8 hr	<sup>a</sup> Time at sacrifice
(Calf No.)	(pH)	(pH)	(pH)
I (long hay)			
43	7.0	6.5	5.7
79	6.8	6.4	5.5
57	5.8	5.6	5.3
89	5.9	5.6	5.4
73	5.4	6.3	5.3
32	6.2	6.0	6.5
75	5.7	6.0	6.6
II (pelleted hay)			
95	5.9	5.9	5.7
65	5.6	5.6	5.3
11	5.7	5.9	5.6
6	5.4	6.2	5.7
90	6.0	5.8	6.0
86	5.7	5.7	5.0
39	5.7	6.3	5.7

<sup>a</sup>Rumen sample taken approximately 30 min following sacrifice.

TABLE XIV  
 THE pH OF RUMEN INGESTA IN TRIAL III TAKEN AT 4 AND 8 HR  
 FOLLOWING FEEDING AND AT TIME OF SACRIFICE

Groups	4 hr	8 hr	<sup>a</sup> Time at sacrifice
(Calf No.)	(pH)	(pH)	(pH)
I (long hay)			
219	6.4	6.3	6.7
25	6.0	6.4	7.0
56	6.0	6.2	6.2
92	5.4	5.5	6.6
3	6.8	6.8	6.3
II (pelleted hay)			
26	5.5	5.5	6.1
42	5.4	5.3	5.8
37	5.2	5.5	5.4
2	5.1	5.9	6.1
67	6.0	6.2	6.3

<sup>a</sup>Rumen sample taken immediately at time of sacrifice.

TABLE XV

FEED CONSUMPTION, BODY WEIGHT GAIN AND ESTIMATED TDN/LB  
GAIN DURING A 2-MONTH EXPERIMENTAL TEST PERIOD

	<u>Feed Consumption</u>			<u>Body Wt. Gain</u>		TDN/lb. gain
	Grain (lbs)	Hay (lbs)	Total (lbs)	Total (lbs)	Daily (lbs)	
Group I						
(long hay)						
98	272.0	268.5	540.5	95.6	1.59	3.40
19	258.0	259.5	517.5	100.0	1.67	3.10
223	312.0	312.0	624.0	95.2	1.59	3.93
18	298.0	298.0	596.0	95.0	1.58	3.76
$\bar{x}$	285.0	284.5	562.8	96.5	1.61	3.55
Group II						
(wafered hay)						
56	293.5	289.2	582.7	111.3	1.86	3.15
9	288.0	287.8	575.8	102.0	1.70	3.39
92	260.0	256.0	516.0	115.7	1.93	2.68
201	263.0	264.0	527.0	105.7	1.76	2.99
$\bar{x}$	276.1	274.3	550.4	108.7	1.81	3.05
Group III						
(pelleted hay)						
91	273.0	261.8	534.8	74.0	1.23	4.35
220	274.0	274.0	548.0	111.3	1.86	2.95
40	268.0	268.0	536.0	88.7	1.48	3.63
63	274.0	274.0	548.0	120.0	2.00	2.74
$\bar{x}$	272.3	269.5	541.7	98.5	1.64	3.42

TABLE XVI  
 FEED CONSUMPTION, BODY WEIGHT GAIN AND ESTIMATED TDN/LB  
 GAIN DURING A 2-MONTH EXPERIMENTAL TEST PERIOD

	<u>Feed Consumption</u>			<u>Body Wt. Gain</u>		TDN/lb. gain
	Grain	Hay	Total	Total	Daily	
Group I	(lbs)	(lbs)	(lbs)	(lbs)	(lbs)	
(long hay)						
43	313.3	79.2	392.5	58	0.97	4.46
79	315.1	78.2	393.3	86	1.43	3.02
57	333.6	84.1	417.7	51	0.85	5.40
89	268.4	64.9	333.3	43	0.72	5.12
73	287.6	71.8	359.4	79	1.32	3.00
32	298.8	74.4	373.2	78	1.30	3.16
75	304.0	81.0	385.0	78	1.30	3.25
$\bar{x}$	303.0	76.2	379.2	67.6	1.13	3.92
Group II						
(pelleted hay)						
95	293.0	75.6	368.6	85	1.41	2.86
65	314.8	78.9	393.7	90	1.50	2.89
11	268.1	68.3	336.4	79	1.32	2.81
6	260.3	62.5	322.8	68	1.13	3.14
90	298.4	74.6	373.0	73	1.22	3.37
86	330.6	82.8	413.4	95	1.58	2.87
39	308.8	82.2	391.0	90	1.50	2.86
$\bar{x}$	296.3	75.0	371.3	82.9	1.38	2.97

TABLE XVII

FEED CONSUMPTION, BODY WEIGHT GAIN AND ESTIMATED TDN/LB  
GAIN DURING A 2-MONTH EXPERIMENTAL TEST PERIOD

	<u>Feed Consumption</u>			<u>Body Wt. Gain</u>		TDN/lb. gain
	Grain	Hay	Total	Total	Daily	
Group I	(lbs)	(lbs)	(lbs)	(lbs)	(lbs)	
(pelleted hay)						
26	312.2	77.3	389.5	94	1.57	2.74
42	320.8	87.0	407.8	112	1.87	2.39
37	262.4	76.8	339.2	85	1.42	2.61
2	289.2	72.3	361.5	82	1.37	2.91
67	323.6	80.9	404.5	102	1.70	2.62
$\bar{x}$	301.6	78.9	380.5	95	1.59	2.65
Group II						
(long hay)						
219	342.0	84.9	426.9	110	1.83	2.56
25	333.8	85.3	419.1	101	1.68	2.74
56	355.2	88.8	444.0	83	1.38	3.53
92	354.0	87.6	441.6	120	2.00	2.43
3	372.0	93.0	465.0	129	2.15	2.38
$\bar{x}$	351.4	87.9	439.3	108.6	1.81	2.73

TABLE XVIII

## ANALYSIS OF VARIANCE ON VFA (ACETIC ACID-8 HR) IN TRIAL I

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	11	351.67	-	-
Blocks	3	107.21	35.74	3.46
Treatment	2	182.43	91.22	8.82*
Error	6	62.03	10.34	

\*P &lt; 0.05

TABLE XIX

## ANALYSIS OF VARIANCE ON VFA (ACETIC ACID-4 HR) IN TRIAL III

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	9	576.96	-	-
Blocks	4	192.17	48.04	1.66
Treatment	1	278.36	278.36	9.66*
Error	4	106.43	28.81	

\*P &lt; 0.05

TABLE XX

## ANALYSIS OF VARIANCE ON VFA (ACETIC ACID-8 HR) IN TRIAL III

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	9	869.02	96.56	
Blocks	4	304.84	76.21	1.60
Treatment	1	373.32	373.32	7.82*
Error	4	190.86	47.72	

\*P &lt; 0.05

TABLE XXI

## ANALYSIS OF VARIANCE ON RUMEN pH TAKEN AT 4 HR IN TRIAL I

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	11	2.77	0.25	-
Blocks	3	0.42	0.14	2.03
Treatment	2	1.94	0.97	13.86**
Error	6	0.415	0.07	

\*\* P &lt; 0.01

TABLE XXII

## ANALYSIS OF VARIANCE ON RUMEN pH TAKEN AT 4 HR IN TRIAL III

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	9	2.74	0.32	
Blocks	4	1.47	0.37	13.21*
Treatment	1	1.16	1.16	41.43**
Error	4	0.11	0.028	

\*P &lt; 0.05

\*\*P &lt; 0.01



TABLE XXIII

## PROXIMATE ANALYSIS OF FEEDS USED IN ALL TRIALS

Nutrients	Trials <sup>a</sup>									
	I				II			III		
	PH	WH	LH	PG	PH	LH	PG	PH	LH	PG
Crude protein <sup>b</sup>	22.0	20.8	22.9	14.6	20.8	17.9	13.9	18.9	17.4	13.9
Fat	2.0	1.9	1.7	3.5	1.8	1.4	2.8	2.4	2.5	2.8
Carbohydrates										
NFE	42.0	38.2	45.3	63.8	45.8	39.8	62.7	42.4	42.3	62.7
Crude fiber	12.8	13.8	8.9	2.8	12.6	21.7	2.9	16.9	21.1	2.9
Moisture	10.2	10.8	12.0	9.6	10.4	11.3	11.9	10.2	9.5	11.9
Ash	11.0	14.5	9.2	5.7	8.6	7.9	5.8	9.2	7.2	5.8

<sup>a</sup>PH = pelleted hay; WH = wafered hay; LH = long hay; PG = pelleted grain.

<sup>b</sup>Crude protein equivalent in Trials II and III.

TABLE XXIV  
 COMPOSITION OF PELLETED RATIONS CAUSING RUMINAL PARAKERATOSIS  
 USED BY VARIOUS RESEARCH WORKERS

Research Workers	Ingredient	%	
Beardsley <u>et al.</u> (15)	Ground snapped corn	Mixture	
	Cottonseed meal		
	Blackstrap molasses		
	Coastal Bermuda hay		
Cullison <u>et al.</u> (22)	Coastal Bermuda hay	30.0	
	Ground shelled corn	50.0	
	Cottonseed meal	10.0	
	Molasses	9.0	
	Defluorinated phosphate	0.33	
	Ground limestone	0.33	
	Salt	0.34	
Garrett <u>et al.</u> (28)	Alfalfa pellet	50.0	
	Ground barley	37.5	
	Molasses beet pulp	12.5	
Haught <u>et al.</u> (32)	Alfalfa pellet	70.0	
	Ground corn	27.0	
	Soybean oil meal	2.5	
	Salt	0.5	
Hopkins <u>et al.</u> (35)	Ground alfalfa hay	Mixture	
	Ground corn		
	Soybean oil meal - 0.1 lb/head daily		
Jensen <u>et al.</u> (40)	Alfalfa, ground	50.0	
	Corn, ground	13.9	
	Barley, ground	13.9	
	Ration, 1st to 36th day	Millet, ground	13.9
		Molasses	8.3
		Alfalfa, ground	40.1
Ration, 37th to 58th day	Corn, ground	17.2	
	Barley, ground	17.2	
	Millet, ground	17.2	
	Molasses	8.3	

TABLE XXIV (Continued)

Research Workers	Ingredient		%
Kunkel <u>et al.</u> (44)	Sorghum grain		40.0
	Molasses		10.0
	Cottonseed hulls		35.0
	Dehydrated alfalfa meal		5.0
	Cottonseed or soybean oil meal		10.0
McClure <u>et al.</u> (49)	Shelled corn		
	Cottonseed meal		Mixture
	Limestone		
McCroskey <u>et al.</u> (50)	Milo	1.0	65.1
	Cottonseed meal	12.0	7.0
	Molasses	7.0	7.0
	Cottonseed hulls	40.0	10.0
	Chopped alfalfa	40.0	10.0
	Limestone	--	0.9
Ward, G. M.	Personal correspondence - Colorado workers. Pelleted and fed to cows in rather large amounts.		
	Ground corn		
	Ground milo		
	Ground barley		Mixture
	Soybean oil meal		
	Molasses		

VITA

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