

Postmortem Ruminal Changes in Sheep and Steers

Key Words: Cattle, acidosis, pH, rumen, lactic acid, volatile fatty acid

Introduction

Beef cattle feedyards typically feed finishing diets containing 90 to 97% concentrate. Due to the high concentrate levels fed, factors such as respiratory disease, weather changes, and errors in feed bunk management can lead to clinical and subclinical cases of acidosis.

Diagnosis of fatal acidosis by visual signs and histological changes is tentative at best. Autolysis can be very rapid in calves that die of acidosis making diagnosis by rumen morphological and histological changes difficult.¹ Interpretation of morphological changes may also be complicated by other pathological alterations, especially in calves that are recovering from respiratory disease, since the presence of severe lung lesions could suggest fatal respiratory disease when the true cause of death was acidosis (D. Griffin, personal communication). Misdiagnosis as bloat may also prevent identification of acidosis as cause of death.

It is generally regarded that acidosis can be diagnosed from the pH of ruminal contents. Thomson² reported that in excised rumens stored at 22 degrees C, the pH of ruminal contents declined only slightly (from 6.6 to 6.2) during the first 24 hours postmortem. Based on those results, Thomson² concluded that ruminal pH would not decline below 6.0 up to 24 hours after death and, therefore, a ruminal pH of 5.0 or less was an indicator of fatal acidosis. However, interpretation of these data are complicated by the fact that the diet of the animals was not known, and because the rumens were excised, thus reducing any insulating effect of the hide, body fat, and offal. Logically, it would be expected that in animals fed a high concentrate diet, ruminal fermentation could continue for several hours after death leading to a decline in the ruminal pH caused by synthesis of volatile fatty acids (VFA) or lactic acid without subsequent absorption or buffering by salivation.

Rapid, simple, and accurate field diagnosis of acidosis is important in determining remedial actions that may be needed to reduce the incidence of the disease. Therefore, these studies were designed to determine the

Five studies were conducted to determine the postmortem changes in ruminal contents of sheep and cattle. Postmortem ruminal pH values decreased and concentrations of volatile fatty acids increased significantly. The decrease in ruminal pH was apparently due to the increased ruminal VFA concentrations because lactate did not accumulate in any of the studies. Postmortem ruminal changes appeared to be dependent upon the concentrate level of the diet as well as feed intake. These results indicate that ruminal fluid pH and/or lactic acid concentrations are potentially poor indicators of fatal acidosis in animals that have been dead for several hours.

effects of subclinical acidosis on postmortem ruminal changes in lambs and steers.

Materials and Methods

All experimental procedures were reviewed and approved by the appropriate Animal Care and Use Committee as to humane treatment of animals.³ All animals were humanely sacrificed using methods approved by the AVMA.⁴

EXPERIMENT 1

Eleven sheep were fed a 65% concentrate, corn/soybean meal/corn based diet for 2 weeks. On the sampling day, sheep were given ad libitum access to the diet and 3 hours postprandial were sacrificed. Ruminal samples were obtained from 3 sheep immediately (T0), from 4 sheep at 6 hours postmortem (T6), and from 4 sheep at 12 hours postmortem (T12). All animals were sampled only one time. Ruminal contents were filtered through cheesecloth and the pH of the ruminal fluid was taken immediately using a combination electrode. Ruminal fluid samples were frozen and later assayed for VFA by gas-liquid chromatography⁵ using a column of 10% SP-1200 on acid-washed Chromosorb W (Supelco, Inc., Bellefonte, PA 16823). Ruminal concentrations of D-lactate⁶ and L-lactate⁷ were determined by spectrophotometric procedures.

EXPERIMENT 2

Sixteen sheep were fed an 83% concentrate, corn/soybean meal/corn based diet for 2 weeks. On the sampling day, sheep were given ad libitum access to the diet and were sacrificed 3 hours postprandial. Ruminal samples were obtained from 4 sheep immediately (T0), from 6 sheep at 6 hours postmortem (T6), and from 6 sheep at 12 hours postmortem (T12). All animals were sampled only one time. Ruminal fluid was treated and analyzed as previously described.

EXPERIMENT 3

Thirteen sheep were fed a 65% concentrate, corn/soybean meal/corn based diet for 2 weeks. After the 2-week adaptation period, sheep were switched to an 83% concentrate diet over a three-day period. On the first day of the dietary change, sheep were fed a diet that contained 2/3 of the 65% concentrate diet and 1/3 of the 85% concentrate diet. On the second day, sheep were fed a diet that contained 1/3 of the 65% concentrate

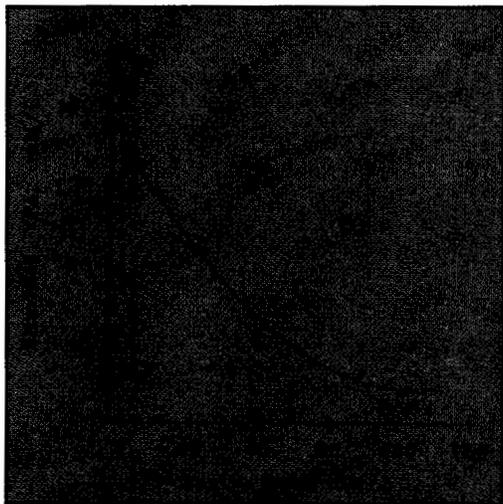


FIG. 1 — Relationship between ruminal volatile fatty acid concentrations and ruminal fluid lactic acid concentrations in Experiments 1, 2, and 3. The quadratic regression equation was as follows: $VFA = 137.5 - 2.01X + 0.01X^2$; $r^2 = 0.81$. Numbers indicate Experiment number.

diet and 2/3 of the 83% concentrate diet. On the third day, the sheep were given ad libitum access to the 83% concentrate diet and at 3 hours postprandial were sacrificed. Ruminal samples were obtained from 4 sheep immediately (T0), from 4 sheep at 6 hours postmortem (T6), and from 5 sheep at 12 hours postmortem (T12). All animals were sampled only one time. Ruminal samples were treated and analyzed as previously described.

EXPERIMENT 4

Eleven mature sheep (3 to 6 years of age, average body weight = 57 kg) with permanent ruminal cannula were used. Seven sheep were allowed ad libitum access (1.4 kg/head/day) to a diet composed of 75% dry rolled grain sorghum, 15% com silage, and 10% supplement. Three sheep were limit-fed (0.45 kg/head/day) the same diet. One sheep was limit-fed (0.55 kg/day) a high-roughage diet consisting of 65% comcobs, 15% molasses, 10% dry rolled com, 9% alfalfa, and 1% supplement. Sheep were adapted to the diets for 60 days.

At the end of the 60-day adaptation period, sheep were sacrificed and strained ruminal fluid samples were taken via the ruminal cannula from each animal immediately after sacrifice and at 6, 12, and 24 hours postmortem. Ruminal fluid was analyzed for pH, VFA, and lactic acid as previously described.

EXPERIMENT 5

Four Hereford steers (average weight 450 kg) were fed a 50% concentrate diet for two weeks. Over an 8-day period, the concentrate level in the diet was increased to 90% by increasing the amount of com in the diet by 5 percentage units per day. The final diet contained 80% dry rolled com, 9.3% cottonseed hulls, and 10.7% protein/vitamin/mineral supplement. The steers were then fed the 90% concentrate diet at 2.5% of body weight (dry matter basis) for 5 days. On the day of sample collection, steers were fed one-half of their daily ration at 0800 hours, and one hour later were sacrificed.

Within 10 minutes of death, a 5-cm transverse incision was made into the dorsal sac of the rumen and a 100 ml sample of ruminal digesta was collected. The incision was closed with a hemostat to prevent further entry of air into the rumen and further loss of fermentation gases. Ruminal and rectal temperatures were obtained with an electronic thermometer. Additional ruminal samples and temperature readings were obtained at 2, 4, 6, and 24 hours postmortem. Ruminal fluid pH and VFA concentrations were determined as previously described.

STATISTICAL ANALYSES

Data were statistically analyzed by analysis of variance using the GLM procedure of SAS.⁸ Time effects were tested by linear and quadratic contrasts.

Results

EXPERIMENT 1

Ruminal pH declined (linear effect, $P < 0.01$) between 0 and 12 hours postmortem (Table 1). There was no significant accumulation of lactic acid in the ruminal fluid, however, total VFA concentrations increased (linear effect, $P < 0.02$) from 61 to 169 mM/L.

Two animals in the group sampled at 12 hours postmortem had ruminal pH values of 5.0, which, under Thomson's² criteria, would indicate potentially fatal acidosis. However, there was no significant accumulation of lactic acid in the ruminal fluid of these sheep (0.6 and 0.5 mM/L), rather, just an abundance of VFA (221 and 186 mM/L).

EXPERIMENT 2

Ruminal pH declined (linear effect, $P < 0.01$) between 0 and 12 hours postmortem (Table 1). In contrast to Experiment 1, ruminal lactate concentrations were relatively high at 0 hours postprandial then decreased (linear effect, $P < 0.10$) at 6 and 12 hours postmortem. Ruminal total VFA concentrations increased (linear effect, $P < 0.001$) from 62 to 203 mM/L.

Four animals in the group sampled at 12 hours postmortem had ruminal pH values of 5.0 or less. As in Experiment 1, there was no significant accumulation of lactic acid in the ruminal fluid of these sheep (0.6 mM/L or less), whereas

Ruminal Changes

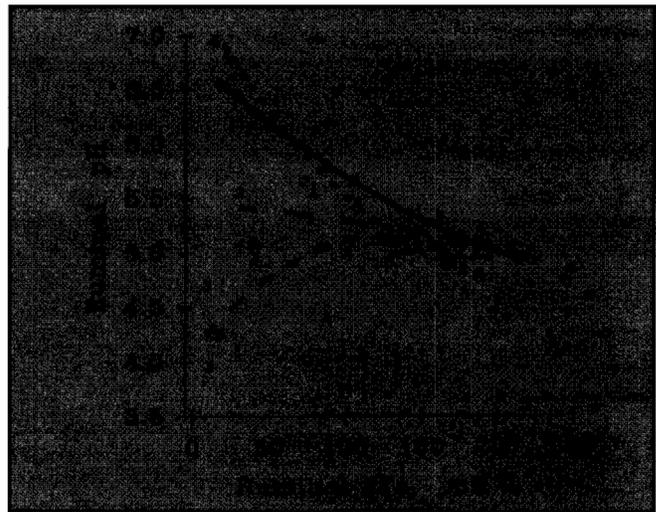


FIG. 2 - Relationship between ruminal volatile fatty acid concentrations and ruminal pH in Experiments 1 to 5. Quadratic regression equations were as follows: Experiment 1 (solid line), $\text{pH} = 6.75 - 0.01X + 0.00002X^2$, $r^2 = 0.81$; Experiment 2 (long dashed line), $\text{pH} = 5.45 - 0.0008X + 0.000007X^2$, $r^2 = 0.46$; Experiment 3 (medium dashed line), $\text{pH} = 4.12 + 0.02X - 0.00006X^2$, $r^2 = 0.56$; Experiment 4 (short dashed line), $\text{pH} = 7.4 - 0.03X + 0.000066X^2$, $r^2 = 0.94$; and Experiment 5 (dotted line), $\text{pH} = 7.43 - 0.02X + 0.000056X^2$, $r^2 = 0.98$. Numbers indicate Experiment number.

there was a marked accumulation of VFA (163 to 250 mM/L).

EXPERIMENT 3

Ruminal pH did not significantly change ($P = 0.62$) between 0 and 12 hours postmortem (Table 1). Ruminal lactic acid concentrations were high (67 mM/L) at 0 hours postmortem then decreased over time (linear effect, $P < 0.10$) to a mean value of 13.5 mM/L at 12 hours postmortem. Total VFA concentrations increased (linear effect, $P < 0.03$) from 37 to 124 mM/L.

Three animals in the group sampled at 0 hours postmortem had ruminal pH values of less than 5.0. These animals also had high ruminal lactic acid concentrations (80 to 93 mM/L) but low VFA concentrations (8.7 to 18.7 mM/L). In addition, two animals sampled at 6 hours postmortem and two animals sampled at 12 hours postmortem also had pH values of less than 5.0. Of the two lambs with low ruminal pH values (4.4 and 3.95) at 6 hours postmortem, both lambs also had elevated ruminal lactic acid concentrations (41.5 and 127.5 mM/L). The lamb with the highest lactic acid concentrations also had the low-

TABLE 1.
POSTMORTEM RUMINAL pH, LACTIC ACID, AND VOLATILE FATTY ACID CHANGES IN EXPERIMENTS 1, 2, AND 3^a (LEAST SQUARE MEANS)

Item	Hours postmortem			
	0	6	12	SEM ^b
EXPERIMENT 1				
No. of sheep	3	4	4	
Ruminal pH	6.27	5.36	5.27	0.16
Lactic acid, mM/L	0.2	0.2	0.5	0.21
Total VFA, mM/L	61.0	121.5	169.0	18.03
EXPERIMENT 2				
No. of sheep	4	6	6	
Ruminal pH	5.40	5.12	5.02	0.08
Lactic acid, mM/L	36.4	13.7	1.2	7.91
Total VFA, mM/L	61.6	138.1	202.6	16.61
EXPERIMENT 3				
No. of sheep	4	4	5	
Ruminal pH	4.71	4.76	5.02	0.13
Lactic acid, mM/L	67.2	43.2	13.5	12.72
Total VFA, mM/L	37.0	85.1	123.5	18.23

^aIn Experiment 1 lambs were fed a 65% concentrate diet, in Experiment 2 were fed an 83% concentrate diet, and in Experiment 3 were switched from a 65% to an 83% concentrate diet.

^bStandard error of the mean. *Significant linear effect ($P < 0.02$). *Significant linear effect ($P < 0.10$).

RUMINAL CHANGES

Continued

Item	Hours postmortem			
	0	6	12	24
AD LIBITUM GRAIN DIET (n = 7)				
Ruminal pH*	5.53	5.01	5.00	5.01
Lactic acid, mM/L	0.26	0.14	0.39	0.34
Total VFA, mM/L*	78.6	139.2	164.1	196.9
RESTRICTED GRAIN DIET (n = 3)				
Ruminal pH*	7.02	6.94	6.94	6.81
Lactic acid, mM/L	0.11	0.13	0.11	0.09
Total VFA, mM/L*	11.6	11.6	14.0	21.3
CORNCOB DIET (n = 1)				
Ruminal pH	7.24	6.38	5.99	5.77
Lactic acid, mM/L	0.11	0.12	0.10	0.11
Total VFA, mM/L	28.7	53.1	68.7	85.7

*Significant linear effect ($P < 0.05$).

est ruminal pH and ruminal VFA concentrations (87.5 and 10.8 mM/L). Of the two lambs with low ruminal pH values (4.90 and 4.95) at 12 hours postmortem, one lamb (pH 4.95) had high ruminal lactic concentrations (59.0 mM/L) accompanied by low VFA concentrations (40.3 mM/L). In contrast, the other lamb (pH 4.90) had relatively low lactic acid concentrations (2.4 mM/L) and relatively high VFA concentrations (128.4 mM/L).

EXPERIMENT 4

In sheep fed the high concentrate diet ad libitum, ruminal pH values declined ($P < 0.05$) from 5.53 to 5.01 within 6 hours postmortem and remained near pH 5 through 24 hours (Table 2). Lactic acid concentrations remained low throughout the 24 hour postmortem period, whereas, VFA concentrations increased ($P < 0.05$) from 79 to 199 mM/L.

Postmortem ruminal pH values also declined ($P < 0.05$) and VFA concentrations also increased ($P < 0.05$) in sheep limit-fed the high concentrate and high roughage diets. However, these changes were not as dramatic as in sheep fed the high concentrate diet ad libitum. This would be expected, since the limit-fed sheep did not have the substrate-loading or the potential for compromised buffering capacity of the sheep fed the high concentrate diet ad libitum.

EXPERIMENT 5

Postmortem ruminal pH changes are presented in Table 3. Two steers had

Item	Hours postmortem					SEM
	0	2	4	6	24	
RUMINAL PH:						
Low (n = 2)	4.70	4.70	4.65	4.55	4.55	0.10
High (n = 2)*	5.50	5.12	5.05	5.05	4.90	0.05
Average (n = 4)*	5.10	4.90	4.85	4.80	4.72	0.22
TEMPERATURE, C DEGREES:						
	37.5	38.8	36.8	36.4	28.7	0.81
	38.7	37.6	35.7	34.6	27.9	0.43
	24	27	30	32	24	

*Significant linear effect ($P < 0.05$).

initial pH values of 4.7, whereas, two steers had initial values above 5.0 (5.6 and 5.4); therefore, values for steers with low and high initial pH values are presented. None of the steers exhibited clinical signs of acute acidosis but the ruminal pH values suggest that at least two steers (pH 4.7) may have had sub-clinical acidosis. Ruminal pH of all steers declined ($P < 0.05$) postmortem but the decline was greatest in steers with high initial pH values.

Ruminal temperature remained fairly constant for the first 6 hours postmortem, whereas, rectal temperatures declined steadily (Table 3: $P < 0.01$).

Total ruminal VFA concentrations (Table 4) increased ($P < 0.05$) postmortem. Molar concentrations of acetate and propionate tended ($P < 0.09$) to decrease postmortem, whereas, molar concentrations of butyrate and minor VFAs increased ($P < 0.05$).

Discussion

In contrast to the results of Thomson,² these studies indicate that, in sheep and cattle, ruminal fermentation continues for 12 to 24 hours postmortem, resulting in significant accumulations of ruminal VFAs and declines in ruminal pH. Thomson² used rumens collected from "apparently normal feedlot cattle" at an abattoir. Thus, the composition and intake of the animals' diets as well as the last feeding time could not be determined. Thomson² reported that most of the sampled rumens had high protozoal counts. The ad libitum feeding of high concentrate diets tends to reduce ruminal protozoal counts to near zero.⁹ Therefore, the high protozoal counts noted by Thomson² would suggest that animals in their study were being fed a low-concentrate diet or were limit-fed a high-concentrate diet. In Experiment 4, when sheep were limit fed a high-concentrate diet, the postmortem changes in ruminal pH were similar to those noted by Thomson.²

In all 5 experiments, the postmortem decrease in ruminal pH values was apparently due to increased ruminal VFA concentrations, rather than to increased lactic acid concentrations. However, in Experiments 2 and 3, the low ruminal pH values noted in some lambs sampled at 0 hours postmortem were apparently the result of pre-mortem accumulation of lactic acid in the ruminal fluid. In Experiments 2 and 3 the mean ruminal lactic acid concentrations were higher in lambs sampled at 0 hours postmortem than in lambs sampled at 6 and 12 hours postmortem. Because different animals were sampled at different times in these experiments, it is not possible to determine if this apparent decline in lactic acid concentrations would have occurred in animals sampled over time. However, if it is assumed that the lambs sampled at 6 and 12 hours also had high ruminal lactic acid concentrations at 0 hours postmortem, and that little or no transport of nutrients or water occurs across the gut wall postmortem, it would suggest that lactic acid present in the rumen at death is metabolized over time.

It is highly probable the lactate that accumulated pre-mortem in Experiments 2 and 3 was converted to propionate or other VFA because some ruminal bacteria may metabolize lactate as an intermediate in the production of VFA.^{10,11} Mackie and associates¹¹ noted that as much as 24% of the acetate, 53% of the propionate, and 18% of the butyrate synthesized in the rumen was formed through lactate. Conversion of lactate to VFA could also explain the lack of a postmortem ruminal lactate accumulation in experiments 1 and 4, as well as the postmortem changes in VFA molar proportions noted in experiment 5.

The data from experiments 2 and 3 also suggest that high ruminal lactic acid concentrations tend to be associated with low ruminal VFA concentrations (Fig. 1). When initial ruminal lactic acid concentrations were high, VFA concentrations tended to be low. With induced acidosis, Wilson and coworkers¹² reported in cattle and Ryan¹³ reported in sheep that ruminal VFA concentrations peaked at approximately 3 hours post-feeding then declined. Concomitant with the decline in ruminal VFA concentrations, lactic acid began to accumulate between 3 and 6 hours after feeding. Wilson and associates¹² also noted that when ruminal VFA concentrations fell below about 50 mM/L, ruminal pH declined below 4.6 to a low of 3.8. High concentrations of VFA in the ruminal fluid actually can prevent the pH of the ruminal contents from falling below approximately 4.8. Because of their high pKa values (4.75 to 4.85), when the pH of the ruminal contents falls below 5.5, the VFA become the major buffering agent in the ruminal fluid. Once the VFA buffer-

ing capacity is overwhelmed, the pH will tend towards the pKa of lactic acid which is 3.73.¹⁴ This effect was apparent in the data from these studies (Fig. 2). When ruminal VFA concentrations were above approximately 125 mM/L, ruminal pH values remained between 4.7 and 5.25. When ruminal VFA concentrations were less than 50 mM/L, ruminal pH values ranged from 3.8 to 7.0. The ruminal pH appeared to be inversely related to ruminal lactic acid concentrations when ruminal VFA concentrations were below 50 mM/L (Fig. 3).

In experiment 5, molar proportions of acetate and propionate decreased and molar proportions of butyrate increased postmortem. These changes in molar proportions of VFA occurred primarily between 6 and 24 hours postmortem. In the live animal, a decrease in acetate (and possibly butyrate) molar proportions and an increase in propionate molar proportions would be expected to occur at 2 to 8 hours post-prandial, followed by a reversal of this trend between 8 and 24 hours post-prandial.¹⁵ As noted previously, these changes could be the result of the conversion of lactate to VFA as well as to the interconversion of VFA.¹⁶

The extent of the postmortem changes in ruminal VFA and lactate concentrations and subsequent decline in ruminal pH values appear to be dependent upon several variables. The concentrate level of the diet, as well as pre-mortem feed intake obviously affected postmortem ruminal VFA, lactate, and pH values. Although not examined in these studies, environmental conditions could also affect postmortem ruminal changes. Under cooler ambient temperatures, ruminal fermentation may decline more rapidly due to a more rapid decrease in ruminal temperature. Other environmental conditions such as wind chill and/or solar radiation heat load could also affect ruminal temperature and thus ruminal fermentation.

Results of these studies indicate that ruminal pH values may be a poor indicator of fatal acidosis. Ruminal pH values obtained several hours postmortem will be equal to or lower than pH values at the time of death. Thus, the use of ruminal pH values alone could lead to an incorrect diagnosis of the cause of death in animals that have been dead for several hours.

The use of ruminal lactic acid concentrations may also produce incorrect diagnosis of fatal acidosis. It appears that in animals with high ruminal lactate concentrations at death, the lactic acid concentration of the ruminal fluid may actually decline due to conversion of the lactate to VFA. Although, ruminal fluid lactic acid concentrations did not increase in any of these experiments, we¹⁷ have noted significant postmortem accumulations of lactic acid. Thus, animals that die of acidosis could conceivably have low ruminal lactate concentrations several hours postmortem, whereas, animals that die of causes other than acidosis could have high ruminal lactate concentrations several hours postmortem.

Conclusion

These studies indicate that in sheep and cattle ruminal pH values decrease and concentrations of volatile fatty acids increase significantly postmortem. The

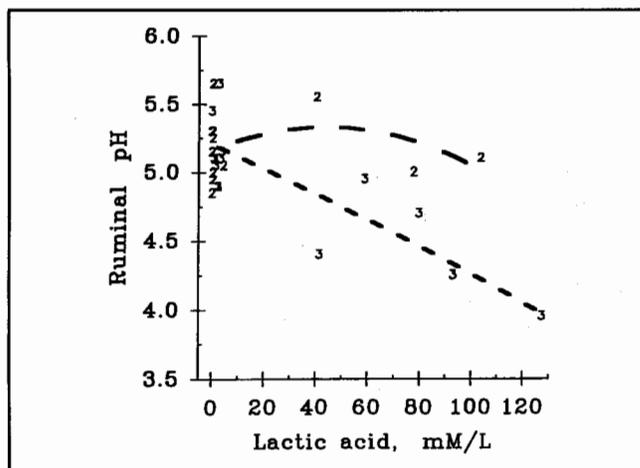


FIG. 3 – Relationship between ruminal lactic acid concentrations and ruminal pH in Experiments 2 and 3. Quadratic regression equations were as follows: Experiment 2 (long dashed line), $\text{pH} = 5.16 - 0.01X + 0.00009X^2$, $r^2 = 0.06$; and Experiment 3 (medium dashed line), $\text{pH} = 5.21 - 0.01X + 0.00008X^2$, $r^2 = 0.76$. Numbers indicate Experiment number.

decreases in ruminal pH were apparently the result of the increased ruminal VFA concentrations because lactate did not accumulate in any of the studies. Postmortem ruminal changes appeared to be dependent upon the concentrate level of the diet as well as feed intake. These results indicate that ruminal fluid pH and/or lactic acid concentrations are poor indicators of fatal acidosis in animals that have been dead for several hours. □

TABLE 4.
POSTMORTEM RUMINAL VOLATILE FATTY ACID CONCENTRATIONS
AND MOLAR PROPORTIONS OF STEERS IN EXPERIMENT 5

Item	Hours postmortem					SEM
	0	2	4	6	24	
Total VFA, mM/L ^a	127	164	177	188	246	18.4
Acetate, molar % ^b	59.1	57.7	57.2	57.2	51.9	2.4
Propionate, molar % ^b	26.0	26.1	25.1	24.2	20.0	1.3
Butyrate, molar % ^a	9.7	10.5	11.9	12.8	19.8	0.9
Minor VFA, molar % ^a	5.3	5.7	5.8	5.8	8.3	0.7

^aSignificant linear effect ($P < 0.05$).

^bSignificant linear effect ($P < 0.09$).

^cIsobutyrate, valerate, and isovalerate.

REFERENCES

- Smith RA: Accurate Diagnosis at Death. Proc. Symp. Rumensin/Tylan For The Professional Feedlot Consultant. Elanco Animal Health, Amarillo, TX., Aug. 25, 1993, p F1-F5, 1993.
- Thomson RG: Postmortem Changes in Rumen Contents. Canadian Vet. Journal. 10:312-313, 1969.
- Consortium. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Consortium for Developing a Guide for the Care and Use of Agriculture Animals in Agricultural Research and Teaching. Champaign, IL, 1988.
- Smith AW, Houpt KA, Kitchell RL, et al: Report of the AVMA Panel on Euthanasia. J. Amer. Vet. Med. Assoc. 188:252-266, 1986.
- Erwin FS, Marco GJ, Emery EN: Volatile Fatty Acid Analysis of Blood and Rumen Fluid by Gas Chromatography. J. Dairy Sci. 44:1768-1771, 1961.
- Ludvigsen CW et al: Kinetic Assay for D(-)-Lactate, With Use of a Centrifugal Analyzer. Clin. Chem. 29:1823-1825, 1983.
- Olsen GF: Optimal Conditions for the Enzymatic Determination of L-lactic Acid. Clin. Chem. 8:1-10, 1962.
- SAS: SAS/STAT User's Guide (Release 6.03 Ed.) SAS Inst. Inc., Cary, NC, 1988.
- Eadle JM, Hyldgaard-Jensen J, Mann SO, Reid RS, Whitelaw FG: Observations on the

- Microbiology and Biochemistry of the Rumen in Cattle Given Different Quantities of a Pelleted Barley Ration. Br. J. Nutr. 24:157-177, 1970.
- Baldwin RL, Wood WA, Emery RS: Conversion of Lactate-¹⁴C to Propionate by the Rumen Microflora. J. Bacteriol. 83:907-913, 1962.
- Mackie RI, Gilchrist FMC, Heath S: An In vivo Study of Ruminant Micro-organisms Influencing Lactate Turnover and Its Contribution to Volatile Fatty Acid Production. J. Agric. Sci. Camb. 103:37-51.
- Wilson JR, Bartley EE, Anthony HD, et al: Analyses of Rumen Fluid From "Sudden Death" Lactic Acidosis and Healthy Cattle Fed a High Concentrate Ration. J. Anim. Sci. 41:1248-1255, 1975.
- Ryan RK: Concentrations of Glucose and Low-Molecular-Weight Acids in the Rumen of Sheep Following the Addition of Large Amounts of Wheat to the Rumen. Am. J. Vet. Res. 25:644-652, 1964.
- Mackenzie DDS: Production and Utilization of Lactic Acid by the Ruminant. A Review. J. Dairy Sci. 50:1772-1786, 1967.
- Phillipson AT: The Fatty Acids Present in the Rumen of Lambs Fed on a Flaked Maize Ration. Br. J. Nutr. 6:190-199, 1952.
- Davis CL: Acetate Production in the Rumen of Cows Fed Either Control or Low-fiber, High Grain Diets. J. Dairy Sci. 50:1621-1628, 1967.
- Cole NA, Richardson LF: Influence of Monensin on Postmortem Ruminal Changes in Sheep. Veterinary Clinical Nutrition, in press 1988.