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Stability of Rumen pH Measurements Obtained Postmortem

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Abstract

Seventeen 350 lb (159 kg) Holstein steers were utilized to determine stability of rumen pH postmortem. Cattle were randomly assigned to two whole-corn ration treatment groups: 1) limit-fed (7.5 lb; 3.4 kg) or 2) fed ad libitum (9.5 lb; 4.3 kg). Calves were humanely euthanized, rumen fluid was collected, and pH measurements were taken at 0, 1, 2, 3, 6, 12, and 24 hours postmortem. A subset of five rumen samples per treatment at each sampling time was collected for later analysis of volatile fatty acid (VFA) content. No significant time by feeding regimen interactions were noted. The average postmortem rumen pH of calves fed ad libitum (5.7) was significantly lower than cattle limit-fed (6.2; $P = 0.03$). The pH of rumen content increased for the first six hours postmortem, then gradually decreased over the next 18 hours back to initial pH readings ($P < 0.01$). Rumen fluid total VFA concentrations tended to increase over time ($P = 0.06$). Rumen fluid acetate concentration significantly increased ($P < 0.05$), and proprionate concentrations tended to increase over time postmortem ($P < 0.08$). These data indicate that postmortem ruminal pH could be a helpful diagnostic tool when used in conjunction with a complete necropsy.

Keywords: necropsy, rumen pH, acidosis

Résumé

On a utilisé 17 bouvillons Holstein de 350 lb (159 kg) afin de déterminer la cinétique du pH dans le rumen post-mortem. Les bovins étaient alloués au hasard dans deux groupes de traitements avec une ration maïs grain entier : 1) restriction alimentaire (7.5 lb; 3.4 kg) ou 2) nourriture ad libitum (9.5 lb; 4.3 kg). Les veaux ont été euthanasiés humainement et le fluide du rumen a été échantillonné pour déterminer le pH à 0, 1, 2, 3, 6, 12 et 24 heures post-mortem. Un sous-ensemble de 5 échantillons du rumen pour chaque traitement à chaque heure d'échantillonnage a été recueilli pour des analyses subséquentes des acides gras volatils. Il n'y avait pas d'interaction entre le traitement d'alimentation et le temps post-mortem. La valeur moyenne post-mortem du pH dans le rumen des veaux du groupe avec nourriture à volonté (5.7) était significativement moins élevée que celui des veaux du groupe avec restriction alimentaire (6.2, $P = 0.03$). Le pH du rumen augmentait pendant les six premières heures post-mortem et décroissait graduellement au courant des 18 heures suivantes pour atteindre de nouveau le niveau initial ($P < 0.01$). La concentration des acides gras volatils dans le fluide du rumen avait tendance à augmenter en fonction du temps ($P = 0.06$). La concentration d'acétate dans le fluide du rumen s'accroissait significativement en fonction du temps ($P < 0.05$) alors que la concentration du proprionate tendait quant à elle à augmenter ($P < 0.08$). Ces données indiquent que le pH ruminal post-mortem peut être un outil diagnostic utile lorsqu'on l'utilise conjointement avec la nécropsie complète.

Introduction

The value of postmortem rumen pH readings as a diagnostic aid in feeder cattle has long been debated among consulting nutritionists and veterinarians. Obtaining a rumen pH measurement as part of a routine postmortem examination reportedly can aid in diagnosing causes of death related to the digestive system, such as ruminal acidosis.[5,7] Ruminal acidosis in cattle results from a decrease in rumen pH due to an influx of readily fermented carbohydrates, microbial utilization of those carbohydrates, and the production and accumulation of organic acids in the rumen.[6,8,9,11] Ruminal acidosis is most often subdivided into two categories: acute (caused by lactic acid accumulation) and subacute (caused by an increase in ruminal volatile fatty acids [VFAs] over time).[8] Acute acidosis increases the risk of other feedlot

ailments, such as laminitis and liver abscesses.[1] Regardless of the type of acidosis observed, economic losses resulting from decreased gain, decreased efficiency, and increased mortality in feedlots can occur.[8]

Experts debate the use of ruminal pH as a ruminal acidosis diagnostic tool. They argue that rumen pH will continue to change after death, yielding inaccurate results. Others have examined the role of the different VFAs, alone or in combinations, and their effects on postmortem rumen pH.[3] Researchers have reported that postmortem rumen pH does not remain stable over a 24-hour period, and that this instability is caused by continued fermentation of rumen contents, the conversion of lactate to proprionate, and the formation of other VFAs.[5] In contrast, others have concluded that rumen pH is stable and remains above 6.0 for up to 24 hours after death.[12] Inadequate sample size and varying methods used to obtain rumen samples likely affected the differences in the outcomes of these studies.

If stable after death, rumen pH measurements could be useful in confirming or refuting death due to digestive disorders. The primary purpose of this study was to compare postmortem rumen pH levels between cattle fed an ad libitum diet versus a limit-fed whole-corn diet for 48 hours, and the ration effect on ruminal pH stability over a 24-hour postmortem period. The secondary purpose of this study was to describe the concentrations of rumen fluid VFAs and lactic acid relative to time postmortem and feeding regimen.

Materials and Methods

General Overview and Diet

This study was conducted at the Kansas State University College of Veterinary Medicine in Manhattan, Kansas, and was consistent with the university's Institutional Animal Care and Use Committee's guidelines. Seventeen 350 lb (159 kg) Holstein steers enrolled in a concurrent model development trial were used in this study. All calves were maintained on a 34% protein pellet diet (1.5 lb; 0.68 kg) and whole-shell corn[a] (6 lb; 2.72 kg). Calves were blocked by previous treatment determined in the aforementioned model development trial, and randomly assigned to two feeding regimens using Excel® software.[b] All cattle were switched to a whole-shell corn diet 48 hours prior to euthanasia by substituting an equal amount of whole-shell corn for commercial pellets on an as-fed basis. Cattle were either assigned to be limit-fed (7.5 lb; 3.4 kg) or allowed ad libitum (9.5 lb; 4.3 kg) access to the diet 48 hours prior to euthanasia. Calves were humanely euthanized using a captive bolt, and transported to the Kansas State University Veterinary Diagnostic Laboratory where they were held for 24 hours at room temperature (72°F; 22.2°C).

pH Measurement

Carcasses were opened following the diagnostic laboratory necropsy protocol. The rumen was visualized, but not penetrated, to ensure proper placement of the needle and to maintain anaerobic conditions. A 14-gauge, 4-inch (10 cm) stainless steel hypodermic needle[c] was inserted into the rumen low enough to enter the fluid area of the rumen. A standard bore three-way stopcock with extension set[d] was then attached to the needle to maintain the anaerobic rumen environment as much as possible. Rumen fluid samples were collected through the apparatus using negative pressure applied by a 20 mL syringe.[e] The first 2 mL of rumen fluid collected was discarded each time to ensure the sample was from the rumen and not the sampling apparatus. One 10-mL sample was taken from each animal at 0, 1, 2, 3, 6, 12, and 24 hours after death, and the pH was recorded. The pH was measured using a portable electronic pH meter,[f] calibrated using a two-step technique using pH 4 and 7 standard solutions.

VFA and Lactic Acid Sampling

Rumen fluid samples were randomly obtained from a subset of five calves in each treatment group at each collection time to measure VFA and lactic acid concentrations using gas-liquid chromatography (GLC). For this analysis, the remainder of the 10 mL sample collected from the selected calves was divided into two 4-mL samples. Each sample was combined with 1 mL of phosphoric acid and frozen overnight at -4°F (-20°C) to complete deproteinization. Samples were then thawed and centrifuged at 30,000 x g for 15 minutes, and the supernatant was collected for GLC analysis. One mL of supernatant was mixed with 50 microliters 6N NaOH and 3 mL pivalic internal standard. Approximately 1 mL of the resulting solution was transferred to a GC vial and stored in a refrigerator until analysis. A standard was prepared using 19.2 mg Li-Lactate and 25 mL VFA standard. Samples were analyzed using a 2m x 2 mm Carbopak B-DA column,[g] with injection and detector temperatures of 392°F (200°C), oven temperature of 374°F (175°C), and a flow rate of 24 mL/min. Results were reported for individual lactate, acetate, butyrate, and proprionate concentrations in the rumen fluid. Also, a calculated total VFA concentration (acetate, butyrate, and proprionate combined) was reported.

Statistical Analysis

Data was analyzed using the Proc Mixed procedures of SAS[h] with a repeated measures statement to account for the multiple samplings within each animal. Time was modeled to account for unequally spaced sampling times, and treatment by time interactions were

examined. Feed regimen treatment and time were the only main effects examined. Significant effects were reported for any effects with a P-value of ≤ 0.05, and effects with a P-value ≤ 0.10 and ≥ 0.06 were reported as trends.

Results

No postmortem time and feeding regimen interactions were observed for pH levels or VFA concentrations ($P > 0.05$). Feeding regimen had a significant effect on ruminal pH of calves (Figure 1; $P = 0.03$). Average ruminal pH of calves fed ad libitum did not exceed 5.9 at any sampling time postmortem. Conversely, the average ruminal pH of calves limit-fed the whole-corn diet never fell below 6.0 at any postmortem sampling time. Ruminal pH of limit-fed calves increased from 6.1 in the first hour to 6.3 at six hours postmortem, while the ruminal pH of calves fed ad libitum increased from 5.6 to 5.8 in the same time period. The pH of both groups then gradually decreased over the next 18 hours back to initial pH readings ($P < 0.01$; Figure 1).

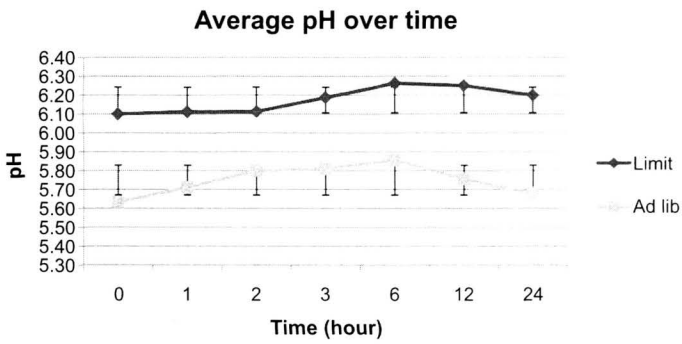

Figure 1. Postmortem ruminal pH values from cattle fed either a limited amount of whole-shell corn or allowed ad libitum access to a diet of whole-shell corn ($P = 0.02$).

No significant differences were found in any rumen VFA concentration, total VFA concentration or lactate concentration between cattle fed ad libitum or limit-fed ($P > 0.05$). Average VFA and lactate concentration for each time period are shown in Table 1. Of individual VFAs analyzed, only rumen acetate concentration changed significantly over time postmortem ($P < 0.05$). There was a trend for proprionate and total VFAs to change over time postmortem ($P = 0.08$ and $P = 0.06$, respectively). Butyrate and lactate concentrations did not change over time postmortem ($P > 0.10$).

Discussion

This study demonstrated that different feeding regimens can significantly alter rumen pH, even after death. However, rumen pH of calves fed different amounts of corn did not approach pH values associated with subacute or acute acidosis.[4,8,9] Alterations in rumen pH due to increased concentrate intake have been reported in several reviews on acidosis in cattle.[5,8,9] Researchers have shown that beef cattle allowed ad libitum access to high-grain diets have average ruminal pH measurements between 5.8 and 6.2. Several researchers have reported rumen pH values to define acute and subacute ruminal acidosis in cattle (Table 2). Increased intake of such concentrates as wheat, corn, and sorghum grain is the most important factor associated with acute and subacute acidosis.[5,8]

Data from the current study also indicate that pH tends to change over time postmortem (Figure 1). Changes in rumen pH could be attributed to continued postmortem anaerobic fermentation with gradual accumulation of acid by-products in the rumen.[3] Average pH in both treatment groups tended to increase during the first six hours postmortem, and then decrease to the initial rumen pH for the remainder of the 24 hour observation period. Total rumen VFA concentrations stayed relatively stable in both treatment groups until six hours

Table 1. Rumen volatile fatty acid (VFA) and lactate concentrations (± standard error of the mean) in feeder calves at different postmortem sampling times.

	Time postmortem, hour							
Acid (mmol/L)	Hour 0	Hour 1	Hour 2	Hour 3	Hour 6	Hour 12	Hour 24	P-value
Acetate	41.82 ± 3.02	43.53 ± 2.73	42.79 ± 2.19	42.41 ± 2.41	43.50 ± 2.31	43.63 ± 2.08	47.32 ± 1.55	0.02
Proprionate	25.73 ± 2.10	27.34 ± 2.14	27.06 ± 2.32	26.89 ± 2.24	28.00 ± 2.46	26.68 ± 1.98	30.87 ± 2.39	0.08
Butyrate	12.88 ± 3.49	13.65 ± 3.66	13.56 ± 3.40	14.16 ± 4.03	14.78 ± 4.18	15.67 ± 4.47	16.68 ± 3.84	0.26
Total VFA	77.53 ± 6.42	81.23 ± 5.87	80.37 ± 5.39	80.22 ± 6.42	82.93 ± 6.46	82.56 ± 5.49	91.16 ± 4.43	0.06
Lactate	0.63 ± 0.34	0.48 ± 0.32	0.64 ± 0.39	0.59 ± 0.39	0.84 ± 0.66	0.45 ± 0.41	0.84 ± 0.71	0.18

Table 2. Previously reported rumen pH ranges for normal, subacute acidosis, and acute acidosis in cattle.

	Normal	Subacute	Acute
Nagaraja & Titgemeyer[a]	5.8-6.5	5.0-5.5	<5.0
Owens *et al*[b]	>5.6	5.2-5.6	<5.2
Goad *et al*[c]	>5.5	5.0-5.5	<5.0

[a]Nagaraja TG, Titgemeyer EC: Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *J Dairy Sci* 90:E17-E38, 2007.
[b]Owens FN, Secrist DS, Hill WJ, Gill DR: Acidosis in cattle: a review. *J Anim Sci* 76:275-286, 1998.
[c]Goad DW, Goad CL, Nagaraja TG: Ruminal microbial and fermentative changes associated with experimentally induced subacute acidosis in steers. *J Anim Sci* 76:234-241, 1998.

Table 3. Volatile fatty acid concentrations obtained from cattle determined to be acidotic in various trials researching ruminal acidosis.

VFA	Current[a] study	Blanch *et al*[b]	Sharp *et al*[c]
Acetate (mmol/L)	42.88	88.5	70.98
Proprionate (mmol/L)	30.32	25.38	48.67
Butyrate (mmol/L)	17.74	22.98	25.33
Total VFA (mmol/L)	90.94	136.86	144.98

[a]Average of individual VFA measurements of cattle (n=9) fed ad libitum at all sample times
[b]Data from cattle (n=6) on day of induced acidosis. Blanch M, Calsamiglia N, DiLorenzo N, DiCostanzo A, Muetzel S, Wallace RJ: Physiological changes in rumen fermentation during acidosis induction and its control using a multivalent polyclonal antibody preparation in heifers. *J Anim Sci* 87:1722-1730, 2009.
[c]Data from acidotic steers (n=2) fed a diet of whole-shell corn for two weeks. Sharp WM, Johnson RR, Owens FN: Ruminal VFA production with steers fed whole or ground corn grain. *J Anim Sci* 55:1505-1514, 1982.

postmortem, then slightly increased until 24 hours after death. This is in agreement with previous reports showing an inverse relationship between postmortem rumen pH and rumen VFA concentration after death.[12] The small changes in rumen pH over time and lack of interaction between time and diet groups indicates that rumen pH could be helpful to diagnose acidosis in feeder cattle. A larger field study is warranted to further define rumen pH changes in cattle under differing field conditions, such as weather extremes, differing postmortem decomposition, and different methods to process grain in the diet.

The average postmortem ruminal pH of cattle fed an ad libitum diet in the current study was below 6.0 for the entire 24 hour period. In a Canadian study, pH values of ruminal content remained above 6.0 up to 24 hours postmortem.[12] Results of that study may be open to interpretation because ruminal fluid was obtained from rumens removed from carcasses at slaughter and transported to a laboratory. Eighteen of these rumens were kept refrigerated, while the remaining 32 rumens were stored at room temperature. Although the effect of temperature on postmortem pH of ruminal samples has not been studied, decreased rumen temperature could result in slower fermentation compared to rumens held at room temperature. In addition, there could have been disruption in the anaerobic environment normally present due to removal of stored rumens and incisions made to obtain rumen fluid samples. Lastly, the diet and quantity of feed consumed by the cattle was unknown.[12]

Concentration of the different VFAs in 10 selected calves did not differ between calves fed ad libitum or limited diets. However, it appeared that rumen proprionate, butyrate, and lactate concentrations tended to be higher in cattle allowed ad libitum access to feed compared to cattle with limited access to feed (data not shown). Total rumen VFA concentration tended to increase over time ($P = 0.06$). Volatile fatty acid concentrations in the present study are compared to VFA concentrations found in live cattle with acidosis in Table 3.

While the individual VFAs proprionate and butyrate are comparable, acetate levels in live cattle are much higher.[2,10] This could be caused by a number of factors. Cattle in the current study were offered a diet of only whole-shell corn, while the cattle in previous studies were fed a total mixed ration and/or ad libitum access to roughage.[2,10] Researchers have suggested that lactic acid can be metabolized to proprionate or other VFAs as time after death increased.[3] In this trial, concentration of lactic acid obtained from all but one of the animals never exceeded 1 mmol/L. This is well below lactic acid levels associated with acute ruminal acidosis in live animals (Table 2).[8]

The slight, yet significant, changes in rumen pH over time postmortem could raise questions about clinical and biological significance as slight fluctuations over time would be expected in a fluid biological system. However, these data indicate that postmortem ruminal pH can be a useful diagnostic tool when used in conjunction with a complete necropsy. Further research in the field with larger numbers of cattle will allow researchers to better answer which diseases or syndromes it would best help diagnose.

Conclusion

Results of this study indicate that postmortem ruminal pH is affected by feed intake, and changes only slightly over a 24 hour postmortem period. The VFA concentrations tended to increase between six and 24 hours postmortem, which correlated with the pH changes at different sample times postmortem. Measurement of ruminal pH may be helpful to diagnose the cause of death in feeder cattle when used in conjunction with a complete necropsy examination.[5] Further field research is recommended to determine if interactions between disease processes, nutritional status, and environmental factors affect the magnitude of ruminal pH changes postmortem.

Endnotes

[a]Kent Feeds, Muscatine, IA
[b]Microsoft Excel, Redmond, WA
[c]JorVet, Loveland, CO
[d]Baxter Healthcare Corporation, Deerfield, IL
[e]Tyco Healthcare Group LP, Mansfield, MA
[f]Twin pH waterproof, Spectrum Technologies Inc, Plainfield, IL
[g]Supelco, Bellefonte, PA
[h]SAS Institute Inc., Cary, NC

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