

Effects of Feeding Virginiamycin and Sodium Bicarbonate to Grazing Lactating Dairy Cows

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ABSTRACT

The effects of virginiamycin, an agent active against Gram-positive lactic acid-producing bacteria, and NaHCO_3 on ruminal and fecal pH, rumen volatile fatty acid proportions, blood metabolites, and milk production and composition were assessed. This study was conducted over 28 d and involved 71 dairy cows that grazed predominantly ryegrass, oats, and clover, and that were fed 10 kg of concentrate pellets/d per head. The pellets contained (per kilogram) no dietary additive, 30 mg of virginiamycin, 20 g of NaHCO_3 , or 30 mg of virginiamycin and 20 g of NaHCO_3 on a DM basis.

Ruminal pH tended to be higher in cows fed pellets containing virginiamycin (7.0 vs. 6.9; SED = 0.16). The results of in vitro incubation of ruminal fluid with glucose found the potential for L-lactic acid accumulation in ruminal fluid to be significantly lower in cows fed virginiamycin (15.5 vs. 35.3 mmol/L; SED = 2.98). Cows fed virginiamycin had significantly higher fecal pH (6.72 vs. 6.57; SED = 0.08) and produced more milk (23.94 vs. 23.32 kg/d) and more lactose than those not fed virginiamycin. No effects of NaHCO_3 on fecal pH, in vitro potential for L-lactic acid accumulation in ruminal fluid, or milk production were observed, but ruminal pH tended to be higher and ruminal acetate proportion was greater for cows fed NaHCO_3 . Milk fat and milk protein percentage did not differ significantly as a result of dietary treatment. These data suggest that the inclusion of virginiamycin in the diet will reduce L-lactic acid accumulation in ruminal fluid and increase fecal pH in grazing dairy cattle fed concentrate supplements. (**Key words:** virginiamycin, sodium bicarbonate, lactic acidosis, ruminal pH)

Abbreviation key: VM = virginiamycin.

INTRODUCTION

The effects on dairy cattle of ruminal acidosis resulting from the rapid fermentation of starch are well recognized. Acute ruminal acidosis causes loss of milk production (4), ruminitis, laminitis (1, 21), and sequelae including hepatic abscesses, portal-caval syndrome, and death. Chronic effects of acidosis in cattle, which include lower fat content of milk (12), reduced rate and extent of roughage digestion (5), lower feed conversion efficiency, and lower DMI (16, 27), may be more prevalent and of greater economic importance than the acute effects. Limited evidence in sheep exists that suggests hindgut acidosis may have significant effects on health and production (10, 11).

Rumen buffering agents such as NaHCO_3 are included in concentrate diets to stabilize ruminal pH, increase acetate production, and increase milk fat percentages (33, 35). Russell and Chow (29) noted the limited capacity of NaHCO_3 to buffer the rumen and suggested that the primary effects of feeding NaHCO_3 may be mediated through increased water intake, decreased ruminal fluid osmolality, increased flow of starch from the rumen, and decreased ruminal propionate production.

Virginiamycin (VM) is an antibiotic active against Gram-positive bacteria, including *Streptococcus bovis* and *Lactobacillus* sp., which produce lactic acid. Inclusion of VM in diets has reduced the risk of lactic acidosis in feedlot cattle (24, 28), stabilizes ruminal pH, and increases digestibility and energy utilization of grains (10). Feeding VM, however, can increase ruminal propionate concentrations (2, 19, 20) and could potentially lower the fat content in milk. The effect of VM on Gram-positive bacteria is similar to that of monensin, although the modes of action differ (17, 18). The effect of feeding VM on ruminal acidosis in dairy cattle has not been described. The effects of feeding NaHCO_3 on ruminal pH and metabolism need to be further examined; this additive should be com-

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TABLE 1. Chemical analysis of dietary supplements fed to grazing dairy cows prior to and during the experimental period (% DM).

Supplement ¹	DM (%)	ME ²	CP	Ca	P	Na
Pretrial pellet	88.1	11.0	18.0	1.42	0.67	0.30
Trial pellet	89.2	12.8	19.2	1.58	0.64	0.28
Cottonseed	92.1	7.8	41.6	0.21	1.27	0.03
Brewers grain	28.2	11.1	20.4	0.25	0.60	0.26
Corn cannery waste	60.0	9.9	9.1	0.08	0.27	0.05

¹Pretrial pellet fed at 6 kg/d (as fed), trial pellet fed at 10 kg/d (as fed). Analysis of the trial pellet is for the Control pellet, i.e., without any dietary additives.

²Metabolizable energy, MJ/kg of DM.

pared with VM to examine whether a synergistic effect may exist in combining these two acidosis treatments that have different modes of action.

The primary objectives of this study were to evaluate the effects of VM, NaHCO₃, and a combination of VM and NaHCO₃ when included in a high grain diet fed to grazing lactating dairy cows, on rumen and fecal pH, blood metabolites, rumen function, milk yield, and milk composition.

MATERIALS AND METHODS

Cows and Pretrial Management

Holstein-Friesian cows, obtained from the University of Sydney herds in Camden, New South Wales, Australia, were used in the trial. The 71 cows were managed to calve year-round and were stratified according to stage of lactation, age, and milk yield, before random allocation within strata to one of four treatment groups. The mean parity of cows used in the trial was 4.5, and stage of lactation was 171 DIM. Cows grazed pasture, predominantly ryegrass (*Lolium perenne*), oats (*Avena sativa*), and clover (*Trifolium repens*) in a rotational grazing system prior to, and during, the trial period. Pasture availability was not considered limiting at any period of the trial. Before the study commenced, cows were fed 6 kg/d (as-fed) of dairy pellets (Millmaster Feeds, Maryland, NSW) and 7 kg/d (as-fed) of a mix of whole cottonseed (2.2 kg/d on a DM basis) and brewers grain (1.25 kg/d on a DM basis) (Table 1). The pellets fed before the trial commenced contained low amounts of grain and were considered to have a limited potential for fermentation and acid production in the rumen (Millmaster Feeds, Maryland, New South Wales) (Table 1). This diet was maintained for 7 d before the trial and the pellet was fed twice daily in equal amounts in the dairy shed at the time of milking. From d 1 of the trial, pellets fed to cows were changed to one of four different treatment pellets, according to group allocation. Diet varied

throughout the trial, depending on pasture quality and availability (Figure 1). On d 13 of the study, the brewers grain and whole cottonseed became unavailable and cows were supplemented instead with corn cannery waste at approximately 7 kg/d as-fed. All cows were offered the same feeds, apart from the additives in the treatment pellets.

Dietary Treatments

The trial pellets fed during the experimental period were formulated to provide a large amount of cereal grain and to possibly cause subacute acidosis. The trial pellets (Table 1) contained wheat (30%), sorghum (13%), wheat millrun (30%), cottonseed meal (22%), limestone (3.5%), salt (0.65%), and a mineral premix (0.5%) and were fed twice daily (5 kg/feeding as-fed). Control cows (n = 17) were fed pellets with no NaHCO₃ or VM; the VM group (n = 18) were fed pellets that contained 30 mg of VM/kg (Eskalin[®], Pfizer Inc., Ltd., New York, NY; Pfizer Pty. Ltd., West Ryde, New South Wales, Australia). The NaHCO₃ group (n = 18) were fed pellets containing 20 g of NaHCO₃/kg (Penrice Soda Products, Osbourne, South Australia); and a group (n = 18) were fed (per kilogram) pellets containing 30 mg of VM and 20 g of NaHCO₃. An equal quantity of wheat millrun was displaced by the addition of NaHCO₃ or VM in the pellets.

From d 1 of the trial, all cows were changed from the pretrial pellet to the treatment pellet in gradual steps. On d 1 the cows received 6 kg of the pretrial pellet and 2 kg of the respective treatment pellet per head. On d 2, the cows received 4 kg of the pretrial pellet and 4 kg of the treatment pellet. Over the next 3 d, the treatment pellet inclusion was increased by 2 kg/d, while the amount of pretrial pellet was decreased by 2 kg/d. On d 5 of the trial cows were receiving 10 kg/d of treatment pellets, and no pretrial pellet, and were maintained on this ration for the remainder of the trial.

Sampling and Measurements

Cows were milked twice daily, and milk yield was recorded (Milk Master Alfa-Laval Agri Pty. Ltd., Richmond, Victoria, Australia). Pellets not consumed at milking were collected for each cow after milking and weighed to calculate daily intake of the pellets. Pellet intake was recorded from d 0 of the study. Milk yield was recorded for each cow at each milking. Mean milk yield for 3 d pretrial on d -2, -1, and 0, provided a baseline measure for milk production. Combined morning and evening milk samples were taken from each cow to determine milk fat, protein, and lactose composition and SCC were taken on d -1, 6, 13, 20, and 27 of the experimental period.

Ten cows from each treatment group were randomly selected for sampling of ruminal fluid, feces, and blood prior to introduction of the treatment pellets and once each week of treatment feeding. These cows were sampled over two consecutive days with five cows sampled from each group each day. Ruminal fluid was obtained from each cow approximately 3 h after pellet feeding by using a shielded stomach tube and analyzed immediately for pH. Any samples that showed evidence of salivary contamination were discarded and the cows were resampled. Samples of ruminal fluid were also taken to determine VFA concentrations and to assess the potential for L-lactic acid accumulation by using an in vitro incubation technique similar to that used by Thorniley et al. (36) as outlined below.

Fecal samples were collected from each cow, and a 20-g subsample was placed in a plastic jar. An equal weight of distilled water was added and pH was determined immediately on this sample. Another subsample of approximately 200-g wet weight was dried in an oven at 80°C until a constant weight was reached for DM determination.

Samples of blood were collected from the coccygeal vessels of cows using Vacutainer (Becton Dickinson, England) tubes. A tube with sodium heparin was used to obtain samples for analysis of BHBA and D- and L-lactic acid; and a 5-ml tube with sodium fluoride was used for samples to determine glucose content. All tubes were centrifuged at $1000 \times g$ for 10 min, and the plasma was frozen pending analysis.

Pasture samples were collected each day of the trial (except d 2, 4, 6, 9, 14, 16, and 29) by cutting 10 random quadrats of pasture before cows grazed the area. Pasture samples were collected from the paddock grazed during the night, from the paddock grazed during the day, and combined to estimate the pasture quality grazed over the whole day. Samples were weighed and dried at 80°C until a constant

weight was reached to estimate DM content. Concentrations of CP and metabolizable energy in dry pasture samples were estimated by NIR analysis.

Analytical Procedures

Potential L-lactic acid accumulation. Four milliliters of fresh ruminal fluid was added to a 10-ml tube containing 1 ml of a solution of 100 mg of glucose/ml. Tubes were shaken and then incubated in a shaking water bath at 39°C for 20 h before fermentation was stopped by addition of 0.7-ml of concentrated sulfuric acid. Samples were frozen and held at -40°C for later analysis of L-lactic acid using a Stat Pack Rapid Lactate Test kit (Cat No. 869218, Behring Diagnostics Inc.) on a Cobas-Bio (Roche Diagnostica Basle, Switzerland).

pH and VFA analysis. The pH in ruminal fluid or feces was measured using a Piccolo 2=ATC pH meter (Hanna Instruments, Woonsocket, RI). Approximately 15 ml of ruminal fluid was added to a glass jar containing 0.7 ml of concentrated sulfuric acid and frozen for subsequent analysis of VFA content. Ruminal fluid was analyzed using a Packard 427 gas chromatograph (Packard Instrument Company, Downers Grove, IL) using isocaproic acid as an internal standard (8). The column was a 1/8th-inch glass column packed with 1.5% w/w orthophosphoric acid plus 17.5% w/w, polypropylene glycol sebacate on a 60-80 chromosol "W" acid wash mesh support.

Blood metabolites. Plasma glucose was determined by glucose phosphorylation and dehydrogenase methods (30) and Boehringer Mannheim GmbH reagents (Boehringer Mannheim GmbH, NSW, Australia) with an autoanalyzer. Concentrations of BHBA in plasma were determined using the colorimetric method of Zivin and Snarr (40). The concentration of D- and L-lactic acid was measured from blood plasma collected with a Stat Pack Rapid Lactate Test kit (Cat No. 869218, Behring Diagnostics Inc.) on a Cobas-Bio (Roche Diagnostica Basle, Switzerland).

Milk composition. Milk samples were preserved with bronopol for later analysis of fat, protein, and lactose percentages using NIR analysis, and SCC (Pacific Milk Analysis Services, Chippendale, NSW). A Delvo-P bacterial inhibition test (Pacific Milk Analysis Services, Chippendale, NSW) was used to test milk obtained on d 27 of the study from 10 cows in each group for inhibitory substances.

Body weight and body condition score. Body weight was assessed immediately before cows were assigned to groups and on d 29 of the study. The cows were weighed on electronic scales (which were tested using a known weight) immediately after milking on

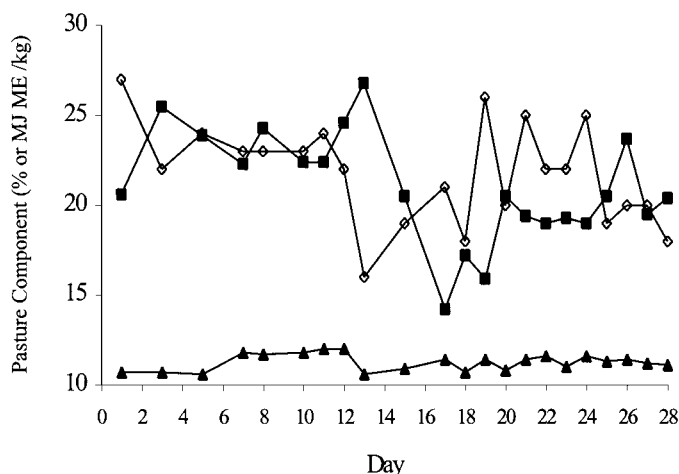


Figure 1. Changes in DM (percentage) (■), CP (percentage) (◇) and metabolizable energy (ME) (MJ/kg DM) (▲) content of pasture offered throughout the 28-d experimental period.

each day. Body condition scores were assessed on the same days as BW using the 1 to 5 scale of extreme cachexia to extreme obesity, respectively (6).

Statistical Analysis

Data were analyzed using repeated measures analysis with the PROC MIXED model in SAS (30). Data were analyzed for the main effects of VM, NaHCO_3 , and time, and the interactions between all main effects. When the interaction between the main effects (i.e., VM and NaHCO_3) was not significant, data are reported showing the main effect. The interaction between VM* NaHCO_3 and time was not significant for any parameter. Therefore, tables only show *P* values for the first-order interactions between main effects.

Pretrial values for most measures were expected to influence subsequent responses to treatments. When a significant linear relationship between pretrial values and subsequent values was identified, the pretrial value was included in the analysis as a covariate. Least squares means have been reported for all data. Standard errors for the differences between least squares means in the model are also reported in tables as the standard error of difference (SED). Significance was assessed and $P < 0.05$.

RESULTS

The quality of pasture changed over the period of the trial (Figure 1). The pasture DM and CP contents decreased over d 13 to 18 of the trial in response to 2 d of wet and cold weather. The pasture content of

CP and metabolizable energy was not considered limiting to cow performance at any stage.

Although pellet intake did not differ between groups, the intake of pellets increased over time as the amount of pellets offered increased from 6 to 10 kg, and there was a significant interaction between the main effects (Table 2). Cows fed VM tended ($P = 0.09$) to have a higher ruminal pH (Figure 2), than did cows not fed VM, and cows tended to have higher ruminal pH than cows not fed NaHCO_3 ($P = 0.11$). The group fed both VM and NaHCO_3 had a higher ruminal pH than the untreated control group numerically; however, there was no evidence of a significant interaction between the NaHCO_3 and VM. L-Lactic acid accumulation in ruminal fluid in vitro was lower in cows receiving VM ($P < 0.001$; Figure 3), but was not affected by NaHCO_3 (Table 2). Fecal pH was higher in cows receiving VM ($P < 0.001$; Figure 4). Fecal DM was not influenced by treatment.

The effects of the treatments on the total rumen VFA concentration and molar proportions of rumen VFA are shown in Table 3. Proportions of rumen acetate were higher ($P = 0.003$) in cows fed NaHCO_3 (Figure 5), although there was no effect of NaHCO_3 on total VFA concentration. There was no effect of VM nor interaction between VM and NaHCO_3 on VFA content or molar proportion in ruminal fluid. The molar proportions of propionate in ruminal fluid increased from 0.27 mol/100 mol pretrial to 0.38 mol/100 mol in wk 2 after treatment was introduced. There was no effect of VM or interaction between VM and NaHCO_3 on VFA content or molar proportion in ruminal fluid. Sodium bicar-

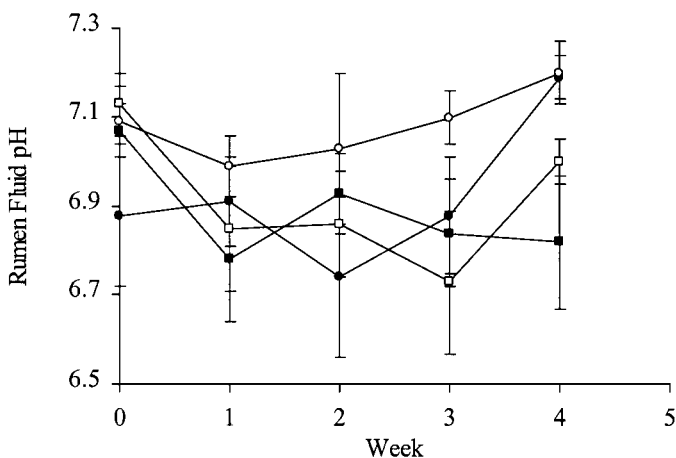


Figure 2. Rumen fluid pH over time in dairy cows fed a high grain pellet containing no dietary additives (■), NaHCO_3 (20 g/kg) (□), virginiamycin (30 mg/kg) (●) or virginiamycin (30 mg/kg) plus NaHCO_3 (20 g/kg) (○). Error bars show standard error of the mean.

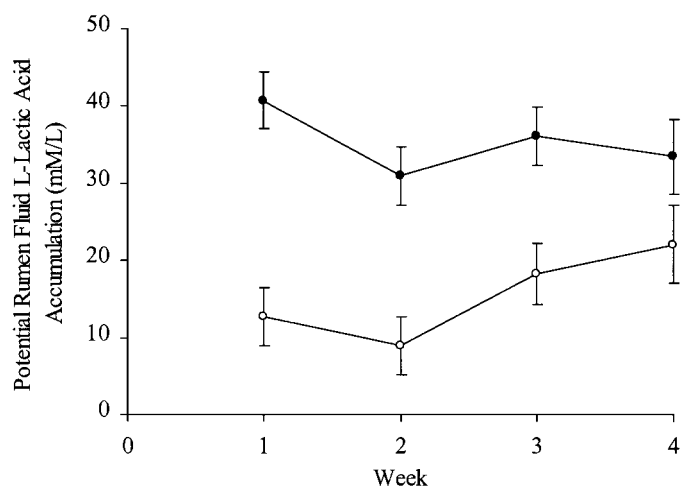


Figure 3. In vitro incubation of rumen fluid with glucose to determine the potential L-lactic acid accumulation in ruminal fluid over time in dairy cows for cows fed a high grain pellet without virginiamycin (●) or with 30 mg/kg of virginiamycin (○). Averaged across the NaHCO₃ treatment, error bars show standard error of the mean.

bonate feeding lowered ($P = 0.046$) valerate proportions. Molar proportions of most rumen VFA changed with time during the study.

The effects of treatment on plasma concentrations of metabolites are provided in Table 4. There were no measurable quantities of D-lactic acid in plasma. The concentrations of plasma L-lactic acid were lower ($P = 0.028$) in cows fed NaHCO₃ than in cows not fed NaHCO₃ (Figure 6). No significant effect of VM and no interactions between main effects and between

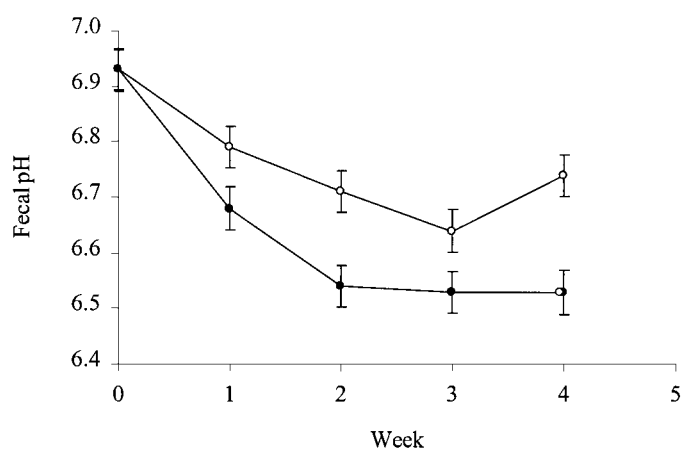


Figure 4. Change in fecal pH over time in dairy cows fed a high grain pellet without virginiamycin (●) or with 30 mg/kg of virginiamycin (○). Averaged across the NaHCO₃ treatment, error bars show standard error of the mean.

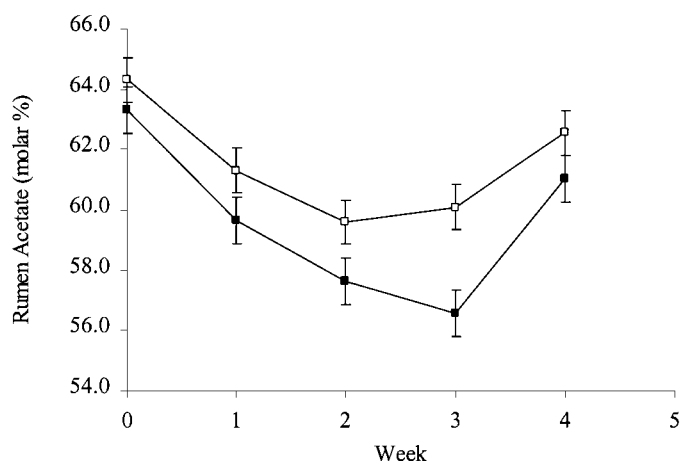


Figure 5. Change in rumen acetate (molar percentage) over time in dairy cows fed a high grain pellet without NaHCO₃ (■) or with 20 g/kg of NaHCO₃ (□). Averaged across the virginiamycin treatment, error bars show standard error of the mean.

main effect and time on blood metabolite concentrations were observed. Blood glucose and BHBA concentrations changed ($P < 0.001$) with time over the study period.

Cows fed VM tended ($P = 0.089$; Figure 7; Table 5) to produce more milk per day (0.62 L) than cows not fed VM and to produce more milk lactose per day ($P = 0.046$). There was no effect of treatment or interaction between main effects on fat, protein, or lactose percentages in milk, or on milk SCC. There was no bacterial inhibition in milk based on the Delvo-P test. Body weights for groups did not significantly differ at trial entry (Table 6) and no differences in change in

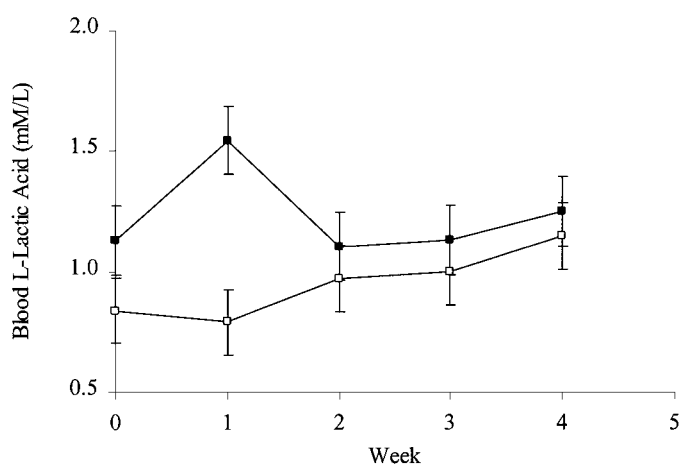


Figure 6. Plasma L-lactic acid concentration (mM/L) over time in dairy cows fed a high grain pellet without NaHCO₃ (■) or with 20 g/kg of NaHCO₃ (□). Averaged across the virginiamycin treatment, error bars show standard error of the mean.

TABLE 2. Least squares means for pellet intake and gastrointestinal acidosis parameters in dairy cows fed high grain pellets containing either no dietary additives, NaHCO₃ (20 g/kg), virginiamycin (VM) (30 mg/kg) or VM plus NaHCO₃ (30 mg/kg and 20 g/kg, respectively).¹

Measurement	Control	NaHCO ₃	VM	VM + NaHCO ₃	SED	<i>P</i> -values					
						VM	NaHCO ₃	VM *	Time	Time *	Time *
Pellet intake, kg/d (as fed)	8.98 ^{ab}	9.14 ^b	9.15 ^b	8.81 ^a	0.12	0.515	0.455	0.036	0.0001	0.710	0.358
Rumen pH	6.89	6.91	6.92	7.08	0.06	0.089	0.109	0.242	0.044	0.087	0.988
Potential rumen L-lactate accumulation, mM/L	35.12	35.54	14.21	16.84	5.92	0.0001	0.718	0.791	0.006	0.215	0.357
Fecal pH ²	6.57	6.57	6.73	6.71	0.03	0.0001	0.667	0.791	0.0006	0.409	0.511
Fecal DM, %	15.86	15.68	15.93	15.70	0.55	0.914	0.590	0.956	0.046	0.766	0.063

^{a,b}Means in each measurement in the same row with different superscript letters differ significantly ($P < 0.05$).

¹Values are the mean of weekly samples for each measurement.

²Analyzed using pretrial value as a covariate.

TABLE 3. Least squares means for rumen VFA in dairy cows fed high grain pellets containing either no dietary additives, NaHCO₃ (20 g/kg), virginiamycin (VM) (30 mg/kg) or VM plus NaHCO₃ (30 mg/kg and 20 g/kg, respectively).¹

Measurement	Control	NaHCO ₃	VM	VM + NaHCO ₃	SED	<i>P</i> -values					
						VM	NaHCO ₃	VM *	Time	Time *	Time *
Total, mM/L	89.06	88.81	88.47	74.17	6.47	0.105	0.117	0.129	0.005	0.090	0.752
	mol/100 mols										
Acetate (A)	59.55	61.40	59.74	61.77	0.90	0.658	0.003	0.888	0.0001	0.001	0.424
Propionate (P) ²	26.30	26.45	24.65	25.78	1.43	0.241	0.551	0.621	0.0001	0.293	0.849
Butyrate (B) ²	10.99	9.50	10.68	10.57	1.02	0.599	0.261	0.348	0.008	0.302	0.762
Iso-Butyrate ²	0.96	0.95	1.06	1.14	0.13	0.114	0.713	0.623	0.138	0.006	0.650
Iso-Valerate ²	1.20	1.11	1.40	1.35	0.21	0.135	0.650	0.890	0.206	0.402	0.414
Valerate	1.75	1.37	1.71	1.30	0.28	0.806	0.046	0.935	0.207	0.186	0.593
P:(A+2B) ²	0.35	0.34	0.31	0.32	0.03	0.123	0.881	0.447	0.0001	0.313	0.934

^{a,b}Means in each measurement in the same row with different superscript letters differ significantly ($P < 0.05$).

¹Values are the means of weekly samples for each measurement.

²Analyzed using pretrial value as a covariate.

TABLE 4. Least squares means for changes in concentrations of blood metabolites in dairy cows fed high grain pellets containing no dietary additives, NaHCO₃ (20 g/kg), virginiamycin (VM) (30 mg/kg) or VM plus NaHCO₃ (30 mg/kg and 20 g/kg, respectively).¹

Measurement	Control	NaHCO ₃	VM	VM + NaHCO ₃	SED	<i>P</i> -values					
						VM	NaHCO ₃	VM *	Time	Time *	Time *
	mmol/L										
D-Lactic acid	0.00	0.00	0.00	0.00
L-Lactic acid	1.32	0.99	1.15	0.91	0.13	0.339	0.028	0.728	0.346	0.319	0.062
Glucose ²	3.44	3.48	3.35	3.43	0.07	0.283	0.366	0.760	0.0006	0.854	0.474
BHBA ²	1.27	1.22	1.38	1.33	0.10	0.177	0.588	0.978	0.0001	0.451	0.254

¹Values are the means of weekly samples for each measurement.

²Analyzed using pretrial value as a covariate.

TABLE 5. Least squares means of milk production and composition for dairy cows fed high grain pellets containing no dietary additives, NaHCO₃ (20 g/kg), virginiamycin (VM) (30 mg/kg) or VM plus NaHCO₃ (30 mg/kg and 20 g/kg, respectively). All measurements are analyzed using pretrial values as a covariate.

	Control	NaHCO ₃	VM	VM + NaHCO ₃	SED	P-values					
						VM	NaHCO ₃	VM * NaHCO ₃	Time	Time * VM	Time * NaHCO ₃
Yield, kg/d	23.30	23.34	23.77	24.11	0.35	0.089	0.593	0.680	0.0001	0.675	0.825
Fat, %	4.31	4.21	4.25	4.27	0.18	0.971	0.772	0.653	0.493	0.470	0.973
Total fat, kg/d	0.93	1.00	1.00	1.00	0.04	0.335	0.325	0.427	0.741	0.800	0.874
Protein, %	3.43	3.44	3.41	3.39	0.03	0.302	0.972	0.771	0.0001	0.439	0.225
Total protein, kg/d	0.77	0.80	0.79	0.81	0.02	0.375	0.077	0.439	0.0001	0.141	0.303
Lactose, %	4.95	5.04	5.01	5.02	0.04	0.605	0.158	0.361	0.292	0.635	0.421
Total lactose, kg/d	1.15	1.18	1.20	1.21	0.02	0.046	0.273	0.624	0.0001	0.648	0.656
SCC, × 1000 cells/ml	273.9	308.0	327.0	375.0	75.8	0.281	0.459	0.900	0.468	0.566	0.655

BW or body condition score between treatment groups (Table 6) were found.

DISCUSSION

Cereal grains are fed to improve the fermentation characteristics of ruminant diets to allow greater feed intake and greater milk production. Grains high in starch that are readily digested can, however, result in digestive disruption and disease if fed in sufficiently high amounts. Both VM and NaHCO₃ in this experiment were used to minimize the adverse effects of acid production during fermentation of supplemental carbohydrates. Sodium bicarbonate was included as a buffer to ameliorate the effects of acid production and maintain a higher ruminal pH. Virginiamycin was used as an antimicrobial agent to limit *Lac-*

tobacillus sp. and *S. bovis* overgrowth, thereby controlling lactic acid production. The accumulation of lactic acid, which is a strong acid, has a number of undesirable effects including lowering of ruminal pH, ruminitis, laminitis, and other sequelae (21).

The basal diets were designed to increase the amount of readily fermentable feedstuffs and reduce pH in the rumen and hindgut. The reduction in ruminal pH and fecal pH in the control group after the experimental diet was introduced is consistent with the rapid fermentation of a grain-based diet with a high content of starch. However, the ruminal pH of all cows was relatively high throughout the trial. This finding may reflect that the sampling time was within 2 to 3 h of feeding; that the diet did not provide an extremely acidotic challenge, or that cows had previously adapted to a grain-based diet. Also, some saliva contamination of ruminal fluid may not have been detected during sampling. Even if this was the case, important observations can still be made about relative differences in groups with respect to pH. Clinical signs of acute or subacute acidosis (9) were not observed in any cow during this study.

Both NaHCO₃ and VM tended to increase ruminal pH ($P < 0.11$). The lack of significant effect of NaHCO₃ on the pH of ruminal fluid is consistent with studies that have examined the effects of NaHCO₃ on ruminal pH on pasture- or alfalfa-based diets (34). The nonsignificant response may reflect the amount of buffer added relative to production of VFA in the rumen. The 2% inclusion of NaHCO₃ in the concentrate diet provided approximately 2.5 mol of buffer per day. This amount can be contrasted with the 2.75 to 7.22 mol of VFA/d produced per kilogram of DMI (15, 25, 38). Russell and Chow (29) concluded that the primary action of bicarbonate in the rumen was unlikely to be that of a buffer. In our study NaHCO₃

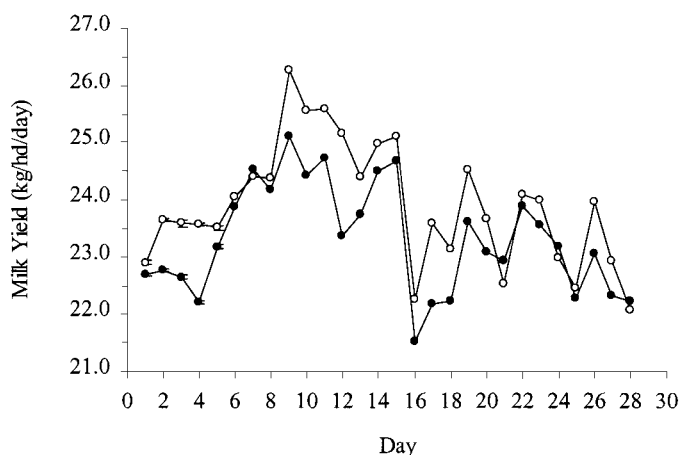


Figure 7. Change in milk yield (kg/d) over time in dairy cows fed a high grain pellet without virginiamycin (●) or with 30 mg/kg of virginiamycin (○).

TABLE 6. Changes in BW and condition score over the trial in dairy cows fed high grain pellets containing no dietary additives, NaHCO₃ (20 g/kg), virginiamycin (VM) (30 mg/kg) or virginiamycin plus NaHCO₃ (30 mg/kg and 20 g/kg, respectively).¹

Group	Control	NaHCO ₃	VM	VM + NaHCO ₃	Group <i>P</i>	Time <i>P</i>
Initial BW, kg	547	548	547	570	0.433	...
Initial CS	2.93	3.09	3.01	3.09	0.425	...
Final BW, kg	574	574	577	600	0.376	...
Final CS ²	2.88	2.96	2.99	3.1	0.323	...
BW Change (kg/28 d)	27	26	30	31	0.423	0.001
CS Change (unit/28 d)	-0.05	-0.13	-0.03	0.01	0.216	0.323

¹Analyzed with one-way ANOVA.

²Condition Score on the 5-point scale (6), 1 = thin, 5 = fat.

had a similar nonsignificant positive effect on ruminal pH, suggesting a similar finding.

The potential for VM to reduce lactic acid accumulation in ruminal fluid was evident from the highly significant reduction in L-lactic acid accumulation in vitro (Figure 3). The L-lactic acid concentration resulting from ruminal fluid incubations was initially, at wk 1 and 2 of sampling, much higher in the cows not fed VM. These differences in L-lactic acid concentrations were less marked by the end of the study, suggesting that some adaptations to the diets may have occurred, although the treatment by time interaction was not significant. There was no effect of NaHCO₃ on L-lactic acid accumulation. This in vitro test highlights an important difference between the rumen modifiers, because VM acts more specifically to reduce lactic acid production and NaHCO₃ acts, in part, to buffer acids produced. Although no chronic or acute lactic acidosis was indicated in cows in this experiment, the in vitro test suggests that VM may be effective in reducing the risk of acidosis, especially with a marked increase in substrate availability for fermentation.

The experimental diets probably increased carbohydrate fermentation and acid accumulation in the hindgut, as indicated by the decrease of fecal pH of all cows over time from the pretrial values in association with the change in diet (Figure 5). The decrease was not as large when cows were fed VM, and the inclusion of VM prevented a decrease as large as non-VM feeding. Godfrey et al. (11) found a strong association between fecal pH and cecal pH in sheep. There may be detrimental effects of uncontrolled hindgut fermentation of carbohydrate on ruminant health. The lack of effect of NaHCO₃ on fecal pH probably reflects the depletion of bicarbonate in the rumen.

Increased concentrations of cereal grain in the diet and, consequently, increased rates of fermentation of

carbohydrate in the rumen normally increase the proportion of propionate relative to other VFA (13). We found increased molar proportions of propionate in response to the change in amount and nature of the concentrate fed at milking (Table 3). Sodium bicarbonate has been used to overcome a negative effect of grain feeding on the concentration of milk fat associated with increased ruminal concentrations of propionate and decreased concentrations of acetate (12, 34). The significant and positive effect of NaHCO₃ on rumen acetate concentrations (Figure 7) was consistent with other studies (25, 31). The reason for a trend toward lower valerate concentrations in the ruminal fluid of cows fed NaHCO₃ is unclear. The effect of VM on the pattern of rumen fermentation in vitro is normally to increase the proportion of propionate relative to other VFA (19, 20). This effect of VM was not observed in the current experiment and may have reflected increased absorption of additional propionate or factors other than VFA production in the animal. The relationship between rumen concentrations of VFA and flux of these from the rumen needs to be considered when interpreting rumen VFA proportions.

Although NaHCO₃ feeding did not increase ruminal pH or fecal pH, blood L-lactic acid concentrations were lower ($P = 0.028$) with NaHCO₃ feeding. These findings contrast other reports (7, 35), and we do not know why this occurred. Plasma L-lactic acid concentrations were large in the control group at the first sampling after treatment, possibly reflecting a degree of challenge from rumen lactic acid accumulation. The lack of detectable D-lactic acid concentrations may reflect the mild acidotic challenge in the study. There were no other effects of VM or NaHCO₃ on blood metabolites. These results suggest that systemic effects of treatments were minor in this experiment or that the crude probes used, i.e., blood metabolite con-

centrations that are under considerable homeostatic control, failed to reflect changes.

The change in concentrate ingredients and the increase in amounts of concentrate fed were expected to cause the observed increase in milk production over the first week of feeding. Variation in milk production (Figure 8) reflected variations in pasture fed and heavy rainfall around d 16 and 17 of the study.

Throughout the experimental period a small, but consistent, increase ($P = 0.09$) in milk production was observed in response to the inclusion of VM in the diet. A higher and more stable ruminal pH facilitates more efficient fiber degradation (22, 37) and more efficient microbial protein synthesis (23). Studies on the effect of VM in sheep fed grain supplements demonstrated an increase in forage intake and less substitution of forage for concentrate (10). If these effects were present in the cows, milk production might have increased. Although NaHCO_3 did not significantly increase milk production, the group fed VM and NaHCO_3 in combination had the largest amount of milk production, a finding consistent with the influence on ruminal pH. Milk production has increased when NaHCO_3 was fed to cows deficient in sodium (3, 7, 39) or when corn silage was the main forage source (34). Our findings of no significant milk production response to NaHCO_3 are consistent with similar studies when cows were fed grass silages or pasture (34).

No significant response to either main effect was observed on milk fat content. This finding is consistent with some, but not all studies, because feeding NaHCO_3 has often increased milk fat content (32, 34). The increase in milk fat content in those studies was consistent with the increase in molar proportions of acetic acid in response to NaHCO_3 feeding. The lack of effect of VM on milk composition or yield of milk components, apart from the increase in lactose yield, was expected. The lack of difference between groups in change in BW and body condition score was expected given the relatively short period of the study and the variance in these measures.

CONCLUSIONS

In this study, grazing lactating Friesian-Holstein dairy cows were fed 10 kg of a pellet high in cereal grains/d, containing 0 or 30 mg of VM/kg and 0 or 20 g of NaHCO_3 /kg. The main effect of either VM or NaHCO_3 tended to show an increase in ruminal pH. Feeding VM maintained higher fecal pH. The results of VM feeding on *in vitro* incubation of ruminal fluid to find the potential production of L-lactic acid sug-

gest that VM reduces the potential for L-lactic acid accumulation in the rumen of cows fed grain. The potential exists for VM to be fed with grain to obtain the benefits from feeding grain and to reduce risks to health of herds arising from acidosis. The feeding of 200 g of sodium bicarbonate/d had limited effects on measures of gastro-intestinal tract acidity.

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