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The influence of virginiamycin on digestion and ruminal parameters under feedlot conditions

Luiz F. Dias Batista,[†,] Madeline E. Rivera,[†] Mozart A. Fonseca,[‡,1,] and Luis O. Tedeschi[†,2,]

[†]Department of Animal Science, Texas A&M University, College Station, TX 77843-2471, USA
[‡]Department of Agriculture, Veterinary, and Rangeland Sciences, University of Nevada, Reno, NC 89557, USA
[1]Present address: Department of Animal and Range Sciences, Clayton Livestock Research Center, New Mexico State University, Clayton, NM 88415, USA

[2]Corresponding author: luis.tedeschi@tamu.edu

Abstract

This experiment aimed to assess the impact of virginiamycin on in vitro gas production dynamics, rumen kinetics, and nutrient digestibility in beef steers fed a grain-based diet. Nine ruminally cannulated British-crossbred steers (596 ± 49 kg) were assigned to this experiment. Animals were housed in three pens (n = 3/pen) equipped with a Calan gate feed system and water troughs. Pens were enrolled in a 3 × 3 Latin square design containing three periods of 16 d, and a 5-d washout interval between periods. Dietary treatments consisted of virginiamycin (**VM**) administration at 0 (VM0), 180 (VM180), or 240 mg/d (VM240). During days 15 and 16 of each period, about 600 mL of rumen fluid and urine samples were collected before (0 h), and at 4, 8, 12, and 16 h after the morning feed (0730 hours), rumen inoculum was used to take pH and redox potential measurements immediately after collection using a portable pH and redox meter, and subsamples were taken for volatile fatty acids (**VFA**) and NH_3–N analyses, and urine samples were composited daily and analyzed for creatinine and purine derivatives (**PD**) content to estimate microbial crude protein flow. During the 4-h post-morning feed rumen collection, rumen inoculum was utilized to perform in vitro gas production measurements. Fecal samples were collected on day 16 of each period to estimate nutrient digestibility using acid detergent insoluble ash as an internal marker. Animals were considered the experimental unit for the statistical analyses, and periods and squares were included as random variables. The total and rate of gas production were similar among treatments ($P \geq 0.17$). The second-pool (i.e., fiber) gas production increased linearly as VM inclusion increased ($P = 0.01$), with VM240 being greater compared to VM180 and VM0 (7.84, 6.94, and 6.89 mL, respectively). Ruminal pH linearly increased as VM increased, with VM240 being greater than VM0 and VM180 intermediate (5.90, 5.82, and 5.86, respectively; $P = 0.03$). The VFA concentrations did not differ ($P \geq 0.13$), but the acetate-to-propionate ratio was the highest in VM240 ($P = 0.005$). Branched-chain VFA increased ($P \leq 0.03$) while lactate concentrations decreased ($P = 0.005$) linearly with VM. The ruminal NH_3–N concentration was the lowest in the VM0 ($P = 0.006$). The estimated absorbed PD, purine derivative to creatinine index, and microbial N flow increased linearly with VM ($P \leq 0.07$). The provision of VM influenced rumen dynamics in a dose-dependent manner.

Lay Summary

This study evaluated the effects of virginiamycin administration at different dosages upon rumen digestion kinetics and fermentation parameters of beef steers receiving a grain-based diet. Ruminal pH increased linearly as virginiamycin dosage amplified from 0 to 240 mg/d. Ruminal NH_3–N and branched-chain volatile fatty acid responded similarly to the virginiamycin inclusion rate, suggesting a better feed N utilization by the rumen microbes. Microbial N flow to the small intestine also improved by including virginiamycin, confirming the increased microbial crude protein (**MCP**) synthesis. Further research should evaluate the possible effects of rumen pH on MCP synthesis and its interaction with virginiamycin supplementation. The improved NH_3–N and estimated microbial N flow could likely be associated with a better rumen health condition through a more stable pH and fermentation profile.

Key words: digestion kinetics, feedlot, liver abscess, ruminal health, virginiamycin

Introduction

In feedlot conditions, finishing cattle involves providing diets with reduced roughage and increased grain content to enhance energy intake and optimize animal performance (Samuelson et al., 2016). Grain-based finishing diets have been widely adopted in feedlot practices since the 1940s. The primary dietary energy source comes from cereal grains as starch, which can represent up to 70% to 50% of the ration in the current feedlot production system to achieve starch levels of 50 to 36%. Further processing of cereal grain involves the exposure of the endosperm, increasing starch digestion in the rumen and thus improving feed utilization by the animal (Owens et al., 1986; Nagaraja and Lechtenberg, 2007). However, increased levels of starch intake contribute to a shift in ruminal microbiota, favoring lactic acid-producing bacteria growth (Nagaraja et al., 1982). Although many ruminal bacteria can utilize starch, lactic acid-producing bacteria, such as *Streptococcus bovis*, tend to prevail due to their rapid growth rate and ability to survive in low pH conditions (Russell, 1991). Even though lactate is an intermediate product from anaerobic fermentation in the rumen, its accumulation can be detrimental to pH homeostasis due to its lower dissociation constant and absorption rate compared to other short-chain fatty acids (SCFA; i.e., acetate, propionate, and butyrate)

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(Williams and Mackenzie, 1965). Thus, controlling lactic acid production in the rumen is vital to avoiding ruminal acidosis in growing and finishing feedlot cattle (Nagaraja and Lechtenberg, 2007).

Virginiamycin is a non-ionophore bactericidal produced by *Streptomyces virginae* that inhibits the growth of Gram-positive bacteria, specifically lactic acid-producing bacteria, by binding to the 50S ribosomal subunit (Cocito, 1979; Nagaraja and Taylor, 1987). Although virginiamycin has shown the potential to alleviate liver abscess incidence in feedlot cattle (Tedeschi and Gorocica-Buenfil, 2018), possibly caused by a reduction in *Fusobacterium necrophorum* counts in the rumen (Nagaraja and Chengappa, 1998), improvements on animal performance when receiving virginiamycin supplementation has been repeatedly reported (Rogers et al., 1995; Tedeschi and Gorocica-Buenfil, 2018). Salinas-Chavira et al. (2009) reported an improvement on gain-to-feed ratio (**G:F**) by 3.97% when virginiamycin was supplemented at 22.5 mg/kg of dry matter (**DM**). Similarly, Carvalho et al. (2022) observed greater average daily gain, G:F, and net energy (**NE**) values for calf-fed Holstein steers supplemented with virginiamycin at the same dosage. However, calf-fed Holstein steers supplemented with virginiamycin at 16 mg/kg of DM had similar performance outcomes to animals not supplemented (Latack et al., 2019). Tedeschi and Gorocica-Buenfil (2018) reported that improvements in animal performance due to virginiamycin supplementation occur dose-dependently when above 18 mg/kg and are likely maximized when fed above 24 mg/kg. However, it remains unclear whether the impacts of virginiamycin supplementation on animal growth and development are solely attributed to a decrease in liver abscess prevalence, which enhances overall animal health status (Batista and Holland, 2022; Dias Batista et al., 2022), a shift in the SCFA profile favoring propionate production in the rumen (Nagaraja et al., 1987), or an improvement in protein flow to the small intestine by sparing protein deamination in the rumen (Ives et al., 2002). The objective of this study was to evaluate the effects of three different doses of virginiamycin supplementation (0, 180, and 240 mg/d) on in vivo and in vitro ruminal digestion kinetics of beef steers consuming a grain-based diet.

Materials and Methods

This experiment was conducted at the Nutrition and Physiology Center, Texas A&M University, College Station, TX. All animals were cared for following acceptable practices and experimental protocols reviewed and approved by the Texas A&M—Institute of Animal Care of Use Committee (AUP #2018-0410).

Description of animals and treatments

Animals and diets Nine ruminally cannulated British-crossbred steers (596 ± 49 kg) were assigned to this experiment. On d-32, steers were ranked by body weight (**BW**) and housed in three pens (three animals per pen) equipped with a Calan gate feed system (American Calan, Northwood, NH) and water troughs. Animals within a pen were enrolled in a 3 × 3 Latin square design containing three periods of 16 d, and a 5 d washout interval between periods, which resulted in a three 3 × 3 Latin square where treatment sequences were designed to balance the carry-over effect. Dietary treatments consisted of virginiamycin (**VM**; V-Max, Phibro Animal Health, Teaneck, NJ) supplementation at 0 mg/d (VM0), 180 mg/d (VM180), or 240 mg/d (VM240). For all treatments, steers were fed a grain-based diet (crude protein [**CP**]: 15.2%, and metabolizable energy [**ME**]: 2.99 Mcal/kg DM basis) consisting of sorghum sudan hay 22.2%, cracked corn 50.7%, dried distillers grains 10.3%, soybean meal 7.1%, dried molasses 5.8%, urea 0.5%, and limestone plus a mineral–vitamin supplement 3.2% (Table 1) formulated using the Large Ruminant Nutrition System (http://www.nutritionmodels.com/lrns.html (Fox et al., 2004). At the beginning of each period (day 0), an animal within a pen was assigned to receive one of three treatments for 14 d, followed by 2 d of collection and 5 d of washout (basal diet with no added virginiamycin). Feed and treatments were offered in equal proportions daily (0730 and 1600 hours), feed bunks were visually evaluated before the morning feed, and feed calls were managed to allow ~3% to 5% residual orts. To ensure the consumption of dietary treatments, preassigned virginiamycin quantities were preweighed and added to empty cellulose pill capsules (XPRS Nutra, South Jordan, UT). At each feeding, animals assigned to receive VM180 and VM240 treatments had the rumen cannula removed, and capsules

Table 1. Ingredients and chemical composition of the basal diet

Items[1]	Basal diet, %
Ingredient composition, % DM	
Sorghum Sudan Hay	22.28
Cracked corn	50.7
Dried distillers grain	10.29
Soybean meal	7.1
Molasses	5.87
Limestone	0.48
Mineral mix	2.79
Urea	0.49
Chemical composition[1,2]	
DM, %	86.6
CP, % DM	15.2
ADF, % DM	12.9
ADIN, % DM	1.25
aNDF, % DM	24
NDIN, % DM	1.55
Lignin, % DM	2.1
ADIA, % DM	1.42
Starch, % DM	37.2
Ether extract, % DM	4.9
Ash, % DM	6.55
TDN, % DM[3]	77.6
ME, Mcal/kg[4]	3.00
NEm, Mcal/kg[4]	2.03
NEg, Mcal/kg[4]	1.37

[1]Items are feed ingredients and chemical composition of diets evaluated by Cumberland Valley Analytical Services (Waynesboro, PA).
[2]DM, dry matter; CP, crude protein; aNDF, neutral detergent fiber with amylase and sodium sulfite; ADF, acid detergent fiber; ADIA, acid detergent insoluble ash, TDN, total digestible nutrients; NEm, net energy for maintenance; NEg, net energy for gain.
[3]Computed using empirical equations as proposed by Weiss (1998).
[4]Computed using empirical equations from calculated TDN as adopted by the NASEM (2016).

were opened and mixed with rumen digesta. Diet samples were collected at the beginning and end (d0 and d15) of each period, composited, dried at 55 °C for 48 h, and stored at –20 °C until further analyses.

Chemical analyses. Diet samples collected throughout the three periods were then composited into one representative sample of the basal diet, and a subsample was shipped to Cumberland Valley Analytical Service (**CVAS**; Waynesboro, PA) for the chemical analysis according to the AOAC (2012) for DM (Goering and Van Soest, 1970), neutral detergent fiber (**NDF**) with the addition of amylase and sodium sulfite (Van Soest et al., 1991), acid detergent fiber (**ADF**; Method# 973.18), lignin using sulfuric acid (Goering and Van Soest, 1970), CP (Method# 990.03) in a Leco FP-528 Nitrogen Combustion Analyzer (Leco Corporation, St. Joseph, MO), soluble CP (Krishnamoorthy et al., 1982), ether extract (Method# 2003.05), starch (Hall, 2009), sugar (Dubois et al., 1956), ash (Method# 942.05), minerals (Method# 985.01) using a Perkin Elmer 5300 DV ICP (Perkin Elmer, Shelton, CT), and calculation of total digestible nutrients (**TDN**), and NE using empirical equations (Weiss, 1998). Individual orts were collected at the end of each period and stored at –20 °C after collection until subsequent analyses.

Rumen sampling and analyses

During days 15 and 16 of each period, about 600 mL of rumen fluid from the ventral sac was collected prior to (0 h) and at 4, 8, 12, and 16 h relative to the morning feed (0730 hours) using a vacuum pump; pH and redox potential (**Eh**) measurements were taken immediately after collection using a portable pH and redox meter (Orion A221, Thermo Fisher Scientific, Waltham, MA) calibrated prior to collections as per manufacturer guidelines. Subsamples were taken and stored at –20 °C using duplicate falcon tubes and preserved for volatile fatty acids (VFA), and NH_3–N analyses. A 0.1M HCl acid solution was used to preserve NH_3–N analyses following a 4:1 ratio of acid and rumen inoculum, respectively. NH_3–N was analyzed via colorimetric methods (Chaney and Marbach, 1962), whereas VFA analyses were analyzed using high-performance liquid chromatography (Shimadzu Scientific Instruments, Columbia, MD) (Weimer et al., 1991), equipped with a temperature-controlled autosampler (Nexera SIL-30AC UHPLC Cooled Autosampler, Shimadzu Scientific Instruments, Columbia, MD), a forced-air column oven (CTO-20A, Shimadzu Scientific Instruments, Columbia, MD), and a UV absorbance detector (SPD-20A UV Detector, Shimadzu Scientific Instruments, Columbia, MD). Briefly, peaks were separated using an Aminex HPX-87H column (Bio-Rad HPX-87H, 300mm × 4.6 mm i.d., Bio-Rad Laboratories Inc., Hercules, CA) and its guard column (Bio-Rad Cation H, Bio-Rad Laboratories Inc., Hercules, CA) and detected at 210 nm UV absorbance over 100 min of retention time.

In vitro gas production measurements

During the 4 h post-morning feed rumen collection time point, the remaining rumen inoculum post-VFA and NH_3–N subsamples collection were utilized to perform in vitro gas production (**IVGP**) measurements as proposed by Tedeschi et al. (2009) with modifications described by Dias Batista et al. (2021). The IVGP measurements were performed for each animal within a pen as the treatment inoculum was incubated with 200 mg of air-dried diet sample ground through a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). A total of 10 replicates per animal was utilized within each run (30 replicates/period/treatment). Briefly, 14 mL of in vitro buffering media (Goering and Van Soest, 1970) was incubated in a wheaton bottle with 2 mL of distilled water and 4 mL of rumen inoculum of the respective animal, and gas pressure was collected every 5 min (Tedeschi et al., 2009). After 48 h of incubation, bottles were placed in an ice bath to cease fermentation and methane concentration was measured by taking a subsample of the bottle headspace and analyzed via gas chromatography (GOW-MC Series 580, Gow-Mac Instrument, Bethlehem, PA) according to Allison et al. (1992). In vitro neutral detergent fiber digestibility (**ivNDFD**) was performed by adding 40 mL of neutral detergent solution (ANKOM Technology, Macedon, NY) in each bottle, autoclaving for 60 min at 105 °C, filtering the samples using a Whatman 54 paper filter, and oven-drying the residue at 55 °C for 48 h. IVGP output data consist of total gas production (**TGP**, mL), fermentation rate (kd, %/h), lag time (h) using exponential curves, asymptote cumulative gas production of nonfiber carbohydrate (**NFC**) pool (mL), fractional rate of degradation of the NFC pool (%/h), lag time to initiation of fiber carbohydrate (**FC**) pool (h), asymptote cumulative gas production of FC pool (mL), fractional rate of degradation of the FC pool (%/h), and equivalent exponential fractional rate of degradation rate (%/h) using nonlinear functions of the 48-h fermentation, where the lowest sum of square errors (Schofield et al., 1994) was utilized to perform and select the functions using a specific R scripts using *nls* and *port* algorithms (Fox et al., 1978; Gay, 1990; Chambers and Bates, 1992), and Gasfit (http://www.nutritionmodels.com/gasfit.html), respectively, as proposed by Tedeschi and Fox (2020).

In vivo digestibility

Spot fecal collections were performed on day 16 of each period before the morning feed. Fecal samples were stored at –20 °C until further analysis. Orts and fecal samples were dried at 55 °C for 48 h, and ground through a 2-mm screen using a Wiley mill (Thomas Scientific). ADF residue was determined by weighing and filling F57 bags (ANKOM Technology; $n = 3$ replicates per sample/animal), and subsequently washed in 100 mL/g DM of acid detergent solution using an ANKOM^{200} fiber analyzer (ANKOM Technology). After cold-water wash, bags were dried at 55 °C for 48 h followed by 24 h at 105 °C, and acid detergent insoluble ash (**ADIA**) was considered as the remaining DM following the combustion of the ADF residue at 525 °C for 4 h as described by Van Soest et al. (1991) and utilized as internal marker to estimate DM, NDF, and ADF digestibilities as described by Adams et al. (2020). Gross energy (**GE**) was obtained on feed, fecal, and orts samples using a bomb calorimeter (Parr adiabatic calorimeter, Parr Instruments Co., Moline, IL). Then GEI (Mcal/d) intake (Mcal/d) was calculated by multiplying the GE value of the basal diet by kilograms offered minus the caloric value of orts multiplied by orts kilograms. Fecal energy (**FE**; Mcal/d) was calculated by multiplying the GE of fecal samples by the estimated daily output from ADIA. Then, digestible energy (**DE**; Mcal/d) and DE to GE ratio were computed by the difference between GE and FE, and the division of DE to GE, respectively.

Urine sampling and analyses

During rumen samples collection (0, 4, 8, 12, and 16 h relative to the morning feed during days 15 and 16), urine spot samples were collected from each animal during the rumen samples collection via mechanical stimulation. Twenty milliliters of urine from each collection were composited in a daily sample (100 mL for days 15 and 16, respectively), acidified with 3M HCl, and stored at –20 °C to prevent purine derivatives (**PD**) degradation. Acidification occurred using 1 mL of HCl solution per 20 mL of urine. If a sample could not be collected at a specific time point, the remaining samples were utilized to reach a total daily volume of 100 mL. Allantoin, uric acid, and creatinine concentrations were determined via high-performance liquid chromatography (Agilent 1100-HPLC System) as described by Shingfield and Offer (1999). Total PD was calculated by summing uric acid and allantoin, considering that xanthine and hypoxanthine are hardly detected in cattle urine (Gonzalez-Ronquillo et al., 2004). Absorbed PD was calculated using Equation 1 (Chen and Gomes, 1992).

$$\text{Absorbed PD} = \frac{\text{Total PD excreted} - 0.385 \times \text{SBW}^{0.75}}{0.85} \quad (1)$$

where total PD excreted is the sum of allantoin and uric acid mmol/d, and SBW is the average shrunk body weight (full BW applied 4% pencil shrink) for the period, kg.

Daily creatinine urinary excretion was calculated assuming a daily excretion rate of 28 mg/kg of animal BW (Lofgreen and Garrett, 1954; Whittet et al., 2019). Daily creatinine excretion was then divided by creatinine concentration in the urine (mg/L) to estimate daily urinary excretion.

Statistical analyses

All statistical procedures were performed using SAS software (SAS Institute Inc., Cary, NC). In vivo and in vitro digestibility variables analyses included treatment as a fixed effect and the random effects of steer within a pen, pen, and period, whereas ruminal pH, Eh, VFA, and NH_3–N variables contained the effects of treatment, hour, and treatment × hour interaction with h being included and analyzed following a repeated measures design using the REPEATED statement in PROC MIXED. The covariance structure for each variable was determined based on the model's Akaike information criterion and covariance structure (Hurvich and Tsai, 1989). The DM intake (**DMI**) was tested in the model as a covariate. Least-square means were obtained using the LSMEANS statement. The linear contrast coefficient was obtained using the PROC IML procedure. The contrast statement included linear and VM0 vs. VM180 + VM240 comparisons. Significance was set as $P \leq 0.05$, and tendencies were considered for $0.1 \geq P > 0.05$.

Results

Table 2 shows the effects of VM inclusion on in vitro gas production dynamics. Exponential TGP, the fractional gas production rate of the exponential nonlinear function, and lag time necessary to commence fermentation did not differ among treatments ($P \geq 0.283$). Similarly, the asymptote cumulative gas production of NFC pool (P1) and fractional rate of degradation of the NFC pool were not altered by VM inclusion ($P \geq 0.941$). However, the lag time required to commence fermentation of the FC pool tended to increase linearly as

Table 2. Effect of virginiamycin on in vitro gas production dynamics of beef steers consuming a high grain diet

	Dietary treatment[1]				*P* value		
Item[2]	VM0	VM180	VM240	SEM	TRT[3]	Linear	VM0 vs. VM180 and VM240
Exponential							
TGP, mL	14.4	14.2	15.2	0.6	0.283	0.269	0.443
kd, %/h	10.3	10.5	10.4	1.2	0.838	0.555	0.812
Lag time, h	-0.7	-0.5	-0.5	0.3	0.382	0.168	0.192
Log. Two-pool							
Asymptote (NFC), mL	7.7	7.7	7.6	0.5	0.959	0.829	0.871
kd (NFC), %/h	20.0	20.2	20.7	2.2	0.941	0.766	0.776
Lag time to FC, h	0.6	0.7	0.7	0.2	0.174	0.066	0.230
Asymptote (FC), mL	6.81[b]	6.85[b]	7.83[a]	0.66	0.006	0.017	0.094
kd FC, %/h	3.32	3.42	3.84	0.37	0.548	0.380	0.247
Exp. kd equivalent, %/h	5.82	6.03	6.86	0.74	0.549	0.384	0.250
Energy estimates							
ivNDFD, 48 h	55.37	55.64	56.09	3.34	0.885	0.806	0.889
Methane, mg/g NDFD	59.13	66.06	59.44	5.97	0.978	0.973	0.520
TDN, %	77.86	78.06	77.92	0.27	0.835	0.767	0.503
ME, Mcal/kg (TDN, 4%)	2.81	2.81	2.81	0.01	0.835	0.767	0.778

[1]VM240, virginiamycin fed at 240 mg/d; VM180, virginiamycin fed at 180 mg/d; VM0, no VM fed.
[2]TGP = Total gas production of the exponential nonlinear function; kd = the fractional rate of gas production of the exponential nonlinear function; lag time = time required to commence fermentation; asymptote (P1) = accumulative gas production of nonfiber carbohydrate pool (**NFC**); kd (P1) = fractional rate of gas production of **NFC** pool; lag time to P2 = time required to commence fermentation of fiber carbohydrate (**FC**) pool; asymptote (P2) = accumulative gas production of **FC** pool; kd P2 = fractional rate of gas production of **FC** pool; Exp. kd equivalent = exponential decay digestion rate (**kd**); ivNDFD = in vitro neutral detergent fiber digestibility; CGP = cumulative gas production, milliliter per gram of NDF digested; TDN = computed TDN; ME = metabolizable energy.
[3]Treatments.
[a,b]Least Squares means in a row with different superscripts differ at $P \leq 0.05$.

VM dosage increased ($P = 0.066$), and the asymptote cumulative gas production of the FC pool was greater ($P = 0.006$) for VM240 compared to VM180 and VM0; and improved linearly as VM dosage increased ($P = 0.017$). Nevertheless, ivNDFD, CH_4 (mg/g), and NDFD were not changed by VM inclusion ($P \geq 0.885$). Correspondingly, estimated dietary TDN and ME were similar among treatments ($P = 0.835$).

Table 3 shows the effects of VM on estimated apparent in vivo digestibility. Neither DMI nor GEI differed among treatments ($P = 0.910$). Apparent DM, NDF, and ADF digestibilities were similar among treatments ($P \geq 0.385$); accordingly, estimated DE, Mcal/d; DE, Mcal/kg; DE, % GE; and fecal GE, % GE did not differ among treatments ($P \geq 0.798$).

Rumen parameters kinetics are shown in Table 4. Ruminal pH linearly increased as VM dosage increased ($P = 0.034$), with VM240 showing greater pH than VM0 and VM180 being intermediate (5.90 vs. 5.82 and 5.86, respectively; $P = 0.038$). However, redox potential did not differ among treatments ($P = 0.947$). Animals that received VM (VM240 and VM180) tended to have greater total VFA concentration compared to animals that did not receive VM ($P = 0.084$). Acetate and propionate concentrations did not differ among treatments ($P = 0.323$). Still, the acetate-to-propionate ratio was greater for VM240 compared to VM180 and VM0 (2.10 vs. 1.81 and 1.90, respectively; $P = 0.005$). Butyrate and isobutyrate tended ($P = 0.057$ and 0.058, respectively) to be increased for animals receiving VM compared to animals that did not receive VM. Valerate tended ($P = 0.072$) to be greater for VM180 compared to VM0, whereas isovaleric and branched-chain VFA (BCVFA) concentration increased ($P = 0.023$ and 0.042, respectively) in a linear fashion as VM dosage increased. Lactate reduced linearly as VM dosage increased ($P = 0.004$), and NH_3–N concentration was greater for VM240 and VM180 compared to VM0 (10.54 and 10.46 vs. 8.49, respectively; $P = 0.006$).

Table 5 shows the effect of VM on urinary parameters. The total PD to creatinine ratio did not differ among treatments ($P \geq 0.102$). However, estimated PD and Microbial N absorbed tended to increase linearly ($P = 0.074$) as VM dosage increased. Accordingly, the purine derivative to creatinine index followed the same trend ($P = 0.079$). Creatine concentration and estimated urinary volume (mmol/L and L/d, respectively) did not differ among treatments ($P \geq 0.256$).

Discussion

The response of supplementing VM in increasing ruminal pH and reducing lactate concentration in grain-based diets has been previously reported in beef (Nagaraja and Taylor, 1987; Van Nevel and Demeyer, 1992; Coe et al., 1999) and dairy cattle (Salgado and Gómez, 2006). On the other hand, Lemos et al. (2016) showed no effect on ruminal pH when VM was provided at 25mg/kg DM (~200 mg/d) for animals receiving a no-roughage diet, similarly Salinas-Chavira et al. (2009) reported no effect on pH when Holsteins steers were supplemented with 22.5 mg/kg (~128 mg/d) of VM when receiving a steam-flaked corn-based diet. Virginiamycin may control the population of *Lactobacillus* spp., *S. bovis*, and *F. necrophorum* during the adaptation period and grain challenge, which aids in reducing lactate production in the rumen (Coe et al., 1999; de Araujo et al., 2016). Although lactate concentration was not altered by VM supplementation in the Salinas-Chavira et al. (2009) study, which disagrees with the present research, the acetate-to-propionate ratio was increased with the inclusion of VM in agreement with the current results. However, the increased levels of acetate-to-propionate ratio reported in previous research were carried by an increased acetate concentration (Nagaraja et al., 1987; Salinas-Chavira et al., 2009), which was not seen in the current research.

Previous in vitro work using rumen inoculum from cannulated sheep reported reduced NH_3–N production with the inclusion of VM and suggested that VM possessed a protein-sparing effect (Van Nevel et al., 1984; Van Nevel and Demeyer, 1992) similar to that observed with the provision of monensin (Russell and Strobel, 1989), which disagrees to the data in our study where NH_3–N concentrations were increased due to VM provision. Despite this, it is essential to note that VM inclusion in these previous research studies was performed in the incubation flask rather than provided to

Table 3. Effect of virginiamycin upon apparent digestibility and energy utilization of beef steers consuming a high grain diet

	Dietary treatment[1]				*P* value		
Item[2]	VM0	VM180	VM240	SEM	TRT[3]	Linear	VM0 vs. VM180 and VM240
DMI, kg	16.08	16.04	16.01	0.38	0.910	0.910	0.916
aDMD, %	76.31	77.52	78.76	1.36	0.560	0.328	0.374
aNDFD, %	71.29	70.17	73.21	2.10	0.669	0.736	0.894
aADFD, %	72.46	71.91	74.86	1.29	0.385	0.435	0.625
GEI, Mcal/d	69.09	69.18	69.37	1.45	0.967	0.822	0.848
ASBW, kg	635.6	636.6	638.8	19.8	0.638	0.413	0.490
Fecal GE, Mcal/d	16.34	15.75	14.44	1.8	0.796	0.573	0.633
DEI, Mcal/d	53.1	53.12	53.90	5.20	0.798	0.637	0.728
DE, Mcal/kg	3.35	3.36	3.37	0.38	0.971	0.841	0.868
DE, % GE	77.14	77.27	77.61	0.08	0.968	0.828	0.857
Fecal GE, % GEI	15.9	15.96	15.65	6.27	0.977	0.910	0.946

[1]VM240, virginiamycin fed at 240 mg/d; VM180, virginiamycin fed at 180 mg/d; VM0, no VM fed.
[2]DMI, dry matter (DM) intake; aDMD, apparent total tract dry matter intake; aNDFD, apparent total tract neutral detergent fiber (NDF) digestibility; ADFD, apparent total tract acid detergent fiber (ADF) digestibility; GEI, gross energy (GE) intake; ASBW, average shrunk body weight (SBW); Fecal GE, fecal gross energy; DEI, digestible energy (DE) intake; DE, digestible energy; % GE, digestible energy as a percentage of gross energy; fecal GE, fecal gross energy as a percentage of total gross energy intake.
[3]Treatments.

the animals, and rumen inoculum was collected from fasted animals receiving a hay-based diet. These factors may have modified the responses that could have occurred in vivo when animals are fed a grain-based diet and continuously provided with VM. Ives et al. (2002) did not observe a difference in NH_3–N concentrations due to VM supplementation (175 mg/d) in animals receiving diets with either soybean meal or wet corn gluten feed as the primary protein source. Still, the authors reported greater α-amino nitrogen and reduced isovalerate concentration in steers receiving VM and suggested that VM supplementation might alter amino acid deamination in the rumen. In the current experiment, NH_3–N and isovalerate concentrations were improved with the provision of VM, which was followed by greater BCVFA concentrations. Because BCVFA is required for several ruminal bacteria, especially the fiber-degrading microorganisms (Cummins and Papas, 1985), the digestion of structural carbohydrates and the synthesis of a microbial protein depend on maintaining a minimum level of BCVFA in the rumen (Wang et al., 2012).

Previous research has reported an increased cellulolytic bacteria population in the rumen of animals receiving a corn-stover-based diet supplemented with isovalerate (Liu et al., 2014). It is suggested that the greater NH_3–N, and BCVFA might have favored the growth of cellulose-digesting bacteria and improved FC digestion, translating into greater asymptote cumulative gas production of the fiber carbohydrate (FC) pool in the IVGP results. However, even if VM provision improves cellulose-digesting bacteria growth, it does not result in greater ivNDF nor apparent total tract NDF digestibilities. However, it might lead to rapid fermentation, as the lag time required to initiate fermentation of the FC pool tends to increase with increasing VM dosage which could suggest that some FC might have been digested at rates alike the NFC pool in our IVGP system. Consequently, assuming a fixed feed passage rate in the rumen, it is possible that VM improves NDF digestibility in the rumen under in vivo circumstances if FC degradation rate is improved. This will lead to less undigested NDF being escaped from rumen digestion; however, it was not confirmed in the current study. It is also possible that VM might have provided a better pH homeostasis for ammonia-producing bacteria that are sensitive to low pH, such as *Megasphaera elsdenii* (Nagaraja and Lechtenberg, 2007) or perhaps the

Table 4. Effect of virginiamycin upon rumen parameters of beef steers consuming a high grain diet

	Dietary treatment[1]				*P* value				
Item[2]	VM0	VM180	VM240	SEM	TRT[3]	Hour	TRT × Hour[3]	Linear	VM0 vs. VM180 and VM240
pH	5.82[b]	5.86[ab]	5.90[a]	0.073	0.038	<0.001	0.668	0.034	0.070
Redox	−300.8	−305.8	−303.5	10.53	0.947	0.033	0.995	0.808	0.773
Total VFA,mmol/L	88.98	97.38	95.70	5.18	0.212	0.110	0.569	0.110	0.084
Acetic, mmol/L	46.59	48.98	50.33	3.47	0.323	0.012	0.654	0.137	0.131
Propionic, mmol/L	25.98	28.18	25.77	1.88	0.377	0.011	0.765	0.546	0.374
Butyric, mmol/L	12.93	15.53	14.73	1.40	0.137	0.076	0.372	0.089	0.058
Isobutyric, mmol/L	0.76	0.99	0.92	0.09	0.136	<0.001	0.934	0.088	0.057
Valeric, mmol/L	1.56	2.05	1.69	0.30	0.072	0.001	0.680	0.231	0.103
Isovaleric, mmol/L	1.44	1.59	1.77	0.22	0.057	<0.001	0.960	0.023	0.042
BCVFA, mmol/L	16.72	20.66	19.60	2.03	0.059	0.003	0.646	0.037	0.022
Lactic, mmol/L	0.42	0.41	0.38	0.07	0.016	0.033	0.644	0.005	0.004
Acetate:propionate	1.90[b]	1.81[b]	2.10[a]	0.16	0.005	<0.001	0.340	0.135	0.461
NH_3–N, mg/dL	8.49[b]	10.46[a]	10.54[a]	1.85	0.006	<0.001	0.227	0.001	0.001

[1]VM240, virginiamycin fed at 240 mg/d; VM180, virginiamycin fed at 180 mg/d; VM0, no VM fed.
[2]VFA, volatile fatty acid; BCVFA, branched-chain volatile fatty acid.
[3]Treatments.

Table 5. Effect of virginiamycin upon urine purine derivatives

	Dietary treatment[1]				*P* value		
Item	VM0	VM180	VM240	SEM	TRT[2]	Linear	VM0 vs. VM180 and VM240
PD:creatinine	2.4	2.7	2.8	0.309	0.250	0.102	0.114
PD absorbed[3], mmol/d	416.47	461.9	484.46	46.71	0.189	0.074	0.088
PDC Index[4]	321.47	352.09	366.69	32.99	0.201	0.079	0.093
Microbial N[5], g/d	302.79	335.8	352.23	33.96	0.189	0.074	0.088
Creatinine, mmol/L	665.57	728.56	624.81	192.55	0.631	0.909	0.906
Urinary volume, L/d	32.65	35.89	37.11	8.29	0.512	0.256	0.272

[1]VM240, virginiamycin fed at 240 mg/d; VM180, virginiamycin fed at 180 mg/d; VM0, no VM fed.
[2]Treatments.
[3]Purine derivative (PD) absorbed using the equation proposed by Chen and Gomes (1992).
[4]Purine derivative to creatinine index calculated using the equation proposed by Chen et al. (2004).
[5]Calculated as proposed by Chen and Gomes (1992).

lower pH might have shifted a greater capture of NH_3 as ammonium ion (NH_4^+) (Owens and Zinn, 1988).

The lack of effect on apparent DM, NDF, and ADF digestibilities is in accordance with previous results (Salinas-Chavira et al., 2009, 2016; da Fonseca et al., 2016) and the in vitro data reported in this study. Comparable to the current results, da Fonseca et al. (2019) reported no difference in DE, % of GE and ME, % of DE when bos indicus influence crossbred bulls were fed a diet containing 30 mg/kg of VM (~200 mg/d). However, Montano et al. (2015) reported improvements in apparent NE for maintenance and growth (NEm and NEg, respectively) by 11% and 9% when crossbred beef steers received diets containing 26 mg/kg of VM (~216 mg/d). Similarly, Carvalho et al. (2022) and Latack et al. (2019) showed an improvement in NEm and NEg values in calf-fed Holstein steers when VM was included in the diet at 22.5 mg/kg and 16 mg/kg, respectively (~184 and 144 mg/d, correspondingly). These results suggest that VM supplementation alters energy utilization by the animal rather than nutrient digestibility, resulting in greater NE available for growth. When performing back-calculation from carcass composition and growth performance in growing and finishing steers, Dias Batista et al. (2022) pointed out that VM supplementation might decrease ME required for maintenance, possibly resulting from improved health status.

It is plausible that VM might improve energy utilization by reducing inflammation response caused by the formation of liver abscess and subacute and acute acidosis. Salgado and Gómez (2006) reported a reduced incidence rate of acidosis in dairy herds supplemented VM. Severe liver abscess has been shown to impact animal performance negatively (Shin et al., 1988; Brink et al., 1990). Batista and Holland (2022) indicated that liver abscesses might increase the NE requirement for maintenance in feedlot cattle. However, evaluations of acute and subacute acidosis's impact on energy utilization have not yet been studied. Zhao et al. (2018) reported greater acute phase proteins (haptoglobin and serum amyloid-A) and inflammatory cytokines in the blood of dairy cows with subacute ruminal acidosis, followed by greater lipopolysaccharide concentration in the blood. Accordingly, Rodríguez-Lecompte et al. (2014) showed greater acute phase proteins in the blood of dairy cows under subacute acidosis. Gifford et al. (2012) indicated that acute inflammation might result in muscle atrophy and lower activity of metabolic enzymes involved in glycolysis, gluconeogenesis, glyconeogenesis, and glycogenesis, while simultaneously promoting glycogenolysis. Therefore, there is a compelling need to enhance our understanding of the effects of acute and subacute acidosis on energy utilization efficiency and explore the viability of nutritional management strategies aimed at minimizing its impact.

The purine derivative results imply that microbial crude protein (**MCP**) flow and absorption might be improved due to VM supplementation (Chen and Gomes, 1992; Shingfield and Offer, 1998; Chen et al., 2004; Crawford et al., 2020), which has not been shown by previous research. Montano et al. (2015) reported a decrease in microbial N to the small intestine when Holstein steers with duodenum cannula were fed a steam-flaked corn-based diet and supplemented with VM at 26 mg/kg of DM, resulting in an average daily intake of 172 mg/d. However, the authors reported an increase in the flow of feed nitrogen due to VM supplementation. On the other hand, Navarrete et al. (2017) found no difference in microbial and feed nitrogen flow to the small intestine when VM was supplemented at 28 mg/kg (resulting in an average intake of 104 mg/d) in Holsteins steers consuming a steam-flacked corn-based diet with two different dietary NEm concentrations (2.10 and 2.22 Mcal/kg, respectively). However, it is reasonable to believe that MCP could be improved due to greater ruminal pH. As ruminal pH decreases, rumen microbes promote an inefficient utilization of carbohydrates and energy to maintain the H^+ balance intracellularly via energy spilling (Strobel and Russell, 1986). Oba and Allen (2003) have shown greater microbial efficiency (g of microbial N/g true ruminally degraded organic matter) as mean ruminal pH is increased in dairy cows.

Some ruminal bacteria species can activate this energy-spilling process when N supply is not synchronized with the carbohydrate fermentation rate (Russell, 2002), resulting in the ruminal fermentation of the NFC without additional microbial protein (Tedeschi and Fox, 2020). This finding could also result from the lower NH_3–N concentration in the VM0 treatment, implying that VM supplementation does not directly impact MCP synthesis but might provide a more suitable environment for microbial growth in the rumen. Perhaps the improved MCP synthesis and metabolizable protein supply to the animal could explain the greater dietary energy values previously reported due to VM supplementation (Rogers et al., 1995; Salinas-Chavira et al., 2009; Navarrete et al., 2017; Latack et al., 2019; Carvalho et al., 2022; Dias Batista et al., 2022). However, it is essential to note that the method utilized in our experiment differs from those once used to categorize the possible effects of VM supplementation on microbial N flow (Montano et al., 2015; Navarrete et al., 2017). Furthermore, the daily VM intake in the current research is greater than that reported by Montano et al. (2015) and Navarrete et al. (2017). Therefore, further research is needed to understand better the effects of VM on MCP synthesis and metabolizable protein supply to the animal.

Overall, the results from our research suggest that VM supplementation neither altered apparent DM, NDF, and ADF digestibilities nor improved dietary DE content but increased ruminal pH and reduced lactate while increasing BCVFA, NH_3–N concentrations, and acetate-to-propionate ratio. Additionally, absorbed PD tended to increase as VM dose increased, which could be indicative of greater MCP flow to the small intestine. It appears that VM supplementation influences bacteria population and fermentation profile, perhaps reducing the energy spilling from the rapid fermentation of starch from some bacteria species. Given the current and previous studies, we believe that animals consuming VM will likely promote rumen health through a more stable pH and fermentation profile in grain-based diets, which could potentially lead to an improvement in MCP synthesis and metabolizable protein supply to the animal. Further research should be encouraged to investigate the effects of rumen pH and VM supplementation on MCP synthesis and absorption of beef steers under feedlot conditions.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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