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Ruminant Nutrition

Combined use of monensin and virginiamycin to improve rumen and liver health and performance of feedlot-finished steers

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Abstract

Monensin and virginiamycin are included in beef cattle finishing diets as prophylaxis to minimize the incidence of ruminal acidosis and liver abscesses. Due to different and probably complementary modes of action, this study aimed to determine the effects of a combination of monensin and virginiamycin, both included in the diet at recommended doses, on ruminal health, the occurrence of liver abscesses, and growth performance of feedlot-finished cattle. One hundred and forty-four steers (6 animals/pen) were fed 1 of 3 corn-based finishing diets containing 30 mg of monensin (**MN**), 25 mg of virginiamycin (**VM**), or 30 and 25 mg of monensin and virginiamycin (**MN + VM**), respectively, per kilogram of dry matter. Ruminal pH probes were inserted into two animals per pen and set to record pH every 10 min. On d 100, animals were slaughtered, and rumens and livers were recovered, on which occurrence and degree of ruminal damage, prevalence and number of liver abscesses, and liver scores (A−: livers with no more than two small abscesses; A+: livers with at least one large abscess or more than four medium abscesses; A: any other abscessed liver) were determined. Simultaneous inclusion of monensin and virginiamycin resulted in a 4.3% decrease ($P < 0.04$) in dry matter intake (**DMI**; 8.8, 9.2, and 9.2 ± 0.19 kg/d for MN + VM, MN, and VM-fed animals, respectively) and similar ($P > 0.13$) average daily body weight gain (**ADG**; 1.49 ± 0.021 kg/d) and hot carcass weight (**HCW**; 269 ± 1.7 kg), compared with feeding diets containing one additive or the other. Therefore, in terms of ADG, a 9.4% improvement ($P < 0.01$) in feed efficiency was observed in MN + VM-fed animals. Backfat thickness (5.6 ± 0.08 mm) and ribeye area (69.9 ± 0.53 cm^2) remained unaffected ($P \geq 0.74$), as well as the minimum (4.98 ± 0.047), mean (6.11 ± 0.037), and maximum ruminal pH (7.23 ± 0.033) values and the time (125 ± 22.3 min/d), area (57.67 ± 12.383 pH × h), and episodes (22 ± 3.8 bouts) of pH below 5.6 ($P \geq 0.12$). Overall, prevalence (24 ± 3.4%) and the number of liver abscesses (1.6 ± 0.14 abscesses/abscessed liver), liver scores (20 ± 3.1% of A− and 4 ± 1.8% of A livers), and prevalence (67 ± 3.5%) and degree of damage to the ruminal epithelium (2.5 ± 0.22% affected surface) were similar ($P \geq 0.18$) across treatments; however, the occurrence of ruminal lesions tended ($P \leq 0.07$) to be associated with that of liver abscesses and reduced ADG when feeding monensin alone.

Lay Summary

Dietary inclusion of significant proportions of rapidly degradable grains may represent a challenge for ruminal and liver health, thus reducing the growth performance of beef cattle. Monensin and virginiamycin have been shown to reduce or inhibit the growth of bacteria responsible for the development of ruminal acidosis and liver abscesses. The present study evaluated the effects of concurrently including monensin and virginiamycin in a dry-rolled corn-based diet on ruminal pH, the occurrence of ruminal lesions and liver abscesses, and the growth and development of feedlot-finished cattle in comparison with including each additive alone. Results indicated that the simultaneous inclusion of monensin and virginiamycin did not affect the growth and development of the animals or their carcass weight, though it reduced their dry matter intake. Thus, feed efficiency improvement was observed for body development. Treatments did not affect carcass characteristics, ruminal pH, and the occurrence of ruminal lesions and liver abscesses.

Key words: beef cattle performance, liver abscess, monensin, rumen health, virginiamycin

Abbreviations: ADG, average daily body weight gain; BW, body weight; DM, dry matter; DMI, dry matter intake; DOF, days on feed; HCW, hot carcass weight; LM, Longissimus dorsi muscle; MN, monensin-containing finishing diet; MN + VM, monensin and virginiamycin-containing finishing diet; VM, virginiamycin-containing finishing diet

INTRODUCTION

Feed additives are widely used in feedlot operations to reduce digestive problems and the occurrence of liver abscesses, as well as to enhance animal performance and reduce production costs. The ionophore monensin and the antibiotic virginiamycin both reduce or inhibit the growth

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of Gram-positive bacteria, such as the fiber-digesting *Ruminococcus* sp., the lactate producers *Streptococcus bovis* and *Lactobacillus* sp., and *Trueperella pyogenes*, the second most common organism in liver abscesses (Nagaraja et al., 1997; Nagaraja and Chengappa, 1998). Additionally, virginiamycin affects the Gram-negative *Fusobacterium necrophorum*, which has been recognized as the primary causative agent of liver abscesses (Nagaraja et al., 1997; Nagaraja and Chengappa, 1998; Amachawadi et al., 2017). Therefore, some of the effects of monensin and virginiamycin on ruminal fermentation and animal performance are similar and may include (Nagaraja et al., 1997) 1) reduced lactic acid ruminal concentration and consequently, reduced risk of ruminal acidosis, and rumen wall lesions; 2) reduced concentration of acetate and therefore, reduced H_2, CO_2 and formate production; 3) greater propionate concentration, possibly due to increased succinate concentration; the later would result from the fibrolytic activity of *Fibrobacter succinogenes* while the first may result from the metabolism of *Selenomonas* sp. and *Prevotella* sp. (Scheifinger and Wolin, 1973), all Gram-negative bacteria neither susceptible to virginiamycin (Nagaraja et al., 1997) nor monensin (Chen and Wolin, 1979; Russell and Strobel, 1989); 4) reduced CH_4 production, as a consequence of 2) and 3); 5) in the case of monensin, reduced dry matter intake (**DMI**), possibly related to the hypophagic effects of increased propionate ruminal concentration and its hepatic oxidation (Allen et al., 2009); 6) in the case of virginiamycin, reduced occurrence of liver abscesses.

Despite similar effects on ruminal fermentation and animal performance, monensin and virginiamycin modes of action differ (Nagaraja et al., 1997). Monensin disrupts membrane physiology and alters the normal internal ion concentration of susceptible cells, thus repartitioning energy away from growth-related functions and eventually resulting in cellular death (Booth, 1985; Russell, 1987; Russell and Strobel, 1989; Russell, 2002). Alternatively, virginiamycin interferes with protein synthesis by binding to the 50S ribosomal subunit, resulting in a bactericidal effect (Cocito, 1979).

Based on the hypothesis that there are benefits derived from the concurrent use of monensin and virginiamycin probably due to complementary modes of action, studies have been performed to evaluate the effect of different combinations of monensin and virginiamycin on ruminal fermentation or animal performance (Sitta, 2011; Lemos et al., 2016; Benatti et al., 2017). However, to our knowledge, no studies have compared the effect of a combination of monensin and virginiamycin, both included in the diet at recommended doses, with each of them fed alone on ruminal and hepatic health and cattle growth and development. Consequently, the objective of this study was to determine the potential beneficial effects of the combined use of monensin and virginiamycin at commercially suggested doses on ruminal health, occurrence, and microbiology of liver abscesses, and performance of feedlot-finished cattle.

MATERIALS AND METHODS

Animal care and handling procedures were approved by the INTA Institutional Animal Care and Use Committee (CICUAE#21-2020). The experiment was conducted at the General Villegas Experimental Station of INTA, Buenos Aires, Argentina (−34.866242, −62.781375).

Dietary Treatments, Cattle Handling, and Feeding Protocol

On d −21, 72 black and 72 red Angus grass-fed steers were weighed after being kept off feed and water for 16 h, allocated by body weight (**BW**) and coat color to 1 of 8 blocks, and group-housed in 24 open soil-surfaced pens (6 steers/pen; 360 m²/pen). Within each block, pens were randomly assigned to 1 of 3 corn-based diets (Table 1) containing 30 mg of monensin (**MN**), 25 mg of virginiamycin (**VM**), or 30 and 25 mg of monensin and virginiamycin (**MN + VM**), respectively, per kilogram of dry matter (**DM**). Diets were formulated to generate a rumen degradable protein balance equal to zero and to meet or exceed metabolizable protein requirements at expected ad libitum DMI and average daily body weight gain (**ADG**), according to Level 1 of the NASEM (2016) model.

Steers were stepped up to the final diets from d −21 to −1 by increasing dietary grain concentration (6 percent units [DM basis] every 4 days) and feed offer (0.5 kg/animal [as-fed basis] every 4 days) from 50% (DM basis) and 1.5% of BW, respectively. The additives monensin, virginiamycin, and a combination of both were fed at a daily rate of 250, 210, and 250 + 210 mg/animal from d −21 to −1. After that, additive intake rose from the combination of resulting DMI and the additive dietary inclusion described above. Animals were fed once daily, starting at 0900 h. During the experimental period (d 1 to 92), bunk scores (0 = licked; 1 = feed crumbs; 2 = more than crumbs) were recorded before feeding. Feed offer was increased by 0.5 kg/animal (as-fed basis) when the bunk scored 0 for two consecutive days, and no additional feed was added to the offer in the first occurrence of score 0 (i.e., the previous day). When the bunk

Table 1. Composition (dry matter basis) of diets containing monensin (MN), virginiamycin (VM), or both (MN + VM)

Item	Diet		
	MN	**VM**	**MN + VM**
Ingredient composition, %			
Dry-roled corn	73.76	73.76	73.75
Corn silage	18.32	18.32	18.33
Whole and raw soybean	4.00	3.99	3.99
Mineral–vitamin supplement[a]	0.92	0.92	0.92
Urea	1.00	1.00	1.00
Additive-containing supplement[b]	2.00	2.01	2.01
Chemical composition[c], %			
Crude protein	12.2	12.2	12.2
Neutral detergent fiber	17.7	17.7	17.7
Starch	62.5	62.5	62.5
Total digestible nutrients[d]	89.4	89.4	89.4

[a]Contained 25% of Ca and 5% of Na; 2,800, 1,800, 1,000, 48, 15, and 11 ppm of Zn, Mn, Cu, I, Co, and Se, respectively, and 220,000 and 44,000 IU/kg of vitamin A and D, respectively.
[b]Contained equal parts of rice bran and oystershell; additionally, it contained 1,483, 1,245, or 1,482 + 1,244 ppm of monensin, virginiamycin, or monensin + virginiamycin when included in diets MN, VM, or MN + VM, respectively.
[c]Calculated based on analyzed chemical composition of each feed ingredient (composite derived from 13 samples resulting from 1 sample collected per week).
[d]Assumed to be equal to in vitro dry matter digestibility, measured after a 30-h incubation.

scored 2, and the weight of the refusal was below 10% of the feed offered, the feed offer was determined as that of the day before minus half the weight of the refusal (visually estimated). In this case, refusal was kept in the bunk, and the amount of feed delivered was calculated as targeted feed offer minus refusal. Refusals greater than 10% were removed, weighed, and sampled immediately, and steers were offered the same amount of feed as the previous day. Otherwise, refusals were kept in the bunk and removed, weighed, and sampled once a week. Dry matter intake was calculated as the difference between total feed delivered and refused from d 1 to 92.

Based on the BW recorded on d −21, two animals per pen, whose BW were closest to the pen average BW, were chosen. On d −10, pH probes (SmaXtec, Graz, Austria) were inserted into those selected animals and set to record ruminal pH every 10 min. On d −1 and 92, animals were kept off feed and water for 16 h (from 1600 h to 0800 h the next day). To determine ADG, initial and final shrunk BW were recorded on d 1 and 93, respectively. On the same days, the Longissimus dorsi muscle (**LM**) area and backfat thickness were recorded by ultrasonography. The gain-to-feed ratio was calculated as the ratio between ADG and DMI.

Animals were kept on their corresponding dietary treatments until d 95 and then kept off feed and water until they were shipped to a commercial abattoir on d 96. Due to a staff shortage from a COVID-19 outbreak (August 2020) within the packing plant, the slaughter was deferred from d 97 to d 100. In the meantime, animals were refed and watered at the abattoir facilities. On d 100, the animals were humanely slaughtered, hot carcass weight (**HCW**) was recorded, and pH probes were recovered. All livers and rumens (drained of digesta and rinsed with water) were properly identified with the carcass number of the animal. Organs were placed in 200-L containers with ice-cold water and transported back to the experimental station in a refrigerated truck.

Data Collection and Analytical Methods

Diet composition. Feed ingredients were sampled weekly to determine DM content and adjust the daily feed offer. After completion of the experimental period, ingredient samples were ground, composited, and analyzed to calculate the chemical composition of the diets (Table 1). The crude protein content was determined by method #46-129 of the AACC (1995). The neutral detergent fiber content was determined using thermostable α-amylase, sodium sulfite, and a fiber analyzer (ANKOM200/220; ANKOM, Macedon, NY) as suggested by Goering and Van Soest (1970). Total starch content was determined enzymatically using method #996.10 of the AOAC (2005). The total digestible nutrient content was assumed to be equal to in vitro DM digestibility, measured after 30 h in an incubator (DaisyII; ANKOM, Macedon, NY), as proposed by Goering and Van Soest (1970).

Ruminal pH data. Minimum, mean, and maximum pH values were determined, and the time that ruminal pH remained below 5.6 was calculated for the entire experimental period. Based on the curve defined by ruminal pH (y-axis) and time in hours (x-axis), the area under a pH threshold of 5.6 was also calculated. Acidosis bouts were calculated as episodes during which ruminal pH remained below 5.6 for at least 180 consecutive minutes (Crossland et al., 2019).

Liver assessment. Number and size (large abscesses: >4 cm diameter; medium abscesses: 4 to 2 cm diameter, small abscesses: <2 cm diameter; scars were considered as small abscesses) of superficial abscesses were recorded on each liver. After that, all livers were sliced every 2.5 cm to record the number and size of internal abscesses. With this dataset, we determined scores for each abscessed liver, as Rezac et al. (2014) suggested. An A− was assigned to livers that displayed no more than two small abscesses, and A+ was assigned when at least one large abscess or more than four medium abscesses were observed. Otherwise, abscessed livers were scored as A. When possible, intact encapsulated abscesses were collected for bacterial isolation, as described by Amachawadi et al. (2017). Abscesses were kept refrigerated at 4 °C and analyzed within 24 h after collection.

Ruminal epithelium assessment. An anteroposterior incision was performed on the dorsal line of the rumen, from the cardia to the lower region of the dorsal sac. Rumens were initially examined on a table with the inner surface facing upward. A 20-quadrant plastic grid was then placed on the extended rumen and used to determine rumen size based on the number of quadrants occupied by the rumen. Each quadrant (16 × 16 cm) was further divided into sixty-four 2 × 2 cm squares to aid in quantifying the affected ruminal area. Within each quadrant, areas devoid of papillae or displaying short papillae (relative to normal), redness, tissue inflammation, petechiae, hyperkeratosis, ulceration, or other insult were visually quantified as the percentage of the total quadrant area. The total affected area was then calculated as $\frac{\sum_{i=1}^{n} Xi}{n}$, where Xi was the affected area (expressed as a percentage) in quadrant i and n was the number of quadrants occupied by the rumen. A similar procedure was followed on the external surface of the rumen.

Experimental Design and Statistical Analyses

The sample size was determined considering a one-tailed test, α and test power set at 5% and 80%, respectively, with a standard deviation of 0.16 for ruminal pH (variable of interest), and an expected difference in ruminal pH between treatment means of 0.20. Data were analyzed as a randomized complete block design with eight pen replications per treatment. All statistical analyses were performed with SAS software using the SAS Studio interface through SAS On Demand for Academics (SAS Institute Inc. 2021). Performance and ruminal pH data were analyzed using PROC MIXED. Correlations between pH data and ADG, the number of abscesses, and the degree of ruminal damage were analyzed using the CORR procedure. Associations between ruminal lesions (presence/absence) and liver abscesses (presence/absence) or between those and ADG (above/below average) were evaluated by a χ^2 test using PROC FREQ. Other variables derived from liver and rumen assessments were analyzed using PROC GLIMMIX.

Treatment effects were considered significant when *P*-values were less than or equal to 0.05 and were considered trends when *P*-values were between 0.05 and 0.10. In any of these cases, treatment means were separated using a *t*-test via the PDIFF option of the LSMEANS statement.

For pen-based variables (i.e., DMI, gain-to-feed, affected rumens or livers as a percentage of total rumens or livers, and liver scores), the statistical model included the effect of the pen within dietary treatment as the random error. Feed DMI was analyzed for the entire experimental period and weekly. For weekly analysis, repeated measures were considered, where time (days on feed, **DOF**) was used in the REPEATED statement, and the subject where measurements were repeatedly recorded was identified as the pen within the dietary treatment. Degrees of freedom were calculated, requesting the SATTERTHWAITE option of the model statement. Unstructured, first-order antedependence, homogeneous and heterogeneous first-order autoregressive, homogeneous and heterogeneous compound symmetry, homogeneous and heterogeneous Toeplitz, and spatial power matrices of variances, covariances, and correlations were requested using the TYPE option of the REPEATED statement. Based on Akaike's information criterion, a first-order antedependence matrix was finally chosen. For variables individually determined (i.e., BW, ADG, HCW, LM area, backfat thickness, ruminal pH-derived variables, number of abscesses per abscessed liver, and degree of damage per damaged rumen), the statistical model additionally included the effect of the animal within the pen and dietary treatment as the random error (St-Pierre, 2007). Due to unbalanced data, the KENWARD-ROGER degrees of freedom calculation was used to analyze the number of abscesses per abscessed liver and the degree of damage per damaged rumen. Backfat thickness and LM area recorded on d 1 were used as covariates in the analyses of the corresponding variables recorded on d 93. Mean ruminal pH was also analyzed on a daily basis, following the repeated measures procedure described for weekly DMI. In this case, the subject where measurements were repeatedly recorded was the animal within the pen and the dietary treatment; a spatial power matrix was chosen.

The significance of random effects (block and pen within treatment) was determined using a χ^2 test between the complete and the reduced models. When the null hypothesis was not rejected (calculated χ^2 value < critical χ^2 value), data were analyzed based on the reduced model.

RESULTS AND DISCUSSION

Performance and Carcass Characteristics

Final BW (468 ± 3.0 kg) was similar ($P = 0.52$) among dietary treatments due to similar ($P \geq 0.25$) initial BW (331 ± 1.6 kg) and ADG (1.49 ± 0.021 kg/d; Table 2). However, similar ADG and HCW (269 ± 1.7 kg, $P = 0.77$) were achieved with reduced ($P < 0.04$) DMI in steers fed the MN + VM diet compared with those fed any of the other diets containing monensin or virginiamycin alone. Decreased DMI in MN + VM-fed animals was observed during the entire finishing period (Treatment, $P = 0.05$; DOF, $P < 0.01$; Treatment × DOF, $P = 0.25$; Figure 1). Therefore, greater ($P < 0.01$) feed efficiency in terms of ADG was observed when feeding monensin and virginiamycin concurrently. Backfat thickness (5.6 ± 0.08 mm) and LM area (69.9 ± 0.53 cm^2) were not affected ($P \geq 0.74$) by dietary treatment. Lack of differences ($P \geq 0.48$) in animal growth and development between MN- or VM-fed animals observed in the present study agrees with results reported by Lemos et al. (2016), who evaluated the same doses as in the present study, though using a no roughage-containing diet. It is also consistent with results reported by Sitta (2011), who evaluated diets containing 30 or 17 mg/kg of DM of monensin or virginiamycin, respectively, fed to Nellore bulls. In contrast, increased ADG for VM-fed animals compared with MN-fed cattle has been reported by Gorocica and Tedeschi (2017a) and Tedeschi and Gorocica-Buenfil (2018) for European and U.S. commercial feedlots, respectively. In the meta-analyses conducted in those studies, virginiamycin dose ranged from 6.6 to 50 mg/kg of DM, and that for monensin ranged from 27.1 to 34 mg/kg of DM, compared with 25 and 30 mg/kg of DM of virginiamycin and monensin, respectively, evaluated in the present study.

Contrasting results have been reported in the literature regarding the effects of feeding monensin and virginiamycin to feedlot cattle concurrently. As in the present study, Benatti et al. (2017) observed reduced DMI, similar ADG, and increased gain-to-feed ratio in Nellore cattle when adding 30 mg of monensin per kilogram to a diet containing 25 mg/kg of DM of virginiamycin. Decreased DMI and similar ADG were observed when feeding a diet containing 30 and 15 mg/kg of DM of monensin and virginiamycin, respectively, compared with diets containing one additive or the other (Sitta, 2011). However, the gain-to-feed ratio was not statistically improved. dos Santos Silva et al. (2018) reported increased observed-net energy for BW gain when adding 25 mg of virginiamycin per kilogram to a diet that contained 30 mg/kg of DM of monensin, resulting from numerically

Table 2. Performance and carcass characteristics of feedlot-finished steers fed diets containing monensin (MN), virginiamycin (VM), or both (MN + VM)

Item	Treatment			SED	*P*-value
	MN	VM	MN + VM		
n	8	8	8	–	–
Initial BW, kg	332	331	330	2.4	0.71
Final BW, kg	467	465	471	5.3	0.52
DMI, kg/d	9.2[a]	9.2[a]	8.8[b]	0.19	0.04
ADG, kg/d	1.47	1.46	1.53	0.048	0.25
ADG:DMI	0.160[a]	0.159[a]	0.175[b]	0.0027	<0.01
HCW, kg	270	268	270	3.5	0.77
Backfat thickness, mm	5.6	5.5	5.6	0.21	0.74
LM area, square cm	70.0	69.8	69.9	1.15	0.99

BW, body weight recorded after the animals were kept off feed and water for 16 h; DMI, dry matter intake; ADG, average daily body weight gain; HCW, hot carcass weight; LM, Longissimus dorsi muscle.
[a,b]Means with uncommon superscripts differ ($P \leq 0.05$).

increased ADG and similar DMI. Based on a meta-analysis of data gathered from five Mexican feedlots, Gorocica and Tedeschi (2017b) concluded that ADG, gain-to-feed ratio, and HCW were greater for cattle fed 400 mg of monensin and 200 to 250 mg of virginiamycin per day than that of cattle fed 400 mg of monensin alone. Contrastingly, including 20 and 25 mg/kg of DM of monensin and virginiamycin, respectively, did not affect Nellore bulls' performance compared with that resulting from feeding 30 or 25 mg/kg of DM of monensin or virginiamycin alone, respectively (Lemos et al., 2016).

Liver and Rumen Assessments

Liver and rumen-derived variables were not affected ($P \geq 0.18$) by dietary treatment (Table 3). Overall, 24 ± 3.4% of livers presented at least one abscess, which falls within the prevalence range reported by Brown and Lawrence (2010), Castillo-Lopez et al. (2014), and Rezac et al. (2014). On average, no more than two abscesses (1.6 ± 0.14) were observed per abscessed liver. A maximum of four abscesses was observed in only one liver. Across treatments, 97% and 3% of observed abscesses were classified as small and medium, respectively, and no ruptured abscesses or A+ scores were observed. Taken together, the prevalence of A− livers was 20 ± 3.1% of total livers or 83% of abscessed livers. This result contrasts with Rezac et al. (2014), where A+ livers represented 4.6% of all livers, and only 26% of abscessed livers scored as A−. Similarly, Castillo-Lopez et al. (2014) reported a prevalence of A+ livers of 10.7% to 14.7% of all livers, which represented 50% to 60% of abscessed livers, though the latter figures might be overestimated since A− livers were merged and reported as normal (no abscesses). Differences in factors that relate to the extent and rate of organic matter digestion and, therefore, to ruminal pH, such as grain processing, amount of grain in the diet, grain type, and vitreousness, as well as DOF may explain differences between studies. At least one superficial abscess or scar was observed in 79% of abscessed livers, those being potentially condemned at the packing plant. However, 21% of abscessed livers showed internal abscesses only; these livers could have successfully passed the visual inspection at the plant.

Intact abscesses from 25 livers were recovered and analyzed for microbial isolation. Abscesses obtained from the same liver were considered as a single sample. Abscesses from 7, 7, and 11 livers corresponding to MN, VM, and MN + VM-fed animals were obtained (Table 4). Due to the reduced number of samples, no statistical analysis was performed. *Fusobacterium necrophorum* and *T. pyogenes* were isolated in 7 and 6 samples, respectively. *Escherichia coli* and *S. faecalis* were present in 3 liver abscess samples, each. Mixed infection of *F. necrophorum and S. faecalis* was observed in a sample from one MN-fed animal, while mixed infections of *E. coli* and *Enterobacter aerogenes*, *E. coli* and *Proteus miriabilis*, and *E. coli* and *Citrobacter intermedius* were observed in samples from MN + VM-fed animals. No bacteria were isolated in 2 samples. *Salmonella enterica* was absent from all livers.

Ruminal lesions were observed on the internal rumen wall of 67 ± 3.5% of cattle, which is greater than the 24.1% reported by Rezac et al. (2014) as mildly and severely affected rumens. This difference could probably be attributed to differences in the ruminal diagnosis procedure. While the ruminal assessment was performed on emptied rumens and at "chain speed" in a commercial slaughter plant in Rezac et al. (2014) study, a

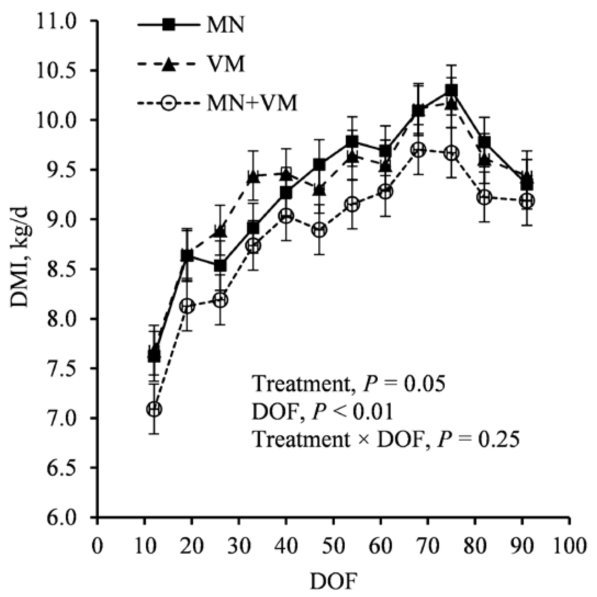

Figure 1. Daily dry matter intake (DMI) is affected by days on feed (DOF) in feedlot-finished steers fed diets (treatments) containing monensin (MN), virginiamycin (VM), or both (MN + VM). The smallest and largest SED between treatment means within each DOF were 0.19 and 0.33 kg/d, respectively; error bars indicate SEM.

Table 3. Liver abscesses and degree of damage to the internal ruminal wall in feedlot-finished steers fed diets containing monensin (MN), virginiamycin (VM), or both (MN + VM)

Item	Treatment			SED	*P*-value
	MN	VM	MN + VM		
n	8	8	8	–	–
Abscessed livers[a], % of total livers per treatment					
Total	21	21	30	7.6	0.43
A–	13	21	26	7.3	0.24
A	8	0	4	4.3	0.18
Abscesses per abscessed liver	1.8	1.5	1.6	0.25	0.70
Damaged rumens, % of total rumens per treatment	65	67	70	8.6	0.82
Damaged area in damaged rumens, % of the total internal ruminal surface	2.6	2.2	2.9	0.51	0.43

[a]*A*–, livers that displayed no more than two small abscesses (<2 cm diameter; scars were considered as small abscesses); *A*+, livers that displayed at least one large abscess (>4 cm diameter) or more than four medium abscesses (4–2 cm diameter); *A*, any other abscessed liver.

more time-consuming evaluation was performed on drained, water-rinsed, and fully opened rumens in the present one, thus possibly allowing for the recognition of more and smaller rumenitis-affected areas. Even though most rumens displayed some damage, the affected area was below 3% of the total internal surface (Table 3), with a maximum of 9% in 3 rumens. Lesions were usually located on the floor of the ventral sac. As ruminal lesions serve as prequels to the development of liver abscesses, a greater prevalence of ruminal lesions (67 ± 3.5%) than that of liver abscesses (24 ± 3.4%) was expected. No damage was observed on the external surface of the rumen, thus leading to the hypothesis that liver abscess-causing bacteria might have exited the rumen through microscopic lesions.

Associations between Rumen- and Liver-derived Variables and between those and ADG

Across treatments, 19% of cattle with normal rumens had abscessed livers (9 out of 47 animals), and among those that displayed some degree of ruminal damage, 26% had abscessed livers (25 out of 96 animals; Table 5). Even though these rates were not different ($P = 0.36$), they agree with those reported by Rezac et al. (2014). When studying the association within dietary treatments, the percentage of MN-fed animals with normal rumens but abscessed livers was 6% (1 out of 17), and the latter tended to increase ($P = 0.06$) almost five times (29%; 9 out of 31) for rumens that displayed some degree of damage. This result indicates that MN-fed animals with ruminal lesions tended to have more liver abscesses than animals with healthy rumens. In this regard, Jensen et al. (1954) reported that the percentage of cattle with liver abscesses increased from 23% to 41% when comparing cattle with normal and damaged rumens, respectively. No association was observed ($P \geq 0.62$) between the occurrence of ruminal lesions and liver abscesses for VM- or MN + VM-fed animals, which could possibly be related to the effects of virginiamycin on *F. necrophorum*, the most common bacteria in liver abscesses.

Similar to the results described above, no association was observed ($P \geq 0.26$) between the occurrence of ruminal lesions and ADG across treatments or when studying this association for VM- or MN + VM-fed animals (Table 6). However, the percentage of MN-fed animals with reduced ADG tended to be greater ($P = 0.07$) in animals with lesion-displaying rumens (68%; 21 out of 31 rumens) compared with those with normal rumens (41%; 7 out of 17 rumens). This result suggests that in MN-fed animals, reduced ADG tended to be associated with the occurrence of ruminal lesions. Thompson et al. (2008) reported a decrease of 46 to 60 g/d in ADG for cattle with a ruminal lesion or scar, while Rezac et al. (2014) reported a reduction of 30 g/d in ADG for animals displaying severe rumenitis, but no association was observed between mild rumenitis and reduced ADG. The occurrence of liver abscesses was not associated with a change in ADG ($P \geq 0.40$; Table 7). In that regard, Rezac et al. (2014) indicated that cattle with severely abscessed livers (A+) gained 100 g/d less than cattle with normal livers; however, no performance loss was observed in cattle with livers classified as A– or A, such as the ones identified in the present study.

Table 4. Number of feedlot-finished steers fed diets containing monensin (MN), virginiamycin (VM), or both (MN + VM), whose livers displayed abscesses that resulted positive for diverse bacterial species

Item	Treatment		
	MN (7)	VM (7)	MN + VM (11)
Bacterial species			
Fusobacterium necrophorum	2	1	4
Escherichia coli	2	0	1
Trueperella pyogenes	2	3	1
Streptococcus faecalis	0	1	2
Mixed infection			
F. necrophorum + *S. faecalis*	1	0	0
E. coli + *Enterobacter aerogenes*	0	0	1
E. coli + *Proteus miriabilis*	0	0	1
E. coli + *Citrobacter intermedius*	0	0	1

Values in parentheses are number of samples cultured; abscesses obtained from the same liver were considered as a single sample.

Table 5. Number of feedlot-finished steers fed diets containing containing monensin (MN), virginiamycin (VM), or both (MN + VM) with or without ruminal lesions or liver abscesses

Treatment	Ruminal lesions	Liver abscesses		Total	*P*-value
		Without	With		
All treatments	Without	38	9	47	0.36
	With	71	25	96	
	Total	109	34		
MN alone	Without	16	1	17	0.06
	With	22	9	31	
	Total	38	10		
VM alone	Without	12	4	16	0.62
	With	26	6	32	
	Total	38	10		
MN + VM alone	Without	10	4	14	0.91
	With	23	10	33	
	Total	33	14		

Ruminal pH

Ruminal pH-derived variables were not affected ($P \geq 0.12$) by dietary treatment (Table 8), which agrees with results reported by Lemos et al. (2016), who evaluated the inclusion of 30, 25, and 30 + 25 mg/kg of DM of monensin, virginiamycin, and both, respectively, in whole corn based-diets fed to cannulated zebu steers.

Across treatments, minimum, mean, and maximum pH values were 4.98 ± 0.047, 6.11 ± 0.037, and 7.23 ± 0.033, respectively. Mean ruminal pH was similar to that reported by Castillo et al. (2014) for a finishing phase (6.11), though the range of variation was narrower in the latter (5.49 and 6.89 for minimum and maximum pH, respectively). It may partially explain the differences between studies when feeding the animals once a day, as in the present experiment, or twice a day, as in Castillo et al. (2014). In that regard, Robles et al. (2007) observed a smaller range of ruminal pH values when heifers were fed a concentrate diet twice compared with once a day. Ruminal pH remained below 5.6 for 125 ± 22.3 min/d and animals experienced an average of 22 ± 3.8 acidosis bouts during the entire feeding period, results that agree with those reported by Castillo et al. (2014; pH < 5.5 for 136 min/d and 21.6 acidosis episodes). None of the ruminal pH variables was directly correlated with ADG, degree of ruminal damage, or the number of liver abscesses ($P \geq 0.12$). Finally, there was an effect ($P < 0.01$) of DOF and a tendency ($P = 0.08$) for an interaction between treatment and DOF on mean ruminal pH (Figure 2). Confirming the results reported in Table 8, there was no effect ($P = 0.46$) of treatment on mean ruminal pH, which stayed in the lower portion of the figure in the case of MN-fed animals, whereas that for the VM-fed ones stayed in the upper part of the figure, and that for MN + VM in between them.

Results from this study indicate that the simultaneous inclusion of monensin and virginiamycin, both at recommended doses, in a dry-rolled corn-based diet, resulted in a 4.3% decrease in DMI and similar ADG, thus improving 9.4% gain-to-feed ratio compared with feeding diets containing one additive or the other. Despite reduced

Table 6. Number of feedlot-finished steers fed diets containing containing monensin (MN), virginiamycin (VM), or both (MN + VM) with or without ruminal lesions or whose average daily body weight gain (ADG) was below or above average

Treatment	Ruminal lesions	ADG		Total	*P*-value
		Below average	Above average		
All treatments	Without	26	21	47	0.80
	With	51	45	96	
	Total	77	66		
MN alone	Without	7	10	17	0.07
	With	21	10	31	
	Total	28	20		
VM alone	Without	11	5	16	0.30
	With	17	15	32	
	Total	28	20		
MN + VM alone	Without	8	6	14	0.26
	With	13	20	33	
	Total	21	26		

Table 7. Number of feedlot-finished steers fed diets containing monensin (MN), virginiamycin (VM), or both (MN + VM) with or without liver abscesses or whose average daily body weight gain (ADG) was below or above average

Treatment	Liver abscesses	ADG		Total	*P*-value
		Below average	Above average		
All treatments	Without	59	50	109	0.90
	With	18	16	34	
	Total	77	66		
MN alone	Without	22	16	38	0.90
	With	6	4	10	
	Total	28	20		
VM alone	Without	21	17	38	0.40
	With	7	3	10	
	Total	28	20		
MN + VM alone	Without	16	17	33	0.42
	With	5	9	14	
	Total	21	26		

Table 8. Ruminal pH in feedlot-finished steers fed diets containing monensin (MN), virginiamycin (VM), or both (MN + VM)

Item	Treatment			SED	*P*-value
	MN	VM	MN + VM		
Minimum	4.93	5.04	4.97	0.103	0.56
Mean	6.07	6.18	6.07	0.085	0.33
Maximum	7.16	7.31	7.20	0.077	0.12
Time pH < 5.6, min/d	162	118	83	57.8	0.42
Area pH < 5.6[a], pH × h	60.07	49.29	64.04	27.137	0.86
Episodes pH < 5.6[b], bouts	25	18	25	8.2	0.63

[a]Calculated based on the curve defined by ruminal pH (y-axis) and time (min; x-axis)
[b]Number of episodes in the entire feeding period during which pH remained below 5.6 for at least 180 consecutive minutes.

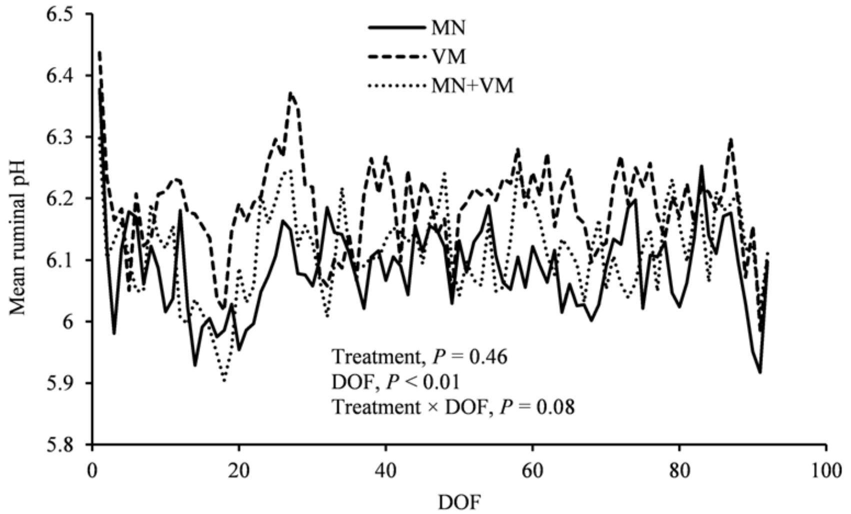

Figure 2. Daily mean ruminal pH is affected by days on feed (DOF) in feedlot-finished steers fed diets (treatments) containing monensin (MN), virginiamycin (VM), or both (MN + VM). Within each DOF, SEM, and SED between treatment means were 0.10 and 0.13, respectively.

DMI when both additives were concurrently fed, HCW resulted similar among treatments. Carcass characteristics remained unaffected, as well as ruminal pH variables. Overall, treatments did not affect liver abscesses and ruminal lesions; however, ruminal lesions tended to be associated with liver abscesses and reduced ADG when feeding monensin alone.

The 67% and 24% of ruminal lesions and liver abscesses indicate room for animal health and welfare improvement, even when feeding additive-containing diets. However, compared with other studies, the severity of damage to the ruminal epithelium (average <3% and maximum of 9% affected area in 3 rumens) and the number of liver abscesses (average <2 and maximum of 4 abscesses in 1 liver) in the present one would be considered small. No benefits on rumen or liver health were observed to support the use of one additive over the other or the combination when feeding a dry-rolled corn-based diet, whereby positive effects of the combination rest on improved gain-to-feed ratio.

SUPPLEMENTARY DATA

Supplementary data are available at *Translational Animal Frontiers* online.

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CONFLICT OF INTEREST STATEMENT

The authors declare no real or perceived conflicts of interest.

SOFTWARE AND DATA REPOSITORY RESOURCES

This research is under INTA Digital Repository regulations.

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