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Substitution of virginiamycin with yeast-based products (*Saccharomyces cerevisiae*) in diets of cattle: digestibility and ruminal parameters

Substituição de virginiamicina por produtos a base de levadura (*Saccharomyces cerevisiae*) em dietas de bovinos: Digestibilidade e parâmetros ruminais

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Abstract: The aim of this study was to evaluate the effect of substituting virginiamycin with yeast-based products in high-energy diets containing monensin on the nutrient digestibility and ruminal parameters of cattle. Five crossbred Nellore × Holstein steers (initial body weight 281 kg ± 9.2 kg) were allotted to a 5 × 5 Latin square design. The diets were composed in dry matter basis of corn silage (355 g kg^{-1}), and concentrate composed of ground corn, soybean meal, soybean hulls,mineral mix, limestone, salt and urea (645 g kg^{-1}). The treatments were: 1 - control, 18 mg kg^{-1} dry matter (DM) virginiamycin (VM); 2 - enriched yeast culture (*Saccharomyces cerevisiae*) at 7 g animal day^{-1} (EYC 7); 3 - enriched yeast culture (*S. cerevisiae*) at 14 g animal day^{-1} (EYC 14); 4 - autolyzed yeast (*S. cerevisiae*) at 7 g animal day^{-1} (AY 7); and 5 - autolyzed yeast (*S. cerevisiae*) at 14 g animal day^{-1} (AY 14). Monensin (18 mg kg^{-1} DM) was added to the mineral mix in all treatments. No significant differences ($P > 0.05$) were found for DM intake (mean 8.36 kg DM), organic matter (OM) digestibility (average of 58.39%), or mean ruminal pH (mean 6.75) among treatments. Moreover, the treatment did not have a significant effect on the short-chain fatty acid or ammonia nitrogen concentrations. These results indicated the possibility of replacing virginiamycin with yeast-based products at the lowest level (7 g of enriched yeast culture or autolyzed yeast per animal day^{-1}) in high-energy diets containing monensin.

Keywords: antibiotics, cattle, enriched yeast culture, autolyzed yeast

Resumo: O objetivo do estudo foi avaliar a substituição de virginiamicina por produtos à base de levedura em dietas de alto grão contendo monensina, sobre a digestibilidade dos nutrientes e parâmetros ruminais. Cinco novilhos cruzados Nelore x Holandês (peso inicial 281 kg ± 9,2 kg) distribuídos em um delineamento Quadrado Latino 5 x 5. As dietas foram compostas por silagem de milho (355 g kg^{-1}) e concentrado composto por: milho moído, farelo de soja, casca de soja, mistura mineral, calcário, sal e

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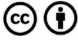
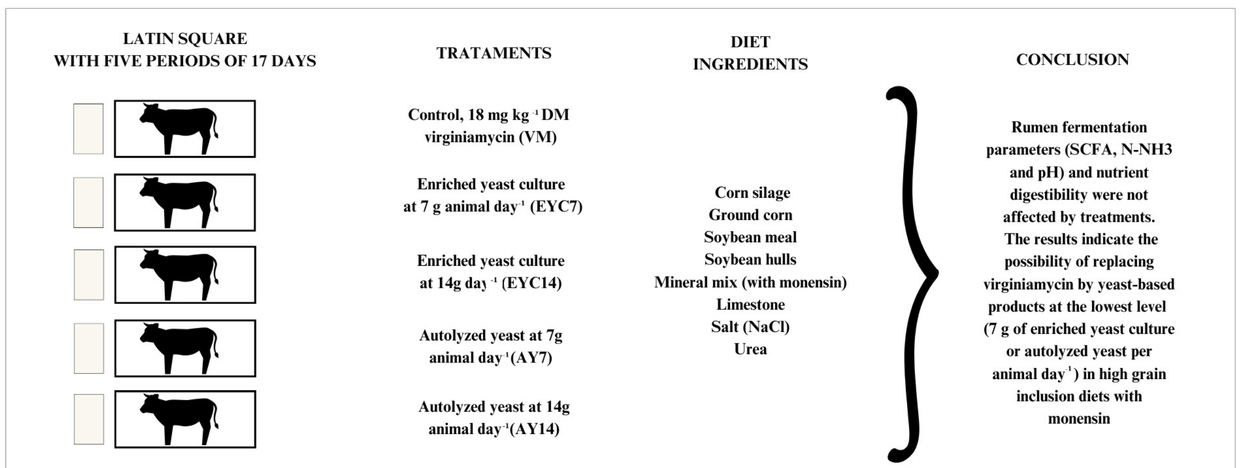


ureia (645 g kg-1). Os tratamentos foram: 1. Controle, 18 mg kg-1 MS de virginiamicina (VM); 2. Cultura de levedura enriquecida (*Saccharomyces cerevisiae*) 7g animal dia-1 (CLE 7); 3. Cultura de levedura enriquecida (*Saccharomyces cerevisiae*) 14g animal dia-1 (CLE 14); 4. Levedura autolisada (*Saccharomyces cerevisiae*) 7g animal dia-1 (LA 7) e Levedura autolisada (*Saccharomyces cerevisiae*) 14g animal dia-1 (LA 14). A monensina (18 mg kg-1 MS) estava presente na mistura mineral vitamínica fornecida em todos tratamentos. Não houve diferença significativa (P>0,05) para o consumo de matéria seca (média 8,36 kg MS); digestibilidade da matéria orgânica (média 58,39%) e média de pH ruminal (média 6,75). Além disso, não foram encontrados efeitos significativos de tratamentos para concentração de ácidos graxos de cadeia curta e nitrogênio amoniacal. Estes resultados indicam a possibilidade de substituir a virginiamicina por produtos à base de levedura nos menores níveis (7g animal dia-1 de cultura de levedura enriquecida ou 7g animal dia-1 de levedura autolisada) em dietas de alto grão com monensina.

Palavras-chave: antibiótico, bovinos, cultura de levedura enriquecida, levedura autolisada



Graphical abstract - Replacement of virginiamycin with an enriched yeast culture or autolyzed yeast (*Saccharomyces cerevisiae*) in cattle diets: digestibility and ruminal parameters

1. Introduction

The risk of ruminal acidosis in cattle increases with high-concentrate diets because of rapid fermentation in the rumen, which yields large amounts of acids, decreases ruminal pH, and may impair proper functioning [(1)]. Monensin is frequently used to modulate rumen fermentation, reduce the incidence of metabolic disturbances such as ruminal acidosis, and improve feed efficiency [(2, 3)].

Similar to monensin, virginiamycin has been reported to act on gram-positive bacteria in the rumen [(4, 5)] but with different mechanisms of action [(6)]. With the ability to penetrate the cell wall of gram-positive bacteria, Virginiamycin reaches its interior, and then the M and S factors bind to the ribosomes irreversibly, thus preventing protein synthesis and cell multiplication, eventually causing the death of the gram-positive bacteria [(7)]. In contrast, monensin acts on the cell membrane of microorganisms, affecting the ionic flow of the membrane and disrupting the gradient of protons and cations responsible for the input of nutrients into the cell [(8)]. Some studies have shown that monensin and virginiamycin can improve feed efficiency in feedlot beef steers [(9, 10)] and increase milk production in high-yield cows [(11)]. This association may have an additive or complementary effect [(11)]. However, the need to reduce the use of antibiotics in animal feed has fostered the development of natural products to replace virginiamycin.

Yeast-based products, such as enriched yeast culture (EYC) and autolyzed yeast (AY), are promising alternatives to commonly used antimicrobials. A yeast culture (YC) is a

combination of yeast biomass and metabolites produced during a specific fermentation process [12]. Although YC contains yeast cells, their viability is either zero or very low [12]. Thus, the physiological effects of YC products are not dependent on live yeast cell viability [13]. Yeast culture drying preserves metabolites, such as peptides, proteins, nucleotides, and B complex vitamins [14, 15], stimulating the growth of ruminal bacteria [15], which are considered prebiotics [16] as well as autolyzed yeasts [17].

Lysed yeasts are yeast cells whose cell walls are ruptured into very small fragments [12]. The smaller the yeast cell wall fragment, the better the immunological stimulation, mycotoxin adsorption, pathogen-binding capacity to bacterial fimbriae, and cell wall carbohydrate availability for beneficial bacterial growth in the intestine [18, 13]. Furthermore, cell wall rupture increases the surface contact area of oligosaccharides and polysaccharides, amplifying their action in the digestive tract of fed animals. Consequently, cytoplasmic content is highly available to rumen microbes that affect rumen fermentation [19, 20].

Previous studies have shown that the benefits of using yeast-based products, including increased dry matter (DM) intake [21], increased concentration of short-chain fatty acids [22], improved rumen pH stability [23, 24], and improved digestibility of organic matter and dietary fiber [25], are similar to that of virginiamycin. These similarities could allow the replacement of virginiamycin with yeast-based products. However, the ruminal effects of the association between yeast-based products and monensin remain unknown. We hypothesized that combining enriched yeast culture or autolyzed yeast and monensin would have similar effects on diet digestibility and ruminal parameters as the combination of virginiamycin and monensin.

Therefore, the aim of this study was to evaluate diet digestibility and ruminal parameters of steers fed different levels of autolyzed yeast (CULTRON PRO) or enriched yeast culture (CULTRON X) as substitutes for virginiamycin in a high-concentrate diet containing monensin.

2. Material and Methods

All the experimental procedures involving animals were approved by the Ethics Committee on Animal Use (protocol no. 055/18). The study was conducted in Goiânia, Goiás, Brazil (16°36’15.621”S; 49°16’51.790”W; 733 m altitude).

Five rumen-fistulated crossbred Nellore × Holstein steers with an initial body weight of 281 kg ± 9.2 kg were allotted to a 5 × 5 Latin square design. The treatments were as follows: 1 - control, 18 mg kg^{-1} DM virginiamycin (VM); 2 - enriched yeast culture (*Saccharomyces cerevisiae*) at 7 g animal day^{-1} (EYC 7); 3 - enriched yeast culture (*S. cerevisiae*) at 14 g animal day^{-1} (EYC 14); 4 - autolyzed yeast (*S. cerevisiae*) at 7 g animal day^{-1} (AY 7); and 5 - autolyzed yeast (*S. cerevisiae*) at 14 g animal day^{-1} (AY 14). Monensin (Elanco, Greenfield, IN, USA) was added to the vitamin-mineral mix of all treatments in accordance with the range suggested by the Rumensin Technical Guide (15–20 mg kg^{-1} DM) [26]. Virginiamycin -Vmax 50% (Phibro, Ridgefield Park, NJ, USA) was weighed separately according to the amount of feed provided. The dosages used for both the yeast and antibiotic products were based on the manufacturer’s recommendations.

The animals were individually housed in stalls covered with concrete floors equipped with automatic feeders and water troughs. All steers received the same diet, with a forage: concentrate ratio of 35.5:64.5. The diets were composed of corn silage formulated with ground corn, soybean meal, soybean hulls, vitamin-mineral mix, urea, and monensin (Table 1) with yeast-based products or virginiamycin added according to the treatment.

Table 1 Ingredients and chemical composition of the diet.

Ingredients (g kg $^{-1}$ DM)[1]	
Corn silage	355.00
Ground corn	361.30
Soybean meal	152.00
Soybean hulls	105.00
Mineral mix[2]	16.90
Limestone	4.20
Salt (NaCl)	2.80
Urea	2.80
Chemical composition of the diet (g kg $^{-1}$ DM)	
Dry matter	480.80
Crude protein	135.50
Neutral detergent fiber	398.40
Organic matter	937.50
Ether extract	24.80
Non-fibrous carbohydrates	378.80

[1]g kg^{-1} DM: grams per kilogram as fed (dry matter). [2]Guaranteed analysis (per kg): Calcium (min) 21%; Phosphorus: 5%; Vitamin A: 250,000 IU kg^{-1}; Vitamin D3: 50,000 IU kg^{-1}; Vitamin E: 2,000 IU kg^{-1}; Sodium: 8%; Magnesium: 1.6%; Sulfur: 1.30%; Manganese: 3,200 mg kg^{-1}; Zinc: 4,000 mg kg^{-1}; Cobalt: 23 mg kg^{-1}; Iodine: 50 mg kg^{-1}; Selenium: 27 mg kg^{-1}; Monensin: 1,080 mg kg^{-1}; BHT: 100 mg kg^{-1}.

The enriched yeast culture or autolyzed yeast was mixed with the concentrate according to the treatment and provided individually to each animal daily. Additionally, virginiamycin was added to the concentrate and administered daily to each animal. The amount of virginiamycin was calculated based on the dry matter intake (DMI) of the previous day, such that the additive corresponded to 18 mg kg^{-1} DM. Monensin (18 mg kg^{-1} DM) was added to the vitamin-mineral mix in all treatments.

The enriched yeast culture - CULTRON X (Aleris Animal Nutrition, Jundiaí, São Paulo, Brazil) used in this study was enriched with a thicker cell wall from autolyzed yeast cells and flavoring agents. Yeast culture (*S. cerevisiae*) was obtained from a corn ethanol production process, using sugar cane molasses and ethanol yeast cream to control yeast biomass growth and generate a high concentration of yeast metabolites. Yeast culture metabolites included amino acids, B-complex vitamins, enzymes, and organic acids, which are important sources of energy, carbon, and nitrogen for rumen bacteria.

Autolyzed yeast - CULTRON PRO (Aleris Animal Nutrition, Jundiaí, São Paulo, Brazil) was obtained from sugarcane fermentation for ethanol production. This product is characterized

by a higher proportion of MOS and B-glucans available through autolysis fractionation of the cell wall compared to most lysed yeasts. The yeast cells are subjected to physical or chemical stress during autolysis. This promotes cell wall thickening, followed by its disruption promoted by endogenous enzymes, thereby releasing the cytoplasmic content and increasing the surface exposure of cell wall components (MOS and β-glucans).

The experimental period lasted 85 days and was divided into five periods of 17 days each (11 days for adaptation and 6 days for data collection) [(27)]. The diets were provided daily at 07h30, which was the reference time for rumen fluid collection. The orts was weighed in advance to adjust the diet. The experimental diets were formulated in accordance with BCNRM [(28)] to provide a weight gain of 1.13 kg d^{-1}.

Feed samples were collected from the 12th to 15th days, whereas orts samples were collected from the 13th to 16th days of each experimental period. The daily samples were mixed to produce composite samples for each treatment during each period.

Fecal samples (200 g) were taken directly from the rectum of the animals four times daily (08h30, 12h00, 16h00, and 18h00) from the 13th to 16th day of each experimental period. The samples were mixed to produce a composite fecal sample for each treatment during each period and frozen at -20°C for subsequent chemical analysis.

After thawing, samples were oven-dried at 55°C and ground in a Wiley mill to pass through a 1 mm sieve for analysis of DM, crude protein (CP), neutral detergent fiber (NDF), ether extract (EE), and ash, as previously described methodology Detmann [(29)].

Fecal excretion was estimated using indigestible NDF fraction (iNDF) as an internal marker. The samples were ground to pass through a 2 mm sieve, transferred to non-woven textile bags (100 g m^2), and incubated for 288 hours in rumen-cannulated animals, as previously described by Detmann [(29)].

Apparent nutrient digestibility (ApND) was calculated as the difference between the amount ingested and the amount excreted in feces, according to the equation of Silva and Leão (30), where NC is the nutrient concentration in the diet or feces:

$$\text{ApND} = \frac{(\text{Intake NC})\ \ (\text{Excretion NC})}{\text{Intake} * \text{NC}} * 100 \quad (1)$$

Fecal output (FO) was estimated using the equation proposed by Ferreira et al. [(31)]:

$$\text{FO (dry-basis)} = \frac{\text{Marker intake}\ (\frac{\text{kg}}{\text{day}})}{\text{Marker concentration in the feces (\%)}} \quad (2)$$

Samples of ruminal fluid were collected from four different regions of the rumen before feeding time (0) and 2, 4, 8, and 12 hours after feeding on days 16th and 17th day of each experimental period to measure the pH and estimate the concentrations of ammonia-nitrogen (N-NH3) and short-chain fatty acids (SCFA). The rumen fluid was filtered through a cotton cloth to obtain a 250 mL sample. The pH of the rumen fluid was measured immediately after sampling using a digital pH meter (PG 1800, Gehara, Ltd., Goiânia, Brazil).

A 50 mL aliquot of rumen fluid was acidified with 1.0 mL of 50% sulfuric acid and then frozen for subsequent N-NH3 analysis. The concentration of N-NH3 was measured using the colorimetric method described by Detmann [29].

A 40 mL aliquot of rumen fluid was acidified with 10 mL of 25% metaphosphoric acid and then frozen for SCFA analysis. After thawing, a subsample of 2 mL of rumen fluid was collected, transferred to a tube, and centrifuged at 15,000 × *g* for 15 min at 4°C. A 0.8 mL sample of centrifuged rumen fluid was transferred to vials to which 0.4 mL of a 3:1 solution of metaphosphoric acid:formic acid was added as a preservative, and 0.2 mL of 2-ethylbutyric acid solution was added as internal standard. The vials were then vortexed and refrigerated until the SCFA content was determined [32]. SCFA levels were determined using a gas chromatograph (GC 2014, Shimadzu Corporation, Tokyo, Japan) equipped with a flame ionization detector and fitted with a polyethylene glycol capillary column (RESTEK, Bellfonte, PA, USA; 30 m length, 320 μm internal diameter, 0.25 μm cyanopropyl polysiloxane). Data were collected using CG Solution Analysis software v. 2.42.00. The samples were run through three consecutive heating cycles: 80°C (1 min), 120°C (20°C min^{-1} and maintained for 3 min), and 205°C (10°C min^{-1} and maintained for 2 min). Helium was used as a carrier gas at a flow rate of 1.0 mL min^{-1}, and the temperature of the injector and detector was 260°C. Helium was used as the carrier gas at a flow rate of 30 mL min^{-1}.

Digestibility-related variables were analyzed in a 5 × 5 Latin square design according to the following model:

$$Yijk = m + ti + Lj + Ck + eijk \quad (3)$$

where Yijk is the observed response for treatments i, animal j and period k, m is the overall mean, ti is the fixed effect of treatment, Lj is the fixed effect of the animal, Ck is the fixed effect of the period, and eijk is the random error associated with the treatment, period and animals.

Variables associated with pH, ammonia-nitrogen concentration, and SCFAs were analyzed in a 5 × 5 Latin square design with repeated measures using mixed models according to the following model:

$$Yijkl = \mu + ti + aj + \delta ij + pk + tel + (tte)\, il + \varepsilon\, ijk \quad (4)$$

where, Yijkl: value observed in treatment i, animal j, period k and time l; μ: overall average of the experiment; ti: fixed effect of treatment i; aj: fixed effect of animal j; δij: random effect of experimental unit j in treatment i; pk : fixed effect of period k; tel: effect of evaluation time l; (tte) il: fixed effect of the interaction between treatment i and assessment time l; ε ijk: random error associated with treatment i, animal j, period k and evaluation time l. Data were tested for the best covariance structure using the lowest-corrected Akaike information criterion. Analysis of variance and Tukey's tests were performed using a 5 × 5 Latin squared design. R software (R Core Team, 2021) and the R package easyanova were used for statistical analysis.

3. Results and Discussion

Dry matter intake (DMI), DM digestibility, and nutrient digestibility were not significantly different among treatments (P > 0.05, Table 2).

Table 2 Average intake and apparent digestibility of dietary components.

Dietary components[2]	Treatments[1]						
	VM	EYC 7	EYC 14	AY 7	AY 14	SEM[3]	P-value[4]
			Intake (kg day^{-1})				
DM	8.22	8.45	8.33	8.51	8.27	0.31	0.96
CP	1.10	1.13	1.11	1.17	1.10	0.04	0.79
NDF	3.34	3.43	3.41	3.27	3.38	0.13	0.90
OM	7.71	7.93	7.81	7.97	7.78	0.29	0.96
EE	0.20	0.21	0.20	0.22	0.21	0.01	0.55
NFC	3.07	3.16	3.09	3.31	3.10	0.17	0.86
			Total apparent digestibility (%)				
DM	61.41	53.79	57.46	54.45	55.83	3.09	0.46
CP	52.75	42.26	49.32	45.76	45.84	3.63	0.36
NDF	61.68	55.28	56.19	49.89	55.49	3.86	0.37
OM	62.85	56.06	59.09	56.4	57.79	3.01	0.53
EE	68.29	66.03	63.9	69.42	66.31	3.35	0.80
NFC	67.22	60.06	65.31	65.62	63.82	3.14	0.58

*[1]Treatments: 1 - control, 18 mg kg^{-1} DM virginiamycin (VM); 2 - enriched yeast culture (**Saccharomyces cerevisiae**) at 7 g animal day^{-1} (EYC 7); 3 - enriched yeast culture (**S. cerevisiae**) at 14 g animal day^{-1} (EYC 14); 4 - autolyzed yeast (**S. cerevisiae**) at 7 g animal day^{-1} (AY 7); and 5 - autolyzed yeast (**S. cerevisiae**) at 14 g animal day^{-1} (AY 14). [2]Dietary components: DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; OM, organic matter; EE, ether extract; NFC, non-fibrous carbohydrates. [3]SEM: standard error of the mean. [4]P-value: Means for significant effects were compared using Tukey's test (P< 0.05).*

The results indicated that enriched yeast culture and autolyzed yeast, tested at two levels, did not change the DMI or digestibility compared with that of diet containing virginiamycin and monensin. In a previous study, hydrolyzed yeast increased DM digestibility (P = 0,0503), although it had no effect on DMI in feedlot diets [33]. In addition, Shen et al. [25] detected no difference in total tract DM digestibility in beef heifers fed yeast culture compared to the control (no feed additive) and antibiotic (monensin plus tylosin) treatments, although yeast culture-fed animals showed greater total tract NDF digestibility.

The absence of differences in NDF digestibility among treatments (P > 0.05) indicated that both enriched yeast culture and autolyzed yeast at the lowest levels, combined with monensin, were satisfactory compared to the monensin–virginiamycin combination. This result was consistent with the similarity in DMI among the treatments. However, previous studies have reported that yeast-based products contain compounds capable of stimulating the ruminal microbiome, especially fibrolytic microorganisms [34, 21], favoring fiber digestibility efficiency [35, 36]. This is in contrast to ionophore and non-ionophore antibiotic additives, which can negatively affect the growth of gram-positive bacteria, including fiber-digesting bacteria [8, 25, 37].

The similarities in NDF digestibility among the treatments suggested that monensin associated with enriched yeast cultures or autolyzed yeast could inhibited the growth of fiber-digesting microorganisms. Future studies on the rumen microbiome could help elucidate the influence of feeding yeast-based products in combination with monensin on fiber-digesting bacterial abundance.

There is no consensus in the literature on how yeast-based products influence DMI. According to Yuan et al. [38], yeast culture does not affect DMI, and the authors reported that DMI was modulated by decreasing meal size, duration, and intervals between meals, leading to increased meal frequency. In contrast, Wagner et al. [39] detected a DMI increase when yeast culture was fed to feedlot steers using a meta-analysis study. Yeast-based products increase the availability of nutrients for the rumen microbiome through autolysis (autolyzed yeast) or metabolite concentrations (yeast culture), thereby fostering the activity of ruminal microorganisms [16, 21], which could explain the positive effects of DMI.

The main effects of monensin in cattle diets are a decrease of DMI and improved feeding efficiency [2, 37]. Similarly, Navarrate et al. [40] observed increased DMI and improved feed efficiency when virginiamycin was used compared with the control treatment. Erasmus et al. [41] demonstrated a positive association between monensin and yeast culture, which alleviated the intake-suppressive effect of monensin and demonstrated a complementary effect. Rigueiro et al.[42] observed a higher DMI of virginiamycin diets compared to monensin diets, and the association of both antibiotics.

There was no significant difference (P > 0.05) among treatments in the mean rumen pH at different times throughout the day (Figure 1). The mean rumen pH was approximately 7.0 at time 0, regardless of treatment. The pH decreased similarly throughout the day across treatments, remaining between 6.3 and 7.2 (P > 0.05), which is adequate for the survival and multiplication of most ruminal microorganisms [1].

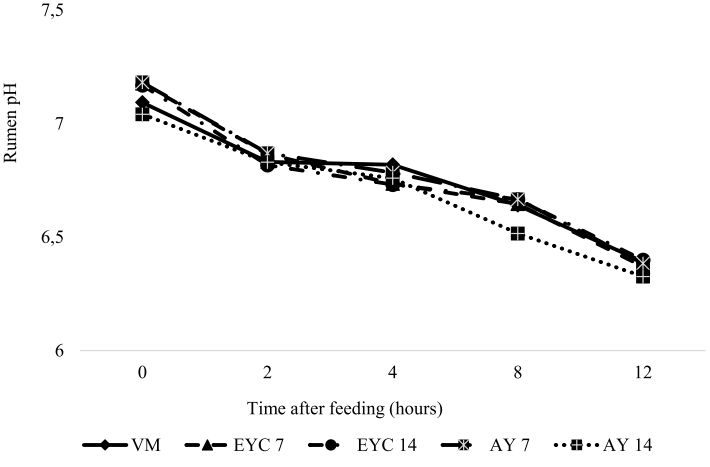


Figure 1 Mean rumen pH levels at different sampling times. 1 - control, 18 mg kg^{-1} DM virginiamycin (VM); 2 - enriched yeast culture (*Saccharomyces cerevisiae*) at 7 g animal day^{-1} (EYC 7); 3 - enriched yeast culture (*S. cerevisiae*) at 14 g animal day^{-1} (EYC 14); 4 - autolyzed yeast (*S. cerevisiae*) at 7 g animal day^{-1} (AY 7); and 5 - autolyzed yeast (*S. cerevisiae*) at 14 g animal day^{-1} (AY 14). Treatment: P= 0.84; interaction (treatment*time): P= 0.99.

The results showed that different levels of enriched yeast culture and autolyzed yeast associated with monensin did not affect rumen pH throughout the day when compared with the combination of monensin and virginiamycin. This was a positive result, as ruminal pH below 6.0 hinders the growth of cellulolytic bacteria, resulting in a reduction in the fiber digestion rate and increased lag time, impairing cell wall degradation [43]. The fact that ruminal fluid pH was higher than 6.2 throughout the day in all treatments explained the lack of difference in NDF digestibility among treatments. High-concentrate diets accelerate short-chain fatty acid production by fermenting rumen bacteria, resulting in a pH below 6.0 [24, 44].

The stabilization of ruminal pH when using yeast culture has been associated with inherent stimulatory factors such as organic acids, B-complex vitamins, and amino acids [25], which promote the growth of *Selenomonas ruminantium* and *Megasphera elsdenii*, which are lactic acid-consuming bacteria [39]. In addition, it has been suggested that yeast culture fosters the development of ruminal protozoa that can engulf starch particles and restrict access of lactic acid-producing bacteria to starch [25]. However, the fact that virginiamycin can interrupt metabolic processes, resulting in inhibition of multiplication and eventually death of lactic acid-producing bacteria, such *as Lactobacillus sp.* and *Streptococcus bovis*, without interfering with the growth of lactic acid-consuming bacteria, such as *Megasphera elsdenii*, could explain the result for the control treatment [5].

Rumen pH decreased linearly throughout the day, reaching a minimum at 12 hours after feed delivery in all treatments. This result coincides with the time of the highest SCFA concentration, which, according to Millen et al. [1], is the main cause of pH reduction. Similar results among treatments in the current study suggested that virginiamycin, autolyzed yeast, and enriched yeast cultures fed in combination with monensin could maintain ruminal pH within the appropriate range.

Ruminal acetate and propionate concentrations varied throughout the day, peaking at 12 hours after feeding, and were similar among treatments (Figures 2 and 3).

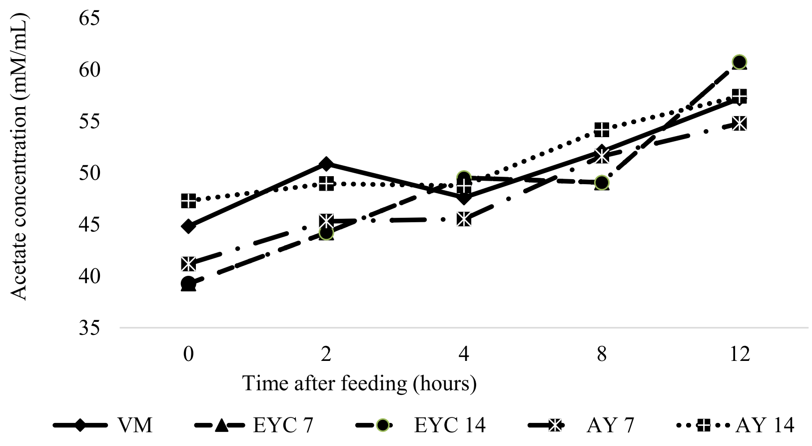


Figure 2 Mean acetate concentrations throughout the day. 1 - control, 18 mg kg^{-1} DM virginiamycin (VM); 2 - enriched yeast culture (*Saccharomyces cerevisiae*) at 7 g animal day^{-1} (EYC 7); 3 - enriched yeast culture (*S. cerevisiae*) at 14 g animal day^{-1} (EYC 14); 4 - autolyzed yeast (*S. cerevisiae*) at 7 g animal day^{-1} (AY 7); and 5 - autolyzed yeast (*S. cerevisiae*) at 14 g animal day^{-1} (AY 14). Treatment: P = 0.3257; interaction (treatment*time): P = 0.5458.

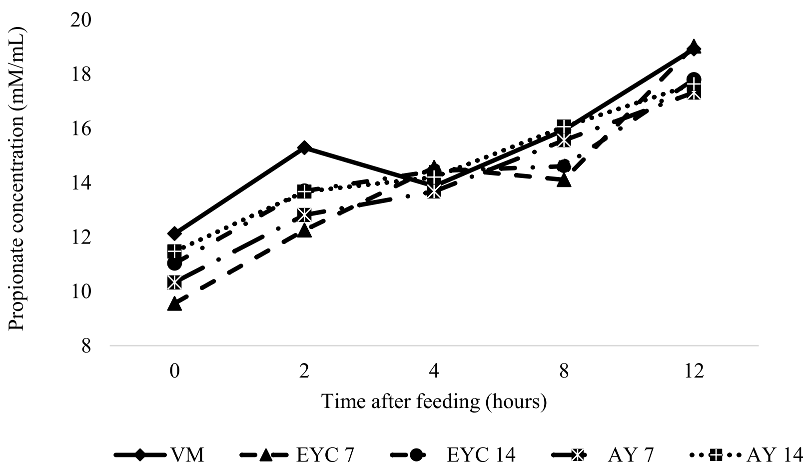


Figure 3 Mean propionate concentrations throughout the day. 1 - control, 18 mg kg^{-1} DM virginiamycin (VM); 2 - enriched yeast culture (*Saccharomyces cerevisiae*) at 7 g animal day^{-1} (EYC 7); 3 - enriched yeast culture (*S. cerevisiae*) at 14 g animal day^{-1} (EYC 14); 4 - autolyzed yeast (*S. cerevisiae*) at 7 g animal day^{-1} (AY 7); and 5 - autolyzed yeast (*S. cerevisiae*) at 14 g animal day^{-1} (AY 14). Treatment: P = 0.2346; interaction (treatment*time): P= 0.6794.

The lack of difference among treatments for SCFA concentrations and profile over time ($P > 0.05$) was consistent with the apparent digestibility of (OM) and NDF, which showed no difference among treatments ($P > 0.05$). According to Metwally and Windisch [45], SCFA production is associated with changes in the ruminal microbial population, with acetic acid being produced to a greater proportion mainly by cellulolytic bacteria, and propionic acid by starch-fermenting bacteria. A similar acetate:propionate ratio among treatments indicated that cellulolytic microorganisms grew satisfactorily despite the high proportion of grain in the diet, showing no significant difference ($P > 0.05$) among additives and levels of both enriched yeast culture and autolyzed yeast tested on the SCFA profile.

Neubauer et al. [36] did not observe the effect of adding autolyzed yeast on acetate and propionate concentrations or the acetate:propionate ratio in the rumen fluid of cows fed high-energy diets. In addition, no difference in the acetate:propionate ratio was detected by Shen et al. [25] although the acetate concentration was higher ($P = 0.06$) in the rumen fluid of heifers fed high-grain finishing diets supplemented with yeast fermentation product compared to the control (no additive) and monensin plus tylosin.

No significant differences were found in the apparent CP digestibility (Table 2) or rumen fluid N-NH3 concentrations at different times throughout the day ($P > 0.05$). Ammonia-nitrogen concentrations were highest 2 hours after feeding (Figure 4).

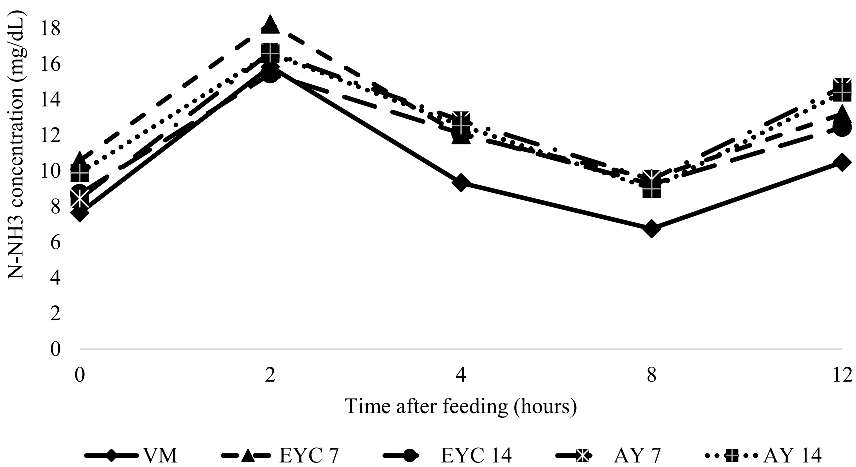


Figure 4 Mean ruminal ammonia-nitrogen concentrations throughout the day. 1 - control, 18 mg kg^{-1} DM virginiamycin (VM); 2 - enriched yeast culture (*Saccharomyces cerevisiae*) at 7 g animal day^{-1} (EYC 7); 3 - enriched yeast culture (*S. cerevisiae*) at 14 g animal day^{-1} (EYC 14); 4 - autolyzed yeast (*S. cerevisiae*) at 7 g animal day^{-1} (AY 7); and 5 - autolyzed yeast (*S. cerevisiae*) at 14 g animal day^{-1} (AY 14). Treatment: P = 0.1447; interaction (treatment*time): P = 0.9692.

Both monensin and virginiamycin have been reported to reduce proteolytic bacterial populations in the ruminal environment, thereby affecting N-NH3 concentration [46, 37]. Yeast-based products can stimulate ruminal microorganisms and thus increase N-NH3 concentrations, however the mechanism has not been completely elucidated [47, 23]. However, Shen et al. [25] reported no differences in ruminal N-NH3 concentrations between the monensin-tylosin association and yeast culture in high-grain-fed heifers. In addition, no effect of feeding yeast culture on ruminal N-NH3 concentrations was observed in steers or dairy cows [48, 49].

An adequate concentration of N-NH3 synchronized with energy can maximize the growth of proteolytic and cellulolytic microorganisms, improve organic matter digestibility, and prevent unnecessary energy expenditure from excreting excess N [50, 51]. Although there was no effect of treatment on ruminal N-NH3 concentration, it was always within or close to the level considered adequate for the growth of ruminal microorganisms and the digestion of OM in the rumen (6.2 mg dL^{-1}) [52]. Thus, ammonia-nitrogen concentration was not a limiting factor for the growth of ruminal microorganisms or ruminal OM digestibility among the treatments in the present study.

4. Conclusion

The levels of both enriched yeast culture and autolyzed yeast showed no effect on the evaluated parameters compared with that of virginiamycin. Therefore virginiamycin can be replaced with enriched yeast culture or autolysed yeast (*S. cerevisiae*) at the lower levels evaluated in this study for cattle fed high-energy diets containing monensin.

Conflict of interests

The authors declare no conflict of interest.

Author contributions

Oliveira, D.S., Silva, L.O.M., Ribeiro, L.G.R., Sousa, D.G., Pereira, L.M.: Investigation, writing – original draft; Fernandes, M.H.M.R., Nascimento, M.Q.: writing – review and editing.

Couto, V.R.M., and Arnhold. E.: Formal Analysis; Manzano, R.P., Fernandes, J.J.R.: Methodology, Conceptualization, Supervision

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