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Use of natural rumen modulators to replace monensin plus virginiamycin in high-grain diets for cattle

Utilização de moduladores ruminais naturais em substituição a monensina mais virginiamicina em dietas de alto grão para bovinos

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ABSTRACT

Thisstudy aimed to evaluate food additives for the replacement of monensin and virginiamycin in high-grain diets for cattle. A Latin square experimental design was adopted, represented by four treatments (functional oil, yeast, tannin and control monensin and virginiamycin) in four experimental periods, with four animals cannulated in the rumen. l The natural ruminal modulators fully replaced the ionophores monensin and virginiamycin in the concentrate. Being evaluated: ruminal pH, apparent digestibility and *in situ* degradability of dry matter. The results showed that the pH variation curve as a function of rumen fluid collection time was not significant (*P*<0.05) among the additives studied. The additives used to replace monensin and virginiamycin in crossbred cattle fed a diet of 70% concentrate and 30% roughage with fractionation five times a day, are efficient in maintaining rumen pH above 6.0. The additives did not affect digestibility of OM, CP, NDF, FDA and EE. The in situ degradability of the soluble and potentially degradable fractions was higher in the yeast and essential oil treatments, respectively. The additives evaluated are efficient in replacing monensin and virginiamycin.

KEYWORDS: ruminants; additives; modulators.

RESUMO

Objetivou-se com o presente estudo avaliar aditivos alimentares em substituição a monensina mais virginiamicina em dietas de alto grão para bovinos. Foi adotado o delineamento experimental em quadrado latino, representado por quatro tratamentos (Óleo funcional, Levedura, Tanino e o controle monensina e virginiamicina) em quatro períodos experimentais, com quatro animais cânulados no rúmen. Os moduladores ruminais naturais substituíram integralmente os ionóforos monensina mais virginiamicina no concentrado. Sendo avaliados: pH ruminal, digestibilidade aparente e a degradabilidade *in situ* da matéria seca. Os resultados mostraram que a curva de variação do pH em função do tempo de coleta de líquido ruminal não foi significativa (*P*<0,05) entre os aditivos estudados. Os aditivos utilizados em substituição a monensina mais virginiamicina para bovinos mestiços com dieta 70% concentrado e 30% volumoso com fracionamento cinco vezes ao dia, são eficientes na manutenção do pH ruminal acima de 6,0. Não diferindo os aditivos na digestibilidade aparente de MO, PB, FDN, FDA e EE. A degradabilidade in situ apresentou maiores valores para a fração solúvel e potencialmente degradável nos tratamentos com levedura e óleos essenciais, respectivamente. Sendo os aditivos avaliados eficientes na substituição a monensina e virginiamicina.

PALAVRAS-CHAVE: ruminantes; aditivos; moduladores.

INTRODUCTION

The use of appropriate feed additives can reduce the excretion of methane and nitrogen and improve ruminant performance. According to studies conducted, ionophores increase the efficiency of feed energy and protein use effectively in the rumen, but not with respect to the Declaration of the use of antibiotics by the European Union in January 2006 and the reduced public acceptability of their compounds for use in animal nutrition, animal nutritionists have tried to use other alternative compounds, control the expenditure of ruminal microbial population, and create appropriate changes in ruminal modification. In recent years, the use of herbs as a growth stimulant has been increasingly considered. The results of this study demonstrate that the use of medicinal plants has a positive effect on ruminal performance and pH, reducing methane production, ammonia nitrogen concentration, and protein metabolism, improving the ruminal microbial population, and increasing the production of volatile fatty acids.

MATERIALS AND METHODS

The work was conducted at the Ruminant Nutrition Sector of the Santa Paula Experimental Farm of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM) (16º21'50" south and 46º54'15" west), in the municipality of Unaí, state of Minas Gerais, Brazil. The altitude of the region is 640 m, and the climate is classified as Aw, tropical, with an average annual temperature of 27 ºC, average annual precipitation of 1,200 mm, and well-defined seasons: rainy in summer and dry in winter (KÖPPEN & GEIGER 1928).

The experiment was conducted in a masonry shed built in an east-west orientation, covered with a colonial-style roof, with 1.10 m high side walls, and open at the ends. The shed was equipped with individual pens measuring 9 square meters each, which were equipped with individual polyethylene feeders and drinkers. The floor of the stall was covered with a bed of wood shavings. The shed has a trunk to contain the animals and a collective stall area (^{30m2}) to assist management.

The experimental design used was the Latin square, represented by four treatments (T1-Functional oil, T2-Yeast, T3-Tannin and monensin control plus virginiamycin) in four experimental periods, with four animals cannulated in the rumen with an average initial body weight of 500±20kg and average age of 30 months. The animals used were from the Ruminant Nutrition Sector belonging to the Experimental *Campus* Fazenda Santa Paula of the Federal University of the Jequitinhonha and Mucuri Valleys. In the pre-experimental period, the animals were dewormed and administered the ADE vitamin complex. The total period of the experiment was 99 days, with four periods of 21 days, 16 days of adaptation, and 5 days of data collection. A pre-experimental period of 15 days was recommended to allow the animals to adapt to the management.

Natural rumen modulators completely replaced the monensin plus virginiamycin ionophores in the concentrate. The concentrations of the additives were as follows: 1) Monensin + Virginiamycin = 30 mg + 25 mg.kg^{-1} DM of the total diet; 2) Functional castor and cashew oils (Activo Premium®) = 400 mg.kg -1 of the total diet; 3) Inactive yeast (*Sacharomyces*) = 1.0 g. kg -1 DM of the total diet and 4) Tannin (ByPro®) = 1.0 g.kg1 DM of the total diet.

The animals were managed in individual stalls (^{9m2}), equipped with a feeder, a drinker and a trough for mineral salt. Water and mineral salt (sodium chloride) were offered "ad libitum" to the animals. Total diets (concentrate + bulky) were provided five times a day, from 7:00 am to 6:00 pm. Food consumption was quantified daily; however, to characterize the experiment and collect data to measure the diet, it was conducted on the 5 days of collection in each experimental period.

Table 1. Chemical composition of silage on a dry matter basis.

Nutrient	%
Crude Protein	4.0
Fiber in Neutral Detergent	75.0
Fiber in Acid Detergent	40.0
Ethereal Extract	2.0
Mineral Matter	11.0
Organic Matter	89.0

At the beginning of each experimental period, the animals were weighed after 12 hours of fasting. Based on individual body weight, the amounts of food offered daily were adjusted for each animal, providing 2.2% of body weight. The percentage composition of the concentrate based on natural matter is shown in Table 2.

Sample collections to determine dry matter intake (DMI) were performed by weighing the diet before supply and the leftovers within 24 hours, for three consecutive days.

Nutrient consumption was calculated as the difference between the amount of nutrients present in the food provided and the amount of nutrients in the leftovers, with the result expressed in Kg.animal.day^{-1}. The foods used to prepare the experimental diets were collected to determine their chemical composition.

The collected samples were identified, weighed, and frozen in a freezer (-10 ºC) and at the end of each period, they were sent to the Ruminant Nutrition Laboratory of the Institute of Agricultural Sciences, UFVJM, for determination of the bromatological composition: dry matter content, ether extract, mineral matter, and crude protein according to AOAC (1995) and Fiber in Neutral Detergent and Fiber in Acid Detergent according to the methodology of SILVA & QUEIROZ (2002) and VAN SOEST (1994).

Table 2. Percentage composition of the concentrate based on natural matter.

Ingredients	%	DM %
Mineral mix*	1.00	99.00
Urea	1.00	99.00
Soy	13.50	89.00
Ground corn	84.50	87.00

*Sodium chloride, 49%; dicalcium phosphate, 49%; zinc sulfate, 1.50%; copper sulfate, 0.40%; cobalt sulfate, 0.05%; potassium iodate, 0.05%.

To obtain the apparent digestibility coefficients of dry matter, crude protein, mineral matter, neutral detergent-insoluble fiber, and acid detergent-insoluble fiber, the total feces collection method was adopted. In the last three days of each experimental period, feces were collected and weighed individually, stored in plastic bags, identified, and properly frozen in a freezer at 10°C. At the end of each period, three composite samples from each day were analyzed.

The diet samples were pre-dried in a ventilated oven at 65 °C for 72 h. They were then ground to a particle size of 5 mm. Forage samples at different cutting ages were incubated in crossbred castrated male cattle (Holstein x Zebu) with rumen fistulas and subjected to the same experimental diet. The methodology used was adapted from NOCEK (1988) and HRISTOV et al. 2019, with the following incubation times in the ruminal environment: 0, 1, 2, 3, 6, 9, 12, 24, 36, 48, 72, 96, and 144 h. TNT bags of 300 gm^{-2} (10 x 15 cm and 50 μmporosity) containing approximately 20 mg of sample per cm^2 were introduced into the rumen. After each incubation period, the bags containing the samples were removed, immediately washed, and dried in a ventilated oven at 65 °C for 72 h. After drying, the samples were weighed to determine the DM degradability.

Food samples, leftovers, and feces were pre-dried in forced ventilation ovens at 55 °C for 72 h and subsequently processed in a knife mill through a 1.0-mm sieve to determine the dry matter (DM), mineral matter (MM), and crude protein (CP) content, according to AOAC (1995).

The levels of insoluble fiber (NDF) in neutral detergent were determined according to the methodology described by MERTENS et al. (2002) and RAPOSO et al. (2020), without the addition of sodium sulfite and using thermostable amylase (Termamyl 120L, Novozymes). For this purpose, the Ankom system was used with modification of the bag used (0.5 x 0.5 cm) with a porosity of 100 μm, which was made with TNT (non-woven fabric / 100 g/m^2).

Acid detergent fiber (ADF) levels were obtained using the sequential method proposed by ROBERTSON & VAN SOEST (1981) and the protocol presented by LICITRA et al. (1996) and DE OLIVEIRA et al. (2020). To estimate the total digestible nutrient (TDN) values of the diets, the equation TDN = 91.0246 0.571588, proposed by CAPPELLE et al. (2001) and CAVALCANTE et al. (2014).

The data were subjected to analysis of variance, and the treatment means were compared using the Tukey test at 5% significance. The results were analyzed using the SISVAR® software (FERREIRA, 2011).

RESULTS

Figure 1 shows the pH variation curve as a function of the ruminal fluid collection time.

Parameters a, b, and c adjusted for the potential degradability of MS and the effective degradability, according to the pre-established passage rates, are listed in Table 3.

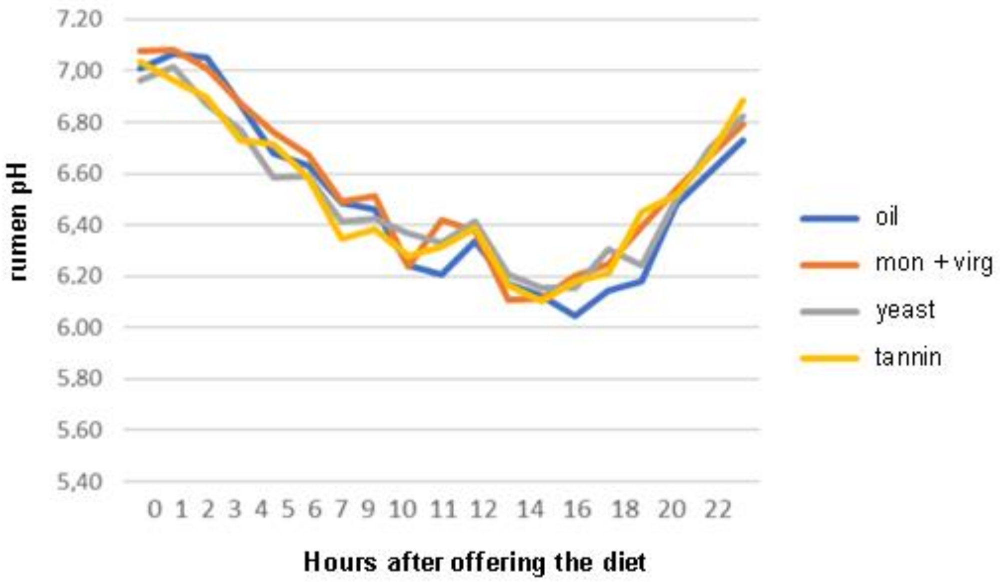

Figure 1. Ruminal pH curve at the time of evaluation according to the diet.

Table 3. Estimation of dry matter degradation parameters (a = soluble fraction, b = potentially degradable fraction and c = degradation rate of fraction b), potential degradability (PD), and effective degradability (De).

Treatment[1]	DM degradability (%)				Effective MS Degradability (%)		
	a[2]	b	c	PD%	0.02[3]	0.05	0.08
oil	26.24	47.93	0.04	73.46	58.38	47.75	42.40
mon + virgin	27.86	42.92	0.03	70.40	55.54	45.92	41.26
yeast	29.08	45.16	0.02	73.42	55.14	45.03	40.56
tannin	28.05	43.54	0.04	71.25	58.57	49.08	44.10

[1]Treatment – Essential oil, Monensin + Virginiamycin, Yeast, Tannin – [2]a = Soluble fraction, b = potentially degradable fraction and c = fraction degradation rate, PD% = Potential degradability – [3]0.02, 0.05, 0.08 approval rate.

Data relating to the apparent digestibility coefficients of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) are presented in Table 4.

Table 4. Averages (%) of apparent digestibility of ingested nutrients, obtained for the different treatments.

Item[2]	Treatment [1]				
	oil	mon + virg	yeast	tannin	CV[3]
DM	66.1	70.0	67.0	66.5	5.0
CP	74.3	76.8	73.1	73.3	3.4
EE	68.1	68.9	71.5	69.8	4.8
NDF	50.7	54.8	48.0	53.9	12.8
ADF	42.8	46.2	40.2	43.8	14.4

Treatment[1] – Essential oil, Monensin + Virginiamycin, Yeast, Tannin. Item[2] - MS dry matter, CP crude protein, EE ether extract, NDF neutral detergent fiber, ADF acid detergent fiber. CV[3] - Coefficient of variation.

DISCUSSION

At all evaluated moments, the means did not differ ($P<0.05$) between the additives studied, and the values were higher than 6.0; probably, the modulation promoted by the additives was efficient in maintaining the pH. Values above 45% of concentrate in the diet already lead to a drop in consumption due to metabolic disorders, one of these factors being the drop in pH to values below 6.0 due to the decrease in the activity of cellulolytic bacteria, altering consumption. With a concentrate/bulk ratio of 70/30% based on dry matter, feedlots without the use of additives and buffers showed a reduction in daily weight gain, final weight, and feed conversion compared with diets with lower concentrate values (VÉRAS et al. 2000, CARVALHO et al. 2007, OLIVEIRA et al. 2013, ABDELLI et al. 2021).

A reduction in pH is generally observed when starch availability increases, which leads to greater production of lactic acid, which can decrease it to values below 5.0, where the animal already has its ruminal activities and consumption interrupted, which did not occur. In the present study, all treatments remained above 6.0 (FIRKINS et al. 2001, OLIVEIRA et al. 2013, ECKERT et al. 2015, SANTOS 2016).

Monensin has been supplied for over 35 years to improve weight gain and prevent/control coccidiosis in feedlot cattle. The additives of monensin and virginiamycin combined have a high pH buffering capacity, and in feedlots with diets with high concentrate values, they maintain average values above pH 6.0 (MACIEL et al. 2019). HARRIS (2014) carried out two experiments to determine whether there is a difference between monensin doses of 360 or 480 mg/steer offered daily during the adaptation period, and this author found a significant interaction between treatment and experiment for body weight, average gain, and weight during the adaptation period. During the adaptation period, cattle fed 360 mg/steer daily showed greater average weight gain, intermediate body weight gain, and better feed conversion than steers fed 480 mg per day, and carcass characteristics were not affected by monensin doses.

However, CARDOZO et al. (2005) and BODAS et al. (2012) evaluated essential oils at a dose of 750 mg/day for dairy cows and found no effect on ruminal microbial fermentation or animal performance. However, the effects of ruminal fermentation of essential oils vary depending on the rumen pH. pH = 5.5 allows changes in ruminal microbial fermentation through essential oils, indicating their use in diets with a concentrate/bulk ratio above 45/55% (BENCHAAR et al. 2007, COBELLIS et al. 2016, MEALE et al. 2014).

JAHANI-AZIZABADI (2014) studied the effect of different doses of some essential oils from seven natural medicinal plants from the semi-arid region on the patterns of ruminal digestion "in vitro" and ruminal fermentation. They found that relative to the control, the addition of all essential oils decreased the disappearance of crude protein and methane. The reduction in methane production in ruminants may be a direct reflection of improvements in the diet and performance of these animals.

Thus, it can be considered that the reduction in methane production is a reflection of the improvement in the diet and the consequent improvement in the performance of ruminants. Because methane is a byproduct of microbial fermentation that occurs in the digestive system of ruminants, mainly in the rumen. The amount of methane produced is directly related to the efficiency of digestion and the composition of the animal's diets.

CARBERRY et al. (2019) investigated the effects of overfeeding and underfeeding on reproduction, mammary gland development, and milk composition in Holstein dairy cows. Although the focus is on dairy production, the article addressed aspects related to animal nutrition and performance, which can also influence methane production. CARDOZO et al. (2005) and RODRÍGUEZ-PRADO et al. (2012) in *in vitro* diets with a concentrate/bulk ratio of 90/10% observed that the addition of oil increased the pH from 5.84 to 6.03 and tended to increase the concentration of VFA, even maintaining the acetate:ratio, the acetate concentration decreased, as well as the rumen ammonia concentration of ruminal ammonia. Similar results were reported by LILLEHOJ et al. (2018).

In the study, the values of the soluble fraction in the treatment with oil obtained a lower value of 26.24, whereas the treatment with yeast obtained 29.08, indicating a greater availability of starch, which can be justified due to the greater colonization of amylolytic bacteria as described by FRANÇA & RIGO (2011) and SARTORI et al. (2017). The values found for the soluble fraction corroborate the data obtained in sheep subjected to monensin and essential oil, where the average value of the readily soluble fraction (a) was 27.7 (OLIVEIRA et al. 2020), observing 23.32 when 1.5 g. kg. DM^{-1} of copaiba oil in the animals' diet.

Therefore, essential oils have the ability to maintain pH values within levels considered ideal in the diets of highly productive animals, with an average pH value of 6.5 due to the possible control of gram-positive bacteria in the rumen, as found by MARTINS (2017). The potentially degradable fraction (b) of dry matter was 47.93% in oil treatment, whereas it was 42.92% in mon+virg. This decrease may have occurred because of a larger portion of the soluble fraction.

DORANTES-ITURBIDE et al. (2022) evaluated the effects of dietary supplementation with essential oils (EOs) on productive performance, ruminal parameters, serum metabolites, and quality of products (meat and milk) derived from small ruminants through a meta-analysis and found that the dietary inclusion of EOs increased ($P < 0.05$) dry matter intake (WMD = 0.021 kg/d), dry matter digestibility (ADM = 14.11 $g.kg^{-1}$ DM), daily weight gain (ADM = 0.008 kg.d-1), and feed conversion (ADM = −0.111) and concluded that diets supplemented with EOs improve productive performance, as well as the quality of meat and milk of small ruminants. Furthermore, EOs improve the antioxidant status of blood serum and ruminal fermentation and decrease environmental impact.

The treatments did not differ for any nutrient evaluated. Alternative feed additives to monensin plus virginiamycin showed the same response for the apparent digestibility of dry matter, crude protein, ether extract, neutral detergent fiber, and acid detergent fiber. In this context, essential oils are indeed a promising option, offering a safer and more "eco-friendly" approach to promoting animal health and well-being.

Recent studies conducted by KHAN et al. (2020) and BENCHAAR et al. (2008) highlighted the potential of essential oils as viable alternatives for traditional feed additives, such as monensin and virginiamycin, in ruminants. The study compared the effects of essential oils with those of conventional substances, showing that both obtained similar responses in terms of performance, apparent digestibility coefficients of dry matter, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, health, and ruminal microbiota. This suggests that essential oils may be as effective as traditional additives, offering a natural solution that is free from potential adverse effects.

In addition to their antimicrobial properties, essential oils have a variety of additional benefits, such as antioxidant and anti-inflammatory activities, which can contribute to the overall health of animals. Furthermore, its use can reduce the dependence on chemical additives in animal production, promoting a more sustainable and environmentally conscious approach.

Evaluation of yeasts and enzymes from *Saccharomyces cerevisiae* in different diets QUEIROZ et al. (2004) found an average increase in the apparent digestibility of EE, NDF, and ADF of 5.0; 8.2; 7.0 and 11.0%, respectively, with the addition of yeast and enzyme. As different diets responded differently, diets containing corn silage and wheat bran were more responsive to treatment. In the present study, essential oils showed similar responses to monensin plus virginiamycin, corroborating what was found by NEUMANN et al. (2022).

Improving ruminant diets can reduce methane production because methane production in ruminants is directly linked to microbial fermentation in the rumen during feed digestion (KNAPP et al. 2014).

Diet composition plays a crucial role in the quantity and quality of gases produced during the process. Therefore, interventions in the diet of ruminants, such as adjustments in diet formulation, selection of high-quality feeds, supplementation with feed additives, or inclusion of ingredients that can reduce methane production, can contribute significantly to improving production efficiency, as well as reducing greenhouse gas emissions associated with livestock (UNGERFELD 2015, DUVAL et al. 2017).

CONCLUSION

The additives used to replace monensin and virginiamycin (30 mg + 25 mg.kg^{-1}), Activo Premium® functional oils (400 mg.kg -1), inactive *Saccharomyces* yeast (1.0 g/kg DM), and Tanin ByPro® (1.0 mg.kg -1) 0 g/kg DM), for crossbred cattle with a diet of 70% concentrate and 30% forage with fractionation five times a day, are efficient in maintaining ruminal pH above 6.0. The apparent digestibility of OM, CP, NDF, ADF, and EE did not differ. *In situ* degradability: Higher values were observed for the soluble and potentially degradable fractions in the respective yeast and essential oil treatments. The additives evaluated are efficient in replacing monensin and virginiamycin.

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