*FULL ARTICLE*

ISSN Online 1678-4456

# Effect of phytogenic additives on ruminal parameters, *in vitro* and *in situ* degradability in beef cattle

## *Efeitos de aditivos fitogênicos nos parâmetros ruminais, degradabilidade in vitro e in situ em bovinos de corte*

Jean Sardinha de Almeida[1]; João Teodoro Padua[1]; Leonardo Guimarães de Oliveira[1]; Daniel Staciarini Correa[1]; Graciele Araújo de Oliveira[1]; Rodrigo Zaiden Taveira[2]; Carlos Henrique Xavier[1]; Reginaldo Nassar Ferreira[1]

[1] Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Ciências Fisiológicas, Goiás – GO, Brazil
[2] Universidade Estadual de Goiás, Departamento de Ciências Animais, Goiás – GO, Brazil

**ABSTRACT**

Technological advances, easily pursued by research on ruminant nutrition, can improve animal production, prevent nutritional disorders, and increase digestibility concomitantly with reducing ingestive discrepancies. This study aimed to investigate the inclusion of crude extract of *Croton urucurana* (Croton) or essential oils of cashew and castor bean on *in vitro* and *in situ* degradability of Tifton 85 hay and metabolic parameters in beef cattle. The experiment was conducted at the Biological Sciences Institute (ICB) and the Veterinary School of the Federal University of Goias, Brazil. The treatments were: Control, Monensin, Croton, and Blend (essential oil composed of cashew and castor bean). We used five replicates to evaluate ruminal parameters and dry matter degradability. Blend increased dry matter degradation, indicating that forage feeds last longer in the rumen and tend to be more degraded with Blend inclusion. The assessments of *ad libitum* ingestion of dry matter showed that monensin treatment reduced feed intake compared to control and Croton treatments. Our results suggest that the inclusion of Blend improves the degradability of dry matter in the diet and could be a strategy of choice in diets based on low-quality forage.

**Keywords:** *Croton urucurana* Baillon. Fatty acids short-chain. Monensin. Protozoa.

**RESUMO**

Os esforços tecnológicos buscados com esforço pelas pesquisas em nutrição de ruminantes são aqueles potencialmente capazes de melhorar a produção animal, prevenir distúrbios nutricionais, aumentar a digestibilidade concomitantemente à redução de discrepâncias ingestivas. Este estudo teve como objetivo investigar a inclusão de extrato bruto de Croton urucurana Baillon (Croton) ou óleos essenciais de caju e mamona na degradabilidade *in vitro* e *in situ* do feno de Tifton 85 e em parâmetros metabólicos em bovinos de corte. O experimento foi conduzido no Instituto de Ciências Biológicas (ICB) e Faculdade de Veterinária da Universidade Federal de Goiás - Brasil. Os tratamentos foram: Controle, Monensina, Croton, Blend (óleo essencial composto de caju e mamona). Foram utilizadas cinco repetições para avaliação dos parâmetros ruminais e degradabilidade da matéria seca. O Blend aumentou a degradação da matéria seca, indicando que a forragem dura mais tempo no rúmen e tende a ser mais degradada com a inclusão do Blend. As avaliações da ingestão *ad libitum* de matéria seca mostraram que o tratamento com monensina foi capaz de reduzir o consumo de ração quando comparado aos tratamentos controle e Croton. Nossos resultados sugerem que a inclusão do Blend melhora a degradabilidade da matéria seca na dieta e pode ser uma estratégia de escolha em dietas baseadas em forragem de baixa qualidade.

**Palavras-chave:** *Croton urucurana* Baillon. Ácidos graxos de cadeia curta. Monensina. Protozoa.

**Correspondence to:**
Reginaldo Nassar Ferreira
Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Ciências Fisiológica
Avenida Esperança, s/n, Câmpus Samambaia
CEP: 74690-900, Goiânia – GO, Brazil
e-mail: reginaldonassar@gmail.com

Received: March 12, 2024
Approved: April 22, 2025

**How to cite:** Almeida JS, Padua JT, Oliveira LG, Correa DS, Oliveira GA, Taveira RZ, Xavier CH, Ferreira RN. Effect of phytogenic additives on ruminal parameters, *in vitro* and *in situ* degradability in beef cattle. Braz J Vet Res Anim Sci. 2025;62:e220460. https://doi.org/10.11606/issn.1678-4456.bjvras.2024.220460.

## Introduction

Intensification of ruminant production requires technologies that increase the feed efficiency of ruminants. Additives in the diet are feasible strategies, although using antibiotics and ionophores as growth promoters has been questioned as producing undesirable side effects (Oliveira et al., 2013). In these regards, natural alternatives such as phytogenic or phytotherapeutic additives are good candidates and deserve the spotlight in the field (Patra, 2010). To modulate ruminal fermentation (McGaw, 2013), oils and plant extracts may represent an alternative since they are considered safe and their use for animal and human consumption is permitted according to the Food and Drug Administration (Bakkali et al., 2008).

In some tropical plants, phytogenic additives such as essential oils and crude extracts contain phenols and flavonoid compounds that potentially modulate ruminal fermentation (Kamra et al., 2006; Patra, 2010). Among the different mechanisms underlying some effects, stimulation of the enzymatic activity of ruminal bacteria associated with the cell membrane, such as electron-carrying proteins, translocation of membrane proteins, and oxidative phosphorylation, may be highlighted (Kamra et al., 2006; Patra, 2012; Patra & Saxena, 2010). Some natural compounds may be found in tropical plants such as sangra d'água (*Croton urucurana* Baillon) (Silva et al., 2009), cashew (*Anacardium occidentale*) (Osmari et al., 2015), and castor bean (*Ricinus communis*) (Naz & Bano, 2012; Salihu et al., 2014; Scarpa & Guerci, 1982), with large amounts of active molecules with great potential as phytogenic additives for use in animal nutrition.

While researching crude extract of Croton urucurana Baillon, Simionatto and colleagues obtained from the bark of the stem, 83 compounds with 94.6% of the analyzed extract, among them borneol (14.7%), bornyl acetate (5.2%), 1-isopropyl-7-methyl-4-methylene-1,3,4,5,6,8 hexahydro-2H-naphthalen-4a-ol (14.7%), sesquicineole (10.5%) and γ-gurjunene epoxide (5.4%), and considering the antioxidant fraction α-bisabolol (38.3%), α-eudesmol (9.3%) and guaiol (8.2%) are the main ones (Simionatto et al., 2007). Literature reported on assessments of the antimicrobial potential of sangra d'água vegetal extract. Nader showed that this extract dramatically reduced Staphylococcus aureus populations in cows with mastitis (Nader, 2014). Soldera and colleagues showed *in vitro* that sangra d'água efficiently reduces growing populations of Staphylococcus aureus (Soldera et al., 2010). Altogether, these findings support the notion that this extract can control microbiomes. Notwithstanding, literature still lacks information on whether these extracts and compounds interfere with the digestibility and performance of beef cattle.

The wide use of monensin in animal feed and the associated risks for human health necessitate the search for alternative additives (European Union, 2015). In this study, we followed the hypothesis that phytogenic additives may replace monensin with similar or better performance. Our attempts were aimed at investigating the effects of the inclusion of crude extract of sangra d'água (Croton) (*Croton urucurana* Baillon), a blend of essential oils of cashew (*Anacardium occidentale*) or castor bean (*Ricinus communis*), and to compare them with monensin on *in situ* and *in vitro* degradability of Tifton 85 hay, and on ruminal parameters of beef cattle.

## Material and Methods

The Ethics Committee approved all procedures in this research on the Use of Animals at the Federal University of Goiás (CEUA-UFG), which generated protocol No. 069/15.

### *Preparing the extract of sangra d'água (Croton urucurana Baillon)*

The crude sangra d'água (Croton) extract was directly extracted from the tree by cutting the trunk and deposited in a stainless-steel container. The crude extract of sangra d'água (Croton urucurana Baillon, is registered in the herbarium of the Federal University of Goiás under number 20,865) was extracted directly from the tree by cutting the trunk and placing it in a stainless-steel container. Subsequently, the container was placed in the refrigerator at a temperature of -8 °C until it reached a significant volume of 100 ml. It was placed at room temperature until the liquid became like a brittle resin, and then macerated until it became a powder.

Cashew and castor bean essential oils evaluated are the active ingredients of a commercial product, which are mixed and microencapsulated by the company Prophytus® (São José dos Campos, State of São Paulo, Brazil).

### *Ruminal parameters in vivo*

Four crossbred, intact male bovines, with an average weight of 523±35 kg and with ruminal cannulas, were housed in individual stalls. The animals were dewormed and vaccinated according to current legislation. The animals were given *ad libitum Brachiaria brizantha* hay with 89.8% dry matter, 9.5% crude protein, 77.7% NDF, and 67.0% TDN in individual troughs, allowing at least 5% leftovers, and the amount ingested by the animal was recorded daily. The animals could access the mineral mix without additives and water *ad libitum*.

The treatments were as showed in Table 1 and as follows: **Control**, without additives; **Monensin**, in the amount of 300 mg/animal/day of monensin (Rumensin, Elanco, Greenfield, IN, USA); **Croton**, Croton extract, 1500 mg/animal/day; **Blend**, commercial blend of essential oils in the amount of 1500 mg/animal/day (Biophytus, São José dos Campos, State of São Paulo, Brazil). For *in vivo* experiments, treatments were administered via ruminal cannula, before the morning's first meal. For *in vitro* experiments, treatments were given in proportion to 10 kg of dry matter ingested during *in vivo* experiments. Doses were chosen regarding the concentrations of the active compounds and based on previous studies (Gurgel, 2005; Oliveira et al., 2008).

The experimental period was 64 days, divided into four rounds of 16 days. The first seven days of each round were used for adaptation to the treatments, three subsequent days were used to estimate voluntary intake, the next three days were used for incubation of the material to determine the in situ degradability, the two subsequent days were used to collect ruminal fluid (RL) to determine parameters, and the last day was used for blood (B) sampling.

The ruminal parameters analysis of bacterial reduction activity, count of protozoa, ammonia nitrogen, quantification of short chain fatty acids and pH were evaluated in the ruminal fluid, which was collected directly from the rumen by the cannula, in four collections per day, with the first occurring immediately before feeding in the morning (0 hr), the second two hours after feeding (2 hr), the third five hours after feeding (5 hr) and the fourth, nine hours after feeding (9 hr).

The ruminal fluid was collected, and the pH was immediately measured with a potentiometer with two decimal places model MPA 201 (MS Tecnopon, Piracicaba, State of São Paulo, Brazil). The analyses of methylene blue reduction activity of bacteria were performed according to Bouda et al. (2000). The protozoan count was performed following the methodology described by Dehority (1984). The determination of ammonia nitrogen in the ruminal liquid was performed according to Chaney & Marbach (1962), the quantification of short-chain fatty acids was performed by HPLC (High Performance Liquid Chromatography) at a wavelength of 210 nm, column: C18 (reverse phase), 30 cm x 4.5 mm in diameter and 0.6 mL/min flow with column pressure of 87 Kgf and water in 1% sulfuric acid and an injected volume of 10 µL.

The *in situ* technique was performed with 12.5 x 10 cm TNT bags containing approximately 5g Tifton 85 hay ground to 2 mm. The bags were inserted into the rumen in descending order of time so that they could be removed all at once at 0, 3, 6, 12, 18, 24, 48, and 72 hr (Nocek, 1985).

After being removed from the rumen, the bags were washed in running water until the residual water was clear. Then, they were dried in a forced ventilation oven at 55 °C for 72 h and weighed on an analytical balance. The estimation of the residual material's dry matter (DM) content was performed according to the methodology described in Latimer Junior (2019).

### *Ruminal parameters in vitro*

A completely randomized design was used for the IVDMD and IVNDFD. The jar was considered the experimental unit, with five rounds for each treatment and all in all rounds. *In vitro* dry matter digestibility (IVDMD) of Tifton 85 hay was determined according to Tilley & Terry (1963) using the equipment TE-150 (Tecnal Scientific Equipment, Piracicaba, State of São Paulo, Brazil). The *in vitro* digestibility estimation was determined at the times of removal of the bags, 0, 3, 6, 12, 18, 24, 48, and 72 hr of incubation. Three bags were used per incubation time, from which an average was obtained. It was repeated 5 times.

The IVDMD test was made up of the same four treatments of the previous test: **Control**, only the ruminal inoculum; **Monensin**, 30 mg sodium monensin (Rumensin, Elanco, Greenfield, USA) per incubation jar; **Croton**, 30 mg crude extract of sangra d'água per incubation jar; **Blend**, 30 mg

**Table 1 –** Treatments and groups: Forage, given *ad libitum* to all groups; Monensin, chosen as positive control; Croton or Blend, as experimental groups

| | CONTROL | MONENSIN | CROTON | BLEND |
|---|---|---|---|---|
| FORAGE | *ad libitum* | *ad libitum* | *ad libitum* | *ad libitum* |
| MONENSIN | - | 300mg | - | - |
| CROTON | - | - | 1500mg | - |
| BLEND | - | - | - | 1500mg |

blend of essential oils composed of cashew and castor bean (Biophytus, Prophytus Agroindustrial, São José dos Campos, Brazil) per incubation jar. These are administered individually directly into each incubation jar.

### *Equipment and solutions*

The IVDMD was determined to maintain the temperature at 39.5 °C in four digestion flasks per round, remaining in constant agitation. Buffer solutions A and B, which compose the Kansas buffer solution, were mixed in the amounts of 266 mL solution B to 1330 mL solution A (1:5). The exact amount of A over B was adjusted to obtain a final pH of 6.8 at 39 °C. 400 mL of ruminal fluid was added to the buffer solution, reaching 2000 mL, which was used for incubation according to the methodology proposed by the equipment manufacturer.

The inoculum of ruminal fluid was obtained from two male Zebu cattle, with an average weight of 584 kg, housed in individual stalls of masonry, dewormed, and vaccinated according to current legislation. The animals were fed *ad libitum* with hay, allowing at least 5% leftovers, with access to the mineral mixture without additives and water *ad libitum*. The diet was exclusively hay, consumed for 30 days before sampling. The ruminal fluid samples were taken before supplying the diet, and the ruminal contents were collected manually at different rumen points, stored in a thermal container until arriving in the laboratory, and placed in a blender for 10 s at high speed with a puncture of $CO_2$ and filtered through two layers of cotton fabric.

### *Incubation*

For the IVDMD, the F57 filter bags were first washed in acetone (P.A. $(CH_3)_2CO$), placed in an oven at 105 °C for 24 hr, identified again in an oven at 105 °C for 24 hr and in a desiccator for 20 min, and individually weighed.

After this process, each bag was filled with approximately 0.5 g of Tifton 85 hay processed in a Wiley mill with a 1 mm sieve. The bags were then sealed and incubated in the jars: 26 bags at the aforementioned times, with a sample and two blanks to make the correction factor, and two bags at time 0 only dipped into the jar to soak in the solution.

Subsequently, the filter bags were washed in running water until the water became clear in the vessel and until all residue was removed. The samples were then dried in an oven at 105 °C for 24 hr, soon after, in a desiccator for 20 min, and then weighed in an analytical balance to determine IVDMD. After that, samples were analyzed for NDF content to determine the in vitro degradability of NDF according to Latimer Jr. (2019).

### *Experimental design and analysis*

The models proposed by Orskov & McDonald (1979) were used to determine the parameters of in situ ruminal degradation of DM and of in vitro digestibility of DM (IVDMD) and of NDF (IVNDFD).

For the IVDMD and IVNDFD, a completely randomized design was used. The jar was considered the experimental unit, with five rounds for each treatment and all treatments in all rounds. The mean values of the bags removed at each time of each round were the subdivision of each replicate for each given removal time.

For the determination of *in situ* ruminal degradation parameters, a 4x4 Latin square design was used. The animal was considered the experimental unit of each subperiod, and the mean of the values of the bags taken at the same time of the same round was used as the subdivision of the incubation time of each round.

The means of *in situ* degradability, IVDMD and IVNDFD were thus adequate to the model proposed by Orskov & McDonald (1979) for the estimation of the parameters of degradation by the least squares method, and the estimated parameters, subjected to one way analysis of variance, with treatment as the variable to be considered. We compared all the means using Tukey's test at a 5% significance level, using the software R (R Core Team, 2020).

Data on DM consumption, pH, ammonia nitrogen, methylene blue reduction time, protozoa count, and short-chain fatty acids were tested by analysis of variance, and the means were compared by Tukey's test at 5% with the aid of the statistical software R (R Core Team, 2020).

## Results

There was a significant difference ($P < 0.05$) in the voluntary intake of DM, evidencing the expected effect of restriction of intake by monensin (20.36%) in relation to control and Croton treatment, which was not observed in the comparison with the treatment Blend (Table 2).

**Table 2 –** Mean dry matter intake (Kg) of cattle fed diets based on hay, including Monensin, Croton, or Blend

| Treatments | | | | SEM | P-value |
|---|---|---|---|---|---|
| Control | Monensin | Croton | Blend | | |
| 1.61a | 9.24b | 1.22a | 0.52ab | 0.35 | 0.02 |

The ruminal pH showed no difference between the treatments, remaining in a range that favors the activity of cellulolytic bacteria (Table 3). The treatments did not influence ammonia/nitrogen levels in the ruminal fluid. However, there was a variation between the collection hours, with an increase in the concentration in the first 2 hr after feeding, followed by a decrease in the subsequent hours. The highest ammonia concentrations in the rumen were observed 2 hr after feeding for all treatments.

Monensin positively influenced the methylene blue reduction time compared to the other treatments, evidencing a negative influence on the bacterial activity. Another behavior observed in the evaluation of the methylene blue reduction time was that in the hours after the feeding, there was an increase in bacterial activity until 5 hr after feeding, followed by a decrease in this activity at 9 hr compared to 5 hr.

The essential oils blend increased the soluble fraction of the hay compared to the other treatments and provided a greater effective degradability in the rate of passage by 2% in relation to monensin (Table 4). Table 4 also lists the IVDMD values, which did not differ. The soluble fraction's mean (of the treatments) was 21.85%, and the potentially degradable fraction was 52.71%, resulting in a potential degradation of the hay of 74.56%.

Any additive did not affect the dry matter degradation rates or the values of the potentially degradable fraction of hay. According to Table 4, the treatments had no significant effect on the in vitro kinetics of NDF at any of the times evaluated.

According to Table 5, there was no significant difference in the total concentration of protozoa per milliliter of ruminal liquid. The concentrations of acetic, propionic, and lactic acids and the acetic: propionic ratio were not influenced by treatments (Table 5), and there was also no interaction between treatments and collection times.

## Discussion

The main results of this study are: i) Monensin promoted a reduction in DM intake; ii) Blend treatment increased DM solubility and effective degradation at a 2% passage rate. The monensin reduced ingestion when compared to the other treatments, which was also described by several authors (Benchaar et al., 2006; McMeniman et al., 2010; Schelling, 1984). The regulation of dry matter intake can be attributed to metabolic factors (Grummer et al., 2004), which are determined by nutrient balancing and physical variables, closely related to the rumen-reticulum capacity and the digestibility of NDF. The contribution of neurohormones, in turn, may arise from inhibitory or stimulating factors in the food, environment, and management. For Albornoz et al. (2019), reported reductions in dry matter intake of animals fed only to meet their requirements is one of the effects of monensin, and, when the energy balance is negative, the additional energy promoted by ionophores is used to improve growth performance and reduce losses of body reserves.

The use of monensin protects the rumen wall, favors the maintenance of the ruminal papillae, thus boosting the absorptive capacity of the rumen with a consequent higher

**Table 3 –** Concentration of pH, $NH_3$, and methylene blue of cattle fed diets based on hay and mineral salt, including Monensin, Croton, and Blend

| Variable | Hours | Treatments | | | | Average hours | SEM hours | T | H |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Monensin | Croton | Blend | | | | |
| pH | 0 | 7.03 | 6.99 | 7.13 | 7.10 | 7.06b | 0.02 | 0.25 | 0.00 |
| | 2 | 7.13 | 7.09 | 7.18 | 7.14 | 7.13ab | | | |
| | 5 | 7.10 | 7.05 | 7.13 | 7.00 | 7.06b | | | |
| | 9 | 7.11 | 7.16 | 7.21 | 7.17 | 7.16a | | | |
| | Treatment average | 7.09a | 7.07a | 7.15a | 7.10a | - | | | |
| | SEM Treatment | | | 0.03 | | | | | |
| $NH_3$ mg/dL | 0 | 4.62 | 4.30 | 4.56 | 4.63 | 4.53ab | 0.26 | 0.85 | 0.00 |
| | 2 | 7.62 | 6.09 | 6.73 | 6.79 | 6.81a | | | |
| | 5 | 5.76 | 5.31 | 5.20 | 5.10 | 5.34b | | | |
| | 9 | 3.35 | 4.16 | 4.24 | 4.50 | 4.06c | | | |
| | Treatment average | 5.34a | 4.96a | 5.18a | 5.25a | - | | | |
| | SEM Treatment | | | 0.33 | | | | | |
| BLUE min | 0 | 1.83 | 2.74 | 1.85 | 1.86 | 2.07a | 0.16 | 0.02 | 0.01 |
| | 2 | 1.29 | 2.32 | 1.32 | 1.57 | 1.62a | | | |
| | 5 | 1.19 | 2.19 | 1.27 | 1.59 | 1.56a | | | |
| | 9 | 2.11 | 3.18 | 1.75 | 1.57 | 2.15a | | | |
| | Treatment average | 1.61b | 2.61a | 1.55b | 1.64ab | - | | | |
| | SEM Treatment | | | 0.18 | | | | | |

SEM: standard error of the mean; P: significant when P <0.05, by Tukey's test; T: treatment; H: hours of collection.

**Table 4 –** Kinetic parameters of the *in situ* degradability of DM, *in vitro,* and IVNDFD of Tifton 85 hay with the addition of Monensin, Croton, and Blend

| Item | Condition | Treatments | | | | SEM | P-value |
|---|---|---|---|---|---|---|---|
| | | Control | Monensin | Croton | Blend | | |
| A (%) | *in situ* | 22.54b | 21.69b | 21.20b | 27.85a | 0.62 | 0,00 |
| B (%) | | 45.96 | 44.46 | 47.74 | 43.98 | 1.86 | 0.53 |
| c (% $h^{-1}$) | | 0.04 | 0.04 | 0.04 | 0.03 | 0.00 | 0.45 |
| ED (k=2%) | | 52.51ab | 51.89b | 52.91ab | 55.09a | 0.49 | 0.04 |
| ED (k=5%) | | 42.29 | 42.19 | 42.32 | 45.25 | 0.70 | 0.10 |
| ED (k=8%) | | 37.27 | 37.23 | 37.04 | 40.64 | 0.75 | 0.08 |
| NF | | 31.5 | 33.85 | 31.06 | 28.17 | 1.62 | 0.26 |
| A (%) | *in vitro* | 21.14 | 23.05 | 21.04 | 22.18 | 2.41 | 0.92 |
| B (%) | | 54.12 | 52.09 | 55.05 | 49.61 | 2.15 | 0.32 |
| c (% $h^{-1}$) | | 3.95 | 3.45 | 3.72 | 4.36 | 0.44 | 0.52 |
| ED (k=2%) | | 56.40 | 55.21 | 56.14 | 55.84 | 2.61 | 0.99 |
| ED (k=5%) | | 44.47 | 43.71 | 43.96 | 44.96 | 2.95 | 0.99 |
| ED (k=8%) | | 38.60 | 38.3 | 38.09 | 39.41 | 2.95 | 0.99 |
| FI | | 24.74 | 24.85 | 23.9 | 28.21 | 1.85 | 0.40 |
| A (%) | IVNDFD | 4.11 | 3.67 | 4.17 | 4.27 | 1.11 | 0.98 |
| B (%) | | 59.84 | 57.12 | 58.72 | 57.17 | 1.63 | 0.60 |
| c (% $h^{-1}$) | | 0.04 | 0.04 | 0.04 | 0.04 | 0,00 | 0.91 |
| ED (k=2%) | | 41.66 | 41.16 | 42.32 | 40.91 | 1.38 | 0.89 |
| ED (k=5%) | | 28.46 | 28.58 | 29.22 | 28.1 | 1.67 | 0.97 |
| ED (k=8%) | | 22.03 | 22.36 | 22.82 | 21.93 | 1.63 | 0.98 |
| NF (%) | | 37.09 | 39.2 | 37.1 | 38.55 | 0.94 | 0.33 |

ED: Effective degradability; k: rate of passage; NF: non-degradable fraction; SEM: standard error of the mean; P: significant when P <0.05, by Tukey's test. A: soluble fraction; B: potentially degradable fraction; c: rumen degradation rate; FI: non-degradable fraction.

absorption of fatty acids (Galyean & Goetsch, 1993). In this case, the changes in *ad libitum* intake by monensin were dissociated from any effects on the concentration of short-chain fatty acids. As an antibiotic and ionophore molecule capable of interfering with anion exchange in the cell membrane, monensin can prevent the effects of excessive ruminal acidosis, and this may be the primary mechanism underlying monensin's benefits in cattle. While assessing the performance of weaned calves on grazing and supplemented with ionophores, Palma et al. (2015) showed a decrease in the consumption of supplements in the monensin treatment, which was attributed to a lower DM passage rate. As a consequence, fiber digestibility was increased. Although our results differed from those above cited, it seems plausible to hypothesize that putative mechanisms related to the efficiency of the ruminal microbiome may be involved. However, it necessitates further investigation.

Although the use of monensin is prevalent, it provides good results. It has been previously reported to provoke damage to human health, and its discontinuation has been a reason for global debate and regulation (European Union, 2015; Food and Drug Administration), which supports the search for alternatives. Including essential oils did not interfere with the ammonia nitrogen concentration in the rumen fluid, probably because the hay had low protein content. In diets with high protein content, the inclusion of cashew nut oil reduced the production of ammonia nitrogen in the rumen fluid by reducing the activity of proteolytic bacteria, as well as reducing the adhesion and colonization capacity of these bacteria to their substrates (Osmari et al., 2015). Another source of variation in the concentration of ammonia nitrogen in rumen fluid is ruminal protozoa, which hinder the use of nitrogen by ruminants by assimilating the bacteria present in the rumen, which displays an important proteolytic activity (Park et al., 2019). This corroborates our findings, showing no differences in the concentration of protozoa and ammonia nitrogen in the rumen fluid.

Literature state that functional oils can improve ruminal fermentation and nutrient digestibility by stimulating enzymatic activity (Patra & Yu (2012) and shows a significant reduction in NH3 production from the analyses of the rumen fluid of animals fed with cashew nut oil. This effect is attributed to the active components of the oil that can inhibit the growth of proteolytic bacteria and reduce the ability of these bacteria to adhere and colonize their substrates (Diaz et al., 2018; Goetz et al., 2023). Effective rumen degradability depends on the inherent characteristics of the feed, and it is also influenced by the interaction between the feed and the microorganisms in the rumen, as well as the additives present in the diet.

**Table 5** – Protozoa in rumen fluid and production of short-chain fatty acids in cattle fed diets based on hay and mineral salt with Monensin, Croton, and Blend

| Protozoa/fatty acid | Hours | Treatments | | | | P-value |
|---|---|---|---|---|---|---|
| | | Control | Monensin | Croton | Blend | |
| Protozoa per mL (x10000) | 0 | 280.80 | 142.00 | 226.00 | 209.48 | 0.43 |
| | 2 | 184.40 | 137.20 | 95.20 | 217.75 | |
| | 5 | 278.60 | 133.00 | 172.40 | 183.62 | |
| | 9 | 197.00 | 161.80 | 215.00 | 179.35 | |
| | Treatment average | 235.20a | 143.50a | 202.15a | 197.55a | |
| | SEM Treatment | | 39.00 | | | |
| Acetic acid | 0 | 3513.00 | 4066.00 | 4197.00 | 3527.00 | 0.16 |
| | 2 | 4187.00 | 4197.00 | 3905.00 | 2929.00 | |
| | 5 | 3285.00 | 3657.00 | 4080.00 | 2571.00 | |
| | 9 | 3509.00 | 3355.00 | 3936.00 | 2324.00 | |
| | Treatment average | 3623.00a | 3819.00a | 4041.00a | 2838.00a | |
| | SEM Treatment | | 290.00 | | | |
| Propionic acid | 0 | 1136.00 | 1230.00 | 1053.00 | 1109.00 | 0.90 |
| | 2 | 1279.00 | 1172.00 | 1026.00 | 983.00 | |
| | 5 | 992.00 | 967.00 | 979.00 | 930.00 | |
| | 9 | 1083.00 | 713.00 | 956.00 | 792.00 | |
| | Treatment average | 1123.00a | 1021.00a | 1003.00a | 953.00a | |
| | SEM Treatment | | 164.00 | | | |
| Butyric acid | 0 | 324.00 | 417.00 | 445.00 | 519.00 | 0.05 |
| | 2 | 412.00 | 510.00 | 537.00 | 406.00 | |
| | 5 | 311.00 | 426.00 | 599.00 | 384.00 | |
| | 9 | 370.00 | 407.00 | 611.00 | 362.00 | |
| | Treatment average | 354.00b | 440.00ab | 547.00a | 418.00ab | |
| | SEM Treatment | | 35.84 | | | |
| Lactic acid | 0 | 121.00 | 148.00 | 109.00 | 93.00 | 0.15 |
| | 2 | 176.00 | 172.00 | 133.00 | 138.00 | |
| | 5 | 132.00 | 139.00 | 107.00 | 112.00 | |
| | 9 | 131.00 | 123.00 | 91.00 | 109.00 | |
| | Treatment average | 130.00a | 145.00a | 109.00a | 113.00a | |
| | SEM Treatment | | 11.19 | | | |
| AC/PR | 0 | 3.34 | 3.35 | 4.43 | 3.48 | 0.54 |
| | 2 | 3.38 | 3.60 | 4.07 | 3.15 | |
| | 5 | 4.71 | 3.93 | 4.39 | 2.93 | |
| | 9 | 3.34 | 4.84 | 4.50 | 3.07 | |
| | Treatment average | 3.69a | 3.93a | 4.34a | 3.16a | |
| | SEM Treatment | | 0.52 | | | |

Different lowercase letters indicate significant differences by Tukey's test ($P < 0.05$). SEM: standard error of the mean; P: significant when $P < 0.05$, by Tukey's test.

However, Blend favored the effective degradation at a low passage rate compared to monensin, probably due to the selection of bacterial species in the rumen (Martínez et al., 2006). Blend provided greater rumen degradation of the dry matter in the 2%/hr rate of passage, which suggests long-lasting effects on the rumen. The greater disappearance of the forage highlights a positive effect on the degradation and supports the Blend as a better choice when compared to Monensin.

Monensin acts on Gram-positive bacteria, which tends to increase the ruminal degradation of the NDF of the feed, a fact that was not observed in the IVNDFD. The study of the action of ionophores on fiber digestion has shown divergent results probably due to the variables related to the interaction between the degradation of the dietary components, the rate of passage of solids and liquids and the interaction in the rumen, these factors influence the response of the additives (Adeniji et al., 2020). Increased degradation rates occurred up to the first 24 hr of incubation, due to the soluble fraction and a higher degradation rate of the potentially degradable fraction. Adding oils can act as a catalyst for fiber degradation, increasing the access of fibrolytic bacteria to polysaccharides of the cell wall present in the diet (Salem et al., 2014).

The effect of ionophores on increasing the proportion of propionate, but not of the acetate butyrate ratio, was not found in our experiment, which is congruent to the results reported by Mourthe et al. (2011). The treatments with

Blend and Croton could decrease the acetic concentration after feeding, but only the Blend showed effects up to 9 hr after the feeding. Control and monensin treatments, in turn, increased the acetic concentration up to 2 hr after treatment, and such effect was stabilized through the subsequent sampling periods.

It is necessary to acknowledge that the effects of feed additives like monensin, phytogenic compounds, and/or croton can be influenced by the forage-to-concentrate ratio in the diet (Chen et al., 2021). However, our study used only forage in the animals´ diet. Forage-based diets have concentrations of short-chain fatty acids ranging from 65 to 70% for acetic acid, from 15 to 25% for propionic acid, and from 5 to 10% for butyric acid (Chilliard et al., 2001), and this resembles the relation found in the present study. Nevertheless, Li et al. (2022) demonstrated that lower acetate: propionate ratios favor the digestibility of organic matter in food, which entirely agrees with the results we obtained during the tests with the Blend.

## Conclusion

The inclusion of a Blend of essential oils at 2% increased the degradability of the diet's dry matter. The study of diet supplements is essential to improving the performance of animals receiving diets with low nutritional value.

## Conflict of Interest

The authors declare that they have no conflict of interest and that they have no financial interests.

## Ethics Statement

The Ethics Committee on the Use of Animals at the Federal University of Goiás (CEUA-UFG) granted ethics approval for this study, which generated protocol No. 069/15.

## Acknowledgements

We thank Dr Leandro do Prado Assunção for the statistical consulting.

## References

Adeniji YA, Sanni MO, Mutassim AM. Review: manipulation of the rumen using additives. Rep Opin J. 2020;12(2):1-6. http://doi.org/10.7537/marsroj120220.01.

Albornoz RI, Harvatine KJ, Allen MS. Diet starch concentration and starch fermentability affect energy intake and energy balance of cows in the early postpartum period. J Dairy Sci. 2019;102(6):5161-71. http://doi.org/10.3168/jds.2018-15634. PMid:30981484.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils: a review. Food Chem Toxicol. 2008;46(2):446-75. http://doi.org/10.1016/j.fct.2007.09.106. PMid:17996351.

Benchaar C, Duynisveld JL, Charmley E. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. Can J Anim Sci. 2006;86(1):91-6.

Bouda J, Quiroz-Rocha GF, Gonzalez FHD. Importância da coleta e análise de líquido ruminal e urina. In: Gonzalez FHD, Borges JB, Cecim M, editors. Uso de provas de campo e laboratório em doenças metabólicas e ruminais de bovinos. Porto Alegre: UFRGS; 2000. p. 13-6.

Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. Clin Chem. 1962;8(2):130-2. http://doi.org/10.1093/clinchem/8.2.130. PMid:13878063.

Chen H, Wang C, Huasai S, Chen A. Effects of dietary forage to concentrate ratio on nutrient digestibility, ruminal fermentation and rumen bacterial composition in Angus cows. Sci Rep. 2021;11(1):17023. http://doi.org/10.1038/s41598-021-96580-5. PMid:34426627.

Chilliard Y, Ferlay A, Doreau M. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest Prod Sci. 2001;70(1):31-48. http://doi.org/10.1016/S0301-6226(01)00196-8.

Dehority BA. Evaluation of subsampling and fixation procedures used for counting rumen protozoa. Appl Environ Microbiol. 1984;48(1):182-5. http://doi.org/10.1128/aem.48.1.182-185.1984. PMid:6476828.

Díaz TG, Branco AF, Ítavo LCV, Santos GT, Carvalho ST, Teodoro AL, Oliveira RL. In vitro gas production kinetics and digestibility in ruminant diets with different levels of cashew nut shell liquid. Semina: Ciênc Agrár. 2018;39(4):1669. http://doi.org/10.5433/1679-0359.2018v39n4p1669.

European Union. Commission notice: guidelines for the prudent use of antimicrobials in veterinary medicine [Internet]. Official Journal of the European Union; Luxembourg; 2015 [cited 14 Mar 2024], nº 299). Available

from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52015XC0911(01).

Galyean ML, Goetsch AL. Utilization of forage fiber by ruminants. In: Hatfield RD, Buxton DR, Jung HG, Mertens DR, Ralph J, Weimer PJ, editors. Forage cell wall structure and digestibility madison. Madison: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 1993. http://doi.org/10.2134/1993.foragecellwall.c2.

Goetz BM, Horst EA, Mayorga EJ, Abeyta MA, Rodriguez-Jimenez S, Carta S, Lourenco JM, Callaway TR, Hikita C, Watanabe T, Baumgard LH. Effects of cashew nut shell extract supplementation on production, rumen fermentation, metabolism, and inflammatory biomarkers in transition dairy cows. J Dairy Sci. 2023;106(12):9843-54. http://doi.org/10.3168/jds.2023-23563. PMid:37641319.

Grummer RR, Mashek DG, Hayirli A. Dry matter intake and energy balance in the transition period. Vet Clin North Am Food Anim Pract. 2004;20(3):447-70. http://doi.org/10.1016/j.cvfa.2004.06.013. PMid:15471620.

Gurgel LA. Estudo das atividades antifúngica, anti-inflamatória intestinal e antinocieptiva visceral do látex do *Croton urucurana* Baill. [thesis]. Fortaleza: Universidade Federal do Ceará; 2005.

Kamra DN, Agarwal N, Chaudhary LC. Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds. Int Congr Ser. 2006;1293:156-63. http://doi.org/10.1016/j.ics.2006.02.002.

Latimer Junior GW, editor. Official methods of analysis of AOAC International. 21st ed. Arlington: AOAC International; 2019. 3390 p. http://doi.org/10.1093/9780197610145.001.0001.

Li MM, Ghimire S, Wenner BA, Kohn RA, Firkins JL, Gill B, Hanigan MD. Effects of acetate, propionate, and pH on volatile fatty acid thermodynamics in continuous cultures of ruminal contents. J Dairy Sci. 2022;105(11):8879-97. http://doi.org/10.3168/jds.2022-22084 PMid:36085109.

Martínez S, Madrid J, Hernández F, Megías MD, Sotomayor JA, Jordán MJ. Effect of thyme essential oils (*Thymus hyemalis* and *Thymus zygis*) and monensin on in vitro ruminal degradation and volatile fatty acid production. J Agric Food Chem. 2006;54(18):6598-602. http://doi.org/10.1021/jf060985p PMid:16939315.

McGaw L. Use of plant-derived extracts and essential oils against multidrug-resistant bacteria affecting animal health and production. In: Rai MK, Kon KV, editors. Fighting multidrug resistance with herbal extracts, essential oils and their components. Amsterdam: Academic Press-Elsevier; 2013. p. 191-203. http://doi.org/10.1016/B978-0-12-398539-2.00013-6.

McMeniman JP, Tedeschi LO, Defoor PJ, Galyean ML. Development and evaluation of feeding-period average dry matter intake prediction equations from a commercial feedlot database. J Anim Sci. 2010;88(9):3009-17. http://doi.org/10.2527/jas.2009-2626. PMid:20453082.

Mourthe MHF, Reis RB, Ladeira MM, Souza RC, Coelho SG, Saturnino HM. Multiple supplement with ionophores for grazing steers: intake, ruminal fermentation, and in situ degradability. Arq Bras Med Vet Zootec. 2011;63(1):129-35. http://doi.org/10.1590/S0102-09352011000100020.

Nader TT. Atividade antibacteriana in vitro de extratos e substâncias isoladas de espécies de Croton frente *Staphybcoccus aureus* causador de mastite bovina. [thesis]. Jaboticabal: Universidade Estadual Paulista; 2014.

Naz R, Bano A. Antimicrobial potential of *Ricinus communis* leaf extracts in different solvents against pathogenic bacterial and fungal strains. Asian Pac J Trop Biomed. 2012;2(12):944-7. http://doi.org/10.1016/S2221-1691(13)60004-0. PMid:23593573.

Nocek JE. Evaluation of specific variables affecting in situ estimates of ruminal dry matter and protein digestion. J Anim Sci. 1985;60(5):1347-58. http://doi.org/10.2527/jas1985.6051347x.

Oliveira AP, Reis RA, Bertipaglia LMA, Melo GMP, Berchielli TT, Oliveira JA, Casagrande DR, Balsalobre MAA. Substituição de monensina sódica por bicarbonato de sódio em dietas de novilhas confinadas. Arq Bras Med Vet Zootec. 2013;65(4):1149-57. http://doi.org/10.1590/S0102-09352013000400030.

Oliveira IS, Lima JCS, Silva RM, Martins DTO. Triagem da atividade antibacteriana in vitro do látex e extratos de *Croton urucurana* Baillon. Rev Bras Farmacogn. 2008;18(4):587-93. http://doi.org/10.1590/S0102-695X2008000400016.

Orskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric Sci. 1979;92(2):499-503. http://doi.org/10.1017/S0021859600063048.

Osmari MP, Matos LF, Salab BL, Diaz TG, Giotto FM. Cashew nut shell liquid: characteristics and applicability

in animal production. Pubvet. 2015;9(3):143-9. http://doi.org/10.22256/pubvet.v9n3.143-149.

Palma ASV, Barra NC, Herling VR, Gomide CA, Netto AS. Supplementation with nutritional additives and organic minerals on the performance of newly weaned Nellore calves on pasture. Pesq Agropec Bras. 2015;50(11):1071-8. http://doi.org/10.1590/S0100-204X2015001100010.

Park T, Yang C, Yu Z. Specific inhibitors of lysozyme and peptidases inhibit the growth of the rumen protozoan Entodinium caudatum without decreasing feed digestion or fermentation in vitro. J Appl Microbiol. 2019;127(3):670-82. http://doi.org/10.1111/jam.14341. PMid:31165532.

Patra AK. Meta-analyses of effects of phytochemicals on digestibility and rumen fermentation characteristics associated with methanogenesis. J Sci Food Agric. 2010;90(15):2700-8. http://doi.org/10.1002/jsfa.4143. PMid:20740549.

Patra AK. Enteric methane mitigation technologies for ruminant livestock: a synthesis of current research and future directions. Environ Monit Assess. 2012;184(4):1929-52. http://doi.org/10.1007/s10661-011-2090-y. PMid:21547374.

Patra AK, Saxena J. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry. 2010;71(11-12):1198-222. http://doi.org/10.1016/j.phytochem.2010.05.010. PMid:20570294.

Patra AK, Yu Z. Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Appl Environ Microbiol. 2012;78(12):4271-80. http://doi.org/10.1128/AEM.00309-12. PMid:22492451.

R Core Team. R: a language and environment for statistical computing [software]. Vienna: R Foundation for Statistical Computing; 2020 [cited 2022 Oct 12]. Available from: https://www.r-project.org/.

Salem AZM, Kholif AE, Elghandour MMY, Buendía G, Mariezcurrena MD, Hernandez SR, Camacho LM. Influence of oral administration of *Salix babylonica* extract on milk production and composition in dairy cows. Ital J Anim Sci. 2014;13(1):10-4. http://doi.org/10.4081/ijas.2014.2978.

Salihu BZ, Gana AK, Apuyor BO. Castor oil plant (*Ricinus communis* L.): botany, ecology and uses. Int J Sci Res. 2014;3(5):1333-41. http://doi.org/10.1007/978-1-4020-9827-7.

Scarpa A, Guerci A. Various uses of the castor oil plant (*Ricinus communis* L.) a review. J Ethnopharmacol. 1982;5(2):117-37. http://doi.org/10.1016/0378-8741(82)90038-1. PMid:7035750.

Schelling GT. Monensin mode of action in the rumen. J Anim Sci. 1984;58(6):1518-27. http://doi.org/10.2527/jas1984.5861518x. PMid:6378867.

Silva LB, Silva W, Macedo MLR, Peres MTLP. Effects of croton urucurana extracts and crude resin on *Anagasta kuehniella* (Lepidoptera: Pyralidae). Braz Arch Biol Technol. 2009;52(3):653-64. http://doi.org/10.1590/S1516-89132009000300018.

Simionatto E, Bonani VFL, Morel AF, Poppi NR, Raposo Júnior JL, Stuker CZ, Peruzzo GM, Peres MTLP, Hess SC. Chemical composition and evaluation of antibacterial and antioxidant activities of the essential oil of *Croton urucurana* Baillon (Euphorbiaceae) stem bark. J Braz Chem Soc. 2007;5(18):879-85. http://doi.org/10.1590/S0103-50532007000500002.

Soldera CC, Zanella GN, Frasson APZ. Avaliação da atividade antibacteriana de *Croton urucurana*. Contexto Saúde. 2010;10(19):25-31.

Tilley JMA, Terry RA. A two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. 1963;18(2):104-11. http://doi.org/10.1111/j.1365-2494.1963.tb00335.x.

**Financial Support:** The researcher Graciele Araújo de Oliveira Caetano received a scholarship from the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES - Grant number: 52001016).