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# Effects of essential oils of Cashew and Castor on intake, digestibility, ruminal fermentation and purine derivatives in beef cattle fed high grain diets

# Efeito de óleos essenciais de Caju e Mamona sobre a ingestão, digestibilidade, fermentação ruminal e derivados de purina em bovinos de corte alimentados com dietas alto grão

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## Abstract

The objective of this study was to determine the effects of essential oils of *Anacardium occidentale* (Cashew) and *Ricinus communis* (Castor) on intake, digestibility, ruminal fermentation and excretion of purine derivatives in beef cattle fed high grain diets. Five Nellore steers fitted with ruminal cannula were used in a 5x5 Latin square design (21 days period). The treatments were control MON (0.2 g monensin animal day$^{-1}$) and 1, 2, 4 and 8 g EO animal day$^{-1}$ (Essential oils - Oligobasics®). All the animals had a basal diet, corn silage-based total mixed ration (TMR) of 80:20 concentrate:forage ratio. Intake, digestibility, ruminal fermentation and excretion of purine derivatives were determined over five consecutive days in each period. Intake and digestibility of dry matter (DM), neutral detergent fibre (NDF) and crude protein (CP) were not influenced by treatments ($P > 0.05$). However, increasing levels of EO showed a quadratic effect ($P < 0.014$) above 2 g animal day$^{-1}$ on the ruminal pH. The excretion of allantoin and uric acid were not influenced by the treatments ($P > 0.05$), but levels above 2 g day decreased the synthesis of microbial proteins ($P < 0.05$). It is concluded that the EO of *A. occidentale* and *R. comunnis* effectively controlled ruminal fermentation as well as sodium monensin at the studied levels. EOs have the potential to be used in place of monensin in the studied levels.
**Key words:** Additives. Antibiotics. Bioactive compounds. Ionophores. Monensin. Plant extracts.

## Resumo

O objetivo deste estudo foi determinar o efeito dos óleos essenciais de *Anacardium occidentale* (Caju) e *Ricinus communis* (Mamona) sobre a ingestão, digestibilidade, fermentação ruminal e excreção de derivados de purina em bovinos de corte alimentados com dietas alto grão. Cinco novilhos da raça Nelore, com cânula ruminal, foram utilizado um delineamento quadrado latino 5x5 (período de 21 dias). Os tratamentos foram controle MON (0,2 g monensina animal dia$^{-1}$) e 1, 2, 4 e 8 g OE animal dia$^{-1}$ (óleos essenciais - Oligobasics®). Todos os animais foram alimentados com uma dieta basal 20:80 volumoso: concentrado. Ingestão, digestibilidade, fermentação ruminal e excreção de derivados de purina foram determinados ao longo de cinco dias consecutivos em cada período. A ingestão e a

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digestibilidade da matéria seca (MS), fibra em detergente neutro (FDN) e proteína bruta (PB) não foram influenciadas pelos tratamentos (P > 0,05). No entanto, níveis crescentes de OE apresentaram efeito quadrático (P < 0,014) acima de 2 g animal dia$^{-1}$ no pH ruminal. A excreção de alantoína e ácido úrico não foi influenciada pelos tratamentos (P > 0,05), mas níveis acima de 2 g dia$^{-1}$ diminuíram a síntese de proteínas microbianas (P < 0,05). Conclui-se que o OE de *A. occidentale* e *R. comunnis* controlam efetivamente a fermentação ruminal, assim como a monensina sódica, nos níveis estudados, podendo substituir a monensina nos níveis estudados.
**Palavras-chave**: Aditivos. Antibióticos. Compostos bioativos. Ionóforos. Monensina. Extratos vegetais.

## Introduction

To improve the productivity in high performance animals is necessary the inclusion of grain in diets. However, in high grain diets the use of additives is necessary to avoid ruminal acidosis. Antibiotics as ionophores have an essential role worldwide to improve feed conversion, decrease methane production (STEINFELD et al., 2006) and control ruminal fermentation in high grain diets to prevent subacute ruminal acidosis (SARA) (RUSSELL; STROBEL, 1989; KRAUSE; OETZEL, 2006). One example is monensin sodium, which has had a proven effect on ruminal fermentation (BERGEN; BATES, 1984; MCGUFFEY et al., 2001). However, to avoid the negative effect of antibiotics, is necessary explore if biocompounds of plants present in the essential oils (EO) can enhance the ruminal fermentation. Antibiotics as regulators of ruminal fermentation can generate residues, with detrimental effects on humans, such as resistance to antibiotics (KHACHATOURIANS, 1998; VAN DEN BOGAARD; STOBBERINGH, 2000; DEWULF et al., 2007; DODDS, 2017; GRENNI et al., 2018, JUTKINA et al., 2018). The bioactive compounds of plants present in the essential oils are an alternative for the control of ruminal fermentation (MEDJEKAL et al., 2017; SOLTAN et al., 2018a). The essential oils (EO) contain secondary metabolites with a modulating effect at rumen fermentation (YANG et al., 2007; BENCHAAR et al., 2008; SOLTAN et al., 2018b). Bioactive compounds of tropical plants have shown a positive effect in methane depression by up to 25%, without negatively affecting digestibility (MEDJEKAL et al., 2017; SOLTAN et al., 2018a) and ruminal modulation (BABAYEMI et al., 2004; KHOLIF et al., 2018), due to bioactive compounds with antimicrobial effects (AUMEERUDDY-ELALFI et al., 2016). The mechanisms that confer EO their antimicrobial properties are still not well understood (LAMBERT et al., 2001) with evidence of change to the bacterial cell membrane due to changes in electron transport and the ion gradient, in the translocation of proteins, phosphorylation and other enzyme-dependent reactions, similar to ionophores (BURT, 2004). Thus, considering the varied range of chemical compounds present in oils, antimicrobial activity may not be mediated by a single specific mechanism, with synergy between modes of action (BAKKALI et al., 2008). The use of EO in mixture can provide potentiated effect. Antimicrobial properties have been described for *A. occidentale* (Cashew) and *R. communis* (Castor) (VIEIRA et al., 2001; MUROI et al., 2004; TREVISAN et al., 2006). Several phenolic compounds and terpenes have been identified in *A. occidentale*, such as cardol, cardonol β-ocimeno, α-copaeno and δ-cadineno (MAIA et al., 2000; ATTANASI et al., 2009; LOMONACO et al., 2009) with antimicrobial activity (HIMEJIMA; KUBO, 1991; NAGABHUSHANA et al., 1995; KUBO et al., 2003) mainly on gram positive bacteria, (MUROI; KUBO, 1993). Controlling Gram positives lactate-producing (*Streptococcus bovis*), lactate rate production decrease, allowing growth of lactic acid – utilizing bacteria (*Selenomonas ruminantium* y *Megasphaera elsdenii*), because the rate of use of lactate usually exceed his production (BELANCHE et al., 2012). Several compounds

with antimicrobial and anti-fungal activity have been associated with *R. communis* (RIBEIRO et al., 2016), such as α-thujone and 1,8-cineole, α-pinene camphor and camphene (KADRI et al., 2011; JENA; GUPTA, 2012; ZARAI et al., 2012). The objective of this study was to determine the effects of EO of *A. occidentale* and *R. communis* in mixture on intake, digestibility, ruminal fermentation and excretion of purine derivatives in beef cattle fed high grain diets.

## Material and Methods

*Animals, experimental design, and diets*

Five Nellore steers fitted with ruminal cannula with an average body weight (BW) of 270 ± 34 kg, housed in individual pens, were fed twice a day with free access to water. A 5x5 Latin square design was used over five periods (21 days each one). All the animals were fed whit the same basal diet containing 16.4 g kg-1 of crude protein (CP) and 79.0 g kg-1 total digestible nutrients (TDN) on a dry matter (DM) basis according to NCR requirements. This constituted of 20 g kg-1 of corn silage and 80 g kg-1 of concentrate corn grain ground, soybean meal, urea and minerals) (Table 1). The Essential-Oligobasics®, containing 11% of cardol, 20% of cardanol and 9% of ricinoleic acid, were placed daily on concentrate. Both oils were produced in Northern Brazil. Vermiculite was used for the solidification of the essential oils formulated by Oligo Basics Agroindustrial Ltd® (Cascavel, Brazil). The treatments were offered to ensure intake of the following amounts of monensin (MON) and EO (Essential oils - Oligobasics®): 1. 0.2 MON g-1; 2. 1 g EO g-1; 3. 2 g EO g-1; 4. 4 g EO g-1 and 5. 8 g EO g g-1.

**Table 1.** Chemical composition of the total mix ration (TMR) and perceptual composition of ingredients used in the experimental basal diet.

| | % of DM |
|---|---|
| Ingredient | |
| Corn Silage | 20.00 |
| Corn grain, ground | 62.18 |
| Soybean meal | 14.84 |
| Urea | 0.74 |
| Dicalcium phosphate | 0.47 |
| Limestone | 0.94 |
| Mineral mixture[1] | 0.83 |
| Chemical composition | |
| Dry matter | 76.99 |
| Crude protein | 16.35 |
| Neutral detergent fibre | 21.95 |
| Ether extract | 3.40 |
| Total digestible nutrients | 79.0 |

[1] Calcium: 130.0g (max.), Phosphorus: 65.0g (min.), Sodium: 135.0g, Sulfur: 12.0g, Magnesium: 12 g, Manganese: 1.050 mg, Cobalt: 63 mg, Iodine: 63 mg, Copper: 1.155 mg, Selenium: 18 mg, Zinc: 3.080 mg and Fluor: 650 mg.

*Intake and digestibility*

Diets were offered twice daily (am/pm). Intake was recorded daily by weighing all the feeds offered and orts left by the steers allow approximately 5% orts. 500 gr of samples of feed ingredients and orts left over were collected daily and were conserved at -20 °C until it was processed by pre-drying in a forced ventilation oven at 55 °C for 72 hours, milled at 1 mm. The experimental periods lasted for 21 days, with the last four days of each period being used to collect at morning samples of feeds, leftovers, ruminal fluid, faeces and urine samples. An external indicator containing chromium oxide to estimate faecal output was placed with the concentrate in the morning feed, at a daily dose of 10 g-1 per animal, from the 7th day of each experimental period. Faecal samples (50 g) in the rectal ampulla, were collected on days 18 and 20 of each experimental period, twice daily at alternate times with 4-hour intervals. The chemical composition was determined using AOAC (2016) methods in dry matter number 930.15, crude protein number 992.15 and ether extract number 920.39, and neutral detergent fibre was determined according to Van Soest et al. (1991) with modification of (MERTENS, 2002). Faecal chromium was determined by atomic absorption spectrophotometry (WILLIAMS et al., 1962) and then used together with the nutrient concentration to determine the flow of nutrients in the faeces (SMITH; REID, 1955). The TDN was calculated according to the equations proposed by Sniffen et al. (1992and the yields are adjusted for maintenance requirements (.05 vs .150 g of cell dry weight per gram of carbohydrate fermented per hour for SC and NSC bacteria, respectively).

*Ruminal fermentation*

Ruminal fluid was collected through ruminal cannula manually within the rumen at 2, 4, 6 and 8 h after the first feeding. Samples of 150 mL were withdrawn. The pH of the ruminal fluid was measured immediately after sampling (Digimed DM20 pH meter). Samples of 50 mL were acidified with 50% H2SO4 (1:1) and frozen at -20 °C for later determination of voltaic fatty acids (VFA) and ammonia N (NH3-N) concentrations (SANTSCHI et al., 2005). Rumen fluid was taken from the middle part of the rumen by a stomach tube connected to a vacuum pump and then filtered through layers of cheesecloth. The concentration of NH3-N of samples was measured by a calorimetric method according to Chaney and Marbach (1962). About 30 mL were centrifuging a 10 ml sample for 15 min at 4000 xg. To 5 ml supernatant, 1 ml of 250 µl metaphosphoric acid was added. After 30 min, if a precipitate formed, it was removed by a brief centrifuging. The determination of VFA was made in GLC (Hewlett Packard 5890 Series II GC) according methodology of Playne (1985).

*Purine derivatives*

Sample spots of urine were collected on days 19 and 20 of each experimental period, 3 to 4 h after the first feeding, during spontaneous urination. Samples of 15 mL were diluted in 135 mL of H2SO4 with 0.036 N (CHEN et al., 1995) and adjusted to a pH of less than 3 to avoid bacterial destruction of purine derivatives, these were then stored at -20 °C. The total daily urine volume was estimated according to the creatinine concentration (mg/L) in the urine spot sample. This volume was then used to estimate the daily excretions of allantoin and uric acid from each animal. The uric acid, xanthine and hipoxantine analyses were perform according (CHEN et al., 1990). The urine allantoin analyses were performed using the colorimetric method (FUJIHARA et al., 1987; CHEN; GOMES, 1992). Estimates of creatinine and uric acid concentrations in urine were performed using commercial Labtest® kits.

*Statistical analysis*

The experimental design was a 5x5 Latin Square. An ANOVA was performed using the mixed

model methodology in MINITAB 17™ (2014). For variables that were repeated over time, a split-plot arrangement was used (subdivided plots), considering the effect of time and the interaction between time and treatment. The effects of inclusion levels of *A. occidentale* and *R. communis* were analysed by polynomial regression models. Monensin was compared with each level of EO by the Tuckey test. The mathematical model used included period, treatment and animal effects, which were considered as random: $Yijk = \mu + Ai + Pj + Tk + eijk$, where $\mu$ = mean of treatments; Ai = effect of animal i, ranging from 1 to 5; Pj = effect of period j, ranging from 1 to 5; Tk = effect of treatment k, ranging from 1 to 5; eijk = random error. Significant differences were accepted if $P \leq 0.05$.

## Results

No differences were observed in intake and digestibility of dry matter (DM), neutral detergent fibre (NDF), crude protein (CP), ether extract (EE) and non-fibre carbohydrates (NFC), between the use of EO and monensin (Table 2). No differences were observed for the inclusions of EO ($P > 0.05$).

**Table 2.** Intake, and digestibility in beef cattle supplemented with Monensin and levels of Essential Oils of *A. occidentale* and *R. communis.*

| Item | MON[1] | +EO g day$^{-1}$ | | | | SEM[2] | P-Value | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 8 | | Linear | Quadratic |
| Intake, (kg day$^{-1}$) | | | | | | | | |
| Dry matter | 6.32 | 6.39 | 6.98 | 6.70 | 6.50 | 0.95 | 0.80 | 0.59 |
| Neutral detergent fibre | 1.59 | 1.56 | 1.77 | 1.66 | 1.63 | 0.23 | 0.95 | 0.67 |
| Crude protein | 1.09 | 1.09 | 1.20 | 1.15 | 1.12 | 0.17 | 0.89 | 0.60 |
| Ether extract | 0.17 | 0.18 | 0.19 | 0.18 | 0.18 | 0.03 | 0.62 | 0.92 |
| Non-fibre carbohydrates | 3.11 | 3.19 | 3.43 | 3.33 | 3.21 | 0.47 | 0.71 | 0.53 |
| Digestibility, (g kg$^{-1}$) | | | | | | | | |
| Dry matter | 688.8 | 580.1 | 686.2 | 654.0 | 580.6 | 4.25 | 0.67 | 0.43 |
| Neutral detergent fibre | 582.3 | 436.1 | 604.2 | 560.3 | 449.5 | 6.42 | 0.73 | 0.42 |
| Crude protein | 660 | 521.1 | 632.9 | 651.3 | 588.8 | 5.28 | 0.77 | 0.28 |
| Ether extract | 717.5 | 644.8 | 760.6 | 734.9 | 612.1 | 3.91 | 0.52 | 0.34 |
| Non-fibre carbohydrates | 780.9 | 720.6 | 767 | 714.2 | 667.9 | 4.64 | 0.19 | 0.71 |
| TDN[3] | 720.1 | 645.1 | 739.9 | 704.8 | 620.4 | 4.12 | 0.50 | 0.42 |

1MON = 0.2 g day-1 Monensin; 2SEM: standard error of means. 3TDN = total digestible nutrients. +Essential oil containing 11% of cardol, 20% of cardanol and 9% of ricinoleic acid.

In ruminal fermentation, the pH was affected by EO inclusion ($P < 0.05$) (Table 3). Ruminal concentrations of ammonia (N-NH3) were not influenced by the treatments (Table 3). No effect was observed for VFA with any inclusion level of EO ($P < 0.05$) (Table 3). The ruminal concentration of VFA not was influenced by EO, with acetate:propionate:butyrate molar ratios close to 50:35:15, respectively (WATKINS et al., 2013).

**Table 3**. Ruminal fermentation in beef cattle supplemented with Monensin and increasing levels of essential oils of *A. occidentale* and *R. communis.*

| Item | +EO Inclusions g day$^{-1}$ | | | | SEM[2] | P-Value | |
|---|---|---|---|---|---|---|---|
| | MON[1] | 1 | 2 | 4 | 8 | | Linear | Quadratic |
| pH | 6.35 | 6.70 | 6.68 | 6.53 | 6.16 | 0.11 | 0.527 | 0.014 |
| $NH_3$-N, mg/100 mL | 22.73 | 23.16 | 22.05 | 22.75 | 23.15 | 2.09 | 0.835 | 0.353 |
| VFA, mM/L | | | | | | | | |
| Acetate | 48.56 | 56.09 | 48.90 | 50.07 | 49.81 | 2.57 | 0.358 | 0.307 |
| Propionate | 27.01 | 59.09 | 48.90 | 50.07 | 49.81 | 3.93 | 0.596 | 0.863 |
| Butyrate | 15.38 | 28.93 | 34.22 | 29.64 | 33.17 | 1.14 | 0.169 | 0.929 |
| Acetate:Propionate | 2.10 | 2.31 | 2.04 | 2.37 | 2.25 | 0.21 | 0.863 | 0.796 |

1MON = 0.2 g day-1 Monensin; 2SEM: standard error of means. +Essential oil containing 11% of cardol, 20% of cardanol and 9% of ricinoleic acid.

Intake, blood nitrogen, daily excretions of allantoin, uric acid and purines, were also not affected by the inclusion of EO (Table 4). Nevertheless, faecal excretion of N and efficiency of microbial protein synthesis (EMPS) were affected by EO levels.

**Table 4.** Excretion of urinary purine derivatives (PD), and microbial N supply in cattle supplemented with Monensin and increasing levels of essential oils of *A. occidentale* and *R. communis.*

| Item | MON[1] | EO Inclusions g day$^{-1}$ | | | | SEM[2] | P-Value | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 8 | | Linear | Quadratic |
| BUN[3], mg dl | 15.3 | 13.4 | 14.4 | 15.4 | 15.0 | 1.6 | 0.131 | 0.237 |
| N intake, g day$^{-1}$ | 174.9 | 174.9 | 191.5 | 184.0 | 179.2 | 26.8 | 0.907 | 0.326 |
| Faecal N, g day$^{-1}$ | 56.3 | 76.6 | 61.8 | 61.5 | 72.1 | 5.5 | 0.764 | 0.040 |
| Allantoin excretion, mmol day$^{-1}$ | 237.2 | 186.9 | 234.9 | 246.4 | 165.6 | 47.5 | 0.824 | 0.122 |
| Uric acid excretion, mmol day$^{-1}$ | 2.7 | 2.4 | 3.3 | 2.4 | 2.2 | 0.7 | 0.562 | 0.525 |
| PD[4], mmol day$^{-1}$ | 239.9 | 189.3 | 238.2 | 248.7 | 167.7 | 48.0 | 0.819 | 0.115 |
| Microbial protein, g N day$^{-1}$ | 186.3 | 142.4 | 184.6 | 193.9 | 124.3 | 41.1 | 0.825 | 0.116 |
| EMPS[5], g N kg TDN | 238.8 | 176.7 | 239.7 | 239.2 | 163.7 | 51.5 | 0.873 | 0.024 |

1MON = 0.2 g day-1 Monensin; 2SEM: standard error of means. 3BUN: Blood urea nitrogen; 4PD: Purine derivates; 5EMPS = efficiency of microbial protein synthesis. +Essential oil containing 11% of cardol, 20% of cardanol and 9% of ricinoleic acid.

## Discussion

In this study the EO had been a similar effect as monensin in the fermentation, without negatively affecting intake or digestibility.

These results are similar to Barreto et al. (2014), who evaluated the inclusion of a mixture of EO from *R. communis* and *A. occidentale* at 3 g day-1 in crossbred bulls, and found no negative effect on intake and digestibility.

EO has a similar effect to monensin (CHAO et al., 2000; GOÑI et al., 2009), which exerts its antimicrobial activity on Gram positive bacteria, acting on the lipid membrane where they accumulate (BENCHAAR; GREATHEAD, 2011). They exert their antimicrobial

effect by altering the permeability of the membrane, interrupting the transport processes of the cells and the interaction with the membrane proteins and other cytoplasmic components (KHORSHIDIAN et al., 2018). EO can improve feed efficiency in a similar way to monensin (MCGUFFEY et al., 2001; BUSQUET et al., 2006; CASTILLEJOS et al., 2006; BENCHAAR et al., 2008).

In beef cattle, Benchaar et al. (2006) evaluated the effects of dietary addition of monensin (33 mg $kg^{-1}$ DM) and different doses of a mixture of EO (2, 3 and 4 g day-1) with thymol, eugenol, vanillin and limonene, and observed that DM intake and feed efficiency (ADG to DM intake ratio) were not affected ($P > 0.05$) by addition of EO, but that EO had a quadratic effect ($P < 0.05$), which was highest at the dose of 2 $gday^{-1}$.

More doses need to be tested, lower doses may have no effect, or the higher doses may have a deleterious effect in ruminal population for the antimicrobial effect of the EO.

In this experiment, different levels of EO showed a quadratic effect ($P < 0.014$) with the inclusions of EO above 2 $g^{-1}$ animal. The ruminal pH remained at an average of 6.48, which is considered high given the high percentage of grains in the diet (80%), demonstrating that both monensin and EO of *A. occidentale* and *R. communis* were efficient in controlling the ruminal acidity. When ruminants are grazing, the pH remains close to neutrality. However, when diets contain large amounts of cereals or grains, the high fermentation rate can lower the pH dramatically, contributing to the appearance of acidosis.

Similar effects have been reported by Benchaar et al. (2006), who observed decreased pH values in cows supplemented with 2 g $day^{-1}$ of a compound with several essential oils (Crina Ruminants®) compared to animals without supplementation; these values were between 6.4 and 6.3, which are similar to values found in the present study. Evans and Martin (2000) also reported an *in vitro* experiment where the addition of 400 mg $L^{-1}$ thymol ruminal liquid increased the pH over 24 hours, yet no effect was observed at lower doses of 50, 100 and 200 mg $L^{-1}$.

In the present study, levels above 2 g EO $day^{-1}$ had a quadratic effect on pH. These results indicate that EO of *A. occidentale* and *R. communis* may have strong antimicrobial activity and lower doses. Patra and Yu (2012) evaluated five EO - clove, eucalyptus, garlic, oregano and mint - in different levels in three doses *in vitro* (0, 25, 0.50 and 1.0 g $L^{-1}$) and found negative effects on the digestibility of DM and fibre in the high doses, possibly due to a negative effect on ruminal populations, since EO decreased the abundance of archaea, protozoa and cellulolytic bacteria. However, bacterial diversity in low and medium doses increase for all EO, except for oregano. They concluded that the effectiveness of EO may be greater at lower doses and as a mixture of EO.

In the present study, the ruminal ammonia concentrations were similar to MON, and not influenced by EO inclusion levels ($P > 0.05$). Observations on the efficiency of the use of EO in reducing the number of hypper-ammonia bacteria, the rate of deamination and consequently the rate of ammonia production, with a corresponding increase in the amount of by-pass protein reaching the small intestine were described (ANDO et al., 2003; MCINTOSH et al., 2003; CASTILLEJOS et al., 2007; SZUMACHER-STRABEL; CIEŚLAK, 2012). Ahmed et al. (2014) conducted *in vitro* and *in vivo* experiments to investigate the potential impacts of different levels of an EO mixture (eucalyptus, cinnamon, mint, thyme and lemon) in three treatments, 0, 0.5 and 1 mL EO $day^{-1}$, and found a decrease in the digestibility of CP without negative impacts on ruminal parameters.

The ruminal concentration of VFA not was influenced by the treatments, probably because the animals was adapted to use of EO. The concentrations and proportions of VFA are not always affected by

the inclusion of EO (BEAUCHEMIN; MCGINN, 2006; NEWBOLD et al., 2004). It is likely that the production and proportion of VFA in the rumen depends on the diet of the animals (BENCHAAR et al., 2008). Data on the effects of EO on VFA are variable, because the ruminal microorganisms can adapt to the use of EO (BENCHAAR, et al., 2008). Soltan et al. (2018b), in canulated sheep with a diet of 50:50 Concentrate:Forage with a microencapsulated blend of EO (cinnamaldehyde, eugenol, carvacrol, and capsicum oleoresin) in three treatments - control, 200 mg EO and 400 mg EO - observed an effect at day 3 for VFA; however, at days 7 and 15, no differences were found. More consistent results on ruminal fermentation were observed using EO of thyme, oregano, cinnamon and garlic or their main components (thymol, carvacrol, cinnamaldehyde and allicin, respectively) (COBELLIS et al., 2016). Kholif et al. (2018) evaluated the effect of an EO blend of capsicum and thymus in ewes with a diet of 60:40 concentrate:forage, and found increased ruminal VFA (13.1%) and increased milk production (11.7%).

Similarly, most of the EO compounds at high doses have demonstrated their antimicrobial activity by decreasing total VFA concentration (CASTILLEJOS et al., 2006; KAHVAND; MALECKY, 2018). In the present study, the VFA have not been affected, this effect can be explained for the lower doses used. However, it is necessary to study the effect major inclusions to observe the effect on the production of VFA.

The intake, faecal and blood nitrogen and the synthesis of microbial protein were also not affected by the inclusion of EO. The microbial protein in the rumen is maximised when the ratio of available energy (fermentable organic matter) to protein (nitrogen) is optimised. When there is an excess of nitrogen relative to energy, or high N degradation for deamination in the rumen, ruminal ammonia concentration increases (BACH et al., 2005). Blood urea nitrogen (BUN) is highly correlated with ruminal ammonia (HAMMOND, 1997). In this study, the effect of EO inclusions of *A. occidentale* and *R. communis* on NH3 and BUN was not observed. Nevertheless, levels above 2 g EO animal day$^{-1}$ presented a quadratic effect ($P < 0.05$) in the efficiency of microbial protein synthesis (EMPS. Like monensin, EO have an effect on the hyper-ammonia producing bacteria, which can be helpful in decreasing N losses (SZUMACHER-STRABEL; CIEŚLAK, 2012).

The allantoin:purine derivatives ratio average was 80%. This value was consistent with the ratio found by (CHEN; GOMES, 1992), who considered that the proportion of allantoin in purine derivatives ranges from 80 to 85%. In relation to the proportion of uric acid in the purine derivatives, Chen and Gomes (1992) considered the ideal value to be 15 to 20%, which is considered constant in one animal, but variable among the animals. In this experiment, the ratio of uric acid to purine derivatives was 20.4%. The mean values for microbial protein synthesis efficiency of 211.6 g TDN kg$^{-1}$ were above the NRC requirement of 130 g TDN kg$^{-1}$ (2006).

## Conclusions

The essential oils of *A. occidentale* and *R. communis* in high grain diets for beef cattle can replace monensin without affecting intake, digestibility, ruminal fermentation or synthesis of microbial proteins. Levels above 2 g animal day$^{-1}$ of essential oils presented effects on ruminal pH and in the efficiency of microbial protein synthesis. Further studies are necessary to evaluate the level in g day$^{-1}$ to reach the potential of these essential oils in diet to avoid negative effects in ruminal microbiota.

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