

# Use of cashew and castor essential oils to improve fibre digestibility in high forage diets: digestibility, ruminal fermentation and microbial protein synthesis

## Uso de óleos essenciais de caju e mamona para melhorar a digestibilidade da fibra: digestibilidade, fermentação ruminal e síntese de proteína microbiana

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### Highlights:

Cashew and castor essential oils at 2 g day<sup>-1</sup> and mixed had enhanced effects.

At 2 g day<sup>-1</sup> this EO improved fibre digestion in high forage diets.

At lower levels EO could modulate ruminal fermentation, increasing the VFA.

### Abstract

High forage diets in the tropics are less efficient and more contaminating due to high energy losses as methane and the low digestibility of tropical forages. The objective of the study was to determine the effects of essential oils (EO) of *Anacardium occidentale* and *Ricinus communis* as additives in high forage diets on intake, digestibility, ruminal fermentation and synthesis of microbial protein. Four 'Holstein' steers fitted with ruminal cannula were used in a 4 × 4 Latin square design (21-day period). The treatments were 1, 2, 4 and 8 g day<sup>-1</sup> EO per animal (essential oils, Oligobasics<sup>®</sup>). The diet was total mixed ration (TMR) of *Brachiaria* hay (*Brachiaria humidicola* cv. Lanero) and concentrate (ground corn, soybean meal, ammonium sulphate, urea and minerals) in an 80:20 ratio. Differences were observed in the digestibility of neutral detergent fibre (NDF) and total digestible nutrients (TDN) in levels above 2 g day<sup>-1</sup> EO (P < 0.05). The VFA (volatile fatty acids) acetate and propionate were higher in diets with 2 g day<sup>-1</sup> EO (P < 0.05), without affecting the acetate:propionate ratio. Levels above 2 g day<sup>-1</sup> EO negatively affected the faecal N excretion (P < 0.05). The inclusions of *A. occidentale* and *R. communis* EOs at 2 g day<sup>-1</sup> per day animal improved fibre digestion and decreased N excretion. These results indicate that this mixture of EOs may have strong antimicrobial activity at lower doses. The N losses, fibre digestion and digestibility could be improved with the use of cashew and castor essential oils in high forage diets.

**Key words:** *Brachiaria humidicola*. Biocompounds. Grazing. Methane. Natural additives.

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## Resumo

As dietas no trópico possuem altas proporções de forragem, sendo menos eficientes e mais contaminantes devido às altas perdas de energia na forma de metano e baixa digestibilidade das forragens tropicais. O objetivo do estudo foi determinar os efeitos dos óleos essenciais (OE) de caju (*Anacardium occidentale*) e mamona (*Ricinus communis*) como aditivos em dietas alto forragem sobre consumo, digestibilidade, fermentação ruminal e síntese de proteína microbiana. Quatro novilhos da raça Holandesa, com cânula ruminal, foram utilizados em delineamento em quadrado latino 4x4. Os tratamentos foram 1, 2, 4 e 8 g dia<sup>-1</sup> OE / animal (óleos essenciais - Oligobasics®). A dieta foi uma ração mista total (TMR) de feno de *Brachiaria* (*Brachiaria humidicola cv Lanero*) e concentrado (grão de milho moído, farelo de soja, sulfato de amônio, ureia e minerais) na proporção 80:20. Foram observadas diferenças na digestibilidade da fibra em detergente neutro (FDN) e dos nutrientes digestíveis totais (NDT) em níveis acima de 2 g dia<sup>-1</sup> OE (P < 0,05). Os AGV (ácidos graxos voláteis) acetato e propionato foram maiores nas dietas com 2 g dia<sup>-1</sup> OE (P < 0,05), sem afetar a razão acetato: propionato. Níveis acima de 2 g dia<sup>-1</sup> OE afetaram negativamente a excreção fecal de N (P < 0,05). As inclusões de OE de *A. occidentale* e *R. communis* em 2 g dia<sup>-1</sup> OE / animal melhoraram a digestão da fibra e diminuíram a excreção fecal de N. Estes resultados indicam que esta mistura de OE pode ter maior atividade antimicrobiana em doses mais baixas. As perdas de N, digestão da fibra e digestibilidade foram melhoradas, com o uso de óleos essenciais de caju e mamona em dietas com alta inclusão de forragem.

**Palavras-chave:** Aditivos naturais. *Brachiaria humidicola*. Bio-compostos. Metano. Pastejo.

## Introduction

Tropical pasture-based systems to raise beef cattle are the most common feeding system (Godde, Garnett, Thornton, Ash, & Herrero, 2018), although forage diets are less efficient (Allen & de Brauw, 2018) due to the low digestibility of tropical forages and high energy losses in form of methane (Archimède et al., 2011). Tropical pastures are characterised by poorly soluble fibrous fractions and low levels of N (Daiyany et al., 2011). Also, to supply energy requirements, proteins in the diet can be fermented as an energy source using the C skeletons and excreting the nitrogen (N) from the NH<sub>2</sub> amino groups (Owens & Mehmet, 2016).

Biocompounds present in the essential oils (EOs) can modify bacterial cell membrane due to changes in electron transport and the ion gradient, the translocation of proteins, phosphorylation, and other enzyme-dependent reactions, similar to ionophores (Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007; McGuffey, 2017) using free H<sup>+</sup> in ruminal liquid (Cobellis, Trabalza-Marinucci, & Yu, 2016; Tomkins et al., 2015), thus

reducing energy losses by redirecting H<sup>+</sup>, limiting CH<sub>4</sub> formation. Diets that favour a lower acetate: propionate ratio decrease the production of CH<sub>4</sub> (Durmic et al., 2014). Poudel, Froehlich, Casper and St-Pierre (2019), in calves fed calf starter pellets and milk replacer supplemented with a commercial blend of EOs (carvacrol, caryophyllene, p-cymene, cineole, terpinene, and thymol) found that propionate concentrations were higher in EO-fed calves compared to controls, and ruminal bacterial communities also differed greatly. The EOs have modulated the degradation of protein and improve fibre digestion in *in vitro* studies (Lin et al., 2013; Nanon, Suksombat, & Yang, 2014; Patra & Yu, 2012) fitted with ruminal and duodenal fistula, were used to investigate the effects of a mixture of essential oils (EO).

Secondary biocompounds of tropical plants can decrease methane production (Goel & Makkar, 2012). Several compounds with antimicrobial mainly on Gram-positive bacteria (Schären et al., 2017) have been associated with cashew (*A. occidentale*) and castor (*R. communis*) (Ajileye, Obuotor, Akinkunmi, & Aderogba, 2015; Ribeiro,

Castro, & Fernandez, 2016) including cardol, cardanol  $\beta$ -ocimeno, ricinoleic acid and others (Ajileye et al., 2015; Jena & Gupta, 2012; Kadri, Gharsallah, Damak, & Gdoura, 2011; Oliveira et al., 2011). Due to the several biocompounds present in Eos, the activity is not mediated by a single mechanism, it is a synergistic and additive effect between biocompounds and the use of EO mixtures can provide a wide spectrum of modulation at low doses (Lv, Liang, Yuan, & Li, 2011; Tak & Isman, 2015).

Several *in vitro* works have shown the effects of the essential oils; however, there is a lack of information to show the effect *in vivo*, and especially with tropical forage (ex. *Brachiaria*) with high fibre and low protein content. The objective of this study was to determine the effects of the essential oils (EOs) of *A. occidentale* and *R. communis* on intake, digestibility, ruminal fermentation, and excretion of purine derivatives in 'Holstein' steers receiving high forage diets.

## Material and Methods

### *Animals, experimental design, and diets*

Four 'Holstein' steers fitted with ruminal cannulae and with average body weights (BW) of  $230 \pm 41$  kg, housed in individual pens, were fed twice a day with free access to water. A  $4 \times 4$  Latin square design was used over four periods (21 days each one). Animals were fed with the same basal diet containing 9.75 % of crude protein (CP), 66.75 %  $\text{g kg}^{-1}$  of neutral detergent fibre (NDF) and 55.60 %<sup>1</sup> total digestible nutrients (TDN) on a dry matter (DM) basis according to National Research Council [NRC] (2006) requirements. This constituted of 80 % of *Brachiaria* hay (*Brachiaria humidicola* cv. Lanero) and 20 % of ground corn, soybean meal, urea and minerals concentrate on a DM basis (Table 1). The Essential-Oligobasics<sup>®</sup>, containing 11% of cardol, 20% of cardanol and 9% of ricinoleic acid, were placed daily on the concentrate. Vermiculite was used for the solidification of the essential oils formulated by Oligo Basics Agroindustrial Ltd<sup>®</sup> (Cascavel, Brazil). Treatments were offered to ensure intake of the following amounts of EOs (Essential oils, Oligobasics<sup>®</sup>): 1, 2, 4 and 8  $\text{g day}^{-1}$  EO.

**Table 1**

**Perceptual composition and chemical composition of the total mix ration (TMR) ingredients used in the experimental basal diet on a dry matter (DM) basis**

Ingredient	TMR
<i>Brachiaria</i> hay, %	80.00
Corn meal, %	14.25
Soybean meal, %	3.95
Urea, %	0.89
Ammonium sulphate, %	0.02
Mineral mixture <sup>1</sup> , %	0.89
<b>Chemical composition</b>	
Crude protein (CP), % DM	9.75
Neutral detergent fibre (NDF), % DM	66.75
Ether extract (EE), % DM	2.07
Total digestible nutrients (TDN), % DM	55.60

<sup>1</sup> Calcium: 130,0  $\text{g kg}^{-1}$  (máx.), Phosphorus: 65,0  $\text{g kg}^{-1}$  (min.), Sodium: 135,0  $\text{g kg}^{-1}$ , Sulfur: 12,0  $\text{g kg}^{-1}$ , Magnesium: 12  $\text{g kg}^{-1}$ , Manganese: 1.050  $\text{mg kg}^{-1}$ , Cobalt: 63  $\text{mg kg}^{-1}$ , Iodine: 63  $\text{mg kg}^{-1}$ , Copper: 1.155  $\text{mg kg}^{-1}$ , Selenium: 18  $\text{mg kg}^{-1}$ , Zinc: 3.08  $\text{mg kg}^{-1}$  and Fluor: 650  $\text{mg kg}^{-1}$ .

### *Intake and digestibility*

Diets were offered twice daily (am/pm). Intake was recorded daily by weighing all the feeds offered to and orts left by the steers. There were 500 g of samples of feed ingredients and remaining orts collected daily, and they were conserved at  $-20\text{ }^{\circ}\text{C}$  until they were processed by pre-drying in a forced ventilation oven at  $55\text{ }^{\circ}\text{C}$  for 72 hours and milled to 1 mm. The experimental periods lasted for 21 days, with the last four days of each period having the collection at morning samples of feeds, orts left, 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  $10\text{ g}^{-1}$  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 Association of Official Analytical Chemists [AOAC] (2016) methods in dry matter number 930.15, crude protein number 992.15 and ether extract number 920.39, and the neutral detergent fibre was determined according to Van Soest, Robertson and Lewis (1991) with the modification of Mertens (2002). Faecal chromium was determined by atomic absorption spectrophotometry (Williams, David, & Iismaa, 1962) and then used together with the nutrient concentration to determine the flow of nutrients in the faeces. The TDN was calculated according to the equations proposed by Sniffen, O'Connor, Van Soest, Fox and Russell.

### *Ruminal fermentation*

Samples were collected at 2, 4, 6 and 8 h after the first feeding. Ruminal fluid was collected through a ruminal cannula using a vacuum pump. 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%  $\text{H}_2\text{SO}_4$  (1:1) and frozen at  $-20\text{ }^{\circ}\text{C}$  for later determination of volatile fatty acids (VFA) and ammonia N ( $\text{NH}_3\text{-N}$ ) concentrations. Ruminal 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  $\text{NH}_3\text{-N}$  of samples was measured by a colourimetric method according to Chaney and Marbach (1962). About 30 mL were centrifuged for 15 min at  $4000 \times g$ . To 5 mL of supernatant, 1 mL of 250  $\mu\text{L}$  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 to the 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  $\text{H}_2\text{SO}_4$  with 0.036 N (Chen, Mejia, Kyle, & Ørskov, 1995) and adjusted to a pH of less than 3 to avoid bacterial destruction of purine derivatives; these were then stored at  $-20\text{ }^{\circ}\text{C}$ . 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 hypoxanthine analyses were performed according to Chen, Methieson, DeB Hovel and Reeds (1990). The urine allantoin analyses were performed using the colourimetric method (Chen & Gomes, 1992; Fujihara, Ørskov, Reeds, & Kyle, 1987). Estimates of creatinine and uric acid concentrations in urine were performed using commercial Labtest<sup>®</sup> kits.

### Statistical analysis

The experimental design was a  $4 \times 4$  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 mathematical model used included period, treatment and animal effects, which were considered as random:  $Y_{ijk} = \mu + A_i + P_j + T_k + e_{ijk}$ , where  $\mu$  = mean of treatments;  $A_i$  = effect of animal  $i$ , ranging from 1 to 4;  $P_j$  = effect of period  $j$ , ranging from 1 to 4;  $T_k$

= effect of treatment  $k$ , ranging from 1 to 5;  $e_{ijk}$  = random error. Significant differences were accepted between treatments if  $P \leq 0.05$  by the Fisher test.

### Results and Discussion

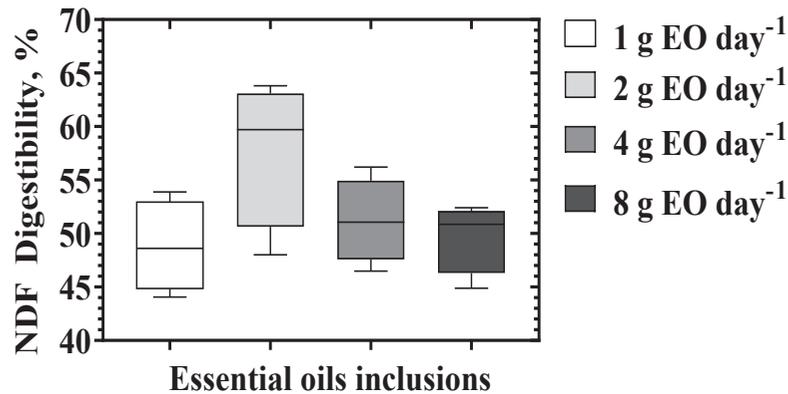
No differences were observed for the inclusions of EO ( $P > 0.05$ ) on intake among treatments (Table 2). In the total apparent digestibility, the neutral detergent fibre (NDF) and total digestible nutrients (TDN) at the level of 2 g day<sup>-1</sup> EO had the highest observed digestibility in comparison with the other treatments ( $P < 0.05$ ).

**Table 2**  
Intake and nutrient apparent total tract digestibility in ‘Holstein’ steers supplemented with increasing levels of cashew (*A. occidentale*) and castor (*R. communis*) EOs in a high forage diet

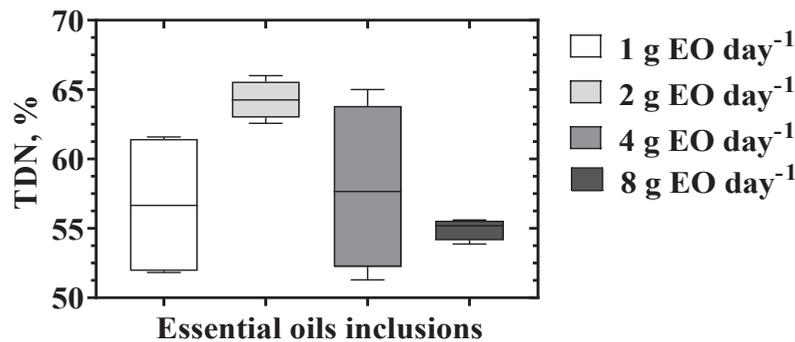
Item	*EO Inclusions g day <sup>-1</sup>				SEM <sup>1</sup>	P-Value
	1	2	4	8		
	Intake kg <sup>-1</sup>					
Dry matter	4.50	4.64	4.56	4.41	0.30	0.953
Crude Protein	0.46	0.46	0.47	0.43	0.03	0.797
Neutral detergent fibre	3.26	3.37	3.29	3.21	0.24	0.971
Ether extract	0.04	0.04	0.04	0.04	0.10	0.968
	Digestibility, %					
Dry matter	51.45	58.31	52.36	49.71	3.69	0.419
Crude Protein	63.06	68.77	58.16	56.58	4.55	0.278
Neutral detergent fibre	48.80b	57.81a	51.20 ab	49.76b	2.40	0.035
Ether extract	72.57	76.89	63.47	63.98	7.27	0.280
TDN <sup>2</sup>	56.68b	64.26a	57.90b	54.96b	2.05	0.004

<sup>1</sup>SEM: standard error of means. <sup>2</sup>TDN = total digestible nutrients. \*Essential oil containing 11% of cardol, 20% of cardanol and 9% of ricinoleic acid. Means with different letters have statistic differences by Fisher test.

In the digestibility, consequently, with the high digestion of fibre, the total digestible nutrients (TDN) in levels above 2 EO g day presented the highest values ( $P < 0.05$ ) (Figures 1 and 2).



**Figure 1.** NDF (Neutral detergent fibre) digestibility in Holsteins steers supplemented with increasing levels of EO of *A. occidentale* and *R. communis* in high forage diet.



**Figure 2.** TDN (total digestible nutrients) in Holsteins steers supplemented with increasing levels of EO of *A. occidentale* and *R. communis* in high forage diet.

Similarly to Michailoff et al. (2020) in sheep with rumen cannulation in a  $4 \times 4$  Latin square, using 2, 4, and 6 EO g day<sup>-1</sup> of Cashew and castor oil, found that intake of digestible organic matter decreased linearly with the inclusion of EO, and at 2 and 4 EO g day<sup>-1</sup>, the treatments did not affect nutrient digestibility. Teobaldo et al. (2020), also found in grazing beef cattle cannulated in a  $4 \times 4$  Latin-square, using a blend of cashew, castor, and copaiba EOs, at 150, 300 and 450 mg kg<sup>-1</sup> of DM in comparison with 150 mg kg<sup>-1</sup> DM of monensin, that in the lower concentration (EO 150) increased NDF digestibility and improved nitrogen utilisation efficiency.

Essential oils could be more efficient at lower doses; high doses could negatively affect digestibility (Benchaar, Duynisveld, & Charmley, 2006), due to an effect on the ruminal population. Patra & Yu, (2012), evaluated five EOs: clove, eucalyptus, garlic, oregano and mint in different levels in three doses *in vitro* (0, 25, 0.50 and 1.0 g L<sup>-1</sup>), and observed negative effects on the digestibility of DM and fibre in the high doses since the EOs decreased the abundance of *Archaea*, protozoa and cellulolytic bacteria.

The effectiveness of cashew (*A. occidentale*) and castor (*R. communis*) EOs may be greater at lower doses and as a mixture. In the present study at 2

g<sup>-1</sup> EO, fibre and TDN digestibility were improved in tropical diets with high forage proportion, in comparison with doses of 1, 4, 8 EO g day<sup>-1</sup>.

No effects of the inclusion levels of EO were observed on the pH and NH<sub>3</sub>-N at the ruminal level ( $P > 0.05$ ) (Table 3). However, in the VFA, acetate and propionate, differences were observed among treatments ( $P < 0.05$ ), without affecting the acetate:propionate ratio. The total VFA and propionate were higher in diets with 2 g day<sup>-1</sup> EO, and the ruminal pH remained at an average of 6.7.

The total VFA increased at the level of 2 g day<sup>-1</sup> EO. According to the substrate of fermentation, the VFA production could be affected. The content of fibre in the diet affects the metabolism of cellulose, degradation and VFA production (Wang, Zhang, Li, & Zhang, 2020), and high forage diets decreased VFA concentration (Penner, Taniguchi, Guan, Beauchemin, & Oba, 2009). The type of diet, fibrous or concentrated, has a direct impact on the use of EO (Coneglian, Castañeda Serrano, Cruz, & Branco, 2019; Molero, Ibars, Calsamiglia, Ferret, & Losa, 2004), due to the retention time of particles of the diet (Owens & Mehmet, 2016), and feed-particle binding properties of rumen microbiota that differs according to diet substrate (Kim, Eastridge,

& Yu, 2014) both alone and together with dietary fat, on ruminal bacterial communities in dairy cattle fed the following 3 diets: a control diet, the control diet supplemented with monensin, and the control diet supplemented with both monensin and fat. Bacterial communities in the liquid and the adherent fractions of rumen content were analyzed using 454 pyrosequencing analysis of 16S rRNA gene amplicons. Most sequences were assigned to phyla Firmicutes and Bacteroidetes, irrespective of diets and fractions. *Prevotella* was the most dominant genus, but most sequences could not be classified at the genus level. The proportion of Gram-positive Firmicutes was reduced by 4.5% in response to monensin but increased by 12.8% by combination of monensin and fat, compared with the control diet. Some of the operational taxonomic units in Firmicutes and Bacteroidetes were also affected by monensin or by the combination of monensin with fat. The proportion of numerous bacteria potentially involved in lipolysis and (or) The EOs have a greater potential to exert a positive effect on fibrous substrates, due to a higher retention rate at the ruminal level (Dijkstra, France, & Davies, 1998). A longer time to expose of EOs could lead to major effects on the fibre-associated rumen microbiota.

**Table 3**  
**Ruminal fermentation characteristics in ‘Holstein’ steers with increasing levels of cashew (*A. occidentale*) and castor (*R. communis*) EOs in a high forage diet**

Item	<sup>a</sup> EO Inclusions g day <sup>-1</sup>				SEM <sup>1</sup>	P-Value
	1	2	4	8		
pH	6.72	6.71	6.71	6.70	0.10	0.938
NH <sub>3</sub> -N, mg 100 mL <sup>-1</sup>	12.68	13.20	10.79	11.98	1.56	0.721
VFA, mM L <sup>-1</sup>						
Acetate	40.12b	44.06a	40.70b	42.59 ab	1.10	0.051
Propionate	13.35b	15.10a	13.62b	14.04b	0.33	0.001
Butyrate	6.86c	8.03a	7.35bc	7.78ab	0.23	0.002
Acetate:propionate	3.02	2.93	3.01	3.05	0.06	0.560

<sup>1</sup>SEM: standard error of means. <sup>a</sup>Essential oil containing 11% of cardol, 20% of cardanol and 9% of ricinoleic acid. Means with different letters have statistic differences by Fisher test.

Most of the EO compounds at high doses have demonstrated their antimicrobial activity by decreasing total VFA concentration (Kahvand & Malecky, 2018). Teobaldo et al. (2020), in *in vitro* experiment using a gas-production (GP) system, with inclusions of a blend of EOs (copaiba, cashew and castor oils), found at low doses (150 mg kg<sup>-1</sup> DM) an improvement in ruminal fermentation, but the medium (300 mg kg<sup>-1</sup> DM) and high (450 mg kg<sup>-1</sup> DM) doses of EOs decreased gas production at 12 and 24 h and decreased DM and NDF digestibility at 48 h compared with the control, Monensina (150 mg kg<sup>-1</sup> DM) and 150 mg kg<sup>-1</sup> EO DM

Nevertheless, Tomkins et al. (2015), in beef cattle fed medium- to low-quality Rhodes grass (*Chloris gayana*) hay using a specific blend of essential oils (CRINA<sup>®</sup> Ruminants at 1 g per day) in a 5 × 5 Latin square, found that EOs significantly increased butyrate and iso-valerate concentrations compared with the control. This suggests that at low levels of EO could improve the VFA concentration.

Akbarian-Tefaghi, Ghasemi and Khorvash (2018), in calves using a blend of EO (containing carvacrol, thymol and limonene), found an increase of molar proportions of acetate and butyrate as well as the acetate: propionate ratio. A higher

acetate:propionate ratio is related to the highest glucose concentration and β-hydroxyl butyrate.

Elcoso, Zweifel and Bach (2019), supplemented 48 Holstein cows with commercial EO Agolin (coriander, eugenol, and geranyl acetate) at 1 g day<sup>-1</sup>, and they found enhanced milk production attributed to an improvement of rumen fermentation by decreases in rumen methane production.

According to our results, the production of propionate improved without affecting the acetate:propionate ratio. Additives could modulate ruminal fermentation, not only by a direct effect of the ruminal population but also through a decrease in substrate availability (Schären et al., 2017). An increase in propionic acid can lead to a reduction in methane (Jouany & Morgavi, 2007). In tropical diets, methane production is elevated (Godde et al., 2018), and the use of cashew and castor EOs could modulate the methane production and energy efficiency, improving ruminal fermentation.

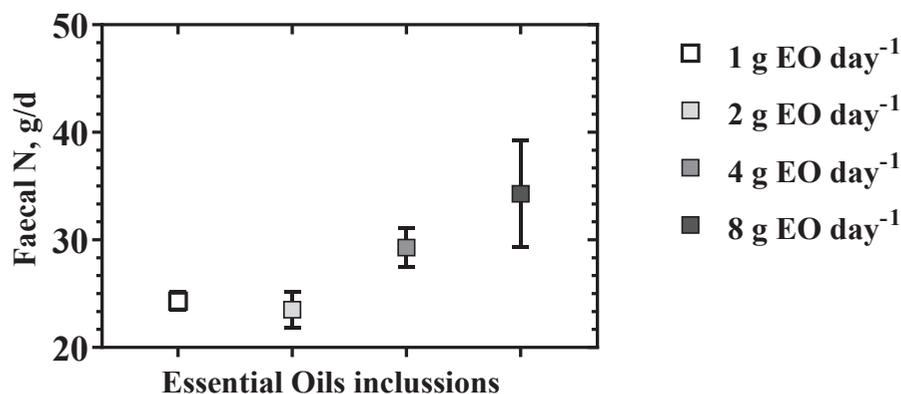
The N Balance and the synthesis of microbial protein were not affected by the inclusion of EO ( $P > 0.05$ ) (Table 4). Nevertheless, among treatments, a tendency was observed in levels above 2 g day<sup>-1</sup> EO affected faecal N excretion ( $P < 0.062$ ).

**Table 4**  
Excretion of urinary purine derivatives (PD) and microbial N supply in 'Holstein' steers supplemented with increasing levels of cashew (*A. occidentale*) and castor (*R. communis*) EOs in a high forage diet

Item	*EO Inclusions g day <sup>-1</sup>				SEM <sup>1</sup>	P-Value
	1	2	4	8		
N intake, g day <sup>-1</sup>	73.51	74.32	74.45	68.69	4.87	0.814
Faecal N, g day <sup>-1</sup>	24.32	23.50	28.28	34.28	2.79	0.062
BUN <sup>2</sup> , mg dl <sup>-1</sup>	13.39	12.51	11.44	12.37	0.76	0.575
Allantoin excretion mmol day <sup>-1</sup>	112.96	121.54	107.67	120.24	14.25	0.916
Uric acid excretion mmol day <sup>-1</sup>	3.51	3.36	2.62	2.77	0.55	0.594
PD <sup>3</sup> , mmol day <sup>-1</sup>	116.47	124.90	110.29	123.01	14.18	0.912
Microbial protein g of N day <sup>-1</sup>	80.62	87.72	75.38	86.09	11.88	0.912
EMPS <sup>4</sup> , g CP kg TDN <sup>-1</sup>	100.01	114.44	99.92	114.62	15.78	0.880

<sup>1</sup>SEM: standard error of means. <sup>2</sup>BUN: Blood urea nitrogen; <sup>3</sup>PD: Purine derivatives; <sup>4</sup>EMPS = efficiency of microbial protein synthesis. \*Essential oil containing 11% of cardol, 20% of cardanol and 9% of ricinoleic acid. Means with different letters have statistic differences by Fisher test.

Levels above 2 g day<sup>-1</sup> EO per day showed a negative effect on the balance of N, increasing the faecal excretion (Figure 3.)



**Figure 3.** Faecal N, g/d in Holsteins steers supplemented with increasing levels of EO of *A. occidentale* and *R. communis* in high forage diet.

Molero et al. (2004), using a blend of essential oils, found that EOs in the high concentrated diet tended to reduce CP degradation of soybean meal. However, Newbold, McIntosh, Williams, Losa and Wallace (2004), in high forage diets, found that EO inhibited ruminal degradation of soybean meal N but not heat-treated rapeseed meal or hay. The EO could inhibit a specific ruminal population that results in an inhibition of deamination and methanogenesis, resulting in lower ammonia N, methane, and acetate, and higher propionate and butyrate concentrations (Calsamiglia et al., 2007; Torres, Moura, Ghedini, Ezequiel and Almeida, 2020). In the present study, cashew and castor EOs at high levels tended to affect N balance, promoting N escape in faeces without effect on synthesis of microbial protein. According to Oh, Harper and Hristov (2019), it is plausible that low doses of secondary metabolites present in tropical plants can have positive effects on the host animal through post-ruminal mechanisms rather than effects on the rumen microbiota.

The allantoin:purine derivatives ratio was more than 90%, considered higher than Chen and Gomes, (1992), who had ranges from 80 to 85%. Relative 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 40%. The microbial protein synthesis efficiency was 107 g kg<sup>-1</sup> of CP per TDN and was below the NRC (2006). However, in Low-TDN diets might reduce passage rate and microbial efficiency.

## Conclusions

Inclusions of 2 g day<sup>-1</sup> of cashew and castor EOs increased the fibre digestibility, the propionate production, and control the rate of N excretion.

These results indicate that these EOs could improve digestibility, reducing potential energy losses by redirecting H<sup>+</sup> for a higher propionate formation and control the fermentation rate of the protein in diets with a high tropical forage ratio.

Further studies are necessary to evaluate the level in g day<sup>-1</sup> to reach the potential of these essential oils in the diet to avoid negative effects at high doses.

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