RBSPA
Revista Brasileira de Saúde e Produção Animal

ISSN 1519-9940
Revista Brasileira de Saúde e Produção Animal
http://doi.org/10.1590/20250012

Received: 03/07/2024
Accepted: 04/11/2024

Animal Production
Editor: Marion Pereira da Costa

Black sucupira seed oil (*Bowdichia virgilioides* Kunth) as a growth-promoting additive for beef cattle

Óleo da semente de sucupira-preta (Bowdichia virgilioides Kunth) como aditivo zootécnico para bovinos de corte

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ABSTRACT The aim of this study was to evaluate the effects of different levels of black sucupira oil (*Bowdichia virgilioides* Kunth) on nutrient intake, digestibility, rumen variables, and serum biochemistry. Four cannulated crossbred steers [body weight (BW) 450 ± 20 kg] were assigned to a 4 × 4 Latin square design with the treatments: (1) control (CON), without oil; (2) inclusion of 1.25 g oil seed (OS) kg^{-1} of dry matter (DM); (3) inclusion of 2.5 g OS kg^{-1} of DM; and (4) inclusion of 3.75 g OS kg^{-1} of DM. Results showed no significant effect ($P > 0.05$) of black sucupira oil seed on rumen pH and N-NH_3 levels. However, the inclusion of 2.5 g OS in the diets significantly decreased ($P \leq 0.05$) crude protein (CP) and DM digestibility. Differences were noted in the concentrations of isobutyric acid (0.67, 0.81, 0.71, 0.80 mmol L^{-1}) and isovaleric acid (0.91, 1.14, 1.21, 1.11 mmol L^{-1}), as well as total volatile fatty acids released in the rumen with OS inclusion. Sampling time significantly affected ($P \leq 0.05$) rumen pH and N-NH_3. Thus, it can be concluded that black sucupira seed oil decreases CP intake and digestibility, acting as a temporary nutritional modulator in beef cattle diets.

Keywords essential oil, natural additives, ruminal fermentation, rumen fatty acids

RESUMO *O objetivo deste estudo foi avaliar os efeitos de diferentes níveis de óleo de sucupira preta (Bowdichia virgilioides Kunth) sobre o consumo de nutrientes, digestibilidade, variáveis ruminais e bioquímica sanguínea. Quatro novilhos mestiços canulados [peso corporal (PC) 450 ± 20 kg] foram distribuídos em um delineamento quadrado latino 4 × 4 com os respectivos tratamentos: (1) controle (CON), sem inclusão de óleo de semente (OS); (2) inclusão de 1,25 g de OS kg^{-1} de matéria seca (MS); (3) inclusão de 2,5 g de OS kg^{-1} de MS; e (4) inclusão de 3,75 g de OS kg^{-1} MS. Os resultados não mostraram efeito significativo ($P > 0,05$) do OS sobre o pH ruminal e os níveis de N-NH_3. Entretanto, a inclusão de 2,5 g de OS nas dietas diminuiu ($P \leq 0,05$) a digestibilidade da proteína bruta (PB) e da MS. Foram observadas diferenças nas concentrações de ácido isobutírico (0,67, 0,81, 0,71, 0,80 mmol L^{-1}) e ácido isovalérico (0,91, 1,14, 1,21, 1,11 mmol L^{-1}). O tempo de coleta afetou ($P \leq 0,05$) o pH ruminal e o N-NH_3. Assim, pode-se concluir que o OS diminui o consumo e a digestibilidade do PB, atuando como modulador nutricional temporário em dietas para bovinos de corte.*

Palavras-chave *óleo essencial, aditivos naturais, fermentação ruminal, ácidos graxos ruminais*

1. Introduction

Plants produce secondary metabolites with diverse chemical structures and biological activities (Yeshi et al., 2022), such as essential oils. There is a wide range of essential oils, and some of these oils have antimicrobial activities and are currently considered safe for human and animal consumption (FDA, 2006).

The black sucupira (*Bowdichia virgilioides* Kunth) is a Fabaceae tree found frequently in the North, Northeast, and Midwest regions of Brazil. The essential oil from fruits contains terpenoids such as geraniol, farnesol, and caryophyllene (Jorge-Neto, 1970) and has been used in the pharmacy industry due to its antimicrobial activity (Almeida et al., 2006). Essential oils are frequently related to changes in the cytoplasmic membrane of the bacteria cell (Calsamiglia et al., 2007), due to the terpenoid contents (Abdillah et al., 2024).

Recent studies have been focusing on understanding the effect of these oils on the rumen environment, whether they have an effect similar to that of ionophores on the concentration of volatile fatty acids (VFA), decrease in methane emissions, and improvement of nutrients utilization (Kara & Pirci, 2024; Luo et al., 2024; Pawar et al., 2021). However, the black sucupira oil seed has not been investigated as a growth-promoting additive to beef cattle. Based on that, the hypothesis is that black sucupira oil seed is a possible modulator of rumen fermentation due to the antimicrobial action on rumen gram-positive bacteria.

The objective of this study was to evaluate the effect of the inclusion of different levels of black sucupira (*Bowdichia virgilioides* Kunth) seed oil on ruminal variables (pH, N-NH_3, and VFA), nutrients intake digestibility of nutrients, and serum biochemical in beef cattle fed on low-quality roughage and concentrated supplementation.

2. Material and methods

The experiment was conducted at Faculdade de Medicina Veterinária e Zootecnia at Universidade Federal de Mato Grosso do Sul in Campo Grande, MS, Brazil (20°30'20" S, 54°37'06" W), according to the institutional committee on animal use (case number 639/2014).

Four cannulated crossbreed steers [body weight (BW), 450 ± 20 kg] were assigned to a replicate 4 × 4 Latin square design. The basal diet was composed of *Megathyrsus maximus* (Syn. *Panicum maximum*) cv Massai hay and 15 g kg^{-1} BW of concentrate. The concentrate was composed of (g kg^{-1}): corn (810 g), soybean meal (160 g), urea (15 g), and mineral mixture (15 g). The black sucupira oil seed (OS) was obtained from Mundo dos Óleos® (Brasília, DF, Brazil; lot no. 0499). The OS treatments were: (1) control (CON), without the inclusion of oil; (2) inclusion of 1.25 g OS kg^{-1} of total dry matter (DM); (3) inclusion of 2.5 g OS kg^{-1} of total DM; and (4) inclusion of 3.75 g OS kg^{-1} of total DM. The ration (Table 1) was formulated to meet the requirements for growth (1.0 kg^{-1} day) of steers (NASEM, 2016).

The experiment lasted for 84 days and was divided into four 21-day periods. The animals were adapted to the diets during the first 14 days of each period, and samplings were collected during the last seven days/period. All steers were housed individually in covered pens with feeders and water troughs.

Table 1 – Chemical composition (g kg^{-1} DM) and ingredients of the experimental diets.

Item	Concentrate	Hay
Dry matter (DM)	943.7	932.4
Mineral matter (Ash)	77.8	63.7
Crude protein (CP)	146.9	27.6
Ether extract (EE)	32.9	9.0
pNDF[1]	193.1	763.2
Acid detergent fiber (ADF)	53.9	437.0
Lignin	60.3	11.9
Non-fiber carbohydrate (NFC)[2]	727.2	136.5
Total digestible nutrient (TDN)[3]	71.02	43.05

[1]pNDF = neutral detergent fiber corrected for protein contents. [2]Concentrate NFC = 100 - (CP from urea + urea inclusion) + Ash + pNDF + EE) and hay NFC = 100 - (CP + Ash + apNDF + EE) (Hall, 2000). [3]Calculated as described by Weiss et al. (1992).

The hay was offered twice daily (at 07:00 and 16:00 h) due to the refusals target of 150 kg^{-1} fed. The OS was infused with 1 kg of concentrate directly into the rumen via cannula on the morning feeding. The concentrate was fed as 15 g kg^{-1} of BW. Before each morning feeding, refusals from each animal were removed and weighed to calculate the intake. The daily dry matter intake (DMI) was calculated as the difference between the dry weight of the feed offered and the amount of refusals.

To determine pH and N-NH_3, ruminal fluid samples were collected at the end of each experimental period at zero hours (before supplementation), 2, 4, 6, 8, 10, and 12 hours after feeding. The ruminal fluid was manually collected (via cannula) from different locations in the rumen and filtered through the cotton fabric. Immediately after sampling, pH was measured using a digital pH meter (Quimis®, model: Q400AS).

A 50 mL aliquot of rumen fluid was stored frozen in plastic tubes after acidification with 5 drops of H_2SO_4 (50%) for subsequent determination of N-NH_3. The rumen fluid was thawed and the supernatant was analyzed for non-protein nitrogen (NPN) content by adapting the method of Fenner (1965).

Samples of 5 mL of rumen fluid were stored frozen in plastic tubes after acidification with 5 drops of HPO_3 for subsequent determination of volatile fatty acids (VFA). The VFA was determined by gas chromatography, according to the method recommended by Barbosa et al. (2001). A gas chromatograph was used (Thermo Finningan, model Trace GC Ultra, with flame ionization detector (FID)), equipped with a Nukol column linked to free fatty acids, 30 m long, 0.25 mm in diameter and 0. 25 µm thick. The carrier gas used was Helium (He) at 0.8 mL min^{-1}.

The apparent digestibility of nutrients was carried out from the 15th to the 19th day of each experimental period and estimated using the total feces collection method, being obtained by the difference between what was consumed in the diet by the animal and what was excreted in the feces. Feces were collected immediately after defecation, weighed and sampled (100 g kg^{-1}) at 6-hour intervals, and subsequently frozen. At the end of the experimental periods, the samples were pre-dried, weighed, and homogenized, followed by grinding (1 mm) for subsequent laboratory analysis.

Chemical analyses of the concentrate, hay, refusals, and feces were carried out according to the official methodologies (AOAC, 1995) for DM (967.03), CP (981.10), ash (942.05), ether extract (EE; 920.29) and acid detergent fiber (ADF; 913.18). The NDF (corrected for ash and protein - apNDF) was performed according to Van Soest et al. (1991) using thermostable alpha-amylase without sodium sulfite and corrected for residual ash (Mertens, 2002) and residual nitrogenous compounds (Licitra et al., 1996).

For analysis of serum concentrations of biochemical variables of ALT (alanine amino transferase), AST (aspartate amino transferase), albumin, GGT (gamma glutamyl transferase), urea, total proteins, and creatinine, 5 mL of blood were sampled at 7:00 am on the last day of each experimental period and were analyzed according to the kits' specifications of Cobas C 111 equipment (F. Hoffmann-La Roche Ltd, Basel, Switzerland) according to the manufacturer's instruction.

Statistical analyses were performed using the SAS Student statistical program. Consumption, apparent digestibility, and serum biochemical data were analyzed using a 4 × 4 Latin Square design. The statistical model used was: $Y_{ijk} = \mu + T_i + P_j + A_k + e_{ijk}$; where: Y_{ijk} is the observation of the effect of treatment i in period j, of animal k; µ is a constant; T_i is the random effect of square "i"; P_j is the random effect of period "j"; A_k is the random effect of steers "k" and e_{ijk} is the random error associated with each observation.

For rumen variables pH, N-NH_3, and VFA, a Latin square design with subdivided plots was considered, where the plots were the treatments and the subplots were the incubation times of the samples in the rumen or the different ruminal fluid sampling times. For the rumen variables pH, N-NH_3, and VFA, the statistical model included the effects of treatment, sampling times, animal, period and, treatment × time. The statistical model used was: $Y_{ijk} = \mu + T_i + H_j + A_k + P_j + (TH)_{ij} + e_{ijk}$, in which Y_{ijk} = observation of the effect of treatment i per collection time (for pH, N-NH_3, and VFA) j in animal k; μ = overall mean; T_i = effect of treatment [i = 1 (0 g), 2 (1.25 g), 3 (2.5 g) and 4 (3.75 g)]; H_j = effect of times of the samples for ruminal parameters (j = 1, ..., 7); A_k = animal effect (k = 1, ..., 4); P_j = the period effect (j = 1, ..., 4); $(TH)_{ij}$ = interaction between treatment i and time j; and e_{ijk} = random error associated with each observation.

All the variables were subjected to analysis of variance based on a simultaneous 4 × 4 Latin squares. When significant differences were observed, the means were compared using the Tukey test at 0.05 significance level.

3. Results

The OS did not affect (P > 0.05) the intakes of DM, NDF, and organic matter (kg day^{-1} and g kg^{-1} BW; Table 2). However, the OS decreased crude protein intake from 1.22 to 1.06 kg day^{-1} in the 0 and 3.75 g of oil diets, respectively. EE intake was highest at levels of 1.25 and 2.5 g of OS in the diet.

Table 2 – Nutrient intake of steers receiving different levels of black sucupira oil seed.

Item	Black sucupira seed oil (g)[1]				SEM[2]	P-value[3]
	0	1.25	2.5	3.75		
Intake, kg day^{-1}						
Dry matter (DM)	11.1	11.4	11.0	10.8	0.36	0.42
Crude protein (CP)	1.22^{a}	1.09^{b}	1.02^{c}	1.06^{bc}	0.05	<0.01
Ether extract (EE)	0.29^{b}	0.32^{a}	0.32^{a}	0.30^{b}	0.01	<0.01
Neutral detergente fiber (NDF)	5.05	5.22	4.99	4.68	0.30	0.35
Organic matter (OM)	10.2	10.5	10.2	9.9	0.49	0.24
Intake, g kg^{-1} BW						
DM	21.9	22.3	22.0	21.3	0.70	0.48
CP	2.41^{a}	2.12^{b}	2.03^{b}	2.08^{b}	0.04	<0.01
EE	0.58^{c}	0.63^{ab}	0.64^{a}	0.59^{bc}	0.01	0.01
NDF	9.9	10.1	9.9	9.3	0.51	0.49
OM	20.2	20.5	20.4	19.7	0.65	0.57
Digestibility, g kg^{-1}						
DM	705.0^{a}	677.5^{ab}	647.5^{b}	670.0^{ab}	0.02	0.03
CP	740.0^{a}	717.5^{ab}	690.0^{b}	702.5^{ab}	0.02	0.02
EE	825.0	745.0	762.5	820.0	0.02	0.10
NDF	650.0	635.0	587.5	602.5	0.03	0.09
OM	727.5	705.0	680.0	702.5	0.02	0.06

[1]Black seed essential oil: 0 g: 0 g essential oil kg^{-1} DM; 1.25 g: 1.25 g of essential oil kg^{-1} DM; 2.5 g: 2.5 g of essential oil kg^{-1} DM and 3.75 g: 3.75 g of essential oil kg^{-1} DM. [2] SEM = standard error of the mean. [3]Means followed by different letters in the same line differ statistically (P ≤ 0.05).

The digestibility of NDF, EE, and organic matter (OM) was not affected (P > 0.05) by the OS inclusion (Table 2). Feeding OS decreased (P ≤ 0.05) the DM and CP digestibility from 705.0 to 647.5 g kg^{-1}, 740.0 to 690.0 g kg^{-1}, in the 0 and 2.5 g of OS, respectively (Table 2).

No interactions were found (P > 0.05) between treatments and sampling times for pH and ruminal N-NH_3 values (Tables 3 and 4). Likewise, OS in the diets did not affect pH (Table 3) and N-NH_3 (Table 4). However, significant differences were found for rumen pH at different sampling times (Table 4), with the highest values observed between 2 and 4 hours after morning feeding (09:00 and 11:00 hours) for all treatments, decreasing after eating time from 6.86 to 6.03, respectively.

Table 3 – Ruminal fluid pH means in steers submitted to different levels of inclusion of black sucupira seed essential oils in the diet.

Sampling time	Black sucupira seed oil (g)[1]				
	0	1.25	2.5	3.75	Average
07:00	6.77 ± 0.12	6.17 ± 0.12	6.74 ± 0.12	6.71 ± 0.12	6.73 ± 0.06^{A}
09:00	6.82 ± 0.12	6.90 ± 0.12	6.83 ± 0.12	6.89 ± 0.12	6.86 ± 0.06^{A}
11:00	6.56 ± 0.12	6.73 ± 0.12	6.63 ± 0.12	6.72 ± 0.12	6.66 ± 0.06^{A}
13:00	6.35 ± 0.12	6.26 ± 0.12	6.23 ± 0.12	6.33 ± 0.12	6.29 ± 0.06^{B}
15:00	5.89 ± 0.12	6.07 ± 0.12	6.12 ± 0.12	6.03 ± 0.12	6.03 ± 0.06^{C}
17:00	6.13 ± 0.12	6.14 ± 0.12	6.23 ± 0.12	6.06 ± 0.12	6.14 ± 0.06^{BC}
19:00	5.98 ± 0.12	6.01 ± 0.12	6.06 ± 0.12	6.15 ± 0.12	6.05 ± 0.06^{C}
Average	6.36 ± 0.05	6.40 ± 0.05	6.40 ± 0.05	6.41 ± 0.05	

[1]Black seed essential oil: 0 g: 0 g essential oil kg^{-1} DM; 1.25 g: 1.25 g of essential oil kg^{-1} DM; 2.5 g: 2.5 g of essential oil kg^{-1} DM and 3.75 g: 3.75 g of essential oil kg^{-1} DM. Means followed by different letters in the columns differ from each other (Tukey, P < 0.001).

Table 4 – Ruminal N-NH_3 (mg dL^{-1}) in steers submitted to different levels of inclusion of black sucupira seed essential oils in the diet.

Sampling time	Black sucupira seed oil (g)[1]				
	0	1.25	2.5	3.75	Average
07:00	5.12 ± 2.80	4.24 ± 2.80	4.03 ± 2.80	5.46 ± 2.80	4.71 ± 1.40^{E}
09:00	27.05 ± 2.80	33.45 ± 2.80	26.04 ± 2.80	30.34 ± 2.80	29.22 ± 1.40^{B}
11:00	31.49 ± 2.80	38.23 ± 2.80	29.72 ± 2.80	36.11 ± 2.80	33.89 ± 1.40^{A}
13:00	24.70 ± 2.80	23.17 ± 2.80	18.60 ± 2.80	14.46 ± 2.80	20.23 ± 1.40^{C}
15:00	12.25 ± 2.80	15.21 ± 2.80	13.97 ± 2.80	12.45 ± 2.80	13.47 ± 1.40^{D}
17:00	6.96 ± 2.80	6.27 ± 2.80	9.18 ± 2.80	8.20 ± 2.80	7.65 ± 1.40^{E}
19:00	5.64 ± 2.80	3.06 ± 2.80	6.18 ± 2.80	4.85 ± 2.80	4.93 ± 1.40^{E}
Average	16.17 ± 1.06	17.66 ± 1.06	15.39 ± 1.06	15.98 ± 1.06	

[1]Black seed essential oil: 0 g: 0 g essential oil kg^{-1} DM; 1.25 g: 1.25 g of essential oil kg^{-1} DM; 2.5 g: 2.5 g of essential oil kg^{-1} DM and 3.75 g: 3.75 g of essential oil kg^{-1} DM. Means followed by different letters in the columns differ from each other (Tukey, P < 0.001).

Sampling time affected (P ≤ 0.05) the N-NH_3 concentrations with a peak between 2 and 4 hours after supplementation (09:00 and 11:00 hours) for all treatments, decreasing until reaching their minimum values 12 hours after morning feeding. Ruminal N-NH_3 ranged from 4.71 to 33.89 mg dL^{-1}, with an average of 16.3 mg N-NH_3 dL^{-1} of ruminal fluid (Table 4).

There was no effect (*P* > 0.05) of OS on the concentrations (mmol L^{-1}) of acetic acid, propionic acid, n-butyric acid, n-valeric acid, n-caproic acid, and on the total sum of short-chain fatty acids (Table 5). However, there was an effect (P ≤ 0.05) on the concentrations of isobutyric acid (0.67, 0.81, 0.71, and 0.80 mmol L^{-1}) and isovaleric acid (0.91, 1.14, 1.21, and 1.11 mmol L^{-1}) with the inclusion of OS in relation to the control treatment (without oil addition) (Table 5). Black sucupira seed oil decreased (P < 0.05) AST and did not affect other biochemical variables (albumin, ALT, creatinine, GGT, total proteins, and urea; Table 6).

Table 5 – Effect of including black sucupira seed essential oils in the diet of cattle supplemented with the concentrate on the production of short-chain volatile fatty acids in the rumen.

Item	Black sucupira oil seed (g)[1]				SEM[2]	P-value[3]		
	0	1.25	2.5	3.75		Treat.	Treat *Hour	Hour
VFA (mmol L^{-1})								
Acetate	85.70	85.04	81.86	84.10	2.09	0.67	0.86	<0.01
Propionate	22.29	20.61	19.09	19.46	2.28	0.78	0.88	<0.01
Isobutyrate	0.66	0.83	0.72	0.81	0.06	0.26	0.96	<0.01
Butyrate	12.72	12.97	12.63	13.75	1.44	0.94	0.68	<0.01
Isovalerate	0.86[b]	1.23[a]	1.03[ab]	1.11[ab]	0.06	0.03	0.76	<0.01
Valerate	0.77	0.84	0.86	0.93	0.05	0.25	0.98	<0.01
Caproic acid	0.22	0.24	0.28	0.33	0.10	0.89	0.77	<0.01
Total VFA	123.16	121.62	116.67	120.24	3.05	0.52	0.85	<0.01
Acetate:Propionate	4.37	4.42	4.46	4.71	0.39	0.92	0.63	<0.01
VFA (mmol 100 $mmol^{-1}$)								
Acetate	69.88	70.05	70.34	70.06	1.00	0.990	<0.01	0.57
Propionate	17.69	16.78	16.26	15.66	1.62	0.859	<0.01	0.67
Isobutyrate	0.56	0.70	0.61	0.69	0.05	0.272	<0.01	0.99
Butyrate	10.45	10.65	10.74	11.57	1.02	0.869	0.06	0.69
Isovalerate	0.74	1.02	0.89	0.99	0.05	0.061	<0.01	0.84
Valerate	0.62	0.69	0.74	0.78	0.04	0.093	<0.01	0.99
Caproic acid	0.18	0.20	0.23	0.26	0.08	0.897	0.02	0.72

[1]Black seed essential oil: 0 g: 0 g essential oil kg^{-1} DM; 1.25 g: 1.25 g of essential oil kg^{-1} DM; 2.5 g: 2.5 g of essential oil kg^{-1} DM and 3.75 g: 3.75 g of essential oil kg^{-1} DM. [2] SEM = standard error of the mean. [3]Means followed by different letters in the same line differ statistically (P ≤ 0.05).

Table 6 – Serum biochemical parameters in steers submitted to different inclusion levels of black sucupira seed essential oils in the diet.

Item	Black sucupira seed oil (g)[1]				SEM[2]	P-value[3]
	0	1.25	2.5	3.75		
Albumin (g dL^{-1})	37.00	35.00	36.50	35.00	2.89	0.45
ALT (UI L^{-1})	26.25	24.72	23.07	22.97	2.99	0.45
AST (UI L^{-1})	69.45[a]	52.90[b]	61.84[ab]	55.35[ab]	7.09	0.05
Creatinine (mg dL^{-1})	1.37	1.32	1.35	1.42	0.08	0.64
GGT (UI L^{-1})	19.22	14.40	15.22	16.75	2.06	0.09
Total protein (g dL^{-1})	73.30	70.27	71.95	73.97	6.78	0.81
Urea (mg dL^{-1})	27.05	20.72	23.15	25.67	2.59	0.15

ALT: alanina aminotransferase; AST: aspartato aminotransferase; GGT: Gama glutamil transferase. Black seed essential oil: 0 g: 0 g essential oil kg^{-1} DM; 1.25 g: 1.25 g of essential oil kg^{-1} DM; 2.5 g: 2.5 g of essential oil kg^{-1} DM and 3.75 g: 3.75 g of essential oil kg^{-1} DM. SEM: standard error of the mean. Means followed by different letters in the same line differ statistically (P ≤ 0.05).

4. Discussion

In the current study, there was no effect on the intake of DM, NDF, and OM but reduced CP intake. Similarly, Hristov et al. (2013) also did not observe a significant effect on the DMI of cows fed with different levels of oregano extract. However, Yang et al. (2010) reported that dietary supplementation with cinnamaldehyde increased the DMI of feedlot cattle at the beginning of the fattening period but did not affect DMI after 4 weeks of feedlot. The effects of essential oils on nutrient

intake are not well established. Overall, the results observed in other studies suggest that the effects of essential oils and their components on ruminant feed intake vary depending on the type of essential oil, the concentration provided in the diets, and the differences in the composition of the basal diet fed to the animals (Pukrop et al., 2019; Torres et al., 2021; Wells, 2024).

Bioactive compounds in essential oils are well recognized for their ability to modify rumen function by promoting digestion (Caroprese et al., 2023) and reducing protein degradation (Macheboeuf et al., 2008). The apparent DM and CP digestibility decreased with the inclusion of black oil seed. Benchaar et al. (2006, 2007, 2008) showed DM, OM, and CP digestibility greater than 60.0% and NDF digestibility of more than 52.0%, while the apparent digestibility resulted in DM, OM, CP, and NDF greater than 58%.

The antimicrobial activity of several essential oils acts as pH-dependent, with its activity potentiated at low pH (close to 5.5) (Castillejos et al., 2006; Rodriguez-Pardo et al., 2012). This is due to the hydrophilic state of the active molecules. As pH decreases, the molecules tend to become undissociated and interact more easily with bacterial cell membranes, exerting their antimicrobial effect (Cardozo et al., 2005). The average of pH in this study remained close to the value of 6.4, which is considered optimal for microbial growth (Van Soest, 1994), suggesting that major changes in the microbial population had not occurred.

The chemical composition and dosage of the essential oil can influence the effects on ruminal nitrogen (N) metabolism. According to the observed results, it was verified that there was a synchronism between the times of higher pH and those of higher concentration of ruminal N-NH_3, demonstrating a relationship with feeding time. The N-NH_3 concentrations remained above 6 mg dL^{-1} for all treatments from 9:00 a.m. to 5:00 p.m. However, at 7:00 a.m. and 7:00 p.m., the observed values (4.71 and 4.93 mg dL^{-1}) suggest that the decrease in ruminal ammonia concentration may indicate an increase in microbial protein synthesis during these times (Tedeschi et al., 2000).

Essential oils may increase VFA (Tomkins et al., 2015), change the microbiota profile in the rumen (Caroprese et al., 2023) due to factors such as the degradation of the compounds in the rumen, microbial adaptation to the essential oil, rate of passage to the duodenum, volatilization, and absorption of the compounds by the rumen wall (Villalba & Provenza, 2010). The black sucupira oil seed did not affect the concentrations of the major VFAs, acetate and propionate, in the rumen. However, it increased the concentrations of isobutyrate (from 0.67 to 0.80 mmol L^{-1} with 0 vs. 3.75 g of black sucupira oil seed) and isovalerate (from 0.91 to 1.11 mmol L^{-1}). The formation of these two branched-chain VFA occurs from the deamination or transamination of branched-chain amino acids (Firkins, 2021). The increases promoted by the black sucupira oil seed are beneficial since it is well-known that these VFA are essential substrates for the growth of bacteria that degrade fibrous carbohydrates (Firkins & Mitchell, 2023).

The AST serum concentrations were higher in the 0 g than when 1.25 g of black sucupira oil seed was provided. This indicates that the inclusion of the oil has no influence on the function of organs (liver and kidneys) associated with the blood substances tested in this study.

The concentration of urea N in the blood is highly correlated with the concentration of N-NH_3 in the rumen (Petit & Flipot, 1992). The synthesis of urea in the liver is carried out from ammonia absorbed by the rumen, so the ruminal ammonia in excess of the microbial requirement is absorbed by the rumen wall into the portal blood, and most of it is converted into urea in the liver. As no changes in N-NH_3 concentration were observed, changes in serum urea concentrations were also not observed.

4. Conclusions

The black sucupira oil seed has a positive effect of modulating rumen fermentation in the first 4 hours and decreases crude protein intake and digestibility. The 1.25 g of black sucupira oil seed

was the best level of inclusion to increase the production of branched-chain VFA. Further studies would be needed to provide data to understand the action of the oil to the bacteria and the form of their supply in order for their effect to be permanent and to have the possibility of preventing acidosis in diets with a high proportion of concentrate.

Acknowledgments

The authors are thankful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant number 564435/2010-4) and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT; TO 014/12) for granting the financial support for this study.

Declaration of Conflict of Interest

The authors declared no conflicts of interest.

CRediT authorship contribution statement

Gumercindo Loriano Franco: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing; **Jocely Gomes de Souza:** Validation, Writing – review & editing; **Gabriella de O. Dalla Martha:** Conceptualization, Data curation, Investigation; **Fábio José Carvalho Faria:** Formal analysis, Supervision; **Marcelo Vedovatto:** Investigation, Writing – original draft; **Luís Carlos Vinhas Ítavo:** Methodology, Validation; **Marcella Cândia D'Oliveira:** Investigation, Project administration, Visualization, Writing – original draft; **Anuzhia Paiva Moreira:** Writing – original draft.

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