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Impact of açaí oil as an additive on *in vitro* ruminal fermentation

Impacto do óleo de açaí como aditivo na fermentação ruminal *in vitro*

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Abstract: This study evaluated effects of açaí pulp oil on *in vitro* rumen fermentation dynamics in cattle. Four treatments (0, 0.3, 3, and 30 mg g^{-1} of açaí oil) were tested using a randomized block design with a split-plot arrangement over time (24 and 48 h). The parameters assessed included *in vitro* dry matter degradability (IVDMD), *in vitro* organic matter degradability (IVOMD), *in vitro* gas production kinetics, short-chain volatile fatty acid (SCVFA) concentrations, and ammonia nitrogen ($N–NH_3$) concentrations. No interaction was detected between açaí oil level and incubation time for IVDMD or IVOMD ($p > 0.05$). However, the 30 mg g^{-1} level significantly reduced IVDMD ($p < 0.05$). SCVFA and $N–NH_3$ concentrations were unaffected across incubation times ($p > 0.05$). *In vitro* gas production increased with açaí oil inclusion ($p < 0.05$), and *in vitro* gas production kinetics indicated similar patterns between 0 vs. 30 mg g^{-1} and 0.3 vs. 3 mg g^{-1} groups ($p < 0.05$). The 30 mg g^{-1} dosage reduced IVDM while increasing total gas production. In contrast, lower levels (0.3 and 3 mg g^{-1}) did not impair rumen fermentation efficiency and warrant further evaluation in animal performance trials.

Key-words: degradability; *Euterpe oleracea*; modulation nutrition; ruminant.

Resumo: Este estudo avaliou os efeitos do óleo da polpa de açaí na dinâmica da fermentação ruminal *in vitro* em bovinos. Quatro tratamentos (0, 0.3, 3 e 30 mg g^{-1} de óleo de açaí) foram testados usando delineamento em blocos casualizados com arranjo de parcelas subdivididas ao longo do tempo (24 e 48 h). Os parâmetros avaliados incluíram degradabilidade *in vitro* da matéria seca (DIVMS), degradabilidade *in vitro* da matéria orgânica (DIVMO), cinética de produção de gás *in vitro*, concentrações de ácidos graxos voláteis de cadeia curta (AGVCC) e concentrações de nitrogênio amoniacal ($N–NH_3$). Nenhuma interação foi detectada entre o nível de óleo de açaí e o tempo de incubação para DIVMS ou DIVMO ($p > 0.05$). No entanto, o nível de 30 mg g^{-1} reduziu significativamente a DIVMS ($p < 0.05$). As concentrações de AGVCC e $N–NH_3$ não foram afetadas ao longo dos tempos de incubação ($p > 0.05$). A produção de gás *in vitro* aumentou com a inclusão do óleo de açaí ($p < 0.05$), e a cinética da produção de gás *in vitro* indicou padrões semelhantes entre os grupos 0 vs. 30 mg g^{-1} e 0.3 vs. 3 mg g^{-1} ($p < 0.05$). A dosagem de 30 mg g^{-1} reduziu a DIVMS enquanto aumentou a produção total de gases. Em contraste, níveis mais baixos (0.3 e 3 mg g^{-1}) não prejudicaram a eficiência da fermentação ruminal e justificam avaliações adicionais em ensaios de desempenho animal.

Palavras-chave: degradabilidade; *Euterpe oleracea*; modulação nutricional; ruminante.

1. Introduction

The growing demand for sustainable livestock production has accelerated the search for feed additives that enhance ruminant efficiency while mitigating environmental impact [1]. Among these, additives from plant extracts have been investigated due to their bioactive compounds, which can modulate rumen fermentation, improve animal health and productivity, and increase feed efficiency [2, 3].

Açaí (*Euterpe oleracea* Mart.), a native Amazonian palm, is widely consumed in human diets for its nutritional value, containing approximately 76% fiber, 24% lipids, 70% unsaturated fatty acids, and 0.4% vitamins, thereby providing 37 kcal $100g^{-1}$ [4]. Açaí pulp is also rich in anthocyanins (3.19 mg g^{-1} DM) and proanthocyanidins (12.89 mg g^{-1} DM), flavonoids with strong antioxidant properties [2]. Owing to these properties, açaí oil supplementation in transition cow diets has been associated with increased milk production [5]. *In vitro* studies further indicate potential benefits, including reduced total gas production [6] and inhibition of gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* [7].

Despite these promising findings, no study has evaluated the effects of açaí oil on nutrient degradability, gas production, and rumen fermentation products. Therefore, we hypothesized that the inclusion of açaí oil modulates ruminal microbiota by lowering the acetate-to-propionate ratio, decreasing ammoniacal nitrogen and gas production, and maintaining or enhancing *in vitro* dry and organic matter degradability. Thus, this study aimed to evaluate the effect of different levels of açaí oil on *in vitro* rumen fermentation parameters in cattle.

2. Material and methods

2.1 Experimental location and animals

The experiment was conducted at the Animal Nutrition and Gas Production Laboratory, Universidade Federal do Norte do Tocantins, Araguaína Campus. All procedures involving animals were approved by the Ethics Committee on the Use of Animals (protocol no. 4028220422).

Four inclusion levels of açaí pulp oil (0, 0.3, 3, and 30 mg g^{-1} of substrate dry matter, DM) were tested for their effects on rumen fermentation and *in vitro* gas production kinetics. Initially, rumen fluid was obtained from four uncastrated male cattle, with three weighing approximately 300 kg and one fistulated bull weighing approximately 500 kg. The animals were housed in paddocks with *Urochloa brizantha* cv. Mombaça grass and supplemented with a concentrate mixture of soybean meal, ground corn, and urea (0.5% body weight day^{-1}) for five days before the rumen fluid was collected.

2.2 Rumen fluid collection and *in vitro* degradability

Rumen contents were collected into thermos flasks preheated with water at 39°C using an esophageal probe and transported immediately to the laboratory for system preparation and assembly. A total of 180 glass bottles (100 mL) were prepared, each containing 1.0 g of substrate (40:60 roughage:concentrate diet; Table 1), 79.2 mL of buffer solution [8], 10 mL of rumen fluid, and açaí oil diluted in 0.8 mL of ethanol. For the 0 mg g^{-1} DM treatment, only ethanol (0.8 mL) was added. The bottles were sealed with silicone stoppers and incubated at 39°C in an oven. To correct for dry and organic matter residues, 36 blank flasks (containing the inoculum without the açaí oil or the substrate) were simultaneously incubated. Substrate chemical composition (dry matter, organic matter, crude protein, neutral detergent fiber, and ether extract) was determined following the method described by Detmann *et al.* [9].

Table 1. Proportion of ingredients and chemical composition of the substrates used in this study.

Item	Composition
Proportion of ingredients in the substrate, g kg^{-1} DM	
Corn silage	400
Ground corn	529
Soybean meal	52.4
Mineral salt	10.2
Urea	8.4
Chemical composition of the substrate, g kg^{-1} DM	
Dry matter, g kg^{-1} natural matter	926.4
Organic matter	964.2
Crude protein	150.3
Neutral detergent fiber	669.4
Ether extract	33.8

After 24 h of incubation, 24 bottles per treatment (eight replicates) were removed. Their contents were filtered under vacuum through No. 2 porous crucibles, dried at 105°C for 24 h, and weighed to determine the *in vitro* dry matter degradability (IVDMD). The samples were then incinerated in a muffle furnace at 400°C for 4 h and reweighed to obtain the *in vitro* organic matter degradability (IVOMD), following the method described by Vargas *et al.* [10]. The same procedures were applied to the bottles incubated for 48 h.

2.3 Determination of ammoniacal nitrogen (N-NH_3) and short-chain volatile fatty acid (SCVFA) in the inoculum

For N–NH_3 analysis, aliquots were filtered and preserved using a mixture of 1.96 mL of rumen fluid and 0.04 mL of 50% sulfuric acid, then stored at –80°C. The samples were then analyzed using UV-Visible spectrophotometry following the method described by Detmann *et al.* [9]. For SCVFA analysis, 1.0 mL of filtered rumen fluid was mixed with 1.0 mL of 0.85% orthophosphoric acid, vortexed, and centrifuged at 4000 rpm for 10 min. The supernatant was recovered, filtered, and analyzed using reverse-phase high-performance liquid chromatography with diode array detection (RP-HPLC-DAD) under the conditions described by Vargas *et al.* [11].

2.4 Gas production kinetics and model evaluation

The gas production kinetics were measured using the semi-automatic technique proposed by Maurício *et al.* [12]. Gas pressure and volume were recorded using a DPI800-P model pressure transducer at 0, 3, 6, 9, 12, 16, 20, 24, 30, 36, and 48 h after fermentation began. Mathematical models were fitted to the gas production curves to describe the *in vitro* gas production kinetics and assess model fit (Table 2).

Table 2. Mathematical models used to fit the *in vitro* gas production curves.

Models	Equation*
Gompertz [1]	$V = V_f(1 - \exp(-kt))$
Exponential [1]	$V = V_f \exp(-\exp(1 - k(t - L)))$
France [2]	$V = V_f(1 - \exp(-k\ (t - L) - d(\sqrt{t} - \sqrt{L})))$
Schofield and Pell [3]	$V = V_{f1}(1 - e(-k_1 t)) + V_{f2} e(e(1 + k_2 e(L - t)))$
Logistic exponential [4]	$V = v_f\ (1 - e(-kt))/1 + e(ln(1/d) - kt)$
Logistic exponential Lag [4]	$V = v_f\ (1 - \exp(-k(t - L)))/1 + e(\ln(1/d) - k(t - L))$

* Parameters for gas production kinetics; V: accumulated gas volume (mL); Vf: asymptotic final gas volume; Vf1: gas volume produced by the rapidly degradable fraction; Vf2: gas volume produced by the slowly degradable fraction; k: gas production rate; k1: gas production rate of the rapidly degradable fraction; k2: gas production rate of the slowly degradable fraction; t: time; L: lag time; 1: Schofield *et al.* [13]; 2: France *et al.* [14]; 3: Schofield and Pell [15]; 4: Wang *et al.* [16].

2.5 Statistical analysis

IVDMD and IVOMD were analyzed using a randomized block design with repeated measures at 24 and 48 h of incubation. Data were evaluated using analysis of variance (ANOVA) with the MIXED procedure of SAS (Statistical Analysis System), adopting $p < 0.05$ as the significance threshold. Fixed effects included treatment (level of açaí oil) and incubation time, and their interaction was also tested.

SCVFA and N–NH_3 concentrations, evaluated at 24 and 48 h, were analyzed using ANOVA with Tukey's test. Treatments (0, 0.3, 3.0, and 30 mg g^{-1} DM açaí oil) were defined as fixed effects, whereas donor animal was defined as a random effect. Differences were considered significant when $p < 0.05$.

Model selection for gas production kinetics was based on the lowest residual sum of squares (RSS), corrected Akaike information criterion (AICc), root mean square prediction error (RMSPE), and highest coefficient of determination (R^2) between observed and predicted values. The parameters derived from each model were compared, and the predicted gas volumes at 0, 3, 6, 9, 12, 16, 20, 24, 30, 36, and 48 h were compared with the observed values.

The selected model was then used to estimate the gas production parameters for each treatment. Model-generated equations were compared using parallelism [17] and model identity [18] tests at a 5% probability.

3. Results

Analysis of variance for IVDMD and IVOMD revealed no effect of interaction between açaí oil dosage and incubation times ($p > 0.05$; Table 3). However, an isolated effect of dosage was observed, with 30 mg g^{-1} DM reducing IVDMD ($p < 0.05$). The 0.3 and 3 mg g^{-1} DM treatments did not differ from the control ($p > 0.05$). Incubation time also had an isolated effect, with mean IVDMD and IVOMD values increasing at 48 h of incubation compared with 24 h ($p < 0.05$).

The concentrations of *in vitro* ruminal fermentation products were unaffected by açaí oil at the different incubation times ($p > 0.05$; Table 4).

Table 3. Effect of açaí oil dosage on the *in vitro* degradability of dry and matter at different incubation times.

Time	Dosages of açaí oil, mg g $^{-1}$ DM				Mean	*p*-value			CV %
	0	0.3	3	30		Dosages	Time	D × T	
In vitro dry matter degradability, %									
24	67.91	66.68	66.76	66.26	66.90 B				
48	74.12	73.63	73.79	73.83	73.84 A	0.046	<0.001	0.845	2.16
Mean	71.01 a	70.15 ab	70.28 ab	70.05 b	70.37				
In vitro organic matter degradability, %									
24	67.33	66.09	66.33	65.52	66.32 B				
48	74.17	74.09	74.02	73.99	74.07 A	0.192	<0.001	0.835	2.59
Mean	70.75	70.09	70.17	69.75	70.19				

Incubation time: 24 and 48 h; Probability of: isolated effect of dosage, isolated effect of incubation time and interaction between açaí oil dosage and incubation times (D × T) with $p < 0.05$. CV: coefficient of variation, %; means followed by different uppercase letters in the columns and lowercase letters in the rows differ at 5% probability according to the Tukey's test.

Table 4. Effect of açaí oil dosage on short-chain volatile fatty acid and ammoniacal nitrogen concentrations at different incubation times.

Item	Dosages of açaí oil, mg g^{-1} DM				SEM	*p*-value
	0	0.3	3	30		
24 h of incubation						
Ammoniacal–N	18.6	16.5	17.1	18.3	3.29	0.977
Total VFA	72.7	73.7	72.0	72.0	3.29	0.977
Acetate	45.5	45.2	46.4	46.3	2.68	0.965
Propionate	25.7	26.4	24.2	25.3	1.11	0.418
Butyrate	2.06	2.10	2.12	2.03	0.20	0.965
Acetate : Propionate	1.68	1.87	1.88	1.91	0.08	0.261
48 h of incubation						
Ammoniacal–N	18.2	18.1	18.8	19.8	2.20	0.761
Total VFA	79.1	73.3	75.2	70.2	3.94	0.334
Acetate	51.8	47.0	47.7	42.5	4.49	0.229
Propionate	24.35	26.3	23.5	24.6	4.20	0.812
Butyrate	2.82	2.50	2.45	2.38	0.17	0.244
Acetate : Propionate	2.03	2.20	2.15	1.99	0.51	0.873

Ammoniacal–N: expressed in mg dL-1; Total VFA, acetate, propionate and butyrate: expressed in mmol mL-1; Acetate : Propionate: expressed in mmol mmol-1; VFA: volatile fatty acids; SEM: Standard error of mean.

Among the statistical models tested, the Schofield and Pell [15] model provided the best fit, with the lowest RSS, AICc, and RMSPE values and the highest R^2 values (Table 5).

Table 5. Statistical criteria and selection of the mathematical model to fit *in vitro* gas production kinetics.

Models	RSS	AICc	RMSPE	R^2
Gompertz	1367.6	280.9	5.45	98.8
Exponential	8556.9	362.7	13.9	88.7
France	3394.9	395.9	8.73	96.7
Logistic exponential	1659.5	290.7	6.05	98.4
Logistic exponential lag	1640.9	292.5	6.01	98.4
Schofield and Pell	1143.7	276.3	4.94	98.9

RSS: residual sum of squares; AICc: corrected Akaike information criterion; RMSPE: root mean square prediction error; R^2: regression of observed versus predicted values.

Curve identity testing showed that the cumulative *in vitro* gas production increased with the inclusion of açaí oil ($p < 0.05$; Table 6). Specifically, 30 mg g^{-1} DM of açaí oil increased gas production parameters, whereas 0.3 and 3.0 mg g^{-1} DM yielded cumulative *in vitro* gas production curves similar to each other.

Table 6. *In vitro* cumulative gas production equations expressed in mL g^{-1} DM of diets including açaí oil.

Treatments [1]	Equations [2]		R^2
0	$V = 55.46(1 - e(0.12 \times t)) + 75.99\ e(e(1 + 0.04e(10.22 - t)))$	aC	0.97
0.3	$V = 63.73(1 - e(0.11 \times t)) + 74.07\ e(e(1 + 0.03\ e(10.16 - t)))$	bB	0.99
3	$V = 60.75(1 - e(0.10 \times t)) + 75.42\ e(e(1 + 0.03\ e(9.75 - t)))$	bB	0.99
30	$V = 74.09(1 - e(0.09 \times t)) + 66.32\ e(e(1 + 0.03\ e(8.46 - t)))$	aA	0.99

1: Expressed in mg g^{-1} DM; 2: Equations estimated using the Schofield and Pell model [15]. Equations followed by equal lowercase letters in the same column are parallel by the parallelism of curves test. Equations followed by equal capital letters are identical by the identity test at 5% probability.

Parallelism testing showed a change in the kinetic behavior of *in vitro* gas production ($p < 0.05$; Table 6; Figure 1a). Comparable kinetic behavior was observed between the 0 and 30 mg g^{-1} DM treatments ($p < 0.05$; Figure 1b) and between the 0.3 and 3 mg g^{-1} DM ($p < 0.05$; Figure 1c).

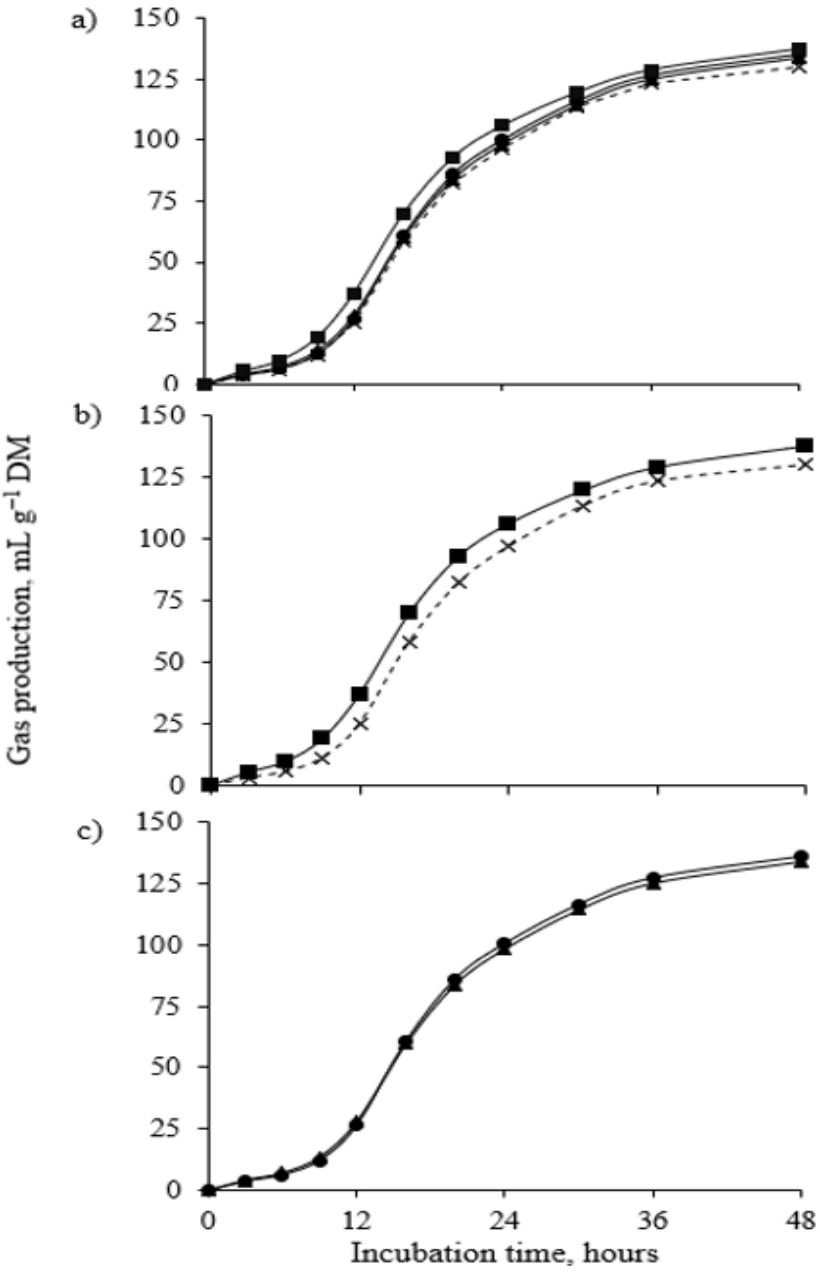

Figure 1. *In vitro* cumulative gas production curves by incubation time: **a**. all dosages of açaí oil; **b**. comparison of 0 and 30 mg g^{-1} DM; **c**. comparison of 0.3 and 3 mg g^{-1} DM.

4. Discussion

The açaí fruit is rich in phenolic compounds, mainly anthocyanins and proanthocyanins, which provide its natural pigmentation and reduce oxidative stress [19]. Based on these properties, this study hypothesized that açaí oil would lower the acetate-to-propionate ratio, N–NH_3 concentration, and total gas production while maintaining or increasing *in vitro* dry and organic matter degradability. This hypothesis was partly supported as IVDMD was not affected with 0.3 and 3 mg g^{-1} DM of açaí oil, but reduced with 30 mg g^{-1} DM, while IVMOD, VFA and N–NH_3 concentrations were not altered.

The higher IVDMD and IVOMD observed at 48 h across all treatments likely reflect microbial colonization and fermentation of the substrate's structural carbohydrate fractions, particularly the slower growth and adhesion of fibrolytic microorganisms that degrade structural carbohydrate fractions [20]. The reduction in IVDMD at 30 mg g^{-1} DM can be attributed to bioactive compounds in açaí oil with

inhibitory effects on gram-positive bacteria, as demonstrated *in vitro* for *S. aureus* and *E. faecalis* [7]. Although these species are uncommon in the rumen, other gram-positive bacteria are present, including Ruminicoccus sp. (degrades cellulose and hemicellulose), *Streptococcus bovis* and *Lactobacillus* sp. (degrade starch), *E. faecium* (hydrolyzes urea to ammonia), and methanogenic archaea, such as *Methanobacterium* sp. and *Methanobrevibacter* sp. (produce methane from CO_2 and H_2, byproducts of ruminal fermentation) [21]. Thus, the possible suppression of these microbial groups by açaí oil may have compromised the fermentation efficiency of fibrous and starchy substrates, reflecting the reduced IVDMD at the highest dose, although the absence of change at 48 h suggests that microbial colonization was ultimately maintained. Thus, açaí oil compounds likely reduced short-term fermentation efficiency at 30 mg g^{-1} DM without exerting significant toxicity on the ruminal microbiota.

The use of plant extracts can have different effects on degradability and rumen fermentation products due to the inhibitory effects of phytochemicals on the rumen microbiota [22]. However, in our study, açaí oil did not alter the profile of VFA and N–NH_3 concentration, indicating that major metabolic pathways of ruminal fermentation were preserved and that the net energy supply to the host animal would likely not be compromised.

However, the changes observed in the rate of gas production, as indicated by the parallelism test, and in gas volume, as indicated by the identity test, revealed an increased production rate with 0 and 30 mg g^{-1} of açaí oil. This indicates that the lower dosages (0.3 and 3 mg g^{-1}) altered the fermentation pattern by slowing the rate of fermentation. Moreover, the increased gas production at 30 mg g^{-1} and the intermediate levels at 0.3 and 3 mg g^{-1} compared with the control treatment indicate that, although lipid inclusion can reduce ruminal fermentation and gas production due to its toxic effects on microorganisms [23], açaí oil may have preserved and stimulated fermentative activity in this study. This effect is likely related to the bioactive compounds in the oil that modulate the ruminal microbiota without compromising the overall fermentation efficiency.

It is well established that rumen fermentation produces microbial proteins, ammonia and VFA. Although these products are vital energy sources for the ruminants, gas formation represents energy loss and inefficient nutrient utilization. Gas production during ruminal fermentation is directly related to organic matter degradation and microbial metabolism, as part of the dietary energy is converted into gases and is not utilized for microbial synthesis or VFA production [24]. Consequently, the aim of manipulating rumen fermentation is to increase the efficiency of nutrient utilization and animal production, as well as to reduce energy loss during fermentation. In agreement with present results, Freitas *et al.* [6] also reported increased *in vitro* gas production following açaí oil supplementation. Despite this, the absence of changes in VFA concentrations in this study suggests that the production of the main degradation end products (acetate, propionate, and butyrate) remained efficient.

From a practical perspective, the findings indicate that higher inclusion levels of açaí oil do not impair *in vitro* fermentation. However, from a nutritional perspective, the increase in gas production without alterations in the VFA concentration or IVOMD likely reflects greater CH_4 or CO_2 release, the main gases produced in the rumen. This represents an increased energy loss without enhancing the production of energetic substrates for the animal. Therefore, even though the VFA profiles were unaffected, the increased gas production and reduced IVDMD observed at 30 mg g^{-1} DM of açaí oil indicate diminished dietary energy efficiency.

5. Conclusion

The findings of this study indicate that açaí oil modulates *in vitro* ruminal fermentation in cattle without compromising fermentative efficiency at dosages of 0.3 and 3.0 mg g^{-1}. In contrast, inclusion at 30 mg g^{-1} reduced IVDM and increased total gas production, resulting in greater energy loss from the diet. Therefore, açaí oil holds promise as a feed additive at moderate levels of inclusion. Future *in vivo* research is encouraged to confirm its effects on animal performance, with particular attention to the role of bioactive compounds in modulation ruminal microbiota and fermentation dynamics.

Conflicts of interest statement

The authors declare no competing interests.

Data availability statement

he data will be provided upon request.

Author contributions

Conceptualization: G. J. Coelho, R. Mezzomo, and R. P. Maciel. Data curation: G. J. Coelho, M. E. Saúde, H, M. F. Martins and S. V. Sousa. Investigation: G. J. Coelho and F. G. Oliveira. Methodology: R. Mezzomo, R. P. Maciel, and L. F. Sousa. Supervision: R. Mezzomo. Writing (original draft): G. J. Coelho. Writing (proofreading and editing): R. Mezzomo and R. P. Maciel.

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