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Inclusion of oils in the *in vitro* fermentation of elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon)

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ABSTRACT - The objective of this study was to assess the impact of incorporating vegetable oils into the *in vitro* fermentation of a tropical grass on the kinetics and digestibility of the fibrous fraction by rumen microorganisms. Treatments consisted of different inclusion levels (0, 2, 4, and 6% dry matter) of lipid sources, namely soybean oil, canola oil, and baru oil, mixed with tropical forage (*Pennisetum purpureum* Schum cv. Cameroon). A completely randomized design was employed, featuring a 3 × 4 factorial arrangement (three lipid sources at four inclusion levels), resulting in 12 treatments, each with three replications. We examined the kinetics of rumen fermentation using the semi-automatic *in vitro* technique of cumulative gas production. Oil inclusion levels had a significant impact on *in vitro* gas production and lag time (P<0.0001), with values decreasing as the oil inclusion levels were increased, with no effect of sources. Furthermore, the digestibility of forage neutral detergent fiber (NDF) was negatively affected by higher oil inclusion levels, with significant differences observed among the lipid sources tested (P<0.0013), with improved digestibility effect for baru oil. The production and profile of short-chain fatty acids were also influenced by oil inclusion level factors; however, the influence of different lipid sources was only evident in treatments with 6% inclusion. Baru oil can be a potential dietary alternative for ruminant supplementation in the intake of tropical forages, as it presented the lowest adverse effect on the *in vitro* digestibility of NDF among the lipid sources used, such as soybean oil.

Keywords: baru, canola, degradability, digestibility, soybean

1. Introduction

Highly productive ruminants require increased energy mobilization to reach their production potential. Using vegetable oils as an energy source can improve performance, whether during the finishing phase (Silva et al., 2020) or in the transition period, of beef cattle and dairy cows (Mikuła et al., 2021).

Indigenous plant-derived oils, like baru oil from the cerrado biome, offer alternatives to traditional oils. The baru tree (*Dipteryx alata* Vog.) produces nuts rich in oleic acid, making it a valuable oil source. Research on cerrado species can promote biome conservation and create jobs through sustainable management (Campidelli et al., 2022).

Elephant grass (*Pennisetum purpureum* Schum.), widely used in ruminant diets, is valued for its high dry matter (DM) productivity, adaptability, and resistance to pests (Araújo et al., 2022). However, ruminants have limitations in metabolizing high-lipid diets (Van Soest, 1994). Lipid supplementation above 5% DM can reduce intake due to intake control mechanisms and fatty acid oxidation limits (Allen, 2020). Excessive lipids may harm the microbiota, reducing fermentation efficiency (Kargar et al., 2023), prompting research into safer lipid sources.

Barueiro almond oil, rich in antioxidants, fatty acids, and bioactive compounds, shows potential for improving lipid markers and reducing cardiovascular risk (Campidelli et al., 2022).

Considering the potential of lipids to support animal production and the challenges ruminants face in utilizing them, it is crucial to study their impact on digestion and effective incorporation into diets as oils. This study aimed to assess the *in vitro* effects of baru oil and common vegetable oils on fermentation, fibrous digestibility, and kinetic parameters in tropical grass.

2. Material and methods

The experiment was conducted in Brasília, Federal District, Brazil (latitude: 15°56'55.2"; longitude: 47°56'03.5"; elevation: 1,100 m) with prior approval from the Ethics Committee on Animal Use (CEUA/UnB, approval no. 21/2019).

We investigated the effects of three lipid sources—soybean oil (SO, *Glycine max*), canola oil (CO, *Brassica napus*), and baru oil (BO)—at four inclusion levels (0, 2, 4, and 6% DM) on the ruminal fermentation of *Pennisetum purpureum* Schum. cv. Cameroon, totaling 12 treatments, each with three replications, in a completely randomized 3 × 4 factorial design. The forage was collected at 70 days (2.65 m high) during the rainy season in Ceres, Goiás, Brazil.

The chemical composition of the forage (Table 1) was analyzed for DM (method 925.45b), crude protein (CP, method 934.13A), mineral matter, and ether extract (EE, method 960.39), following AOAC (2019). Neutral detergent fiber (NDF, method F-002/2) and acid detergent fiber (ADF, method F-004/2) followed INCT-CA (Detmann et al., 2021).

Table 1 - Chemical composition of the forage (*Pennisetum purpureum* Schum. cv. Cameroon)

Component	Concentration (g/kg DM)
Dry matter (DM)	159
Crude protein	112
Mineral matter	57
Ether extract	22
Neutral detergent fiber	658
Acid detergent fiber	299

The semi-automatic *in vitro* gas production technique (Mauricio et al., 1999) was applied using 160-mL glass vials. Three consecutive incubations were performed with ruminal fluid from two adult sheep (60 kg) with permanent ruminal cannulas, grazing Tifton 85 grass (*Cynodon* spp.) and receiving 100 g of concentrate daily.

For each treatment, 1 g DM of forage or its combination with oil levels was placed in 160-mL bottles, followed by 90 mL of medium B and reducing solution (Theodorou and Zhu, 1993). After CO_2 saturation,

bottles were sealed, incubated at 39 °C, and inoculated with 10 mL of rumen fluid. Initial pressure readings were standardized, and three blank bottles were incubated.

Gas pressure readings were taken at 0, 1, 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60, 72, and 96 h due to continued fermentation beyond 48 h. Pressure data (PSI) were converted to gas volume using Guimarães Júnior et al. (2008) (Eq. 1), considering Brasília's temperature and atmospheric pressure. Bottles were manually shaken after each measurement.

$$\text{Volume (mL)} = 4.50231 \times \text{Pressure} + 0.05164 \times \text{Pressure}^2 \ (R^2 = 0.996) \quad (1)$$

The gas volume from control bottles was subtracted from sample bottles at each reading, with adjusted values cumulatively added to form an overtime soluble fraction curve. Gas production kinetics were analyzed using the model France et al. (1993) (Eq. 2).

$$V(t) = Vf \{1 - e^{[-b(t-\lambda)-c(\sqrt{t}-\sqrt{\lambda})]}\} + \varepsilon \quad (2)$$

in which V(t) represents the cumulative gas volume at time (t), Vf indicates the total gas volume; parameters b and c have no biological interpretation and are the exponential function (−e), and ε denotes the experimental error.

To assess ammonia nitrogen (N-NH_3) and short-chain fatty acids (SCFA), 500 mg of forage samples or forage-oil combinations were placed in 5 × 5 cm non-woven fabric (NWF) filter bags. The bags were sealed and incubated in 100-mL glass bottles containing 45 mL of medium B. The bottles were inoculated with 5 mL of rumen fluid (1:9 ratio, inoculum/medium) and incubated at 39 °C (Chaney and Marbach, 1962). Gases were released every three hours for 12 h, and every six hours for 36 h. Samples were collected at 24 and 48 h, processed, and analyzed (Erwin et al., 1961).

The *in vitro* NDF digestibility (IVNDFD) was measured by incubating 500 mg of forage or forage-oil combinations in 5 × 5 cm NWF filter bags, placed in 100-mL bottles with 10 mL rumen inoculum and 40 mL McDougall's buffer (1:4), incubated at 39 °C, and the gas produced was removed periodically (Tlley and Terry, 1963). After incubation, bags were autoclaved, rinsed, dried, and weighed to determine NDF residue (Detmann et al., 2021).

The experiment followed a completely randomized 3 × 4 factorial design (three lipid sources and four inclusion levels) with three replications, using bottles as the experimental units. Statistical analyses were performed with the PROC MIXED procedure in SAS software (Statistical Analysis System, 2004), with a significance level of 0.05. Interactions were analyzed based on significance as determined by the statistical model (Eq. 3).

$$Y_{ijkl} = \mu + A_i + B_j + AB_{ijk} + E_{ijkl} \quad (3)$$

In the model, Y_{ijkl} represents the observation on lipid sources (i), inclusion levels (j), and replication (k); μ is the fixed general mean effect; A_i and B_j are random effects for lipid sources (soybean, canola, baru oil) and oil inclusion levels (0, 2, 4, 6% DM); AB_{ijk} is the interaction effect; and E_{ijkl} is the random error associated with repetitions.

3. Results

The examined oil inclusion levels had a significant linear effect (P<0.0001) on total gas production (Table 2). As oil inclusion increased, gas production decreased across all lipid sources. Average gas production values were 263.2, 261.83, 253.37, and 243.05 mL of gas/mg DM incubated for treatments with 0, 2, 4, and 6% oil inclusion, respectively.

Gas production was significantly affected by the treatments and their interactions at 72 and 96 h, with inclusion levels showing a quadratic effect (P<0.05) regardless of the lipid source. Additionally, oil inclusion levels had a linear effect (P<0.05) on lag time, accelerating colonization as lipid inclusion increased.

Table 2 - Kinetic parameters and cumulative i*n vitro* gas production as a function of the inclusion of different oil sources and levels

Parameter	Soybean oil (% DM)				Canola oil (% DM)				Baru oil (% DM)				SEM	P-value		
	0	2	4	6	0	2	4	6	0	2	4	6		Source (S)	Level (L)	S×L
Vf[1]	261.93	259.73	257.46	240.56	262.26	263.50	251.03	243.00	266.96	262.26	251.63	245.60	2.01	0.7952	<0.0001	0.7651
Lag (h^{-1})	1.12	0.95	1.03	0.97	1.17	1.04	1.06	0.87	1.20	1.02	0.95	0.76	0.05	0.7978	0.0252	0.8468
GP6h[1]	30.45	35.74	33.65	32.03	30.10	29.54	35.11	34.77	27.34	31.92	32.18	33.70	1.27	0.6423	0.1569	0.6418
GP12h[1]	78.49	83.42	79.00	74.50	76.36	73.82	77.96	80.91	71.49	77.24	80.61	83.36	2.80	0.9276	0.7923	0.7308
GP24h[1]	157.21	162.88	153.95	145.75	154.89	149.82	154.89	158.60	149.92	153.74	158.36	160.82	3.59	0.9735	0.9917	0.5566
GP48h[1]	229.18	232.89	224.08	207.11	226.66	222.79	223.87	217.28	223.65	225.45	226.13	220.74	3.16	0.9560	0.0971	0.6465
GP72h[1]	256.72	257.39	250.50	229.75	255.06	254.42	250.22	237.42	254.73	254.22	249.91	241.21	2.51	0.9094	0.0002	0.7895
GP96h[1]	267.49	266.84	257.62	240.55	263.05	266.31	257.91	246.17	266.14	267.71	258.28	249.92	2.70	0.7914	0.0002	0.9587
IVNDFD96h	53.93	44.78	40.48	34.05	53.56	43.43	39.29	32.66	54.58	45.92	41.46	36.14	1.38	0.0013	<0.0001	0.8290

Vf - final gas volume; GP - gas production; IVNDFD - *in vitro* neutral detergent fiber digestibility (%); SEM - standard error of the mean; S×L - interaction between oil sources and levels.
[1] mL/g DM incubated.
Significant at the 5% level (P<0.05).

Oil inclusion levels significantly reduced IVNDFD in a linear manner (P<0.0001) as levels increased. The lipid source also had a linear effect (P<0.05) on IVNDFD at 96 h of incubation. Baru oil treatments consistently showed higher IVNDFD values, with a significant difference at the 6% inclusion level: baru oil (36.14%) was higher compared with soybean oil (34.05%) and canola oil (32.66%).

The sources, oil levels, and their interaction affected ruminal N-NH_3 concentration at 24 h (Table 3). Baru oil showed higher values (6.75 mg/dL) compared with canola (4.47 mg/dL) and soybean (4.73 mg/dL) oils, with significant differences (P<0.0001) at 2, 4, and 6% levels. A quadratic effect was observed, with the highest N-NH_3 concentration in treatments without oil (9.11 mg/dL).

At 48 h, oil inclusion levels showed a significant linear effect (P<0.0001) on ruminal N-NH_3 concentration, with higher inclusion levels resulting in the highest mean values for this parameter.

Oil inclusion levels caused a quadratic effect (P<0.001) on SCFA production at 24 h (Table 3). Acetate and propionate production decreased, while butyrate production increased across all oil treatments. There was an interaction effect (P<0.05) on butyric acid, with baru oil leading to lower values at 2% inclusion and canola oil at 6%. The C2:C3 ratio also showed a significant linear effect (P<0.0001), increasing with higher oil inclusion levels.

Within 48 h, oil levels significantly affected all fermentation profile variables (P<0.0001). Oil treatments showed lower acetate and propionate production, a lower C2:C3 ratio compared with treatments without oil, and higher butyrate production.

4. Discussion

Soybean, widely used in Brazil in ruminant diets as oil and grain, contrasts with canola oil, which, though less common, holds potential due to its favorable fatty acid profile and local production. Baru oil, as an alternative lipid source, aids Cerrado biome conservation, where Brazil's largest cattle herd is located. Typically, lipid inclusion in animal diets follows international guidelines (Elsabaawy and Gad, 2021), often neglecting the distinct chemical and nutritional characteristics of regional feed ingredients. Literature suggests lipid inclusion should remain between 5 to 7% DM to avoid negative effects on rumen microbiota, which can reduce fiber and organic compound digestion (Ibrahim et al., 2021).

Higher oil levels likely interfered with bacterial activity on forage particles, reducing fermentative activity and fiber digestibility. This led to a decrease in final gas volume (Vf), with lipids modulating ruminal microbiota, particularly those that metabolize carbohydrates (Hassan et al., 2020). All oil sources negatively impacted NDF digestion, with the highest reductions observed in canola oil (39.54%), soybean oil (36.97%), and baru oil (33.10%). Lipids disrupt ruminal fermentation, decreasing digestion of non-lipid energy sources, with structural carbohydrate digestion reduced by 50% or more with less than 10% added fat (Ibrahim et al., 2021). However, for IVNDFD, baru oil showed the highest value (44.52%), followed by soybean oil (43.31%) and canola oil (42.23%).

Vegetable oils generally contain more unsaturated fatty acids, with canola and soybean oils having higher unsaturation levels, impacting rumen fermentation kinetics (Joy et al., 2021). Soybean oil is rich in polyunsaturated linoleic acid (51%), while canola oil has a higher proportion of monounsaturated oleic acid (53.8%) (NRC, 2016). Baru oil contains mainly oleic acid (47.1%) and less unsaturated fatty acids, likely contributing to its milder negative effects on IVNDFD (Kargar et al., 2023).

Gas production at 48 h showed no significant differences between oil sources or levels. However, at 72 and 96 h, lower oil levels led to increased gas production, suggesting biohydrogenation had reduced the negative impact of unsaturated fatty acids on fermentation. The lag period, or time before gas production begins, was reduced with increasing oil doses, with an average of 0.87 h for treatments with 6% oil, likely due to rapid fermentation of glycerol from oils (Castañeda-Rodríguez et al., 2023). Lipids, though a valuable energy source for ruminants, do not serve the same purpose for rumen microbiota, which rely more on proteins and carbohydrates.

Table 3 - *In vitro* fermentation profile depending on the inclusion of different oil sources and levels

Parameter	Soybean oil (% DM)				Canola oil (% DM)				Baru oil (% DM)				SEM	P-value		
	0	2	4	6	0	2	4	6	0	2	4	6		Source (S)	Level (L)	S×L
24 h																
N-NH_3[1]	9.13	5.22	1.68	2.88	9.06	2.41	2.59	3.80	9.14	4.13	5.98	7.74	0.15	<0.0001	<0.0001	<0.0001
Acetic acid[2]	72.63	71.28	71.78	71.77	72.70	71.44	71.53	72.26	72.60	71.64	71.25	71.58	0.09	0.2929	<0.0001	0.1627
Propionic acid[2]	20.55	19.13	18.84	18.70	20.32	18.81	18.94	18.89	20.39	19.24	19.34	18.94	0.0954	0.2042	<0.0001	0.5626
Butyric acid[2]	6.80	8.31	8.13	8.20	6.97	8.32	8.27	7.54	6.99	7.86	8.08	8.16	0.0592	0.4987	<0.0001	0.0017
C2:C3	3.53	3.72	3.81	3.83	3.57	3.79	3.77	3.82	3.56	3.72	3.68	3.77	0.0224	0.1981	<0.0001	0.6612
48 h																
N-NH_3[1]	2.62	5.54	5.65	5.45	2.00	3.54	4.75	6.05	2.47	4.85	4.51	6.01	0.19	0.0502	<0.0001	0.0672
Acetic acid[2]	68.02	66.06	67.01	67.00	68.08	65.70	66.13	66.03	68.05	66.06	66.97	66.86	0.13	0.0103	<0.0001	0.4448
Propionic acid[2]	24.54	23.14	22.51	21.92	24.49	23.62	22.81	23.05	24.54	22.83	22.45	22.11	0.15	0.0461	<0.0001	0.5149
Butyric acid[2]	7.43	8.39	8.08	8.49	7.41	8.28	8.55	8.36	7.39	8.52	8.27	8.63	0.07	0.6397	<0.0001	0.4061
C2:C3	2.77	2.85	2.97	3.05	2.78	2.78	2.89	2.86	2.77	2.90	2.98	3.02	0.02	0.0187	<0.0001	0.4168

C2:C3: acetic to propionic acids ratio; SEM: standard error of the mean; S×L: interaction between oil sources and levels.
[1] mg/dL DM incubated.
[2] mM/100 mM.
Significant at the 5% level (P<0.05).

Nitrogen ($N\text{-}NH_3$) availability for microbial synthesis depends on forage digestion, medium composition, and microbial recycling (Ortega et al., 2022). Oil inclusion lowered $N\text{-}NH_3$ concentrations at 24 h compared with treatments without oil, likely due to lipid inhibition of protozoa. By 48 h, treatments with oil showed higher $N\text{-}NH_3$ concentrations, possibly due to delayed protein release from forage (Muñoz-Cuautle et al., 2022). This increase could also be linked to reduced microbial growth from glycerol fermentation, leading to less $N\text{-}NH_3$ utilization. Higher oil inclusion levels further increased $N\text{-}NH_3$ concentrations, suggesting a role in nitrogen utilization and fiber digestion. Consequently, variations in $N\text{-}NH_3$ concentrations may be closely associated with the level of lipid inclusion and their degree of unsaturation, potentially contributing to a decrease in the microbial population (Steinbuch et al., 2018).

Short-chain fatty acid production is crucial, providing 60-80% of the host's dietary energy. Lipid supplementation affects SCFA molar ratios depending on diet composition, lipid inclusion rate, and basal lipid content (Bai et al., 2024). Treatments with oil inclusion showed lower proportions of acetic (C2) and propionic (C3) SCFA at 24 h, with higher butyric (C4) acid. At 48 h, the trend continued, with canola oil resulting in the lowest acetate and highest propionate concentrations at 6% oil inclusion. This shift is likely due to fatty acid inhibition of cellulolytic microorganisms, which produce acetate, coupled with lower fermentation quality of tropical forage.

The C2:C3 ratios showed significant linear increases ($P<0.05$) with higher inclusion levels across all tested oil sources, whereas the effect on ammonia concentration was only observed after 48 h. Current research indicates that vegetable oils can increase proteolytic activity through alterations in the microbial population, leading to increased protein degradation and subsequent ammonia release (Nurfatahillah et al., 2022; Zhang et al., 2022). These microbial alterations simultaneously reduce methane production (Muñoz-Cuautle et al., 2022) and acetate (C2) accumulation. The observed effects are primarily attributed to the high unsaturated fatty acid content in the oils, which selectively inhibits methanogenic archaea and stimulates propionate (C3)-producing bacteria.

The precise mechanism behind increased butyrate production from oil supplementation remains unclear. However, oil inclusion alters the microbiota profile, promoting butyrogenic bacteria, while inhibiting fibrolytic species (Gruninger et al., 2022). Unsaturated fatty acids serve as precursors for microbial hydrogenation, a key butyrate-generating process, while released glycerol from lipid hydrolysis provides additional fermentation substrate (Amanullah et al., 2022).

The increasing linear effect observed for the C2:C3 ratio with higher oil inclusion levels contrasts with conventional literature, which often reports a reduction in this ratio due to an increase in propionate production (Gunun et al., 2024). The result found in the present study may be related to the selective inhibition of C3-producing bacteria, alterations in biohydrogenation pathways, or specific characteristics of the tested oil.

The $N\text{-}NH_3$ concentration, butyrate production, and C2:C3 ratio exhibited contrasting temporal patterns between 24 and 48 h across oil treatments. While these parameters decreased at 24 h, they increased significantly by 48 h ($P<0.05$). We hypothesize that this shift reflects microbial adaptation dynamics: initial oil exposure transiently suppressed activity (lag phase, 24 h), followed by population shifts and enzymatic acclimation enhancing fermentation at 48 h. This aligns with ruminal microbial colonization (lag time), in which fibrolytic microbes require prolonged exposure to degrade lipid-coated feed (Zhang et al., 2022).

5. Conclusions

In vitro testing revealed that baru oil is the most suitable option for supplementing ruminants due to its lesser effect on fiber digestibility, likely because of its lower unsaturated fatty acid content. Fiber digestibility is most reduced with canola oil (39.54%), followed by soybean oil (36.97%) and baru oil (33.10%).

The SCFA profiles from the *in vitro* study show decreased acetic and propionic acids and increased butyric acid, with more pronounced effects at higher inclusion levels, especially with canola oil. The study suggests keeping lipid inclusion below 5% DM to avoid negative impacts on rumen function, highlighting the milder impact of baru oil compared with soybean and canola oils.

Data availability

The datasets supporting the results of this study are included in the article itself.

Author contributions

Conceptualization: Ribeiro, M. D. **Data curation:** Cabral Filho, S. L. S.; Fabino Neto, R.; Di Calaça, A. M. M. and Arnhold, E. **Formal analysis:** Fabino Neto, R.; Di Calaca, A. M. M.; Zacaroni, O. F. and Arnhold, E. **Funding acquisition:** Ribeiro, M. D. **Investigation:** Cabral Filho, S. L. S.; Silva, C. J.; Zacaroni, O. F. and Silva, N. B. S. **Methodology:** Ribeiro, M. D.; Fabino Neto, R.; Di Calaça, A. M. M. and Silva, C. J. **Project administration:** Ribeiro, M. D. and Cabral Filho, S. L. S. **Resources:** Cabral Filho, S. L. S.; Silva, C. J. and Castro, M. A. O. **Software:** Arnhold, E. **Supervision:** Ribeiro, M. D.; Silva, C. J. and Zacaroni, O. F. **Validation:** Fabino Neto, R. **Visualization:** Fabino Neto, R.; Silva, N. B. S.; Ferreira, D. J. and Zanine, A. M. **Writing – original draft:** Silva, C. J. **Writing – review & editing:** Ribeiro, M. D.; Cabral Filho, S. L. S.; Fabino Neto, R.; Di Calaça, A. M. M.; Silva, N. B. S.; Ferreira, D. J. and Zanine, A. M.

Conflict of interest

The authors declare no conflict of interest.

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References

Allen, M. S. 2020. Review: Control of feed intake by hepatic oxidation in ruminant animals: Integration of homeostasis and homeorhesis. Animal 14(S1):s55-s64. https://doi.org/10.1017/S1751731119003215

AOAC. 2019. Official methods of analysis of the Association of Official Analytical Chemists. 21st ed. AOAC International, Washington, D.C.

Amanullah, S. M.; Lee, S. S.; Paradhipta, D. H. V.; Joo, Y. H.; Kim, D. H.; Seong P. N.; Jeong, S. M. and Kim, S. C. 2022. Impact of oil sources on *in vitro* fermentation, microbes, greenhouse gas, and fatty acid profile in the rumen. Fermentation 8:242. https://doi.org/10.3390/fermentation8050242

Araújo, J. S.; Araújo, C. A.; Macedo, A.; Silva, C. S.; Novaes, J. J. S.; Lima, D. O.; Borges, E. N.; Gois, G. C.; Araújo, G. G. L. and Campos, F. S. 2022. Fermentation dynamics, nutritional quality, and heating capacity of mixed silages of elephant grass (*Pennisetum purpureum* Schum) and Leucaena (*Leucaena leucocephala*). Brazilian Journal of Veterinary Research and Animal Science 59:e189466. https://doi.org/10.11606/issn.1678-4456.bjvras.2022.189466

Bai, H.; Wang, L.; Lambo, M. T.; Li, Y. and Zhang, Y. 2024. Effect of changing the proportion of C16:0 and *cis*-9 C18:1 in fat supplements on rumen fermentation, glucose and lipid metabolism, antioxidation capacity, and visceral fatty acid profile in finishing Angus bulls. Animal Nutrition 18:39-48. https://doi.org/10.1016/j.aninu.2024.04.010

Castañeda-Rodríguez, C. S.; Pámanes-Carrasco, G. A.; Páez-Lerma, J. B.; Herrera-Torres, E.; Araiza-Rosales, E. E.; Hernández-Vargas, V.; Medrano-Roldán, H. and Reyes-Jáquez, D. 2023. Effect of vegetable oils or glycerol on the in vitro ruminal production of greenhouse gases. Ruminants 3:140-148. https://doi.org/10.3390/ruminants3020013

Campidelli, M. L. L.; Carneiro, J. D. S.; Souza, E. C.; Vilas Boas, E. V. B.; Bertolucci, S. K. V.; Aazza, S.; Oliveira, R. R.; Chalfun-Junior, A.; Reis, G. L.; Seixas, J. N.; Nelson, D. L. and Pereira, L. J. 2022. Baru almonds (*Dipteryx alata* Vog.) and baru almond paste promote metabolic modulation associated with antioxidant, anti-inflammatory, and neuroprotective effects. Innovative Food Science and Emerging Technologies 80:103068. https://doi.org/10.1016/j.ifset.2022.103068

Chaney, A. L. and Marbach, E. P. 1962. Modified reagents for determination of urea and ammonia. Clinical Chemistry 8:130-132. https://doi.org/10.1093/clinchem/8.2.130

Detmann, E.; Silva, L. F. C.; Rocha, G. C.; Palma, M. N. N. and Rodrigues, J. P. P. 2021. Métodos para análise de alimentos. 2.ed. Suprema, Visconde do Rio Branco.

Elsabaawy, E. H. and Gad, S. M. 2021. Lipids in ruminant nutrition and its effect on human health. p.344-367. In: Abd El-Kader, S. M. and Mohammad El-Basioni, B. M. (Orgs.). Precision agriculture technologies for food security and sustainability. IGI Global Scientific Publishing. https://doi.org/10.4018/978-1-7998-5000-7.ch015

Erwin, E. S.; Marco, G. J. and Emery, E. M. 1961. Volatile fatty acid analyzes of blood and rumen fluid by gas chromatography. Journal of Dairy Science 44:1768-1771. https://doi.org/10.3168/jds.S0022-0302(61)89956-6

France, J.; Dhanoa, M. S.; Theodorou, M. K.; Lister, S. J.; Davies, D. R. and Isac, D. 1993. A model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feeds. Journal of Theoretical Biology 163:99-111. https://doi.org/10.1006/jtbi.1993.1109

Gunun, N.; Kaewpila, C.; Khota, W.; Kimprasit, T.; Cherdthong, A. and Gunun, P. 2024. The effect of supplementation with rubber seed kernel pellet on *in vitro* rumen fermentation characteristics and fatty acid profiles in swamp buffalo. BMC Veterinary Research 20:177. https://doi.org/10.1186/s12917-024-04017-8

Gruninger, R. J.; Zhang, X. M.; Smith, M. L.; Kung Jr, L.; Vyas, D.; McGinn, S. M.; Kindermann, M.; Wang, M.; Tan, Z. L. and Beauchemin, K. A. 2022. Application of 3-nitrooxypropanol and canola oil to mitigate enteric methane emissions of beef cattle results in distinctly different effects on the rumen microbial community. Animal Microbiome 4:35. https://doi.org/10.1186/s42523-022-00179-8

Guimarães Júnior, R.; Cabral Filho, S. L. S.; Fernandes, F. D.; Vilela, L. and Martha Júnior, G. B. 2008. Relação entre pressão e volume para implantação da técnica *in vitro* semiautomática de produção de gases na Embrapa Cerrados. Comunicado Técnico, 144. Embrapa, Planaltina. Available at: <https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/571896/1/comtec144.pdf>. Accessed on: Apr. 25, 2025.

Hassan, F.; Arshad, M. A.; Ebeid, H. M.; Rehman, M. S.; Khan, M. S.; Shahid, S. and Yang, C. 2020. Phytogenic additives can modulate rumen microbiome to mediate fermentation kinetics and methanogenesis through exploiting diet–microbe interaction. Frontiers in Veterinary Science 7:575801. https://doi.org/10.3389/fvets.2020.575801

Ibrahim, N. A.; Alimon, A. R.; Yaakub, H.; Samsudin, A. A.; Candyrine, S. C. L.; Wan Mohamed, W. N.; Abidah, M. N.; Amirul, F. M. and Mookiah, S. 2021. Effects of vegetable oil supplementation on rumen fermentation and microbial population in ruminant: A review. Tropical Animal Health and Production 53:422. https://doi.org/10.1007/s11250-021-02863-4

Joy, F.; Johnson, J. A.; Górka, P.; McKinnon, J. J.; Hendrick, S. and Penner, G. B. 2021. Effect of dietary lipid inclusion from by-product-based pellets on dry matter intake, ruminal fermentation, and nutrient digestion in finishing beef heifers. Canadian Journal of Animal Science 101:481-492. https://doi.org/10.1139/cjas-2020-0133

Kargar, S.; Taasoli, G.; Akhlaghi, A. and Zamiri, M. J. 2023. *In vitro* rumen fermentation pattern: Insights from concentrate level and plant oil supplement. Archives Animal Breeding 66:1-8. https://doi.org/10.5194/aab-66-1-2023

Mauricio, R. M.; Mould, F. L.; Dhanoa, M. S.; Owen, E.; Channa, K. S. and Theodorou, M. K. 1999. A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. Animal Feed Science and Technology 79:321-330. https://doi.org/10.1016/S0377-8401(99)00033-4

Mikuła, R.; Pruszyńska-Oszmałek, E.; Pszczola, M.; Rząsińska, J.; Sassek, M.; Nowak, K. W.; Nogowski, L. and Kołodziejski, P. A. 2021. Changes in metabolic and hormonal profiles during transition period in dairy cattle – the role of spexin. BMC Veterinary Research 17:359. https://doi.org/10.1186/s12917-021-03069-4

Muñoz-Cuautle, A.; Ortega-Cerrilla, M. E.; Herrera-Haro, J. G.; Ramírez-Bribiesca, J. E. and Zetina-Córdoba, P. 2022. *In vitro* rumen fermentation and methane production of sheep diets with the inclusion of condensed tannins and increasing levels of oregano oil (*Lippia graveolens*). Tropical and Subtropical Agroecosystems 25(2). https://doi.org/10.56369/tsaes.4245

NRC - National Research Council. 2016 Nutrient requirements of beef cattle: 8th rev. ed. National Academies Press, Washington, D.C. https://doi.org/10.17226/19014

Nurfatahillah, R. K.; Cusiayuni, A.; Jayanegara, A.; Wiryawan, K. G. and Evvyernie, D. 2022. Effect of palm oil supplementation level on in vitro ruminal fermentability. IOP Conference Series: Earth and Environmental Science 1041:012066. https://doi.org/10.1088/1755-1315/1041/1/012066

Ortega, R. M.; Moreno, D. S. and Angúlo, L. M. 2022. Protein-energy supplementation on productive and nutritional performance of grazing beef heifers. Semina: Ciências Agrárias 43:297-310. https://doi.org/10.5433/1679-0359.2022v43n1p297

Silva, P. I. J. L. R.; Zervoudakis, J. T.; Cabral, L. S.; Hatamoto-Zervoudakis, L. K.; Freiria L. B.; Silva, Y. R. V. B.; Paulino, P. V. R.; Tsuneda, P. P. and Possamai, A. J. 2020. Effects of rumen-protected oil supplementation on finishing grazing beef cattle. Tropical Animal Health and Production 52:763-769. https://doi.org/10.1007/s11250-019-02067-x

Steinbuch, K. B.; Benhamou, R. I.; Levin, L.; Stein, R. and Fridman, M. 2018. Increased degree of unsaturation in the lipid of antifungal cationic amphiphiles facilitates selective fungal cell disruption. ACS Infectious Diseases 4:825-836. https://doi.org/10.1021/acsinfecdis.7b00272

Theodorou, M. and Zhu, Y. 1993. A new laboratory procedure for determining the fermentation kinetics of ruminant feeds. Ciencia e Investigacion Agraria 20:332-344.

Tilley, J. M. A. and Terry, R. A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science 18:104-111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Cornell University Press, Ithaca. https://doi.org/10.7591/9781501732355

Zhang, H.; Lang, X.; Li, X.; Chen, G. and Wang, C. 2022. Effect of Zanthoxylum bungeanum essential oil on rumen enzyme activity, microbiome, and metabolites in lambs. PLoS ONE 17:e0272310. https://doi.org/10.1371/journal.pone.0272310