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Supplementation of a Basal Goat Diet With Incremental Doses of Canola Essential Oil Modulates In Vitro Rumen Fermentation and Microbial Diversity

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ABSTRACT

Background: Canola essential oil (CEO) contains linoleic and oleic fatty acids that can inhibit the growth of pathogenic microorganisms and alter microbial digestion to increase ruminal fermentation and nutrient utilisation.

Objectives: The study evaluated the effect of supplementing a basal goat diet with incremental doses of CEO on chemical constituents and in vitro ruminal fermentation parameters and microbial diversity.

Methods: Experimental treatments were a basal goat diet containing 0.0025% antibiotic growth promoter (AGP) without CEO (POSCON), a basal diet without AGP and CEO (NEGCON), and NEGCON supplemented with 0.5 (CEO5), 1.0 (CEO10), 1.5 (CEO15), and 2.0% (v/w) CEO (CEO20). The treatment samples were homogenised, oven-dried, milled and analysed for chemical constituents. For the in vitro experiment, each sample (1 g) was weighed into serum bottles containing a pre-mixed phosphate buffer solution (pH 6.8) and pre-warmed (39°C) overnight. Ruminal inoculum from three donor goats was used for the incubation. Rumen fermentation parameters and volatile fatty acids were determined and the 16s rRNA gene of the fermentation medium was sequenced and amplified to detect the archaea and bacteria abundance.

Results: Dry matter and organic matter contents were lower ($p < 0.05$) for CEO15 and CEO20. Crude fat increased with CEO doses with the highest value recorded for CEO20. Treatment CEO20 produced the highest ($p < 0.05$) value for the immediately fermentable fraction, effective gas production and 96-h partition factor. Lag time had a positive quadratic effect whereas acetic and butyric acids conferred a positive quadratic effect in response to CEO inclusion. A total of 15 phyla, 46 genera and 65 species were identified. The *Firmicutes*, *Bacteroidetes* and *Actinobacteria* predominated the phyla groups while unclassified microbes, *Prevotella* and *Succiniclasticum* across all treatments predominated the genera and species. The genus *Methanobrevibacter* and *Ruminococcus* reduced significantly at CEO15 and CEO20.

Conclusion: The inclusion of CEO in a basal goat diet increased gas production, partition factor at 96 hour of incubation and decreased total volatile fatty acids. However, 1.5% CEO level enhanced the abundance of fermentative bacteria such as *Firmicutes* and *Actinobacteria* while 1.5% and 2% CEO levels reduced the abundance of methanogenic microbes.

1 | Introduction

The world is experiencing a growing consumer awareness that increases the demand for healthy foods (Mazhangara et al. 2019). This has created a market demand for healthier meat including chevon, which is high in protein and low in fat and cholesterol (Lalhriatpuii et al. 2021). Goat production plays important socio-economic and nutritional roles, especially in empowering rural communities in Africa. Despite its socio-economic potential and nutritional advantages, chevon production is lagging due to limited scientific interventions and poor intensification at a commercial level (Van Wyk et al. 2020). Disease outbreaks, high cost of medications and vaccines, poor quality forage, and high feed costs compromise productivity, causing farmers to rely on antibiotics to promote growth and prevent diseases (Nwachukwu and Berekwu 2020). Farmers use antibiotic growth promoters (AGPs) to manipulate rumen metabolism for optimal fermentation efficiency and productivity (Hassan et al. 2020; Arsène et al. 2021).

However, the use of AGP could jeopardise public safety due to the development of antimicrobial resistance by pathogenic bacterial strains (Gunnarsson and Mie 2018). This is concerning due to its negative effects on environmental, human and animal health (Rahman et al. 2022). The World Health Organisation (WHO 2015) estimated that up to 50,000 deaths in Europe and United states was caused by antibiotic microbial resistance. Although there are no sufficient data for the African continent, human mortality and morbidity due to antibiotic resistance is on the rise (Tadesse et al. 2017) and this has caused several countries to ban the use of AGP (Nehme et al. 2021).

However, the complete withdrawal of AGP without alternatives is a major problem that threatens the economic viability of livestock farming (Cardinal et al. 2019). Thus, the use of herbal plants and their extracts (e.g., essential oils) as feed additives has gained worldwide research interest (Kholif and Olafadehan 2021). Phytogenic products have bioactive compounds (flavonoids, terpens, alkaloids, carvacrol, eugenol, etc.) that are noted for their antioxidant, antibacterial and antifungal activities (Upadhaya and Kim 2017). These bio-compounds can improve feed utilisation, ruminal fermentation products and reduce methane emissions in ruminants (Kholif and Olafadehan 2021; Corrêa et al. 2021). Essential oils can impact the rumen digestion to favour propionate production, which is a glucogenic precursor that increases the supply of glucose and, ultimately improve nutrient utilisation (Hausmann et al. 2018; Mottin et al. 2022). Although some studies have investigated the potential of essential oils as alternatives to AGP in animal feeds, no study has evaluated the effectiveness of canola essential oil (CEO) in place of AGP in a basal goat diet. Thus, this study evaluated the effect of supplementing a basal goat diet with incremental doses of CEO on chemical composition and in vitro ruminal fermentation and microbial diversity. The study aims to provide insights into how CEO inclusion in goat diet influences fermentation parameters and the rumen microbiome community, compared to an AGP.

2 | Materials and Methods

2.1 | Treatment Formulation

A total mixed ration was formulated as the basal diet to meet the nutritional requirement for growing goats (NRC 2007). Six isonitrogenous and isocaloric experimental treatments were formulated as follows: a basal goat diet containing 0.0025% standard inclusion level of Flavomycine AGP (POSCON); a basal diet without AGP (NEGCON), and NEGCON with 0.50 (CEO5), 1.00 (CEO10), 1.50 (CEO15), and 2.00% (v/w) (CEO20) of CEO, replacing AGP as shown in Table 1. Exactly 100 g of the basal diet was proportionally mixed with the AGP and CEO to form six treatments that were each replicated nine times, producing a total of 54 independent samples. Samples were milled through a 1 mm screen and then stored in labelled sample bottles pending analysis.

2.2 | Chemical Composition

The chemical composition of the replicate samples was analysed for dry matter (method: 945.15), ash (method: 942.05), crude protein (CP; method: 979.09), and crude fat (method: 920.39) according to the Association of Official Analytical Chemists (AOAC 2005). The CP was measured through the standard macro-Kjeldahl method (AOAC 2005, method no. 984.13). Following the detergent methods by Van Soest et al. (1991), an ANKOM^{2000} Fibre Analyzer (ANKOM Technology, New York, USA) was used to determine neutral detergent fibre (NDF) and acid detergent fibre (ADF). Acid detergent lignin (ADL) was analysed by submerging the ADF residue into 72% sulphuric acid for 3 h while agitating every 30 min. The samples were washed thoroughly with water, submerged into acetone, air dried, then oven dried before weighing. The difference between NDF and ADF was used to estimate hemicellulose, while cellulose was estimated as the difference between ADF and ADL.

2.3 | Ruminal Inoculum

Three donor Boer goats (29–32 kg) were fed a diet containing 30% forage (Blue Buffalo grass) and 70% concentrate (corn bran, palm kernel cake, wheat bran, soybean meal, molasses, vitamins and minerals) for 2 weeks. Thereafter, the goats were slaughtered at an abattoir (Zeerust, South Africa) and the rumen inoculum was collected immediately into pre-warmed insulated flasks, transported to Animal Science laboratory at the University research farm. A four-layer muslin cloth (1 mm pore size) was used to filter the rumen fluid into pre-warmed Erlenmeyer flask. This was done under continuous flushing with CO_2 and kept in a 39°C water bath to simulate rumen conditions (Fievez et al. 2005).

2.4 | In Vitro Gas Production Experiment

Approximately 1 g of each replicate sample was carefully weighed into 125 mL airtight serum bottles and flushed with CO_2. Exactly

TABLE 1 | Ingredient composition (g/kg *as is* basis) of the experimental treatments.

Ingredients	[a]Treatments					
	NEGCON	POSCON	CEO5	CEO10	CEO15	CEO20
Yellow maize (8.0%)	433	433	402	350	307	288
Wheat bran 15%	–	–	27.6	88.1	132	135
Molasses	80.0	80.0	80.0	80.0	80.0	80.0
Canola essential oil	–	–	5.00	10.0	15.0	20.0
Flavomycine	–	2.50	–	–	–	–
Soya oilcake (47%)	20.9	20.9	–	–	–	–
Sunflower oilcake (38%)	130	130	152	139	131	133
Eragrostis hay	150	150	150	150	150	150
Lucerne meal	150	150	150	150	150	150
Acid buffer	6.00	6.00	6.00	6.00	6.00	6.00
Ammonium chloride	10.0	10.0	10.0	10.0	10.0	10.0
Ammonium sulphate	3.00	3.00	3.00	3.00	3.00	3.00
Monodicalcium phosphate (21%)	0.45	0.45	–	–	–	–
Limestone powder	9.09	9.09	9.17	9.06	11.4	20.0
Magnesium oxide (51%)	0.42	0.42	0.15	–	–	–
Salt course (2 mm)	5.00	5.00	5.00	5.00	5.00	5.00
Summer lick premix	0.33	0.33	0.33	0.33	0.33	0.33
Melis P	0.16	0.16	0.16	0.16	0.16	0.16
Total	1000	1000	1000	1000	1000	1000

[a]Treatments:CEO5, canola essential oil at 0.5% inclusion level in the basal diet; CEO10, canola essential oil at 1.0% inclusion level in the basal diet; CEO15, canola essential oil at 1.5% inclusion level in the basal diet; CEO20, canola essential oil at 2.0% inclusion level in the basal diet; NEGCON, a basal diet without flavomycine or canola essential oil; POSCON, a basal diet with flavomycine.

90 mL phosphate buffer solution (pH 6.8) was added into the bottles and pre-warmed in an incubator at 39°C (Menke and Steingass 1988). Thereafter, 25 mL of rumen fluid was carefully injected into serum bottles. Gas pressure (psi) peak readings were recorded up to 96 h post-incubation and corrected using two blank serum bottles without the samples (Theodorou et al. 1994). Gas pressure was recorded for each sample by inserting a 23-gauge needle attached to a pressure transducer (model PX4200-015GI, Omega Engineering, Inc., Laval, QC, Canada) through the rubber stoppers that were used to close the serum bottles. The needles were left on the serum bottles after insertion to allow all available gas to escape. The gas pressure readings (psi) were then converted to gas volume (mL) using the following laboratory-specific equation:

$$y = 0.034x^2 + 6.2325x + 1.8143$$

where y is the gas volume (mL) and x is the measured gas pressure (psi).

Cumulative gas production parameters were estimated by fitting data into the Ørskov and McDonald (1979) non-linear model:

$$y = a + b(1 - e^{-c(t-lt)}$$

where y is the gas produced per period 't'; a is the gas produced from the immediately fermentable fraction (mL/g OM); b is the gas produced from the slowly fermentable fraction (mL/g OM); c is the gas production rate constant for the insoluble fraction b (%/h); t is the incubation time (h); and lt is the lag time (h). Potential gas production (Pgas) was calculated by adding fractions a and b, while effective gas production (Egas) was calculated using the formula below:

$$\text{Egas} = a + \frac{bc}{K + c}$$

where K is the rumen outflow rate assumed to be 2.5% per h, and a, b and c are the Ørskov–McDonald parameters already described above.

Three serum bottles per treatment were opened at 24 h post-incubation and 2 mL of the medium was sampled and centrifuged using a Cryste S4100G centrifuge (Model: Varispin 4 with Max RPM of 4000; Gyeonggi-do, Republic of Korea). The samples were stored at 4°C for volatile fatty acid (VFA) analysis using a gas chromatography (GC-MS Laboratory, Stellenbosch University, South Africa) as described by Sompong et al. (2009). Triplicate samples of rumen fermentation mixture were collected into 0.5 mL micro plastic tube and DNA/RNA shield was added (200 µL of Shield to 100 µL of rumen fermentation mixture) and sent to Inqaba Biotec (Inqaba Biotec, Pretoria, South Africa) for genomic DNA extraction, 16S (v4) bacterial/archaea and NGS DNA sequence analysis.

TABLE 2 | Chemical composition of a basal goat diet supplemented with varying levels of canola essential oil.

	[1]Diets							*p* value			Contrast
Parameters (%)	**POSCON**	**NEGCON**	**CEO5**	**CEO10**	**CEO15**	**CEO20**	**SEM**	**GLM**	**Linear**	**Quadratic**	**PvsN**
Dry matter	91.9[a]	91.3[a]	91.2[ab]	91.9[a]	90.1[b]	91.1[ab]	2.450	0.001	0.214	0.820	0.049
Organic matter	83.4[a]	82.6[ab]	83.6[a]	83.2[a]	81.7[b]	82.4[ab]	3.000	0.002	0.090	0.330	0.049
Crude protein	13.4	13.7	13.7	13.6	13.8	13.6	0.270	0.867	0.630	0.882	0.024
Crude fat	3.12[c]	3.32[bc]	3.81[bc]	4.06[abc]	4.55[ab]	5.15[a]	0.290	0.001	0.0001	0.691	0.547
NDF	31.1	32.2	30.5	28.4	28.9	30.0	0.860	0.055	0.029	0.015	0.415
ADF	12.9	12.7	13.3	11.6	12.4	13.2	0.390	0.060	0.980	0.115	0.397
ADL	3.39	3.24	2.85	3.53	3.20	3.95	0.750	0.940	0.387	0.599	0.903
Cellulose	18.3	19.5	17.2	16.9	16.6	16.8	0.750	0.060	0.005	0.033	0.397
Hemicellulose	9.47	9.48	10.4	8.03	9.18	9.24	0.570	0.158	0.292	0.362	0.999

[a, b, c] in a row, dietary treatment means with common superscripts do not differ ($p > 0.05$).

[1]Diets: ADF, acid detergent fibre; ADL, acid detergent lignin; CEO5, canola essential oil at 0.5% inclusion level in the basal diet; CEO10, canola essential oil at 1.0% inclusion level in the basal diet; CEO15, canola essential oil at 1.5% inclusion level in the basal diet; CEO20, canola essential oil at 2.0% inclusion level in the basal diet; NDF, neutral detergent fibre; NEGCON, a basal diet without flavomycine nor canola essential oil; POSCON, a basal diet with flavomycine; PvsN, preplanned orthogonal contrasts for POSCON and NEGCON; SEM, standard error of the mean.

The Illumina NextSeq sequencing of the V4 region of 16S rRNA gene was amplified with the prokaryotic primer pair 515F-806R to detect both archaea and bacteria at the phylum, genus and species level (Wasimuddin et al. 2020).

2.5 | Measures of Fermentation Efficiency

After the incubation period, the residues were decanted into pre-weighed crucibles and dried in the oven at 105°C for 12 h. The dry residues were weighed and incinerated at 600°C for overnight to determine ash residue content. Organic matter was calculated as the difference between dry residue and ash residue and used to calculate organic matter digestibility (OMD) as follows:

$$\text{OMD}\left(\frac{\text{g}}{\text{kg}}\right) = \frac{\text{OM content of incubated sample} - \text{OM content of residues}}{\text{OM content of incubated sample}} \times 1000$$

$$\text{Partition factor (mL/mg OM)} = \frac{\text{96 h cumulative gas production}}{\text{96 h OMD}}$$

2.6 | Statistical Analysis

The data were assessed for linear and quadratic coefficients using Response Surface Regression (PROC RSREG) in SAS version 9.4 (SAS Institute Inc. 2013). A non-linear model was used to determine the level of CEO inclusion that maximised and minimised the response variables.

$Y = ax^2 + bx + c$ where y is the dependent variable, a and b are the coefficients of the quadratic equation, and c is the dietary SMS levels. The x value that minimised or maximised the response variables was determined as: $\frac{-b}{2a}$.

The data were further subjected to the procedure of general linear model (PROC GLM) in SAS version 9.4 (SAS Institute Inc. 2013) to account for treatment differences. Significant means were separated using the option of probability of difference in SAS. Pre-planned orthogonal contrast statements were used to compare the performance of POSCON against NEGCON. The level of significance was considered at $p < 0.05$ for all variables.

3 | Results

Table 2 shows that crude fat ($p = 0.0001$) linearly increased with CEO inclusion levels. Significant linear decrease and quadratic effects were identified for cellulose ($p = 0.033$) and NDF ($p = 0.015$). The POSCON had the lowest ($p < 0.05$) crude fat while CEO20 had the highest. CEO15 recorded the least ($p < 0.05$) DM and OM when compared to POSCON, which did not vary significantly with the other treatments. Orthogonal contrasts showed that POSCON promoted a higher ($p < 0.05$) dry matter (DM) and organic matter (OM) while NEGCON had higher CP values.

Table 3 shows that Cumgas96 ($p = 0.019$), Egas ($p = 0.006$) and partition factor at 96 h ($p = 0.03$) linearly increased with CEO inclusion. A positive quadratic effect was observed for lag time ($p = 0.007$) in response to CEO inclusion. CEO20 resulted in the highest ($p < 0.05$) fraction a and effective gas production (Egas) and the least was from POSCON. The partition factor at 96 h increased with CEO inclusion when compared to POSCON. The orthogonal contrast for the rumen fermentation parameters showed no treatment variation.

Table 4 shows that negative linear and positive quadratic effects were observed for acetic ($p < 0.05$) and butyric acids ($p = 0.050$). There were negative linear effects for propionic ($p = 0.002$), iso-valeric ($p = 0.004$) and valeric acids ($p = 0.003$) in response to CEO inclusion. Total volatile fatty acids (TVFAs) showed negative linear and positive quadratic effects ($p < 0.05$) to CEO

TABLE 3 | Effect of supplementing a basal goat diet with varying levels of canola essential oil on in vitro ruminal fermentation parameters.

[2]Parameters	[1]Treatments						*p* values			Contrast
	POSCON	NEGCON	CEO5	CEO10	CEO15	CEO20	GLM	Linear	Quadratic	PvsN
a (mL/g OM)	45.1 ± 4.120^{b}	49.0 ± 3.760^{b}	56.3 ± 4.120^{ab}	58.4 ± 4.610^{ab}	63.2 ± 4.120^{ab}	68.2 ± 4.120^{a}	0.005	0.174	0.166	0.638
b (mL/g OM)	661 ± 55.59	744. ± 50.75	814 ± 55.59	831 ± 62.15	873 ± 55.59	873 ± 55.59	0.582	0.155	0.529	0.470
c (%/h)	0.05 ± 0.010	0.03 ± 0.010	0.04 ± 0.010	0.04 ± 0.010	0.03 ± 0.010	0.04 ± 0.010	0.582	0.241	0.621	0.302
Lag time (h)	1.54 ± 0.120	1.39 ± 0.110	1.04 ± 0.12	1.13 ± 0.140	1.16 ± 0.120	1.10 ± 0.120	0.055	0.116	0.007	0.556
Pgas	706 ± 58.21	793 ± 53.13	871 ± 58.21	890 ± 65.08	936 ± 58.21	942 ± 58.21	0.060	0.147	0.474	0.474
Egas	430 ± 30.39^{b}	459 ± 27.74^{b}	529 ± 30.39^{ab}	549 ± 33.98^{ab}	551 ± 30.39^{ab}	598 ± 30.39^{a}	0.006	0.006	0.097	0.569
Cumgas96 (mL)	717 ± 66.05	722 ± 73.85	761 ± 147.7	794 ± 85.27	648 ± 104.43	786 ± 73.85	0.870	0.019	0.161	0.964
ivOMD96 (g/kg)	633 ± 68.02	458 ± 58.91	464 ± 58.91	507 ± 68.02	466 ± 58.91	484 ± 68.02	0.443	0.859	0.409	0.291
PF96 (mL/mg OM)	1.17 ± 0.170^{b}	1.70 ± 0.150^{ab}	1.99 ± 0.150^{a}	1.76 ± 0.170^{ab}	2.03 ± 0.150^{a}	2.13 ± 0.170^{a}	0.010	0.030	0.720	0.121

[a, b, c] in a row, dietary treatment means with common superscripts do not differ ($p > 0.05$).

[1]Treatments: CEO5, canola essential oil at 0.5% inclusion level in the basal diet; CEO10, canola essential oil at 1.0% inclusion level in the basal diet; CEO15, canola essential oil at 1.5% inclusion level in the basal diet; CEO20, canola essential oil at 2.0% inclusion level in the basal diet; NEGCON, a basal diet without flavomycine nor canola essential oil; POSCON, a basal diet with flavomycine. Parameters: *a*, the gas production from the immediately fermentable fraction (mL); *b*, the gas production from the slowly fermentable fraction (mL); *c*, the gas production rate constant for the insoluble fraction; Pgas, potential gas production; Egas, the effective gas production; ivOMD96, in vitro organic matter degradability; PF96, partition factor at 96 h of incubation; Cum96, cumulative gas production at 96 h of incubation; PvsN, preplanned orthogonal contrasts for POSCON and NEGCON.

inclusion. CEO15 produced the lowest ($p < 0.05$) TVFA compared to the other treatments which were statistically similar. There was no treatment variation observed for the orthogonal contrast of the VFAs.

Table 5 shows that there were negative quadratic effects for *Firmicutes* ($p = 0.022$) and *Spirochaetes* ($p = 0.022$) and a positive quadratic effect for *Verucomicrobia* ($p = 0.011$). *Planctomycetes* ($p = 0.009$) linearly decreased while *Chloroflexi* ($p = 0.0003$) linearly increased with CEO inclusion levels.

Thirteen phyla were identified in this study, and only the phylum *Euryarchaeota* was identified in the Archaea domain. CEO5 reduced ($p < 0.05$) the abundance of *Euryarchaeota* compared to POSCON and other treatments. CEO5 and CEO15 promoted the highest ($p < 0.05$) ruminal abundance of *Firmicutes* while CEO20 had the least and was statistically similar to POSCON. CEO5 caused an increase in *Proteobacteria* abundance ($p < 0.05$) compared to other treatments. CEO20 had the highest ($p < 0.05$) *Planctomycetes* abundance compared to POSCON and NEGCON while CEO5 had the least abundance. The abundance of *Spirochaetes* was higher ($p > 0.05$) for CEO10 and CEO15 while NEGCON and POSCON had the lowest values. The lowest abundance of *Actinobacteria* was caused by CEO10 and CEO20 while the highest abundance was caused by CEO15 and POSCON. CEO20 caused the highest abundance of *Chloroflexi*. compared to all other treatments.

Figure 1 shows the abundance of microbes identified at Genera level. Table S1 shows that CEO doses caused positive linear and negative quadratic effects for *Prevotella* ($p = 0.006$), *RFN20* ($p = 0.026$] and *Treponema* ($p = 0.002$). CEO inclusion linearly reduced ($p < 0.05$) the abundance of *Succiniclasticum* ($p = 0.044$), *Bifidobacterium* ($p = 0.0003$), *Ruminococcus* ($p = 0.015$), *CF23* ($p = 0.001$), *Adlercreutzia* ($p = 0.004$) whereas a linear increase was observed for *Pseudoramibacter_Eubacterium* ($p = 0.029$). *Clostridium* ($p = 0.023$) and *Coprococcus* ($p = 0.025$) showed negative quadratic effects, while *vadinCA11* showed a positive quadratic effect ($p = 0.001$) to CEO inclusion.

CEO20 resulted in the highest ($p < 0.05$) levels of unclassified microbes compared to POSCON. CEO5 increased the population of *Selenomonas* while other treatment groups did not show any significant variation. POSCON caused the highest ($p < 0.05$) *Bifidobacterium* abundance followed by CEO20 and the lowest abundance was from CEO5. CEO10 promoted the higher ($p < 0.05$) *Desulfovibrio* abundance than POSCON. The abundance of *Fibrobacter* was increased ($p < 0.05$) by CEO20 compared to other treatments which do not vary ($p > 0.05$). CEO5 reduced *Methanosphaera* abundance while CEO15 caused the highest abundance. CEO15 caused higher ($p < 0.05$) *Lactobacillus* abundance compared to POSCON, which did not vary ($p > 0.05$) with other treatments. CEO5 promoted the highest ($p < 0.05$) *Anaerovibrio* abundance followed by CEO20, and the lowest abundance was from POSCON. CEO5 caused the lowest ($p < 0.05$) *Methanobrevibacter* abundance compared to POSCON which promoted the highest abundance. CEO20 and CEO15 reduced ($p < 0.05$) *Ruminococcus* abundance, followed by CEO15, CEO10 and POSCON, while CEO5 and NEGCON had the highest abundance ($p < 0.05$).

TABLE 4 | Effect of supplementing a basal goat diet with varying levels of canola essential oil on in vitro ruminal volatile fatty acids.

	[1]Treatments							*p* value			Contrast
VFAs (mg/L)	POSCON	NEGCON	CEO5	CEO10	CEO15	CEO20	SEM	GLM	Linear	Quadratic	PvsN
Acetic	1412[a]	1370[a]	1266[ab]	1104[ab]	992[b]	1179[ab]	75.10	0.007	0.015	0.042	0.589
Propionic	699[a]	702[a]	642[a]	595[ab]	463[b]	601[ab]	36.00	0.002	0.012	0.068	0.918
Butyric	462[a]	503[a]	452[a]	415[a]	313[b]	413[a]	21.90	0.0002	0.002	0.039	0.384
Iso-butyric	77.3[a]	83.2[a]	72.8[a]	64.9[a]	38.0[b]	61.0[ab]	5.230	0.0002	0.002	0.074	0.064
Iso-valeric	68.6[a]	73.1[a]	66.6[a]	60.2[a]	37.6[b]	58.0[a]	4.130	0.0002	0.004	0.091	0.327
Valeric	96.2[a]	105[a]	93.2[a]	87.1[a]	52.2[b]	82.4[a]	5.430	< 0.0001	0.003	0.082	0.162
[2]TVFA	2814[a]	2835[a]	2593[a]	2325[ab]	1895[b]	2393[ab]	143.0	0.002	0.006	0.042	0.883

[a, b, c]in a row, dietary treatment means with common superscripts do not differ ($p > 0.05$).

[1]Treatments: CEO5, canola essential oil at 0.5% inclusion level in the basal diet; CEO10, canola essential oil at 1.0% inclusion level in the basal diet; CEO15, canola essential oil at 1.5% inclusion level in the basal diet; CEO20, canola essential oil at 2.0% inclusion level in the basal diet; NEGCON, a basal diet without flavomycine nor canola essential oil; POSCON, a basal diet with flavomycine.

[2]PvsN, preplanned orthogonal contrasts for POSCON and NEGCON; SEM, standard error of the mean; TVFA, total volatile fatty acid.

TABLE 5 | Effect of varying levels of canola essential oil inclusion in a basal goat diet on in vitro microbial abundance.

	Treatments							*p* value			
Phyla	POSCON	NEGCON	CEO5	CEO10	CEO15	CEO20	SEM	GLM	Linear	Quadratic	PvsN
Firmicutes	32.8[b]	32.8[b]	35.1[a]	32.4[b]	36.3[a]	31.8[b]	0.431	< 0.0001	0.756	0.022	0.945
Unknown	5.51[ab]	5.70[ab]	6.49[a]	6.65[a]	5.07[b]	6.70[b]	0.314	0.003	0.638	0.974	0.817
Actinobacteria	10.1[a]	6.55[ab]	6.16[ab]	5.20[b]	10.1[a]	3.97[b]	0.969	0.0002	0.742	0.202	0.247
Euryarchaeota	2.26[a]	2.60[a]	1.13[b]	2.72[a]	2.77[a]	2.46[a]	0.216	< 0.0001	0.131	0.461	0.705
Verrucomicrobia	1.94[c]	2.78[bc]	2.02[c]	3.22[ab]	1.93[c]	4.02[a]	0.256	< 0.0001	0.024	0.011	0.309
Planctomycetes	1.01[bc]	1.04[bc]	0.65[c]	1.63[ab]	0.66[c]	2.06[a]	0.182	< 0.0001	0.009	0.070	0.956
Proteobacteria	0.64[b]	0.84[b]	1.40[a]	0.88[b]	0.80[b]	0.93[b]	0.096	0.0002	0.274	0.369	0.501
Lentisphaerae	0.21	0.30	0.25	0.32	0.31	0.30	0.024	0.036	0.176	0.897	0.413
Others	0.24[ab]	0.23[ab]	0.14[b]	0.34[a]	0.18[b]	0.22[ab]	0.033	0.007	0.849	0.506	0.842
Spirochaetes	0.48[b]	0.33[c]	0.54[b]	0.70[a]	0.72[a]	0.60[ab]	0.035	< 0.0001	< 0.0001	< 0.0001	0.842
Fibrobacteres	0.14	0.14	0.14	0.14	0.09	0.17	0.021	0.255	0.965	0.229	0.969
Synergistetes	0.08[ab]	0.11[ab]	0.14[a]	0.08[ab]	0.07[b]	0.10[ab]	0.015	0.038	0.163	0.485	0.602
Cyanobacteria	0.03[b]	0.04[ab]	0.07[a]	0.04[b]	0.04[b]	0.05[ab]	0.007	0.011	0.311	0.902	0.639
Bacteroidetes	44.5	45.4	46.1	45.7	41.0	44.9	1.632	0.4241	0.200	0.601	0.540
Chloroflexi	0.01 [b]	0.01 [b]	0.02[b]	0.02[ab]	0.02[b]	0.03[a]	0.002	0.0014	0.003	0.588	1.000

[a, b, c] in a row, dietary treatment means with common superscripts do not differ ($p > 0.05$).

[1]Diets: CEO5, canola essential oil at 0.5% inclusion level in the basal diet; CEO10, canola essential oil at 1.0% inclusion level in the basal diet; CEO15, canola essential oil at 1.5% inclusion level in the basal diet; CEO20, canola essential oil at 2.0% inclusion level in the basal diet; NEGCON, a basal diet without flavomycine nor canola essential oil; POSCON, a basal diet with flavomycine.

Figure 2 shows the abundance of microbes identified at the species level. Table S2 shows that there was a linear decline ($p < 0.05$) in the population of *Adleurtezia* spp., *Bifidobacterium breve*, *Bulledia* spp., *Prevotella* spp., *Prevotella rumicolla*, *Pseudobacterium* spp., *Succinivibrio* spp., and *Selenomonas ruminantum* in response to CEO doses. *Atopium* spp. ($p = 0.039$) and *Shuttleworthia* spp. ($p = 0.042$) linearly increased, while *Anaerovibrio* spp. showed a negative quadratic effect ($p = 0.003$) in response to CEO doses.

CEO5 caused higher abundance of *Ruminobacter* spp. and *Prevotella ruminocola* ($p < 0.05$) compared to NEGCON, while other treatments had similar values ($p > 0.05$). All CEO inclusion levels promoted higher ($p < 0.05$) abundance of *Pseudobutyrivibrio* spp. compared to NEGCON and POSCON. CEO5 promoted the lowest abundance of *Methanosphaera* spp. and the highest was from CEO15.

4 | Discussion

Quantifying the proximate components of feed is essential to ensure nutrient availability and supply in other to predict performance of animals (Baris 2023). The DM and OM recorded in

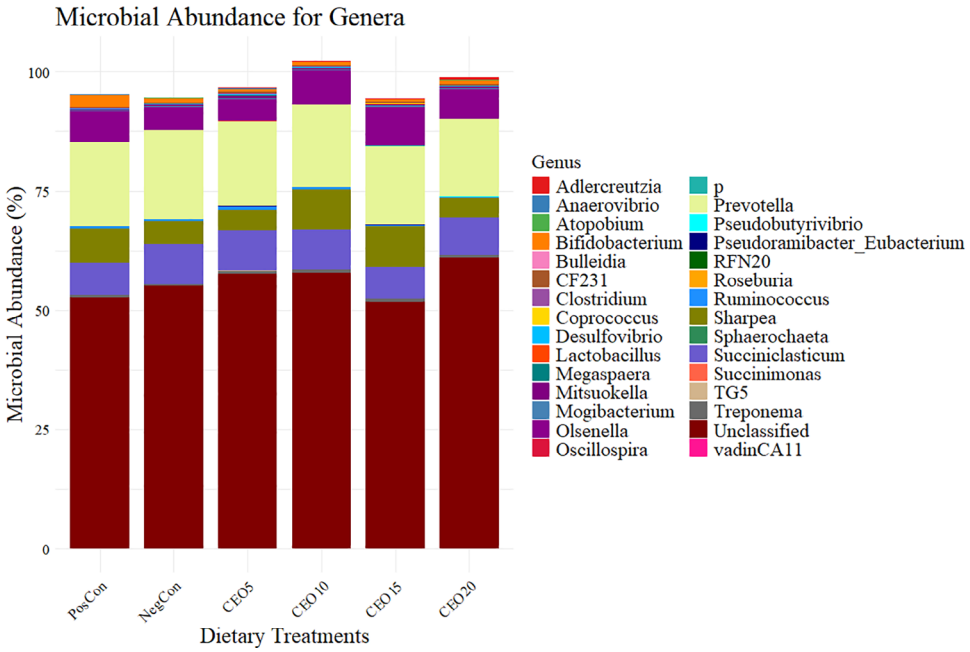

FIGURE 1 | The effect of supplementing a basal goat diet with varying levels of canola essential oil on microbial abundance at a genera level. CEO5, canola essential oil at 0.5% inclusion level in the basal diet; CEO10, canola essential oil at 1.0% inclusion level in the basal diet; CEO15, canola essential oil at 1.5% inclusion level in the basal diet; CEO20, canola essential oil at 2.0% inclusion level in the basal diet; NEGCON, a basal diet without flavomycine nor canola essential oil; POSCON, a basal diet with flavomycine.

this study ranged from 90.1% to 91.9% and 81.7%–83.6% with the lower values recorded at CEO15 and CEO20. The high level of DM and OM indicates higher nutritive value which means there will be more nutrient uptake per unit of feed consumed by the animal. Higher levels of crude fat recorded for the diets with CEO inclusion were expected due to the inclusion of oil components. The reason for the variations observed in proximate composition following CEO supplementation is unclear; however, the reduction in NDF and cellulose contents may be due to the oil dilution effect. Fibre (an energy source) was reduced as the energy value increased with inclusion of CEO in the treatments (Adeyemi et al. 2016). Xu et al. (2022) reported that lower fibre content in feed can lead to higher gas production as it facilitates higher fermentation rates. This is consistent with the higher *a* and Egas recorded for treatments with CEO inclusion when compared to both NEGCON and POSCON. The increased *a* and Egas suggest higher digestibility of substrates and availability of nutrients to ruminal microbes (Beyzi 2020). Molho-Ortiz et al. (2021) reported that the essential oils of garlic, cinnamon, rosemary included at 900 mg/L in a basal diet reduced gas production while *Eucalyptus* essential oil increased gas production volume with high values similar to those recorded in this study. Partitioning factor (PF) is a good measure of microbial efficiency that indicates efficient energy utilisation (Benetel et al. 2022). In this study, PF was calculated as the ratio of cumulative gas produced to the organic matter of residue after 96 h of incubation. This mean that a higher cumulative gas compared to the organic matter value will result in high PF value which will indicate low efficiency. This study shows that the inclusion of CEO increased PF96 and promoted contrasting effect when compared with POSCON. The increased PF96 recorded in this study is caused by the increased cumulative gas production from rapid fermentation as recorded with *a*. This leads to decreased feed efficiency and reduced energy supply as observed with the TVFA result recorded in this study. Our findings agree with the findings of Valenzuela-Rodríguez et al. (2021) which evaluated the effect of organic oils (2% fish oil, 2% fish oil + 1.5% soybean oil and 2% fish oil + 3% soybean oil) on the in vitro fermentation of cattle diet. The authors found that the oils increased gas production, and decreased lag time and production of short chain fatty acids. In addition, Castañeda-Rodríguez et al. (2023) evaluated the effect of canola oil at 0%, 2% and 4% inclusion level using a basal diet and rumen inoculum from a donor sheep and reported an increase in gas volume at 2% and 4% compared to control. Lag time shows the rate at which substrates are being colonised by the rumen microbes and an increase in lag time means delay in colonisation of substrates by ruminal microbes (Kahvand and Malecky 2018). The results of this study showed a positive quadratic response to CEO inclusion, suggesting that CEO10 might be the level that hasten microbial attachment to substrates. This shows that levels beyond 1% CEO could delay the time the microbes are able to act on the substrate and consequently reduce fermentation efficiency.

In this study, the production of acetate and other VFA declined as CEO levels increased. This corroborates the high gas production recorded because high gas production indicates energy loss in a form of VFA, which are considered the major energy source for ruminants. Similarly, the use of nut meg essential oil in feed consisting of forage and concentrate in the ratio 60:40 reduced the in vitro acetate, propionate, butyrate and TVFA levels (Abdillah et al. 2024). This result is consistent with the report of Chahaardoli et al. (2018) who observed that all levels (0, 250, 500, 750 and 1000 μL/30 mL rumen fluid) of anise essential oil reduced TVFAs in Sanjabi sheep. Similarly, the findings of Nehme et al. (2021) reported a significant increase in gas production and decrease in total VFA when *Thymbra capitata* essential oil was

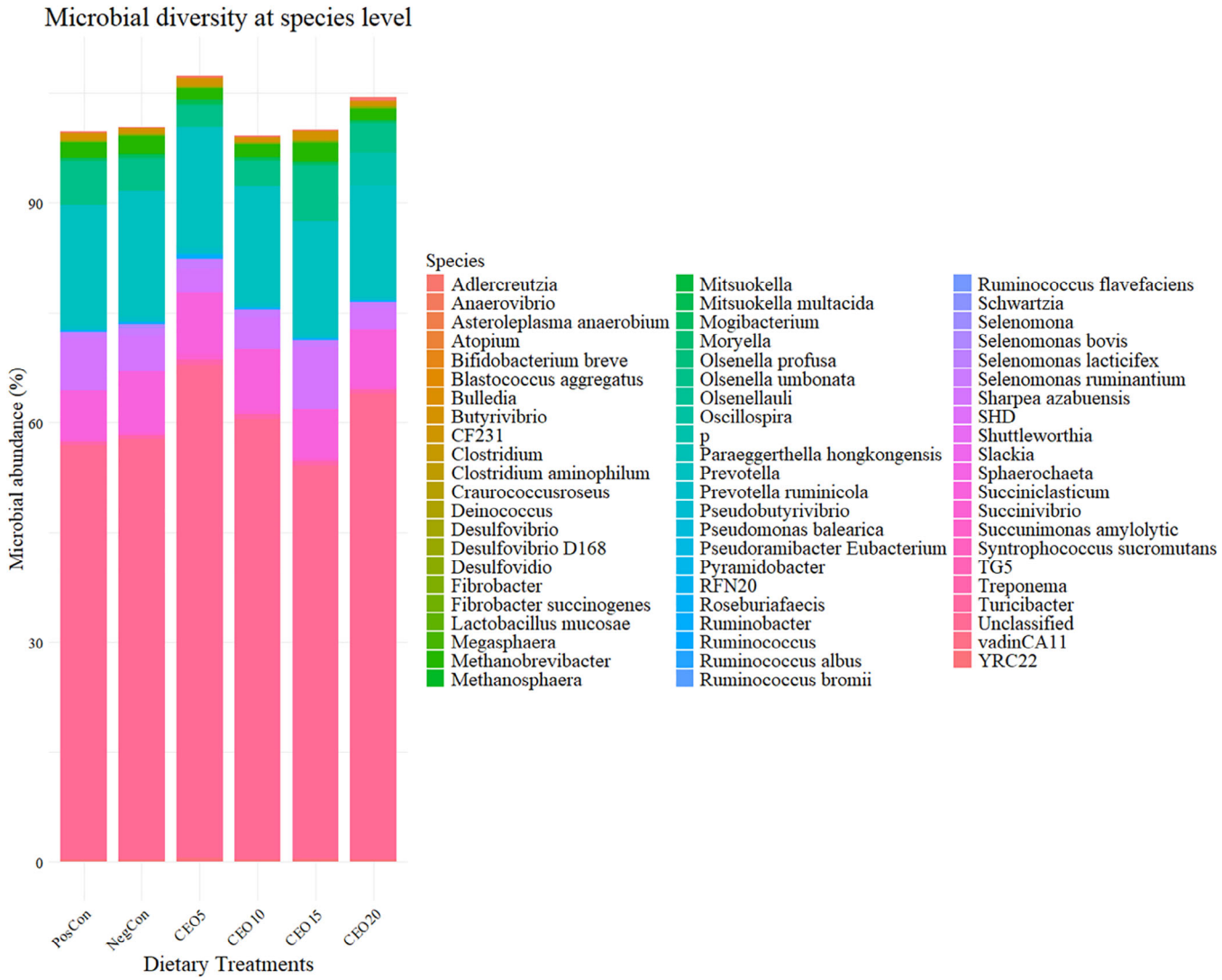

FIGURE 2 | The effect of supplementing a basal goat diet with varying levels of canola essential oil on microbial abundance at a species level. CEO5, canola essential oil at 0.5% inclusion level in the basal diet; CEO10, canola essential oil at 1.0% inclusion level in the basal diet; CEO15, canola essential oil at 1.5% inclusion level in the basal diet; CEO20, canola essential oil at 2.0% inclusion level in the basal diet; NEGCON, a basal diet without flavomycine nor canola essential oil; POSCON, a basal diet with flavomycine.

included in a high concentrate diet with 75 mg carvacrol/L and incubated for 24 h in vitro. The reduction in VFA was thought to be due to the depression of acetic acid production. Contrary to the current findings, Nur Atikah et al. (2018) reported that feeding a diet containing olive oil and sunflower oil at 6% included in total feed ingredient fed to mature local Katjang-crossed male goats increased total VFA and acetate concentration. The reduction of VFAs in this study suggests that CEO inhibited microbial action as crude fat increased in the substrate (Kholif and Olafadehan 2021) and since VFA is a major energy source, this is not considered a favourable outcome nutritionally (Palmonari et al. 2023).

This study identified 15 phyla and 46 genera. The phyla groups were majorly Firmicutes, *Bacteroidetes*, and *Actinobacteria* while the genera were predominated by unclassified microbes, *Prevotella*, and *Succiniclasticum* across all treatments. This is similar to the report of Ramos et al. (2021) who reported that these microbes along with *Ruminococcus* dominated the genera abundance in dairy cows transitioned from high forage diet to high concentrate diet. The lack of treatment variation between the POSCON and NEGCON recorded in this study was surprising because we expected POSCON to supress the abundance of certain microbes because of its AGP content. Genera *Methanobrevibacter* and *Ruminococcus* were suppressed by the supplementation with CEO, indicating that CEO can replace the use of AGP to inhibit methane-producing microbes. *Methanosphaera* had similar result with NEGCON but increased at 1.5% showing that CEO did not inhibit this methanogenic archeon at the inclusion level. However, this may not suggest an inefficiency because *Methanosphaera* has a lower methane production efficiency (1 mol of methanol gives 0.75 mol of methane) when compared to *Methanobrevibacter* which produces 1 mol of methane from 1 mol of CO_2 (Cunha et al. 2019). Furthermore, there was an increase in *Megasphaera* population at CEO15 suggesting a competitive shift in the rumen fermentation pathways where *Megasphaera* favours lactate utilisation for propionate production thereby reducing the amount of lactate available for methanogenesis (Susanto et al. 2023).

Ruminococcus albus, *Prevotella rumicola* and *Ruminobacter* increased at 0.5% CEO and declined at 2% CEO, which means these microbes are adversely affected by higher concentrations of CEO because it contains more phenolic compounds (Sharma et al. 2022). These compounds cause the leakage of the cell membrane as they interact with the lipid layer of the bacterial cell and consequently lead to cell death (Nourbakhsh et al. 2022). Furthermore, the abundance of *Ruminococcus flavefaciens* increased with all CEO treatments promoting similar result as POSCON while there were no values recorded for NEGCON. This result shows that the response of some of the fibrolytic bacteria to essential oils is selective and may be dose dependent (Kim et al. 2018). *Selenomonas ruminantum* reduced with increasing levels of CEO, which explains the decline in TVFA at higher inclusion levels because this species is associated with starch digestion to produce acetic and propionic acids (Halfen et al. 2021). *Prevotella* spp. are proteolytic bacteria that produces different degrading extracellular enzymes. They recorded a linear decrease with CEO inclusion in this study. This decrease can be attributed to the effect of the bioactive component in CEO which have negative effects on proteolytic bacteria (Abdillah et al. 2024).

5 | Conclusion

Gas production and microbial activity increased with CEO inclusion and was associated with increased partition factor and decreased VFAs signalling a potential loss of energy from the diet. The supplementation of the diet with 1% CEO shortened lag time and is deduced to be the level that can promote rapid substrate colonisation by microbes. Moreover, dietary inclusion of CEO increased fermentative bacteria (*Firmicutes* and *Actinobacteria*) and reduced the abundance of methanogenic archaea (genus *Methanobrevibacter* and *Ruminococcus*) at 1.5% and 2% inclusion levels, respectively. Although, CEO can reduce methanogenic bacteria better than the antibiotic-containing treatment, its impact on energy utilisation efficiency needs careful consideration to balance the benefits and the inefficiencies of its inclusion in ruminant diets. Future studies should investigate the functions of the unclassified microbes as they constituted a significant part of the rumen microbial diversity.

Author Contributions

Adeola P. Idowu: Conceptualisation, methodology, investigation, data curation, data analysis, visualisation and writing. **Lebogang E. Motsei:** Project administration, Methodology, Funding acquisition and supervision. **Chidozie F. Egbu:** Methodology, Review and Project administration. **Caven M. Mnisi:** Data curation, software, visualisation, review and editing.

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Ethics Statement

Ethical clearance (approval no. NWU-0081623A5) was granted by the Animal Production Research Ethics Committee of North-West University.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/vms3.70283.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.