International Journal of Agriculture and Biosciences 2026 15(2): 636-645.
https://doi.org/10.47278/journal.ijab/2025.213

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Rumen Environment and Enteric Methane Emissions of Dohne Merino Wethers Fed Fossil Shell Flour Diets Supplemented with Varying Inclusion Levels of Baobab Oil Seed Cake

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ABSTRACT

Sustainable livestock production requires reducing enteric methane emissions. Enhancing rumen fermentation through dietary supplements is a promising strategy to achieve this goal. Therefore, this study investigated the effect of fossil shell flour (FSF) diets supplemented with varying inclusion levels of baobab oil seed cake (BOSC) on the rumen environment and enteric methane emissions of Dohne Merino wethers. Twenty-four, five-month-old wethers (weighted 25±0.5kg) were kept in individual pens for 90 days of successive feeding. The wethers were randomly allocated to four dietary treatments in a completely randomised design, with each treatment consisting of six animals. Fossil shell flour was included in all diets at 4% and baobab oil seed cake at incremental levels of 0%, 5%, 10%, and 15%. Rumen environment (rumen pH, temperature (°C), ammonia concentration (mg/dL), total volatile fatty acids (TVFA), acetate, propionate, butyrate, valerate, isobutyrate, isovalerate, and acetate: propionate ratio) and enteric methane emissions (ppm-m, g/day, L/day, g/kg DMI) were determined. Wethers fed 10% BOSC had the highest TVFA, acetate, propionate, butyrate, isobutyrate, and isovalerate concentration and the lowest A:P ratio.

Baobab oil seed cake supplementation did not significantly affect the rumen pH, temperature, ammonia, and VFA concentration (P>0.05). Enteric methane output was highest in wethers fed 0% BOSC during feeding and resting (P<0.05). Enteric methane output decreased with increasing BOSC supplementation level across all activities. It can be concluded that diets with 4% FSF supplemented with 10% BOSC maintain the rumen environment for fermentation and reduce enteric methane emission in Dohne Merino wethers, thereby promoting sustainable agriculture.

Keywords: Rumen environment; Enteric methane emissions; Baobab oil seedcake; Fossil shell flour; Dohne Merino.

Article History
Article # 25-483
Received: 20-Aug-25
Revised: 07-Oct-25
Accepted: 15-Oct-25
Online First: 10-Dec-25

INTRODUCTION

Ruminants account for approximately 39% of animal methane production and 13% of global methane emissions (Gere et al., 2022). Methane has a global warming potential 23 times greater than carbon dioxide in a 100-year horizon (Jafari et al., 2020; Flores-Santiago et al., 2022). Therefore, methane is a significant contributor to climate change. Methane production signifies that approximately 8% of the gross energy has been lost at the maintenance level of intake and 6% when feed intake increases (Lakamp et al., 2022; Parnian-Khajehdizaj et al., 2023). Studies have shown that sheep produce methane ranging from 20 – 55L/day. According to Flores-Santiago et al. (2022), sheep can produce 10 – 16kg of methane per head annually. Methane production contributes to environmental pollution and affects energy utilization in sheep. Methane is produced during rumen fermentation, a process that

Cite this Article as: Clyde H, Ikusika OO, Mpendulo CT and Fon F, 2026. Rumen environment and enteric methane emissions of dohne merino wethers fed fossil shell flour diets supplemented with varying inclusion levels of baobab oil seed cake. International Journal of Agriculture and Biosciences 15(2): 636-645. https://doi.org/10.47278/journal.ijab/2025.213

A Publication of Unique Scientific Publishers

produces energy from volatile fatty acids (acetate, propionate, and butyrate) together with ammonia and carbon dioxide (Løvendahl et al., 2018). Methane is eliminated through eructation (Getabalew et al., 2019). Enteric methane emission is affected by several factors, including the quantity of feed consumed, the composition of the diet (forage-to-concentrate ratio), and the animal's physiological state (Van Gastelen et al., 2019). Therefore, it is imperative to modify rumen fermentation and decrease enteric methane emissions in sheep. Among other strategies, diet manipulation has been shown to alter rumen fermentation and reduce enteric methane production (Tseten et al., 2022).

One effective method is supplementing diets with feed additives and protein-rich ingredients. This improves microbial growth efficiency, which alters rumen fermentation and reduces enteric methane output in animals (Getabalew et al., 2019; El-Obied et al., 2024). Utilizing agro-industrial by-products such as baobab oil seed cake (BOSC) is essential in livestock production (Chisoro et al., 2018; Ayssiwede et al., 2023). Baobab oil seedcake contains considerable amounts of nutrients, including proteins (20–36% CP), energy, vitamins, minerals, and amino acids, especially lysine and methionine, which are not available in most cereal grains (Chisoro et al., 2019; Nkosi et al., 2019; Babalola et al., 2021). The protein content of BOSC may increase propionate production while decreasing the availability of hydrogen ions for methane production (Saho et al., 2020). Baobab oil seed cake also contains 2–12% tannin, 2% phytate, and 10% oxalate concentration (Ujor et al., 2020). A study by Saho et al. (2020), showed reduced enteric methane emission with increasing levels of baobab oil seed cake in goats. Puchala et al. (2005) and Nawab et al. (2020), showed that forage that contains condensed tannins can potentially minimize enteric methane emission in ruminants. This could be due to the presence of tannins, which alter microbial fermentation in the rumen by reducing protein fermentation, thereby preventing methanogenesis.

On the other hand, Ikusika et al. (2019), supplemented sheep diets with fossil shell flour (FSF), an organic feed additive with physical and chemical characteristics that make it suitable for use in livestock feeds. Fossil shell flour is a mineral-rich feed ingredient made from fossilized remains of diatoms and can be found in many countries (Isabirye et al., 2020; Ikusika & Mpendulo, 2023), including South Africa. Belanger (2015) reported a significant decrease in enteric methane emission in diets with 75g/L and 100g/L of fossil shell flour. This was attributed to reduced hydrogen ions, which altered methane production. However, Ikusika & Mpendulo (2023), reported increasing methane output with increasing fossil shell flour inclusion in sheep diets. The variability in the FSF impact could be attributed to variations in diet composition, specifically the proportion of other feed ingredients, varying inclusion levels, and the duration of the feeding trial.

As a result, based on the available literature where fossil shell flour and baobab oil seed cake were included individually in livestock diets to determine their effect, combining them may produce a positive outcome due to their chemical, physical, and nutritional composition. There is little or no literature available where fossil shell flour and baobab oil seed cake were incorporated together in diets and to determine their influence on the rumen environment and enteric methane emissions. These feed resources can complement each other by altering the rumen environment and reducing enteric methane emissions, promoting sustainable agriculture. Therefore, the current research determined the effect of fossil shell flour diets supplemented with varying inclusion levels of baobab oil seed cake on the rumen environment and enteric methane emissions in Dohne Merino wethers. It was hypothesized that FSF and BOSC could have a synergistic effect by improving the stability and efficiency of the rumen environment and reducing enteric methane emissions in Dohne Merino wethers.

MATERIALS & METHODS

Study Site

The experiment was conducted at Fort Cox Agriculture and Forestry Training Institute in Middledrift, Eastern Cape, South Africa. The college lies along 580 m above sea level and has a latitude and longitude of 27.03' N and 32.45' E, respectively. The farm is located at about 34km from the University of Fort Hare, Alice (Akuru et al., 2021). The area receives around 500–600mm of rainfall, which falls mainly during the summer season, with a mean temperature of 22.9°C (Gajana et al., 2011).

Animals, Experimental Design, and Management

Twenty-four (power analysis), five-month-old Dohne Merino wethers (weighted 25±0.5kg) were used in this study as they are a dual-purpose breed well adapted to South Africa's harsh conditions and contribute significantly to the livestock industry (Dzomba et al., 2021). The wethers were purchased from a commercial farm in Eastern Cape Province, South Africa, and were used for the study in a completely Randomized Design. On arrival, all animals were allocated randomly to four treatments; hence, there were six animals per treatment. The wethers were confined individually in well-ventilated pens (1.5×1m) with concrete floors. The wethers were allowed to adapt to the feed and environmental conditions for 7 days. The feeding trial lasted for 90 days, without considering the 7 days for the adaptation period. For easy identification, the wethers were ear-tagged. Clean, fresh water was made available to the wethers ad libitum daily, and they were fed twice a day at 8:00hrs and 16:00hrs at 5% of their body weight.

Experimental Diets

Table 1 shows the inclusion levels of feed ingredients in the experimental diets. Fossil shell flour was included in all experimental diets at a 4% level; this is the recommended level for optimum performance, based on previous research studies (Ikusika et al., 2019; Ikusika et al., 2020; Mwanda et al., 2020). Treatment 1 had 0% BOSC, treatment 2 had 5% BOSC, and treatments 3 and 4 had 10% and 15% BOSC, respectively; the levels were chosen based on nutritional requirements, previous research, and cost-effectiveness. Fossil shell flour (Food - Grade) was bought from Eco-Earth (Pty) Ltd. in Port Elizabeth. Baobab oil seed cake was purchased from Eco Products in Louis Trichard, Limpopo province, South Africa. Concentrate ingredients were purchased from Umtiza Agricultural Products, and lucerne from a farm in Queenstown, South Africa. The dietary nutritional requirements of the animals were considered during feed formulation. Experimental diets (as fed) are shown in Table 2.

Table 1: Experimental diets fed to animals

Ingredients (%)	Dietary Treatments			
	0% BOSC	5% BOSC	10% BOSC	15% BOSC
Maize	5	5	5	5
Soybean Meal	15	10	5	0
Wheat bran	15	15	15	15
Leucine	60	60	60	60
Molasses	5	5	5	5
Mono-dicalcium phosphate	0.5	0.5	0.5	0.5
Sheep premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Fossil shell flour	4	4	4	4
Limestone	1.5	1.5	1.5	1.5
Chemical composition				
DM	88.96	88.72	88.73	88.83
OM	79.68	79.88	79.95	79.52
CP	13.34	12.91	12.67	11.57
CF	26.25	27.05	26.92	28.99
EE	1.79	1.82	2.04	1.98
ADF	17.47	21.34	31.08	41.70
NDF	39.11	51.09	51.22	59.10
Nitrogen	2.13	2.07	2.03	1.85
Starch	10.68	10.02	10.62	9.79

BOSC = baobab oil seed cake, DM = dry matter, OM = organic matter, CP = crude protein, CF = crude fiber, EE = ether extract, ADF = acid detergent fiber, NDF = neutral detergent fiber.

Table 2: Proximate composition of baobab oil seedcake

Nutrient (%	Baobab oil seed cake (%)
DM	90.5
Moisture	9.53
OM	83.8
CP	25.3
CF	12.9
EE	5.92
NDF	43.5
ADF	25.2
Total N	4.05
Starch	16.4
Phosphorus (%)	0.07
Calcium (%)	0.79
Magnesium (%)	0.41
Potassium (%)	0.76
Sodium (mg/kg)	8
Copper (mg/kg)	12
Iron (mg/kg)	65
Manganese (mg/kg)	7

DM = dry matter, OM = organic matter, CP = crude protein, CF = crude fibre, EE = ether extract, ADF = acid detergent fibre, NDF = neutral detergent fibre, N = nitrogen, mg/kg = milligrams per kilogram.

Proximate Analysis of Experimental Diets and Baobab Oil Seedcake

Proximate analysis was done to determine the chemical composition of experimental diets and baobab oil seed cake, as shown in Table 1 and 2. The dry matter (DM) was determined by drying the samples for 24hrs. at 105°C in an air-forced oven, according to the Association of Analytical Chemists methods (AOAC, 2005). A muffle furnace was used to measure the ash content of the samples at 550°C for 5 hrs. According to the AOAC (2005) method 938.08. The difference between the DM and the ash content of the samples was regarded as organic matter (OM) (Al-mentafji, 2006; Nurfeta, 2010). Ether extract (EE) was determined using the Soxhlet extraction method with dimethyl ether (method 920.85) (AOAC, 2005). Crude fibre (CF) was determined by boiling samples in 1.25% dilute H_2SO_4, washing with water, further boiling in NaOH, and drying the samples at 65ºC for 3 hours (AOAC, 2005).

The Kjeldahl method was used to determine the nitrogen (N) content of the samples, and the crude protein (CP) content was calculated by multiplying the N content in the samples by 6.25 (Orlandi et al., 2020). The neutral detergent fiber (NDF) was determined by using sodium sulfite and a heat-stable α-amylase and acid detergent fiber (ADF), according to the methods described by Van Soest et al. (1991).

Measurement Parameters

The Rumen Environment

Twelve animals were chosen randomly, three per treatment, to collect rumen fluid. The animals were slaughtered at the East London abattoir at the end of the feeding trial, and 200ml of rumen fluid was taken from each animal (Wanapat et al., 2014). A digital pH meter (HI8424, Singapore) was used to determine the pH immediately after collecting the rumen fluid (Abdel-Raheem and Hassan, 2021). Temperature (°C) was measured immediately by a digital thermometer from each rumen fluid sample. Rumen fluid from each animal was filtered through four layers of cheesecloth and 40mL of the samples were mixed with H_2SO and stored at -20ºC for VFA (Acetate, butyrate, propionate, valerate, isovalerate, isobutyrate) analysis (Grimsell, 2020). Rumen fluid samples (40mL) collected per animal were mixed with 1 ml of HCl 6N and stored at -20ºC for ammonia analysis (Reis et al., 2016). The VFA concentration was determined by gas chromatography as described by El-Essawy et al. (2021), and the ammonia concentration was determined by colorimetry as described by Broderick and Kang (1980) and Zhang et al. (2017).

Enteric Methane Emission

A Laser Methane Detector (LMD) (LMm-g®; Tokyo Gas Engineering Solutions, Ltd) (Roessler & Schlecht, 2021) was used to measure the amount of methane produced. The measurements were taken at different activities when the animals were feeding, resting, and standing weekly from the start of the experimental trial and daily in the last week. The measurements were taken at a 1.5m distance from the animal to avoid disturbing the animals and interfering with the activity of wethers. The laser beam of the hand-held LMD machine was aimed at the nostrils of the wethers. The machine was reset before taking measurements for each day to adjust it to ppm-m (parts per million-meter) (Saho et al., 2020). The offset function of the laser methane detector was used to discount the atmospheric methane effect before the machine recorded methane concentration from the animals. Multiple measurements were taken from the same distance at approximately the same time of day to ensure precision (Chagunda & Yan, 2011). The methane concentrations were measured in ppm-m for the weekly measurements, and the daily values were converted to g/day using a formula by Chagunda et al. (2009). Enteric methane emission was also determined based on the dry matter intake of the animals in g/kg dry matter intake (DMI) and litres/day using formulas by Shibata et al. (1993) as described by Ikusika and Mpendulo (2023).

Statistical Analysis

Recorded data on rumen pH, temperature, ammonia, volatile fatty acids, and enteric methane emissions were analysed using the Generalised Linear Model (GLM) procedure of SAS (2010) (version 9.0). Data were presented as Least Square Means (LSMs) with respective Standard Error of the Means (SEMs). Differences amongst least square means were tested using the probability of difference (PDIFF) option of SAS (2010) and were considered significant at $P<0.05$.

The statistical model used is as follows:

$Y_{ijk} = \mu + A_i + P_j + (A \times P)_{ij} + \varepsilon_{ijk}$; where,

Y_{ijk} = response variable (rumen environment parameters, enteric methane emissions)

μ = overall mean

A_i = effects of diet

P_j = period effect

$(A \times P_{ij}$ = Interaction of diet and period effect.

ε_{ijk} = Random error. For i = 1, 2, 3, 4; j = 1, 2, 3, 4…

RESULTS

The Rumen Environment

The effect of fossil shell flour diets supplemented with varying inclusion levels of baobab oil seed cake on rumen pH, temperature, ammonia and volatile fatty acids in Dohne Merino wethers is shown in Table 3. Rumen pH was lowest in Dohne Merino wethers fed 5% BOSC and highest fed 0% BOSC ($P>0.05$). Rumen temperature (°C) was highest in wethers in wethers fed 5% and 15% BOSC ($P>0.05$). Dohne Merino wethers fed 10% BOSC had the highest ammonia (mg/L) ($P>0.05$). Total volatile fatty acids were highest in wethers fed 10% BOSC ($P>0.05$). Acetate, propionate, butyrate, isobutyrate, and isovalerate production were highest in wethers fed 10% BOSC ($P>0.05$). Dohne Merino wethers fed 0% BOSC had the highest valerate production ($P>0.05$). A:P ratio was highest in Dohne Merino wethers fed 15% BOSC ($P<0.05$). The rumen pH, temperature, ammonia, and volatile fatty acid concentration were not significantly affected ($P>0.05$) by BOSC inclusion in Dohne Merino wethers (Table 3).

Enteric Methane Emissions

Table 4 shows enteric methane emissions of Dohne Merino wethers fed fossil shell flour diets supplemented with baobab oil seed cake during different activities (feeding, standing, and resting). Enteric methane output (ppm-m) during feeding was highest in wethers fed 0% BOSC ($P<0.05$). Methane produced (ppm-m) during resting was highest in wethers fed 5% BOSC and 0% BOSC ($P<0.05$). Enteric methane output (ppm-m) decreased as the BOSC inclusion level increased during resting and standing. Enteric methane output (ppm) was highest in wethers during resting than during feeding and standing ($P<0.05$). Fig. 1 shows enteric methane output (ppm-m) by Dohne Merino wethers over 9 weeks during different activities. Overall methane output during standing, feeding, and resting constantly decreased across all weeks except in week 5 during feeding and week 6 during standing and resting. Dohne Merino wethers fed fossil shell flour diets supplemented with varying inclusion levels of baobab oil seed cake produced the highest methane values during week 1 and the lowest methane during week 9 across all activities ($P<0.05$).

Table 3: Rumen environment parameters of Dohne Merino wethers fed diets with fossil shell flour supplemented with varying baobab oil seedcake inclusion levels

Parameter	[1]Treatment				[2]SEM	P-value
	0%	5%	10%	15%		
Rumen pH	6.76	6.62	6.70	6.67	0.08	0.688
Temp (°C)	30.7	31.7	31.3	31.7	0.62	0.643
Ammonia (mg/dL)	7.55	8.72	10.2	7.63	2.29	0.828
Total VFA	69.7	57.8	76.5	56.3	24.2	0.919
Acetate	51.7	43.2	55.3	42.7	18.6	0.949
Propionate	10.4	8.04	12.3	7.87	3.40	0.766
Isobutyrate	1.60	1.07	1.78	0.99	0.48	0.584
Butyrate	4.07	4.06	4.99	3.46	1.31	0.870
Isovalerate	0.98	0.69	1.19	0.61	0.27	0.449
Valerate	0.99	0.73	0.93	0.68	0.34	0.898
A:P ratio	5.05^{ab}	5.44^{ab}	4.25^{a}	5.60^{b}	0.37	0.115

^{a,b} Means in the same row with different superscripts are significantly different ($P<0.05$). [1]Treatments: 0% = diet containing 4% FSF, 0% BOSC, and 15% Soybean meal; 5% = diet containing 4% FSF, 5% BOSC, and 10% soybean meal; 10% = diet containing 4% FSF, 10% BOSC and 5% soybean meal, 15% = diet containing 4% FSF, 5% BOSC and 0% soybean meal. Total VFA (Total Volatile fatty acids) = Acetate + propionate + valerate + butyrate + isovalerate + isobutyrate. [2]SEM = standard error of the mean.

Table 4: Enteric methane emissions (ppm-m) of Dohne Merino wethers fed fossil shell flour diets supplemented with varying inclusion levels of baobab oil seed cake during different activities

Activity	[1]Treatments				[2]SEM	P-value		
	0%	5%	10%	15%		T	W	T×W
Feeding	25.7^{b}	23.5^{ab}	22.2^{a}	22.6^{a}	1.14	0.138	$<0.0001^{**}$	0.156
Standing	25.1	24.9	21.5	21.5	1.56	0.268	$<0.0001^{**}$	0.838
Resting	40.4^{b}	41.3^{b}	32.5^{a}	32.2^{a}	2.34	0.006^{**}	$<0.0001^{**}$	0.045^{*}

^{a,b} Means in the same row with different superscripts are significantly different ($P<0.05$). $^{*}P<0.05$; $^{**}P<0.01$. [1]Treatments: 0% = diet containing 4% FSF, 0% BOSC, and 15% Soybean meal; 5% = diet containing 4% FSF, 5% BOSC, and 10% soybean meal; 10% = diet containing 4% FSF, 10% BOSC and 5% soybean meal, 15% = diet containing 4% FSF, 5% BOSC and 0% soybean meal. T = Dietary treatment effect, W = Week effect, T×W = interaction between treatment and week. [2]SEM = standard error of the mean.

Enteric methane was also measured in g/day during different activities, as shown in Table 5 Dohne Merino wethers fed fossil shell flour diets supplemented with varying inclusion levels of baobab oil seed cake produced the highest methane while resting than feeding and standing. Enteric methane output was highest in wethers fed 0% BOSC during feeding and resting ($P<0.05$). Increasing supplementation levels of BOSC decreased enteric methane output when the wethers were standing, with wethers fed 0% BOSC having the highest methane output ($P>0.05$). Table 5 shows that the dry matter intake was highest in wethers fed 15% and 10% BOSC ($P<0.05$). Enteric methane output in L/day and g/kg DMI was highest in wethers fed 15 and 10% BOSC ($P<0.05$). Enteric methane output increased with increasing DMI in Dohne Merino wethers.

Table 5: Enteric methane emissions of Dohne Merino wethers fed fossil shell flour diets supplemented varying inclusion levels of baobab oil seedcake

[1]Parameter	[2]Treatments				[3]SEM	P-value		
	0%	5%	10%	15%		T	D	T×D
DMI (kg/day)	1.20[b]	1.11[a]	1.19[ab]	1.21[b]	27.77	0.048*	0.583	0.992
Methane (L/day)	32.2[b]	29.3[a]	31.7[ab]	32.4[b]	0.85	0.048*	0.583	0.992
Methane (g/kg DMI)	22.6[b]	20.8[a]	22.3[ab]	22.7[b]	0.52	0.048*	0.584	0.992
Methane (g/day)								
Feeding	0.62[b]	0.48[a]	0.43[a]	0.50[a]	0.04	0.004**	0.003**	0.067
Standing	0.63[b]	0.55[ab]	0.52[ab]	0.49[a]	0.04	0.122	0.066	0.347
Resting	1.29[b]	1.16[ab]	0.97[a]	0.99[a]	0.08	0.013*	0.056	0.006**

[a,b] Means in the same row with different superscripts are significantly different (P<0.05). [1]Treatments: 0% = diet containing 4% FSF, 0% BOSC, and 15% Soybean meal; 5% = diet containing 4% FSF, 5% BOSC, and 10% soybean meal; 10% = diet containing 4% FSF, 10% BOSC and 5% soybean meal, 15% = diet containing 4% FSF, 5% BOSC and 0% soybean meal. DMI = dry matter intake, kg/day = kilograms per day, L/day = litres per day, g/kg = grams per kilogram, g/day = grams per day. T = Dietary treatment effect, D = Day effect, T×D = interaction between treatment and day. [2]SEM = standard error of the mean.

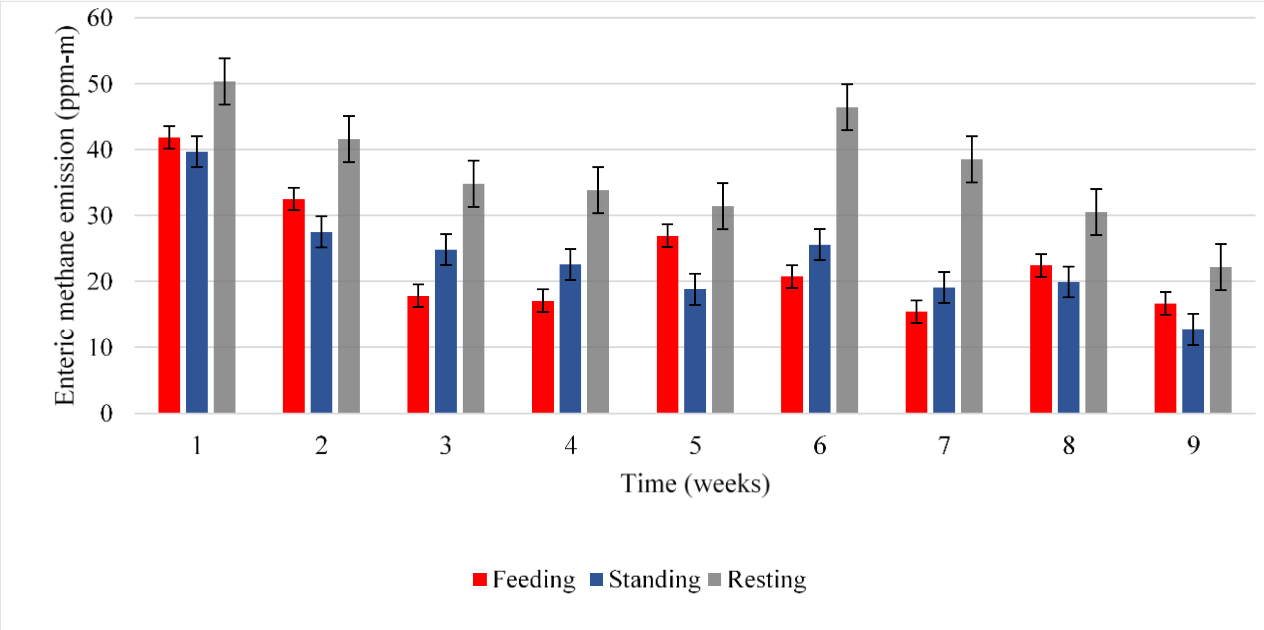

Fig. 1: Enteric methane emissions of Dohne Merino wethers fed diets with fossil shell flour supplemented with varying inclusion levels of BOSC over 9 weeks.

Table 6: Relationships between baobab oil seed cake inclusion and weeks of feeding on enteric methane emissions of Dohne Merino wethers during different activities

Activity	Week	Diets				SEM	R^2	P-value		
		0%	5%	10%	15%			Diet	Linear	Quadratic
Feeding	1	44.0	40.7	39.0	43.7	3.51		0.7077	0.8617	0.2597
	2	31.3	25.3	38.0	35.3	4.12		0.2279	0.2650	0.7278
	3	16.0[ab]	24.3[b]	14.7[a]	16.3[ab]	2.87		0.1497	0.5912	0.3682
	4	17.0	17.7	18.0	15.7	2.61		0.9226	0.7471	0.5580
	5	26.0	27.0	29.7	25.0	3.40		0.7909	0.9828	0.4088
	6	26.0	21.3	16.7	19.0	4.70		0.5676	0.2301	0.4526
	7	17.0	18.7	11.0	15.0	3.02		0.3706	0.3713	0.7277
	8	32.7	19.0	20.7	17.3	2.77	0.47	0.0166*	0.0095**	0.1211
	9	21.3	17.3	12.3	15.7	3.23		0.3280	0.1499	0.2708
Standing	1	46.0	35.0	38.7	39.0	5.88		0.6272	0.5141	0.3467
	2	27.3	27.7	27.0	28.0	4.78		0.9989	0.9488	0.9428
	3	26.0	28.3	22.0	23.0	5.93		0.8683	0.5636	0.9098
	4	27.7	23.0	17.0	22.7	4.99		0.5456	0.3545	0.3107
	5	16.7[a]	28.7[b]	13.0[a]	17.0[a]	1.95		0.0024**	0.4311	0.3911
	6	22.3	32.2	24.7	23.0	5.79		0.6141	0.8301	0.3356
	7	18.7[b]	22.7[a]	17.7[a]	17.3[a]	3.72		0.7340	0.5933	0.5679
	8	22.0	17.7	23.0	17.0	4.96		0.7760	0.6715	0.8696
	9	19.0	8.33	10.7	13.0	2.13	0.43	0.0373*	0.1512	0.0173*
Resting	1	34.3[a]	66.7[c]	40.0[ab]	60.3[bc]	7.90		0.0549	0.3179	0.5939
	2	42.3	50.0	38.7	35.3	5.98		0.4005	0.2579	0.3821
	3	51.7[c]	38.7[b]	28.3[ab]	20.7[a]	3.28	0.85	0.0008**	<0.0001**	0.4113
	4	43.0	26.7	29.0	36.7	9.97		0.6563	0.7024	0.2359
	5	29.3	36.7	31.3	28.3	6.51		0.8069	0.7719	0.4289
	6	58.7	61.7	30.3	35.0	10.4		0.1146	0.0694	0.9418
	7	45.7	38.0	37.0	33.3	8.28		0.7624	0.3065	0.8043
	8	39.3[b]	29.7[ab]	30.0[ab]	32.0[a]	3.52	0.51	0.0640	0.0137*	0.7168
	9	19.7[ab]	24.0[bc]	28.0[c]	17.0[a]	1.62	0.64	0.0061**	0.6447	0.0027**

[a,b] Means in the same row with different superscripts are significantly different (P<0.05). *P<0.05, **P<0.01. [1]Treatments: 0% = diet containing 4% FSF, 0% BOSC, and 15% Soybean meal; 5% = diet containing 4% FSF, 5% BOSC, and 10% soybean meal; 10% = diet containing 4% FSF, 10% BOSC and 5% soybean meal, 15% = diet containing 4% FSF, 15% BOSC and 0% soybean meal. [2]SEM = standard error of the mean.

Table 6 shows the relationships between enteric methane emissions during different activities (standing, feeding, and resting) on a weekly basis with baobab oil seed cake inclusion in Dohne Merino diets. There was a linear relationship between baobab oil seed cake inclusion and enteric methane output in week 8 during feeding (P<0.01). There was a quadratic relationship between baobab oil seed cake inclusion and enteric methane emissions, while Dohne merino wethers were standing in week 9 (P<0.05). Enteric methane emissions during resting decreased linearly with baobab oil seed cake inclusion in Dohne Merino wethers (P<0.01). There was a linear relationship between baobab oil seed cake inclusion in Dohne Merino diets and enteric methane emissions during resting in week 8 (P<0.05). There was a quadratic relationship between baobab oil seed cake inclusion in Dohne Merino diets and enteric methane emissions during resting in week 9 (P<0.01).

DISCUSSION

The Rumen Environment

Rumen temperature and pH are crucial factors that rely on the fermentation of ingested feeds in the rumen (Fu et al., 2024). The findings of this research show no significant differences in BOSC inclusion in rumen pH, temperature, ammonia levels, and volatile fatty acids of Dohne Merino wethers. Ruminal pH is the initial indicator of the fermentation qualities in the rumen (Malebana, 2018). This study found that the rumen pH level (6.62 – 6.76) remained within the normal range of 6.1 – 6.8, which is optimal for microbial fermentation. Additionally, according to a study by Karimizadeh et al. (2017), the ideal ruminal pH range for the growth of fibre-digesting microbes is between 6 and 6.8. The rumen pH values observed in this research were lower compared to the pH values (7.86 – 8.70) reported by Binuomote et al. (2022), in sheep, suggesting that the diets used in this study create an optimal rumen environment for microbial growth and fermentation. In this study, the ruminal pH values are slightly lower than those reported by Malebana (2018), in Dorper lambs that were given diets containing Marula seedcake. However, they are comparable since no significant differences were observed across all diets. Osman et al. (2020), attributed a significant decrease in rumen pH to an elevation in volatile fatty acid production, reduced fibre, and increased sugar content in the diet. Nevertheless, while slightly lower ruminal pH values were observed in wethers fed 5% BOSC in this research, BOSC inclusion did not significantly affect ruminal pH. This indicates that the supplementation of BOSC did not have an adverse effect on the rumen environment and fermentation.

Ruminal ammonia represents a balance of dietary nitrogen (N) breakdown, microbial protein synthesis, use, and N uptake (Wahyono et al., 2022). If the rate of protein degradation exceeds carbohydrate fermentation, a significant amount of nitrogen is converted into ammonia-N (Zurak et al., 2023). The mean ammonia concentration in this study was above the minimal 5mg/dL (Osman et al., 2020) and fell within the ideal range of 5mg/dL – 25mg/dL for proper microbial growth and function in the rumen. Rumen ammonia values in this study are slightly lower than Shen et al. (2015), who reported 10.1mg/dL to 15.7mg/dL in dairy cows. These findings suggest that BOSC supplementation maintained optimal microbial growth and efficient rumen fermentation (Osman et al., 2020). The highest rumen ammonia concentration in wethers fed 10% BOSC in this study may indicate the level of crude protein degradability and nitrogen uptake by the rumen microbes over amino acids, ultimately leading to an increase in the supply of metabolizable protein for the animals (Wahyono et al., 2022). Additionally, this might be attributed to the rumen microbiota influenced by the diet composition to degrade and hydrolyse protein (Cai et al., 2021).

Ruminants rely on volatile fatty acids, such as acetic acid, butyric acid, and propionic acid, to meet about 70% of their energy needs (Reddy & Hyder, 2023). These acids are considered fundamental end-products of anaerobic microbial fermentation of carbohydrates in the rumen (Binuomote et al., 2022). The rumen's concentration of volatile fatty acids is significantly influenced by the animals' diet, dry matter intake, and feed digestibility (Malebana, 2018). Diets that increase the synthesis of volatile fatty acids can improve sheep performance and productivity because they provide ruminants with more energy (Penner, 2014). Fibre-rich diets promote acetate production, while concentrates in diets promote propionate and butyrate production. Furthermore, diets high in protein increase the production of valerate and iso-butyrate acid. The total volatile fatty acid values observed in this study exceeded the 23.69–34.37mol/L range reported by Binuomote et al. (2022). Additionally, the values Okoruwa et al. (2016), reported ranged from 66.00 – 72.00mmol/L and were within the range of the total volatile fatty acid values observed in this study.

When the production rate of volatile fatty acids is higher than the clearance rate, there will build up in the rumen. This would decrease rumen pH and trigger a metabolic imbalance called rumen acidosis (Okoruwa, 2015; Binuomote et al., 2022). Nevertheless, the optimal pH values observed in this study indicate that there was a metabolic balance between volatile fatty acid production and clearance in the rumen. The total volatile fatty acids values of Dohne Merino wethers fed 0% and 10% BOSC fell within the normal range of 60–150mmol/L (Martínez-Fernández et al., 2014; Rad et al., 2016). The highest total volatile fatty acids, acetate, butyrate, isobutyrate, propionate, and isovalerate concentration, and the lowest A:P ratio observed in wethers fed 10% BOSC could be attributed to an ideal balance of nutrients that led to better fermentation of diets by rumen microbes and improved nutrient utilization for energy production (Malebana, 2018; Binuomote et al., 2022). The antioxidants and bioactive compounds of BOSC might have been at a concentration that maximizes their beneficial effect. According to Binuomote et al. (2022), the differences in volatile fatty acid concentrations in various research studies could be attributed to the physical fibrous nature, levels of starch content and solubility of carbohydrates in the different dietary treatments used in those studies.

Overall, volatile fatty acid concentration was inconsistent with increasing inclusion levels of BOSC in this study. Regardless, the lowest total volatile fatty acids, acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations, were observed in wethers fed 15% BOSC. Increased levels of BOSC may have resulted in protein–tannin and tannin-carbohydrate bonds that are not easily digestible, decreasing ammonia and volatile fatty acid production (Ibrahim & Hassen, 2022). The volatile fatty acid production is primarily dominated by acetate, followed by propionate and butyrate. The findings from this study indicate that acetate is the most prevalent, followed by propionate and butyrate. Propionate concentration increased as acetate concentration increased. However, wethers fed 10% BOSC had the lowest A:P ratio. The lower A:P ratio could be due to an increase in the concentration of propionic acid as acetic acid increased and serves as a positive indicator for rumen fermentation efficiency (Chen et al., 2021) and a shift towards propionic acid production reduces methane output, which is beneficial to the animals and the environment (Pal and Paul 2015; Soltan & Patra 2021).

Additionally, all diets in this study included FSF, a feed additive at a 4% inclusion level, with the ability to buffer and stabilize the rumen pH (Ikusika & Mpendulo, 2022). FSF also enhances the breakdown of proteins in other feed ingredients, especially BOSC in this study, where the protein was broken down to ammonia, which is used for microbial protein synthesis, enhancing the growth and activity of rumen microbes. Although there was a decrease in TVFAs, acetate, butyrate, and isobutyrate in their study, the values still fell within the optimal range. The addition of fossil shell flour in all diets and BOSC supplementation may have provided a balanced nutrient profile that contributed to maintaining the rumen pH for the proper growth of rumen microbes and improved nutrient availability for volatile fatty acid production.

Enteric Methane Emission

Ruminants produce methane, a greenhouse gas that contributes to environmental pollution and affects energy utilization in sheep (Van Gastelen et al., 2019). Dry matter intake (DMI) is one of the determinants of enteric methane output in animals (Du Toit et al., 2013; Ramin & Huhtanen, 2013; Washaya et al., 2018). The more feed is consumed, the more methane is produced by animals (Maigaard et al., 2024). The findings from this study confirm this since Dohne Merino wethers fed 15% BOSC had the highest DMI and methane (L/day) and methane (g/kg DMI). The findings of this study are consistent with Washaya et al. (2018) and Saho et al. (2020), who observed increased enteric methane output due to increased dry matter intake in goat diets supplemented with baobab oil seed cake. Similarly, Bhatt et al. (2020), also observed increased enteric methane output in lambs with high dry matter intake. The enteric methane output (L/day and g/kg DMI) in this study was higher than that observed by Washaya et al. (2018), in goat diets supplemented with BOSC. The lower methane output was attributed to the effect of forage legumes supplemented in goat diets. In this study, the higher methane values may be attributed to Dohne Merino wethers increased dry matter intake.

Enteric methane emission in livestock varies based on feed consumed, diet composition, physiological condition, and activities such as standing, feeding, and resting (Silvestre et al., 2021). Flores-Santiago et al. (2022), reported that animals produce more methane while resting than when standing or feeding, which is consistent with the findings from this study. Chagunda et al. (2009), Washaya et al. (2018) and Ikusika & Mpendulo (2023), also reported similar findings in cows, goats, and sheep, respectively. Microbial activities during resting result in more methane production in the rumen than when feeding. During resting, animals regurgitate, which reduces the particle size of the feed and increases the surface area of the feed for microbial fermentation (Washaya et al., 2018). The lowest overall enteric methane emissions in Dohne Merino wethers fed 10% BOSC may be attributed to the lowest A:P ratio and the highest propionate concentration than those fed other diets in this study.

A study by Saho et al. (2020), showed reduced enteric methane emission with increasing levels of baobab oil seed cake in goats. This is in line with the findings from this study. The reduction in enteric methane output observed with increased BOSC inclusion levels may be attributed to some secondary metabolites, such as tannins, which modify microbial fermentation in the rumen by inhibiting protein fermentation and blocking the activity of methanogenic bacteria. According to Magonka et al. (2018), BOSC contains anti-nutritional factors such as tannins (2–12%), oxalates, and saponins, which are relatively below levels toxic to animals. A study by Puchala et al. (2005) and Nawab et al. (2020), showed that forage that contains condensed tannins can potentially minimize enteric methane emission in ruminants. Similarly, Van Gastelen et al. (2019), reported that including tannin-rich forage in sheep diets reduces methane production. According to Njidda & Oloche (2022), the tannin content of browsable species reduces methane production. Uushona et al. (2022), alluded that tannins indirectly limit fibre digestion and inhibit the activity of methanogens.

Considering methane emission over time, it was observed in this present study that enteric methane emissions decreased as the feeding period increased. This contradicts Saho et al. (2020), who reported that goats emitted more methane with time with BOSC supplementation. Reduced methane emission with time could be due to the animals adapting to the feed and the continuous action of anti-nutritional factors coupled with the increased production of propionate and reduced hydrogen ions available for methane production (Bhatt et al., 2023). The acetate: propionate ratio is also hypothesized to be positively associated with methane emissions (Negussie et al., 2017; Washaya et al., 2018).

Additionally, Ikusika & Mpendulo (2023), supplemented diets with FSF and observed increased methane output with increasing inclusion levels in sheep, which contradicts the findings from this study. This can be attributed to the variations in the proportions of other ingredients, such as the inclusion of BOSC in this study, which might have a synergistic effect to reduce enteric methane emissions. The reduced enteric methane emission is beneficial to the livestock industry and aligns with the global efforts to mitigate climate change.

Conclusion

Fossil shell flour diets supplemented with varying inclusion levels of baobab oil seed cake had no effect on ruminal pH, temperature, ammonia concentration, and volatile fatty acid concentration in Dohne Merino wethers. However, enteric methane emission was significantly affected. Including fossil shell flour at 4% supplemented with 10% level of BOSC gives the best rumen environment for fermentation and reduces enteric methane emission in Dohne Merino wethers. The synergistic effect of these diets on rumen fermentation and enteric methane emission offers a sustainable and cost-effective approach to improving Dohne Merino's productivity while mitigating environmental impact thereby promoting sustainable agriculture. Therefore, farmers can include these feed resources to supplement livestock and increase production because they are viable alternatives to chemical-based feed additives and traditional feed sources.

DECLARATIONS

Funding: The authors acknowledge the National Research Foundation for funding this research.

Acknowledgement: The authors acknowledge the members of our research group, the SAMRC, and the Research and Innovation Office at UFH.

Conflict of Interest: All authors declare that there is no conflict of interest regarding the publication of this article.

Data Availability: Data will be available upon request

Ethics Statement: This study adhered to the procedures and standards required and approved by the University of Fort Hare, Animal Research Ethics Committee (Approval number MPE011SHAR01/22/A_U).

Author's Contribution: Conceptualization, C.H. and O.O.I; Methodology, C.H., O.O.I and C.T.M; Validation, O.O.I, C.T.M and F.O.A; Investigation, C.H. O.O.I and F.O.A; Resources, O.O.I and C.T.M; Data curation, C.H. and F.O.A; Writing-original draft and presentation, C.H and O.O.I; Writing-review and editing, O.O.I, C.T.M and F.F; Visualization, C.H, O.O.I and C.T.M; Supervision; O.O.I, F.O.A, C.T.M and F.F. Project administration; O.O.I and C.T.M; Funding acquisition, C.T.M. All authors have read and agreed to the published version of the manuscript.

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