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INFLUENCE OF *Odontonema Strictum* OIL ON THE GROWTH PERFORMANCE AND RUMINAL FERMENTATION OF BARBARI BUCKS

Hernández Morris[1], Alagbe John Olujimi[2*]

[1]Department of Ruminant Nutrition, Gandhi College of Agriculture, Rajasthan, India
[2]Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India

Abstract. *Odontonema strictum* oil is rich in several bioactive compounds with medicinal properties which are less toxic, environmental friendly and can promote food safety. To further examine their effects, a 60 days' experiment (excluding 14 days' quarantine period) was carried out using 28 Barbari bucks of about 8 months of age with an initial body weight of 30.55 ± 0.20 kg. Animals were stratified by live weight and randomly assigned to four treatments (n=7 goats per treatment). A completely randomized design was adopted and animals in treatment 1 (T^1) which also served as the control received basal diet only while T^2, T^3 and T^4 were fed basal diet supplemented with 0.1 mL, 0.2 mL and 0.3 mL *Odontonema strictum* oil respectively. *Odontonema strictum* oil supplementation enhanced ($p<0.05$) the average body weight gain and average daily feed intake. High *Odontonema strictum* oil supplementation decreased ($p>0.05$) the feed conversion ratio, methane enteric emission and improved the concentrations ($p<0.05$) of total volatile fatty acids, acetate, propionate and ruminal ammonia nitrogen compared to the control. Ruminal temperature, butyrate and pH values were not ($p>0.05$) affected by the treatment. In conclusion, *Odontonema strictum* oil supplementation at 0.3 mL/kg DM feed improved weight gain, feed intake, feed conversion ratio and also have positive effects on ruminal fermentation without causing any detrimental effect on the performance of animals.

Keywords: *Goats, fermentation, degradation, food safety, performance, phytocomponents.*

[*]***Corresponding Author:*** *Alagbe John Olujimi, Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India, e-mail: demsonfarms00@gmail.com*

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1. Introduction

Globally, there is a clear demand to reduce antibiotic use in ruminants and in this way reduce the risk of antimicrobial resistance (Bashir *et al*., 2020). This scenario has led animal scientist in search for natural and sustainable alternative to antibiotics that are eco-friendly, less toxic, effective to improve human and human health (Adewale *et al*., 2021). The use of herbal plants and their extracts (essential oils) have been identified as natural alternative to antibiotics because they possess antimicrobial properties and have been used as a rumen modifier to reduce greenhouse gas emissions (Matlop *et al*., 2017). However, the bioactive compounds in essential oils are influenced by processing technique, age of plant, geographical location, parts of plant used (seed, stem bark, leaves, flowers, buds) as well as specie (Alagbe, 2024).

Odontonema strictum are shrubs belonging to the family Acanthaceae and genus Odontonema. The plant has medicinal properties and are widely found in tropical regions of the world (Luhata *et al*., 2015; Félix *et al*., 2008). The family Acanthaceae has over 2000 species (Ouattara *et al*., 2013; Caballero *et al*., 2011). Pharmacological reports on the plant showed that it possesses, antioxidant, antimicrobial, anti-cancer, cytotoxic, anti-helminthic, anti-infertility, immune-stimulatory anti-inflammation, hepato-protective bronchodilatation, gastro-protective, vasodilatation, hypolipidemic, anti-viral, analgesics, anti-helminthic, antifungal, anti-diarrhea, antiplasmodial amongst others (Caballero *et al*., 2015). Phytochemical analysis of the leaves shows that they are loaded with terpenoids, alkaloids, flavonoids, tannins and saponins (Corrêa & Alcântara, 2012; Nabèrè *et al*., 2013). Phyto-components such as, stigmasterol, β-sitosterol, verbascoside, isoverbascoside and α, β-unsaturated δ–lactone terpenes and deacetylboronolide have been found to be abundant in essential oils of *Odontonema strictum* (Luhata & Usuki, 2022). These compounds have been reported to inhibit the growth of some species of gram +ve and gram –ve bacteria (Ouédraogo *et al*., 2018). *Odontonema strictum* aqueous leaf extract has also widely been used in folk medicine for the treatment of pyrexia, skin disease, malaria, high blood pressure, intestinal parasites, gastro-intestinal infections, wounds, snake bite, sexually transmitted infections, tooth ache and body pains (Luhata & Usuki, 2021).

Previous studies have revealed that essential oils from coriander, celery, oregano, peppermint and lemon grass have been shown to have a positive effect on growth performance, nutrient digestibility, feed intake and rumen fermentation and ecology, nitrogen utilisation, body morphometry of goats (Matlop *et al*., 2017; Khattab *et al*., 2020; Zhou *et al*., 2022; Wallace *et al*., 2022). Excessive supplementation of essential oils in the diet of goat's can become toxic especially if an appropriate dose has not been established. However, there is a dearth of information on the dietary supplementation of *Odontonema strictum* essential oil on the growth performance and rumen fermentation of goats. This information will help to promote food security, potentials in *Odontonema strictum* oil and boost livestock production.

2. Materials and methods

2.1. Study location

The study was carried out at the Ruminant unit, Ganhi College of Agriculture located at Rajasthan, India. Management of experimental animals was done in accordance to the guidelines outlined by Indian Society of sheep and goat production, India. All laboratory analysis was carried out at Sumitra Research Institute, Gujarat India.

2.2. Experimental goats and their management

28 Barbari bucks of about 8 months of age with an initial body weight of 30.55 ± 0.20 kg was used for this study. Animals were stratified by live weight and randomly assigned to four treatments (n=7 goats per treatment) and individually housed in semi-open pens measuring 1.8 square meters equipped with iron feeders and buckets for water. Before the arrival of goats, pens were thoroughly disinfected with Aquaclean and Morigad plus mixture in ratio 1:3 and fed basal diet which meets the nutritional requirement of goats according to NRC (2007) recommendations throughout the 14 days' quarantine period. Deworming against external and internal parasites as well as vaccination against *Peste Des Petits* (PPR) was done during the adjustment period. Goats in treatment one received basal diet without *Odontonema strictum* oil while those in treatment two, three and four were given basal diet supplemented with 0.1 mL, 0.2 mL and 0.3 mL in that order. 500 g of basal fed was first mixed with the concentration of oil in each treatment to ensure that goats consumed their full dose. Animals was fed twice

daily between 8:00 H and 14:00 H. Fresh water was served ad libitum and experimental period was 60 days excluding 14 days' acclimatization period.

2.3. Odontonema strictum leaves collection, extraction and analysis of oil

Fresh leaves of *Odontonema strictum* were collected from Rajasthan in the month of August 2023. Botanical authentication and identification was carried out at the department of Biological Sciences, College of Agriculture, Gandhi in Rajasthan and assigned a reference number (DET/BB01/2023). Leaves were air dried under shade for ten days to retain the bioactive components in plants before they were pulverized into powder using an electric grinder. Oil extraction from the pulverized powder was done using steam-distillation method with Clavenger apparatus as described by John (2024). Briefly, 200 g *Odontonema strictum* powder was measured into a round bottom flask, placed on a heating mantle and heated at 60°C for 30 minutes the steam produced passes through the glass condenser mounted on the top of the round bottom flask. After cooling it is collected into a beaker and transferred into a separator to separate the oil from other liquid.

Bioactive compounds in *Odontonema strictum* oil was analyzed using Noble gas chromatography and mass spectrometry (GC-MS 096H-TT0, Australia). Kit was operated according to the manufacturers recommendation for precision, Briefly, repeatability and repeatability of machine was set at < 0.3 % and < 0.006 %. Column oven is maintained at a temperature setting accuracy of ≤ 0.2°C supported carrier and make up gas of nitrogen, helium with two injectors ports, split ratio up to 11000:1 to avoid column overload, flow control range between 0 to 300 mL/min (N_2), 0 to 800 mL (H_2 and He). While the mass spectrometry was adjusted to an ionization energy (0-240 eV), ion source temperature (50 to 350°C) and transfer line temperatures of 50 to 400°C. Identification of bioactive components in the essential oil was based on comparison with their retention time and mass spectra by matching with those of the National Institute of Standard and Technology (2011) database.

2.4. Evaluation of growth parameters and analysis of basal diet

Weight of goats were taken weekly before feeding using digital scale. Feed intake of each goat was estimated as the difference between the feed offered and left over from the previous day. Body weight gain was taken as by subtracting the initial body weight of animals from their final weight. Chemical analysis of basal diet was analyzed according to AOAC (1997). Acid detergent fibre and Neutral detergent fibre were determined using alpha amylase and sodium sulphite and each parameter was expressed exclusive of residual ash. Organic matter, cellulose, hemicellulose, lignin and non-structural carbohydrates were estimated using the formula below:

Organic matter (g/kg DM) = 1000 - ash content

Non-structural carbohydrate (g/kg DM) = 1000 - (Crude protein + ether extract + ash + NDF)

Hemicellulose = Neutral detergent fibre - acid detergent fibre

Cellulose (g/kg DM) = ADF - lignin

Lignin (g/kg DM) = ADF - cellulose

2.5. Rumen liquor collection and analysis

Rumen liqor was collected on the last day of the experiment from each goat in the morning 3 hours after feeding to determine the total volatile acid and ammonia nitrogen (NH_3-N). The procedure was carried out using by inserting a stomach tube and pump. The pressure created by a suction pump makes it easier to collect 100 mL ruminal fluid. After collection, temperature was immediately recorded using a digital thermometer while the pH reading was done using an automated digital pH meter (model VB08-AD, China). Samples were placed in a closed sided warmer and transferred to the laboratory for further test. For the analysis of NH_3-N, 5 mL of sample was preserved in 5mL concentrated hydrochloric acid. Ammonia nitrogen (NH_3-N) concentrations were measured by using the micro diffusion method while total and proportional volatile fatty acid concentrations (acetate, butyrate and propionate) were conducted by using Press auto-gas chromatography (model BGH009-BA, China). Methane (CH_4) estimation was calculated from the molar proportion of volatile fatty acid using the formula:

$$CH_4 = 0.45 \times \text{acetate} - 0.275 \times \text{propionate} + 0.4\ (\text{butyrate})$$

3. Data analysis

In Statistical Analysis for Social Sciences (SPSS), data on growth performance and rumen fermentation were subjected to analysis of variance. To test the significant differences Duncan multiple range test was employed where $p<0.05$ level was declared significant.

Table 1. Ingredient and chemical composition of basal diet (g/kg of DM)

Components	Quantity (g/kg DM)
Maize (890 g/kg CP)	310.0
Corn barn	250.0
Cowpea husk	225.0
Groundnut cake (400 g/kg CP)	210.0
Growers Mineral/Vitamin Premix	2.00
Limestone	2.00
Salt	1.00
Total	1000.0
Chemical composition (g/kg dry matter - DM)	
Dry matter	910.0
Organic matter	920.1
Crude protein	160.0
Ether extract	30.06
Ash	70.90
Non-structural carbohydrate	350.36
Acid detergent fibre	220.72
Acid detergent lignin	300.11
Neutral detergent fibre	370.66
Cellulose	190.61
Hemicellulose	14.94
Energy (Kcal/kg)	2556.7

Note: 2.5 kg Growers premix contained: 15,000 I.U. vitamin A; 8000 mg vitamin B1; 3000 I.U. vitamin D3; 60.0 mg vitamin E; 15 mg Choline; 0.96 mg Cobalt; 2.00 mg I; 50 manganese Mn; 0.50 mg Selenium; 250 mg

Table 2. Major bioactive compounds in *Odontonema strictum* oil

Compounds	Retention time (min)	Composition (%)
α-Pinene	220.5	21.40
β-Guaiene	250.8	6.31
Palmitic acid	290.1	5.26
n-Hexadecanoic acid	330.2	4.10
Pentadecanoic acid	356.8	4.55
Humulane-1,6-dien-3-ol	400.1	3.03
β-Linalol	439.6	15.67
α-Cadinol	475.4	10.13
D-Limonene	494.5	9.52
2,4,6-Trimethyldecane	503.6	3.05
β-Caryophyllene	511.4	3.61

Table 3. Growth performance of Barbari bucks fed diet supplemented with *Odontonema strictum* oil

Parameters	T^1	T^2	T^3	T^4	[4]SEM
[1]Initial body weight (kg)	32.00	32.09	32.05	32.02	0.12
Final body weight (kg)	34.00^b	36.00^a	36.01^a	36.00^a	0.18
Body weight gain (kg)	2.00^b	3.91^a	3.96^a	3.98^a	0.02
[1]Average daily weight gain (kg)	0.052^b	0.065^a	0.066^a	0.068^a	0.01
Average total feed intake (kg)	16.00^b	18.00^a	18.01^a	18.02^a	0.07
[2]Average daily feed intake	0.26^b	0.30^a	0.30^a	0.30^a	0.01
[3]Feed conversion ratio	5.10^a	5.00^b	5.00^b	5.00^b	0.02

Note: Means with different superscripts in a row are significantly different ($P<0.05$); T^1: basal diet with no oil; T^2: basal diet with *Odontonema strictum* oil at 0.10 mL/kg diet; T^3: basal diet with *Odontonema strictum* oil at 0.20 mL/kg diet; T^4: basal diet with *Odontonema strictum* oil at 0.30 mL/kg diet;
SEM: standard error of mean

Table 4. Rumen fermentation of Barbari bucks fed diet supplemented with *Odontonema strictum* oil

Parameters	T^1	T^2	T^3	T^4	[4]SEM
Temperature (°C)	38.6	39.7	39.0	38.5	0.25
Rumen pH	5.92	5.95	6.02	6.01	0.02
NH_3-N (g/L)	79.7	81.0	81.3	81.9	1.61
TVFA (Mm/L)	108.3^c	125.2^b	128.7^b	130.4^a	2.03
Acetate (Mm/L)	56.22^b	75.90^a	77.1^a	77.9^a	1.05
Propionate (Mm/L)	20.6^b	29.3^a	30.8^a	30.9^a	0.66
Butyrate (Mm/L)	11.71^a	8.76^b	8.51^b	8.44^b	0.01
Methane ([CH_4 (Mol)])	17.89^a	14.21^b	14.08^b	14.00^b	0.02

Note: Means with different superscripts in a row are significantly different ($P<0.05$); T^1: basal diet with no oil; T^2: basal diet with *Odontonema strictum* oil at 0.10 mL/kg diet; T^3: basal diet with *Odontonema strictum* oil at 0.20 mL/kg diet; T^4: basal diet with *Odontonema strictum* oil at 0.30 mL/kg diet;
SEM: standard error of mean

4. Results and discussion

The major bioactive compounds in *Odontonema strictum* oil (Table 2) are: α-pinene with 21.40 %, β-linalol (15.67 %), α-cadinol (10.13 %), D-limonene (9.52 %), β-guaiene (6.31 %), palmitic acid (5.26 %), pentadecanoic acid (4.55 %), hexadecanoic acid (4.10 %), β-caryophyllene (3.61 %), 2,4,6-trimethyldecane (3.05 %) and humulane-1,6-dien-3-ol (3.03 %). The presence of these compounds suggests that *Odontonema strictum* oil possess medicinal properties such as, anti-inflammatory (Klavina *et al*., 2015; John, 2024a), antidiabetic, anti-cancer (Alagbe, 2022), antidiarrheal (John, 2024b; Zakariya *et al*., 2017), anti-helminthic (Daniel *et al*., 2023), cardio-protective (Choi *et al*., 2013), cytotoxic (Aworinde *et al*., 2016), immuno-modulatory (Bazie *et al*., 2014; John, 2024c), hepato-protective, derma-protective (Singh *et al*., 2022), gastro-protective (Prabhu & Guruvayoorappan, 2012), antifungal (Alagbe *et al*., 2022), antioxidant (Ajiboye *et al*., 2013), antiviral, antimicrobial (Omokore & Alagbe, 2019; Shittu *et al*., 2024), antibacterial (John, 2024d; Bashir *et al*., 2020) and hypolipidemic (Ojediran, 2024a, 2024b; Adewale *et al*., 2021). Luhata et al. (2015a, 2015b) reported a lower concentration of 0.44 % for hexadecanoic acid in *Odontonema strictum* leaf extracts. Similarly, Felix et al. (2008) found out that extracts from *Odontonema strictum* contained 0.62% of pentadecanoic acid. Variations in these result could be attributed to age of plants, geographical location as well as processing method adopted (John, 2024e; Bashir *et al*., 2020).

Growth performance of Barbari bucks fed diet supplemented with *Odontonema strictum* oil (Table 3) showed that body weight gain was lower in T^1 (2.00 kg) than T^2 (3.91 kg), T^3 (3.96 kg) and T^4 (3.98 kg) ($p<0.05$) whereas total feed intake was higher in T^4 (18.02 kg), T^3 (18.01 kg), T^2 (18.00 kg) than in T1 (16.00 kg). According to Kholif et al. (2021), essential oils possess numerous bioactive components or compounds having the capacity to modulate gut microbiota, inhibit pathogenic bacterial growth, thus leading to improved growth performance. Higher body weight observed in goats fed diet supplemented with *Odontonema strictum* oil strongly suggests that it has antimicrobial properties. This outcome is in agreement with the reports of Khattab et al. (2020) who replaced antibiotics with thyme and celery seed mixture on the performance of Barki ewes. *Odontonema strictum* supplementation influenced feed intake; this suggests that it had a positive effect on the flavor, odor and palatability. This result is in agreement with the report of Shaaban et al. (2021) who recorded an increased in feed intake with thyme and celery supplementation in Barki lambs. The decreased in feed conversion ratio as the level of *Odontonema strictum* supplementation increased in the diet is in line with the observations of Matloup et al. (2017) who noted improved a decreased in feed conversion ratio in lactating cows fed diet supplemented with crude coriander. The presence of bioactive compounds in *Odontonema strictum* oil are probably the main reason for the decreased in feed conversion ratio.

Rumen fermentation of Barbari bucks fed diet supplemented with *Odontonema strictum* oil (Table 4) revealed that temperature, pH and ammonia nitrogen (NH_3-N) values which varied from 38.5-39.7 (°C), 5.92-6.02 and 79.7-81.90 (g/L) were not affected ($p>0.05$). Values obtained were within the range 38.2-40.0 (°C), 5.88-6.13 and 70.00-95.6 g/L reported by Zhou et al. (2019) when oregano essential oil was supplemented in the diet of sheep. The ruminal pH was within the minimum range required for the normal activities of microorganisms in the rumen and their growth to facilitate nutrient digestion (Kong *et al*., 2022). Rumen ammonia nitrogen values were not significantly ($p>0.05$) affected. However, values obtained in this study was greater than 50.9 g NH_3-N/L reported by Matloup et al. (2017) when coriander oil was supplemented in the diet of Friesian cows. Kong et al. (2022) recorded a lower value of 9.2 g NH_3-N/L in goats fed diets containing different levels of essential oil. Variation in results could be due to composition, processing method as well as dose supplemented in the diet of animals (John, 2024d). Total volatile fatty acids (TVFA) was higher in T^4 (130.4 Mm/L), similar ($P>0.05$) in T^2 (125.2 Mm/L) and T^3 (128.7 Mm/L) and lower in

T^1 (108.3 Mm/L) ($p<0.05$) in (T^1) 102.17 Mm/L. Result on the TVFA suggests that *Odontonema strictum* oil supplementation can improve apparent nutrient digestibility which translates to a better growth and weight gain in animals (Lin *et al*., 2013). This outcome is in agreement with the reports of Ando et al. (2003) who observed a significant ($p<0.05$) influence when peppermint was fed to goats. Acetate and propionate values follow similar trend as records were lowest in T^1 (56.22 Mm/L; 20.60 Mm/L) than in T^2 (75.90 Mm/L; 29.30 Mm/L), T^3 (77.10 Mm/L; 30.80 Mm/L) and T^4 (77.90 Mm; 30.90 Mm/L) ($p<0.05$) respectively. Increased acetate production observed among bucks fed with *Odontonema strictum* oil suggests it can improve the degradation of fibre in the rumen (Wang *et al*., 2022). Similarly, improved propionate may also result from degradation of non-structural carbohydrate as well as organic matter (Zhou *et al*., 2020). Result obtained is in consistent with the findings of Ribeiro et al. (2020) who observed a significant ($p<0.05$) in bucks fed different levels on thyme essential oil. Butyrate and methane values follow similar pattern, data obtained were higher in T^1 (11.71 Mm/L; 17.89 Mol) than in T^2 (8.76 Mm/L; 14.21 Mol), T^3 (8.51 Mm/L; 14.08 Mol) and T^4 (8.44 Mm/L; 14.00 Mol) ($p<0.05$). Values for butyrate was within 9.82-15.51 Mm/L reported by Khateri et al. (2017) when specific blend of essential oil was fed to sheep. Similarly, supplementation of oils also reduced enteric methane emission in bucks. According to (Wallace *et al*., 2014), there is a correlation between enteric methane emissions and the relative abundance of rumen protozoa and methanogens. This result is consistent with the findings of Cobellis et al. (2016); Khateri et al. (2017) who reported that essential oil is capable of modulating methane production in sheep.

5. Conclusion

In conclusion, *Odontonema strictum* oil is rich in several bioactive compounds with medicinal or pharmacological properties. The oil also posses antimicrobial, antioxidant, anti-inflammatory amongst others. The experiment has revealed that dietary supplementation of the oil up to 0.30 ml/kg had a positive impact on the body weight gain and feed intake of goats and was also able to modulate fermentation in the rumen.

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