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In Vitro Evaluation of Bitterleaf and Moringa Leaves as Anti-Methanogenic Plants and Their Nutritive Potentials in Ruminants' Nutrition

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Abstract

An *in vitro* study was conducted to investigate the effects of graded inclusion levels of *Vernonia amygdalina* and *Moringa oleifera* leaf meals in ruminants' diet on nutritive composition, extent and rate of gas and short-chain fatty acid (SCFA) production. Fresh *V. amygdalina* and *M. oleifera* leaves were harvested, air-dried, milled to make respective leaf meals, designated as VALM and MOLM. The leaf meals were added to formulated concentrate diet (FCD) at 0, 5, 10, 15, 20%, respectively, and were analyzed for nutrient (proximate, fibre fraction, macro-minerals and phytochemicals) compositions. The suitability/feeding value of these test diets were evaluated using in vitro gas production techniques. During the incubation, the gas production was measured at regular intervals from 0 to 24 hrs. The treatments were assigned to completely randomize design (n=3) and all data generated were subjected statistical analysis. The leaf meals significantly improved the nutritive quality and mineral concentration of the feed. The *in vitro* characteristics results suggest that the high inclusion of VALM and MOLM enhanced the extent and rate of fermentation, while decreasing the methane production. 20% VALM inclusion level with the FCD had the least methane gas production (3.10 ml), thus, suggestive of the potentials of VALM as anti-methanogenic plants. Hence, the plants could be incorporated in ruminant feed up to 20%

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inclusion level for optimum feed utilization and methane emission reduction. However, *in vivo* study is hereby recommended for validation of these *in vitro* results.

Key words: methane, protein quality, small ruminant, tannin, tropical leaves.

Introduction

The amount of gases (carbon-dioxide and methane) emitted by ruminants is relational and an indicator acids produced by fermentation (digestion). Meanwhile, amount of gas produced predicts nutrient fermentation and measures the digestibility of ruminant feeds, i.e., the extent and rate of feed digestion, which can be measured using *in vitro* gas production technique (Tedeschi *et al*., 2009). In order to produce organic animal products free of possible dietary antibiotics residues, especially for consumers, while raising them in an eco-friendly environment (mitigating methane emission) which contributes significantly to greenhouse gas (GHG) emissions, there is dire need to strengthen the development of modern alternative natural feed additives and use of ionophores as dietary antibiotic growth promoters on ruminants. It is conceivable that moringa *(Moringa oleifera*) and bitterleaf (*Vernonia amygdalina)* could play a valuable role in achieving this. Because they are popular multipurpose trees, naturally cultivated in many developing countries and recognized historically to have many nutritional and pharmacological, anti-inflammatory, anti-bacterial and antimicrobial properties (Dewangan *et al*., 2010; Sholapur and Patil, 2013). The tropical plants are rich source of protein, crude fibre and mineral content and contain negligible amount of anti-nutritive compounds such as tannin, terpenoids and glycosides as abundant active components (Soliva *et al*., 2005; Sholapur and Patil, 2013; Babiker *et al*., 2017). The crude protein (CP) content of Moringa ranges from 7.12 to 39.17% (Ayasan, 2015). *V.*

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amygdalina has an astringent taste. According to Aregheore *et al.* (1998), bitterleaf was reported to contain 20-34% (CP) with about 200 species and can be used as an antioxidant, anti-diarrhoea, growth promoter and for medicinal purposes (Githiori, 2004; Calsamiglia *et al*., 2007; Cieslak *et al*., 2013). With these attributes *M. oleifera* and *V. amygdalina*, it is capable of influencing the ruminal ecosystem, affect methane production, and yet support the performance of ruminant, if fed. Hence, this paper evaluated the potentials and efficacy of these two tropical leaves (*Vernonia amygdalina* and *Moringa oleifera*) as anti-methanogenic plants and their suitability (feeding values) in small ruminants' production, using *in vitro* gas production techniques.

Materials and Methods

Experimental site

The study was carried out in the Department of Animal Production and Health, Federal University of Technology, Akure (FUTA), Nigeria. The University is located in the humid rain forest zone of Western Nigeria, with the tropical climate of broadly two seasons, which are rainy season (April – October) and dry season (November – March). Temperature throughout the year ranges between 21^0C and 29^0C with relatively high humidity. The mean annual rainfall is about 1,500mm and located within the coordinates $7^0$10'N $5^0$12'E (Nigerian Meteorological Agency, 2014).

Sourcing and processing of *Vernonia amygdalina, Moringa oleifera* leaves and feed

The *Vernonia amygdalina* and *Moringa oleifera* leaves, after identification by the Department of Crop, Soil and Pest Management, FUTA. *Vernonia amygdalina* and *Moringa oleifera* leaves

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were harvested within Akure metropolis, air-dried, milled and stored in dessicator for analytical use. The cassava peels were collected at cassava processing industries in Akure, Ondo State. The cassava peels were sun-dried for 3-5 days depending on the intensity of the sun to reduce the cyanide content and moisture content while other convectional feed ingredients were bought at a reputable feed mill industry in Akure.

Diet formulation

A concentrate was formulated to meet NRC (2007) nutrient requirements recommended for growing goats (Table 1). While the leaf meals (*V. amygdalina* leaf meal (VALM) and *M. oleifera* leaf meal (MOLM)) were added at T1 control diet (0% leaf meal), T2 (5% VALM), T3 (10% VALM), T4 (15% VALM), T5 (20% VALM), T6 (5% MOLM), T7 (10% MOLM), T8 (15% MOLM) and T9 (20% MOLM) to make nine (9) test diets.

Table 1: Gross composition of the basal diet

Ingredients	Quantity (kg)
Cassava peels	55.00
Brewer dried grain	14.00
Wheat offal	20.00
Palm kernel cake	7.00
Urea-molasses	1.00
Bone meal	1.00
Salt	1.00
Vitamin-mineral Premix	1.00
Total	100.00

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Chemical compositions determination

The *V. amygdalina* leaf meal (VALM), *M. oleifera* leaf meal (MOLM), and the test diets were analyzed for chemical (proximate, fibre fractions, minerals, phytochemicals) compositions and suitability as ruminants' diet, by determining the methane gas production, short chain fatty acid, organic matter digestibility and metabolizable energy, using standard procedural methods. Proximate and fibre fractions – Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL), and phytochemical analyses were determined according to standard procedures of AOAC (2006). Mineral components were determined using Atomic Absorption Spectrophotometer (AAS) Buck Scientific 210 VGP & Lamotte Spectro 2 while calcium and potassium contents were determined by flame photometry and phosphorus by the Vanado-molybdate method.

***In vitro* degradability of the leaf meals and the test diets**

***In vitro* protocol**

Samples of leaf meals and test diets were milled using hammer mill of 1 mm sieve, 200 mg dry matter each of the samples were weighed carefully into a fiber bag, and the bag was sealed and carefully placed in the cylinder of the syringe of 120 mL capacity. Each syringe with the sample was replicated thrice and the blank. The plunger was greased with Vaseline to ensure easy movement and precise fitting. It was then pushed down the cylinder gently to avoid thrusting out the sample through the syringe nozzle. The silicon tubes were fitted to the syringe, thereafter tightened by a metal clip so as to prevent escape of gas.

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Collection and preparation of the rumen fluid

Rumen liquor was collected from rams through suction tube, after the rams have been fed (5% body weight) for two (2) weeks with 60% *Panicum maximum* and 40% formulated concentrate feed. The rumen liquor was collected before feeding between (7:00 am – 8:00 am) into the pre-warmed thermo-flask to a temperature of 39°C.

Preparation of the buffer solution

McDougall's buffer solution was prepared according to the procedures of Babayemi and Bamikole (2006) to consist, solution (g/litre) of 9.8 $NaHCO_3$ + 2.77 Na_2HPO_4 + 0.57 KCl + 0.47 NaCl + 2.16 $MgSO_3.7H_2O$ + 16 $CaCl_2.2H_2O$ (1:4 v/v) under continuous flushing with CO_2 (to minimize changes in microbial populations and to avoid O_2 contamination), this was added using another 50 mL plastic calibrated syringe. The rumen liquor-buffer solution was mixed in the ratio 1:4 (v/v) for this incubation.

Preparation of the syringes for incubation

Incubation procedure was carried out using 120 ml calibrated transparent plastic syringes with fitted silicon tube. The sample weighing 200 mg (n=3) was carefully dropped into syringes and thereafter, 30ml each of the inoculum containing cheese cloth strained rumen liquor and buffer solution was added. To eliminate the air in the inoculum, syringe was tapped and pushed upward using piston. The silicon tube fitted to the syringe were then tightened by a metal clip to prevent escape of gas. Incubation was carried out at 39±1°C and the volume of gas production was measured at 3 hours interval for 24 hrs, as described by Omotoso (2019).

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Methane gas determination

At the end of the termination hour, 4 mL of NaOH (10M) was introduced to estimate the methane production according to Fievez *et al*. (2005); the methods of Menke and Steingass (1988) were used to evaluate the metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA). The average of the volume of gas produced from the blanks were deducted from the volume of gas produced per sample.

Calculations

ME (MJ/Kg DM) = 2.20 + 0.136GV + 0.057CP + 0.0029 CF, OMD (%) = 14.88 + 0.889GV + 0.45CP + 0.651XA according to Babayemi and Bankole (2006).

SCFA = 0.0239 V - 0.0601 according to Getachew *et al*. (1999).

Where; GV, CP CF and XA are total gas volume, Crude protein, crude fibre and ash, respectively.

Experimental Design and Statistical Analysis

The experimental design was Completely Randomized Design and all data generated were subjected to one-way analysis of variance (ANOVA) using Minitab Statistical Package and where significant differences were found, the means were compared using New Duncan Multiple Range Test of the same package. Confidence interval was set at 0.05.

Results

The Chemical composition of *Vernonia amygdalina* and *Moringa oleifera* leaf meal is presented in Table 2. *V. amygdalina* leaf meal (VALM) and *M. oleifera* leaf meal (MOLM) had dry matter values ranged from 86.98 – 88.21% while crude protein ranged from 22.86% (MOLM) to 26.10% (VALM). Conversely, the crude fibre in MOLM was the higher (8.58%).

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Ash content in VALM (6.50%) was higher than of MOLM (6.16%). The neutral detergent fibre and acid detergent lignin values (60.34% and 27.98%, respectively) in MOLM was the higher than values (52.81% and 16.67%) recorded for VALM. However, the mineral (Ca, P, Na and Mg) concentrations in this present study were higher in MOLM, except for K which was higher in VALM (75.10ppm). Tannin (0.048mg/g), phytate (33.43mg/g) alkaloid (1.98%) was higher in VALM while MOLM had higher oxalate (20.01mg/g) and saponin (6.16%).

Table 2. Chemical composition of *Vernonia amygdalina* and *Moringa oleifera* leaf meal

Parameters (%)	*Vernonia amygdalina* leaf meal	*Moringa oleifera* leaf meal
Proximate		
Dry matter	86.98	88.21
Crude protein	26.10	22.86
Crude fibre	7.20	8.58
Ether extract	6.50	6.16
Ash	7.56	7.01
Fibre fractions		
Neutral detergent fibre	52.81	60.34
Acid detergent fibre	33.77	32.88
Acid detergent lignin	16.67	27.98
Minerals		
Calcium	0.39	0.43
Phosphorus	0.21	0.22
Sodium (ppm)	50.90	53.00
Potassium (ppm)	75.10	73.50
Magnesium (ppm)	60.10	64.00
Anti-nutrients		
Tannin (mg/g)	0.048	0.040
Phytate (mg/g)	33.43	25.02
Alkaloid, %	1.98	1.02
Oxalate (mg/g)	10.23	20.01
Saponin, %	4.19	6.16

Nutrient composition of varying levels of VALM and MOLM as supplements to basal diets is presented in Table 3. The inclusion of leaf meals to the formulated concentrate diet (FCD) significantly ($P<0.05$) influenced the nutrient compositions except DM, CF, Ash and acid

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detergent fibre. FCD + 20% VALM had the highest CP (18.21%). Though CP ranged from 17.22% (FCD) to 18.21% (FCD+20%VALM).

Table 3. Nutrient composition of varying levels of VALM and MOLM as supplements to basal diets

Parameters (%)	FCD	FCD + 5% VALM	FCD + 10% VALM	FCD + 15% VALM	FCD + 20% VALM	FCD + 5% MOLM	FCD + 10% MOLM	FCD + 15% MOLM	FCD + 20% MOLM	p-value
Dry matter	89.67	88.99	88.57	89.01	88.99	88.87	88.56	88.48	88.45	0.61
CP	17.22^{c}	17.58^{bc}	17.77^{b}	17.98^{b}	18.21^{a}	17.42^{bc}	17.59^{bc}	17.83^{b}	18.00^{b}	0.03
Crude fibre	8.37	8.39	8.43	8.47	8.49	8.37	8.41	8.46	8.48	0.52
EE	6.68^{a}	6.47^{b}	6.48^{b}	6.51^{b}	6.53^{b}	6.32^{b}	6.37^{b}	6.38^{b}	6.43^{b}	0.41
Ash	11.22	11.09	11.10	11.14	11.18	11.11	11.12	11.14	11.17	0.52
NFE	56.51^{a}	56.47^{a}	56.22^{a}	55.90^{b}	55.59^{b}	56.78^{a}	56.51^{a}	55.77^{b}	55.92^{b}	0.43
NDF	53.57^{b}	53.04^{b}	53.64^{b}	53.89^{b}	53.97^{b}	54.78^{b}	55.01^{ab}	55.55^{a}	55.56^{a}	0.03
ADF	34.02	33.33	33.63	33.68	33.87	33.45	33.56	33.59	33.87	0.54
ADL	18.76^{a}	17.67^{b}	17.67^{b}	17.88^{b}	17.98^{b}	18.43^{a}	18.45^{a}	18.66^{a}	18.69^{a}	0.01

abcdef: means on the same row with different superscript are significantly (P<0.05) different. CP – Crude protein, EE – Ether extract, NFE - Nitrogen free extract; NDF - Neutral detergent fibre; ADF - Acid detergent fibre; ADL - Acid detergent lignin, VALM – Vernonia amygdalina leaf meal; MOLM – Moringa oleifera leaf meal; FCD – Formulated concentrate diet.

From Table 4, the minerals and anti-nutrients of the FCD were significantly (P<0.05) influenced by the inclusion of the leaf meals at graded levels, except for sodium, Na. The Ca and P contents of the diets ranged from 0.40% to 0.43% and 0.22% to 0.25%, respectively.

Table 4. Macro-minerals and anti-nutrient composition of varying levels of VALM and MOLM as supplements to basal diets

Parameters	FCD	FCD + 5% VALM	FCD + 10% VALM	FCD + 15% VALM	FCD + 20% VALM	FCD + 5% MOLM	FCD + 10% MOLM	FCD + 15% MOLM	FCD + 20% MOLM	P-value
Minerals (ppm)										
Calcium, %	0.40^{c}	0.40^{c}	0.41^{b}	0.41^{b}	0.42^{a}	0.41^{b}	0.41^{b}	0.42^{a}	0.43^{a}	0.01
Phosphorus, %	0.25^{a}	0.22^{c}	0.22^{c}	0.23^{b}	0.23^{b}	0.22	0.22^{c}	0.23^{b}	0.23^{b}	0.02
Sodium	52.10	51.87	51.89	51.98	52.00	51.87	51.89	52.11	52.14	0.52
Potassium	73.80^{b}	74.44^{a}	74.45^{a}	74.54^{a}	74.59^{a}	73.45^{b}	73.49^{b}	73.68^{b}	73.69^{b}	0.03
Magnesium	62.90^{a}	61.19^{b}	61.23^{b}	61.27^{b}	61.30^{b}	63.00^{a}	63.11^{a}	63.24^{a}	63.31^{a}	0.02

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Anti-nutrients (mg/g)										
Tannin, %	0.018^{f}	0.021^{e}	0.027^{b}	0.029^{b}	0.034^{a}	0.023^{d}	0.025^{c}	0.027^{b}	0.028^{b}	0.01
Phytate	37.08^{a}	35.22^{b}	35.57^{b}	35.77^{b}	35.98^{b}	28.90^{c}	28.94^{c}	28.99^{c}	29.07^{c}	0.01
Alkaloid, %	0.87^{b}	0.90^{b}	0.93^{b}	0.97^{b}	1.12^{a}	0.89^{b}	0.94^{b}	0.95^{b}	0.99^{b}	0.01
Oxalate	6.89^{d}	7.89^{c}	7.99^{c}	8.13^{c}	8.34^{c}	9.13^{b}	9.57^{a}	9.67^{a}	9.89^{a}	0.02
Saponin	5.48^{b}	5.01^{f}	5.05^{f}	5.18^{e}	5.21^{e}	5.67^{b}	5.68^{b}	5.77^{b}	6.01^{a}	0.01

abcdef: means on the same row with different superscript are significantly (P<0.05) different.

The *in vitro* characteristics of VALM, MOLM and the test diets is presented in Table 5. All parameters observed were significantly (P<0.05) influenced. The methane gas produced ranged from 0.66ml (MOLM) to 3.73ml (FCD). MOLM had the least methane, organic matter digestibility (OMD), short chain fatty acid (SCFA) and carbon dioxide (CO_2) values (0.66ml, 33.73%, 0.05 µm, 4.14MJ/kgDM and 3.84ml, respectively).

Table 5: *In vitro* characteristics of *Vernonia amygdalina* leaf meal, *Moringa oleifera* leaf meal and test diets

Feed samples	**Methane (ml)**	**OMD (%)**	**SCFA (µm)**	**ME (MJ/KgDM)**	**CO_2**
VALM	1.06^{e}	35.99^{b}	0.06^{c}	4.39^{d}	3.94^{d}
MOLM	0.66^{f}	33.73^{c}	0.05^{d}	4.14^{e}	3.84^{d}
FCD	3.73^{a}	39.89^{a}	0.21^{a}	4.73^{a}	7.48^{b}
FCD + 5% VALM	3.40^{c}	39.61^{a}	0.20^{b}	4.70^{c}	7.40^{c}
FCD + 10% VALM	3.33^{c}	39.70^{a}	0.20^{b}	4.71^{b}	7.48^{b}
FCD + 15% VALM	3.28^{c}	39.82^{a}	0.20^{b}	4.72^{b}	7.53^{b}
FCD + 20% VALM	3.10^{d}	39.86^{a}	0.20^{b}	4.72^{b}	7.60^{a}
FCD + 5% MOLM	3.61^{b}	39.82^{a}	0.21^{a}	4.73^{a}	7.49^{b}
FCD + 10% MOLM	3.51^{b}	39.81^{a}	0.20^{b}	4.72^{b}	7.49^{b}
FCD + 15% MOLM	3.49^{b}	39.93^{a}	0.20^{b}	4.74^{a}	7.51^{b}
FCD + 20% MOLM	3.35^{c}	40.03^{a}	0.20^{b}	4.75^{a}	7.65^{a}
SEM	0.19	0.14	0.02	0.11	0.46
P-value	0.02	0.01	0.01	0.03	0.02

abcdef: means on the same row with different superscript are significantly (P<0.05) different. OMD- Organic Matter Digestibility; SCFA- Short Chain Fatty Acid; ME-Metabolizable Energy; VALM – Vernonia amygdalina leaf meal; MOLM – Moringa oleifera leaf meal; FCD – Formulated concentrate diet.

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Figure 1 shows the graphical representation of in vitro gas production per 3-hours interval over a 24-hour period. The gas productions were at their peak at 18th hour, thereafter maintained a static movement and declined afterwards.

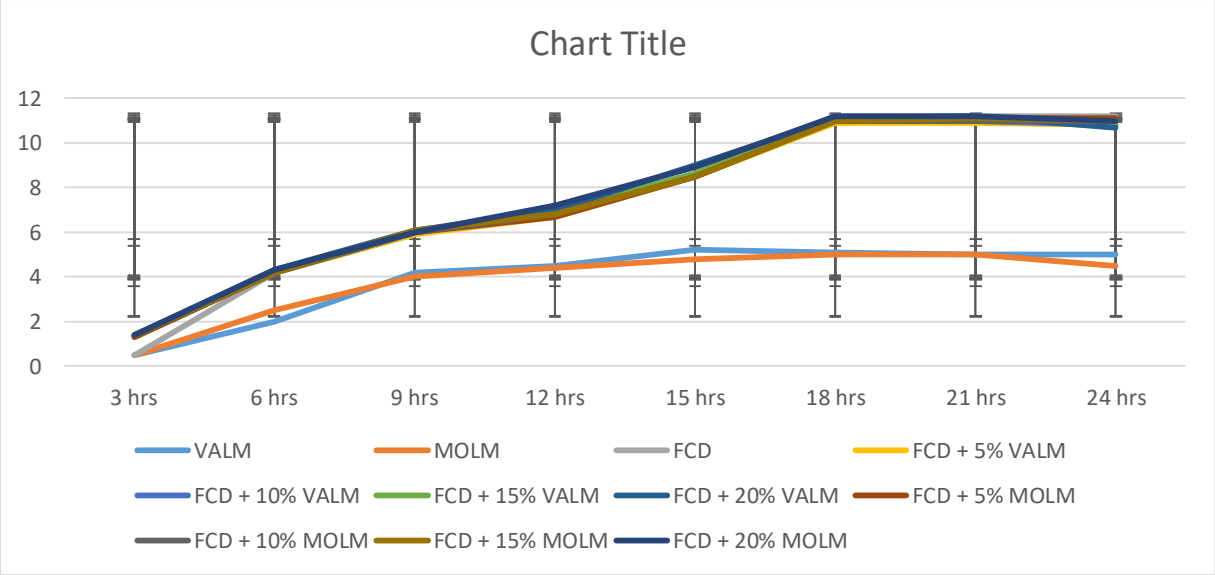

Figure 1: Graphical representation of *in vitro* gas production of *V. amygdalina* and *M. oleifera* leaf meal

Discussion

The leaf meals and test diets under consideration had rich nutrients (Table 2 and 3) to support the growth and maintenance of growing goats (NRC, 2007). The protein content of VALM and MOLM used in this study were comparable with the values reported by Soliva *et al*. (2005) Fadiyimu *et al*. (2010) for *M. oleifera* meals. The leaf meals (VALM and MOLM) revealed the leaves are rich in nitrogen (contained 26.10% and 22.86% CP, respectively), which agrees with the report of Aregheore *et al.* (1998) and Ayasan (2015) and hence, positively influenced the protein quality of the test diets. The dry matter recorded might be

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attributed to the leaf-stem ratio, age and time of harvest of the plants. By implication, the feed samples cannot be easily prone to microbial deterioration/spoilage and hence, could prolong its shelf life. Further, the dietary DM and CF contents would also encourage voluntary intake by ruminants, rumination, gut motility/fill and consequently, enhance their growth, because a low content of crude fibre encourages good palatability for animals. The rich ash content in the plants is an indication that the leaf meals are good source of mineral and would be helpful for acid-base balance, proper bone formation and growth (Table 2 and 3). The anti-nutritional factors considered in this study were within the threshold that could easily be degraded in the rumen and would not hinder nutrient availability and utilization by the animals, if fed. Phytochemicals such as tannins, alkaloids, saponins, phytates are naturally occurring polyphenolic compounds found in plants, which form complexes with feed and microbial proteins (Getachew *et al*., 2004). Consumption of these leaves are capable of modulating the rumen ecosystem, through the bio-availability of the complexes formed. However, the relatively high concentration of alkaloid in VALM could explain the bitter and astringent taste of the leaf (Table 4).

The *in vitro* gas produced by the leaf meals and feed samples better explained the quality of the feed, as the amount of gas produced during incubation/fermentation (Table 5) is dependent on the nature, level of fibre, anti-nutrients and potency of the rumen liquor used for the incubation. The gas production at 18th hour predicted digestibility, fermentation end-product and microbial protein synthesis of the diets by rumen microbes at that hour, if fed to ruminants. Further, could be an indirect measure of dry matter degradability. The reduced methane gas volume produced in this study, especially at 20% VALM supplementation, could

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be attributed to the protein quality, lower fibre levels and anti-nutritional contents of the diets. As VALM had the lowest NDF content. Thus, VALM is more easily degradable than other test diets because lower NDF showed higher potential extent of gas production (Table 2 and 5). Further, from Figure 1, it revealed the potentials of VALM and MOLM to lower methane production, when compared with the formulated concentrate diet (FCD). The lowered methane gas produced (3.10 ml) at 20% VALM inclusion level in this study is suggestive of the potentials of VALM as anti-methanogenic plants and thus, would be effectively utilized and reduce methane emission, if fed to ruminants. The leaves of VALM and MOLM proved to be alternative natural feed additives capable of altering the rumen fermentation pathway, inhibit methanogen efficiently and improve ruminant production (Soliva *et al*., 2005) which has direct relationship with greenhouse gas emissions. The progressive production rate of organic matter digestibility and metabolizable energy, is an indication of mutual co-existence between them. The fermentation of the insoluble fraction of the diets is an indication their potentials as ruminant feed. The low SCFA reported in this study were due to the lower methane gas production which was evident within the 24 hours incubation period. Hence, 20% VALM as supplement to the basal diet are digestible and could support the growth of ruminant animals.

Conclusions

From the foregoing, *V. amygdalina* and *M. oleifera* leaf meals are rich in nutrients and macro-minerals to support the nutrient requirements by small ruminants for optimum growth. More importantly, bitterleaf (*V. amygdalina)* better reduced the methane production, when

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included to the formulated concentrate diet at 20%. However, *in vivo* study is recommended to validate the *in vitro* result.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The materials and datasets used and/or analysed during this current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' Contributions

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References

AOAC (2002): Association of official analytical chemists. Official Methods of Analysis, 17th ed. Published by Association of Official Analytical Chemists, Washington D. C.

Aregheore E.M, Makkar H.P.S and Becker K (1998) Feed value of some browse plants from the central zone of Delta State, Nigeria. *Tropical Science,* 38(2): 97–104.

Ayasan, T. (2015). Use of Moringa Oleifera in Poultry and Ruminant Nutrition. *Turkish Journal of Agriculture*. 3(6): 425 -429. https://doi.org/10.24925/turjaf.v3i6.425-429.327

Babayemi, O. J. and Bamikole, M. A. (2006). Effect of *Tephrosia candida* DC leaf and its mixtures with Guinea grass on in vitro fermentation changes as feed for ruminants in Nigeria. *Pakistan Journal of Nutrition,* 5(1): 14-18.

Babiker, E.E., Juhaimi, F.A.L., Ghafoor, K., and Abdoun, K.A. (2017). Comparative study on feeding value of Moringa leaves as a partial replacement for alfalfa hay in ewes and goats. Livestock Science, 195, 21–26.

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., and Ferret, A., (2007). Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. 90, 2580–2595

Cieslak, A., Szumacher-Strabel, M., Stochmal, A. and Oleszek, W. (2013). Plant components with specific activities against rumen methanogens. Animal, 7, 253–265

Dewangan, G., Koley, K.M., Vadlamudi, V.P., Mishra, A., Poddar, A. and Hirpurkar, S.D. (2010). Antibacterial activity of Moringa oleifera (drumstick) root bark. J. Chem. Pharm. Res. 2, 424–428.

Fadiyimu A.A., Alokan J.A., and Fajemisin A.N. Digestibility, nitrogen balance and haematological profile of West African dwarf sheep fed dietary levels of Moringa oleifera as supplement to Panicum maximum. *J Am Sci.* 2010;6(10):634–643.

Fievez, V., Babayemi, O. J. and Demeyer, D. (2005): Estimation of direct and indirect gas production in syringes: A tool to estimate short chain fatty acid that requires minimal laboratory facilities. *Animal Feed Science and Technology,* 124 (1): 197-210

Getachew G., DePeters E.J. and Robinson P.H. (2004). In vitro gas production provides effective method for assessing ruminant feeds. California Agriculture, 58(1): 54-58. DOI: 10.3733/ca.v058n01p54

Getachew, G., Makkar, H.P.S. and Becker, K. (1999): Stoichiometric relationship between short chain fatty acids and in vitro gas production in presence and polythyleneglycol for tannin containing browses. *European Association for Animal Production* satellite symposium.

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Githiori J.B (2004) Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by pastoralists and small holder farms in Kenya. PhD Thesis, Swedish University of Agricultural Science, Sweden.

Menke, K.H and Steingass, H. (1988): Estimation of the Energetic Fed Value from Chemical Analysis and In Vitro Gas Production Using Rumen Fluid. *Animal Research and Development*, 28: 7-55.

Nigerian Meteorological Agency (2014). Archives Pp. 1:1 – 2. Nimet.gov.ng/akure-weather/12.5

NRC, (2007). National Research Council. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelid. The National Academy of Sciences, Washington, DC.

Omotoso, O. B. (2019). Nutritional potentials and in vitro estimation of composite cocoa pod husk-based diets for ruminants. *Bulletin of the National Research Centre*, 43:150. https://doi.org/10.1186/s42269-019-0189-4.

Sholapur, H.P.N., and Patil, B.M. (2013). Pharmacognostic and phytochemical investigations on the bark of Moringa oleifera Lam. Indian J. Nat. Prod. Resour. 1, 96–101

Soliva, C.R., Kreuzer, M., Foid, N., Foid, G., Machmüller, A., and Hess, H.D., (2005). Feeding value of whole and extracted Moringa oleifera leaves for ruminants and their effects on ruminal fermentation in vitro. Anim. Feed Sci. Technol. 118, 47–62

Tedeschi L.O., Kononoff P.J., Karges K., and Gibson M.L. (2009). Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co)products. J. Dairy Sci., 92: 401-413.

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