EFFECTS OF GUAVA LEAF (*PSIDIUM GUAJAVA*) SUPPLEMENTATION IN THE DIET OF RUMINANT ON *IN VITRO* METHANE PRODUCTION, DEGRADABILITY AND PROTOZOA POPULATION

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ABSTRACT

Guava leaf is a sustainable and locally accessible forage that can serve as a valuable supplement to the diet of ruminants due to their rich nutritional profile and appreciable secondary metabolites which support methane reduction for effective nutrient utilization. This study investigated the effects of guava leaf supplementation in the diet of ruminant on in vitro methane production, degradability and protozoa population. Guava leaves (GL) was substituted for Panicum hay (PH) at 0, 15, 30, 45 and 60% levels as roughage source in the diet of West African dwarf goats with roughage to concentrate ratio of 60:40. In vitro gas production technique was carried out by incubating 200 mg of samples from each treatment with 30 mL inoculum. Total gas production, Methane production, diet degradability and protozoa population were evaluated at the end of 48 hours incubation. Guava leaves supplementation improved the proximate composition of the diets especially the crude protein content which was higher ($p < 0.05$) in diet containing 30 to 60% GL supplementation. In vitro gas and methane production decreased drastically ($P < 0.05$) with guava leaves supplementation. Diet with 30% GL caused 59% methane reduction with minimal effect on diet degradation. Protozoa count reduced with increase in the level of GL inclusion. It can be concluded from this study that guava leaves should form 30% of diet for West African dwarf goats for effective methane reduction with minimal effect on feed degradability.

Keywords: Guava leaves, methane, degradability, protozoa, rumen fermentation

INTRODUCTION

A significant population in Africa depends on livestock industry directly or indirectly for food, social and economic means. In Nigeria, especially in the Southwestern part, small ruminant production plays a critical role in rural livelihood sustenance and overall economic development. Livestock production in the urban areas has increased as a means of sustenance due to increasing demand for animal products such as meat and milk by the burgeoning population. Production of ruminant is one of the major factors that contribute to global environmental degradation. About 80% of total greenhouse gas and 75% ammonia emissions were attributed to beef, mutton and milk production in the livestock sector (Behera *et al*., 2013). Estimation by Herrero *et al*. (2008) showed that Africa cattle, sheep and goat with production of about 7.8 million tonnes of methane in the year 2000 are likely to increase to 11.1 million tonnes by 2030. This may leads to serious challenge with respect to adaptation to climate change in the years to come. Dry season poses a lot of challenges on ruminant production system since most grasses during this season are highly fibrous with low crude protein contents resulting to inefficient dry matter fermentation in the rumen. This affects animal productivity by generating enormous enteric methane emission which may account up to 12% loss of gross energy of feed (Johnson and Johnson, 1995). However, several plant secondary metabolites such as saponin, tannins, flavonoids and essential oil have been identified to mitigate methane emission in order to achieve economic and environmental benefits when included in ruminant diet.

Guava (*Psidium guajava*) is an important traditional plant that is grown in most tropical countries for its nutritive and medicinal properties. It belongs to genus *Psidium* and family *Myrtaceae*. Guava leaves have been reported to contain tannins and flavonoids as its major predominant polyphenolic compounds and inclusion of tannin containing plant in the diet of ruminants reduced methane emission (Hariadi and Santoso, 2010). This study therefore, aims to investigate the effects of guava leaves supplementation in the diet of ruminants on *in vitro* methane production, degradability and protozoa population.

MATERIALS AND METHODS

Air dried guava leaves (GL) was combined with Panicum hay (PH) at varying levels (0, 15, 30, 45 and 60%) as roughage source. Roughage to concentrate ratio of 60:40 was used. In addition, guava leaves and Panicum hay were also incubated alone as sole substrates. The diets were as follows:
Diet 1: 100% GL; Diet 2: 100% PH; Diet 3: 0% GL + 60% PH + 40% concentrate (GL_0PH_{60});
Diet 4: 15% GL + 45% PH + 40% concentrate ($GL_{15}PH_{45}$); Diet 5: 30% GL + 30% PH + 40% concentrate ($GL_{30}PH_{30}$); Diet 6: 45% GL + 15% PH + 40% concentrate ($GL_{45}PH_{15}$); Diet 7: 60% GL + 0% PH + 40% concentrate ($GL_{60}PH_0$).

In vitro gas production of the different experimental diets was determined using the procedure of Menke and Steingass (1988). Two hundred milligram (200 mg) of each experimental diet was incubated in 100 mL glass syringes with 30 mL incubation medium (buffer solution and rumen fluid in the ratio 2:1). Three blanks containing only 30 mL buffered rumen fluid were incubated along the treatments for correction of gas produced. Each treatment was replicated eight times in a completely randomised design and gas production was measured at 3 hours interval for 48 hours. At the end of 48 hours incubation period, methane gas production was determined by introducing 4 mL of 0.1N NaOH into three of the incubated syringes per treatment. The added NaOH absorbed the Carbon dioxide (CO_2) content of the gas produced with a pop sound and the remaining gas in the syringes was measured as methane gas.

The *in vitro* dry matter degradation of the substrate was determined by subtracting the residue (which was recovered by filtering after incubation and drying in the oven at 105^0C overnight) of each sample from the initial quantity incubated.

In vitro DM degradation = $\frac{(DM_{substrate} - (DM_{residues} - DM_{blank})}{DM_{substrate}} \times 100$

The protozoa population of the incubation fluid was determined by mixing the fluid with 10% formalin solution in a sterilized saline solution and total direct count of protozoa was made by the method of Baker and Breech (1986).

The proximate composition of the experimental diets were done (AOAC, 2005) while the tannin and flavonoids contents were determined diets were determined according to Do *et al*. (2014) and Nasseri *et al*. (2019), respectively

Data were subjected to one –way analysis of variance in a Completely Randomized Design (SPSS, 2016). Effect of PH and GL on all parameters measured was analysed using t-test. Linear and quadratic effects of increasing guava leaves was done using orthogonal polynomial contrast of the same statistical procedure. The mathematical model is as follows:

$Y_{ij} = \mu + T_i + \Sigma_{ij}$ where Y_{ij} = observed values of dependent variables, μ = population mean, T_i = effect of different levels of guava leaves and Σ_{ij} = random residual error, $P < 0.05$.

RESULTS AND DISCUSSION

Substituting guava leaves for Panicum hay affected the diet composition as crude protein, tannins and flavonoid content of the diets increased with increase in guava leaves substitution (Table 1). This was in agreement with previous studies (Al-Sagheer *et al*., 2018). Secondary metabolites in plants support effective energy utilization while reducing rumen gas and methane production. It was evident in this study that *in vitro* gas production reduced drastically when guava leaves alone was incubated compared to PH. Similar trends was observed when GL was substituted for PH in the diet; gas production decreased as level of GL increased in the diet. The decrease in gas production may be as a result of different plant metabolites present in the leaves whose concentration increases as the level of guava leaves increased. Effect of medicinal plant species especially the tannin containing plants on rumen fermentation has been reported to vary according to their chemical composition (Tiemann *et al*., 2008). Guava leaves supplementation also affected the protozoa population of the incubated fluid which reduced with increase in GL in the diet. Protozoa are important hydrogen producers in the rumen with produced hydrogen mostly converted into methane by methanogens (Morgan *et al*., 2010) and methane emission represent 2-12% dietary energy loss in ruminants (Johnson and Johnson, 1995). Therefore, dietary manipulation that reduce protozoa population as observed in the current study will affect animals' energy efficiency positively.

In vitro dry matter digestibility decreased with increase in guava leaves supplementation (Figure 1). The reduced degradability as a result of guava leaves may be associated with the effect of

phytochemicals on protozoans. This is evident in this study as incubation of guava leaves alone reduced protozoa population by 50% with subsequent reduction as inclusion levels in the diet increases. Impairment in dry matter degradation with inclusion of medicinal plant materials or extract in ruminant diet has been reported in previous studies (Patra *et al*., 2006). However, more than 60% dry matter degradation was obtained with about 59% methane reduction when 30% of the diet was guava leaves. This will better animal performance with improve environmental health.

Table 1: Chemical composition of guava leaves supplemented diets

	Parameters				
Treatments	Dry matter	Crude protein	NDF	Tannin	Flavonoids
Evaluation of pH and GL					
PH	88.97	8.41^{b}	85.33^{a}	227.719^{b}	53.431^{b}
GL	87.09	14.14^{a}	66.50^{b}	1477.865^{a}	1795.679^{a}
SEM	1.39	0.31	0.14	1.13	2.37
P-values	0.433	<0.001	<0.001	<0.001	<0.001
Substitution levels of GL					
GL_0MH_{60}	92.03^{ab}	10.88^{c}	62.40^{a}	127.719^{e}	13.431^{d}
$GL_{15}MH_{45}$	92.27^{a}	12.52^{b}	53.70^{b}	772.408^{b}	1316.400^{c}
$GL_{30}MH_{30}$	92.63^{ab}	13.02^{ab}	51.30^{c}	1151.788^{c}	1523.051^{b}
$GL_{45}MH_{15}$	86.40^{c}	13.62^{ab}	52.20^{bc}	1372.002^{b}	1721.094^{a}
$GL_{60}MH_0$	90.90^{b}	14.33^{a}	48.60^{d}	1427.865^{a}	1735.679^{a}
SEM	0.69	0.87	1.28	128.33	172.90
P-values					
Linear	0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.686	0.009	<0.001	<0.001	<0.001

[abcd]Means on the same column having different superscripts are significantly different (<0.05), SEM – Standard error of means, PH – Panicum hay, GL – Guava leaves, GL_0PH_{60} – 60%PH + 40% concentrate, $GL_{15}PH_{45}$ – 15%GL + 45%PH + 40% concentrate, $GL_{30}PH_{30}$ – 30%GL + 30%PH + 40% concentrate, GL_{45}PH15 – 45%GL + 15%PH + 40% concentrate, $GL_{60}PH_0$ – 60%GL + 40% concentrate, NDF – neutral detergent fibre

Table 2: Total gas volume, methane production and protozoa count of diets supplemented with guava leaves

	Parameters			
Treatments	TGV (mL/g DM)	Methane (mL/g DM)	%Methane	Protozoa (x 10^5/mL)
Evaluation of pH and GL				
PH	103.33^{a}	35.00^{a}	33.81^{a}	7.50^{a}
GL	35.00^{b}	5.00^{b}	14.29^{b}	3.50^{b}
SEM	1.67	1.56	1.54	0.29
P-values	<0.001	0.004	0.013	0.001
Replacement levels of PH with GL				
GL_0MH_{60}	125.00^{a}	37.00^{a}	27.89^{a}	5.50^{a}
$GL_{15}MH_{45}$	103.33^{b}	26.67^{a}	25.87^{a}	5.00^{ab}
$GL_{30}MH_{30}$	75.00^{c}	15.00^{b}	20.00^{b}	4.00^{b}
$GL_{45}MH_{15}$	63.33^{cd}	11.67^{b}	19.41^{b}	3.50^{b}
$GL_{60}MH_0$	61.67^{d}	8.33^{b}	13.25^{c}	3.20^{b}
SEM	2.56	3.00	2.06	0.28
P-Values				
Linear	0.017	0.275	0.015	0.010
Quadratic	<0.001	0.001	0.804	0.254

[abcd]Means on the same column having different superscripts are significantly different (<0.05), SEM – Standard error of means, TGV- Total gas volume, PH – Panicum hay, GL – Guava leaves, GL_0PH_{60} – 60%PH + 40% concentrate, $GL_{15}PH_{45}$ – 15%GL + 45%PH + 40% concentrate, $GL_{30}PH_{30}$ – 30%GL + 30%PH + 40% concentrate, GL_{45}PH15 – 45%GL + 15%PH + 40% concentrate, $GL_{60}PH_0$ – 60%GL + 40% concentrate,

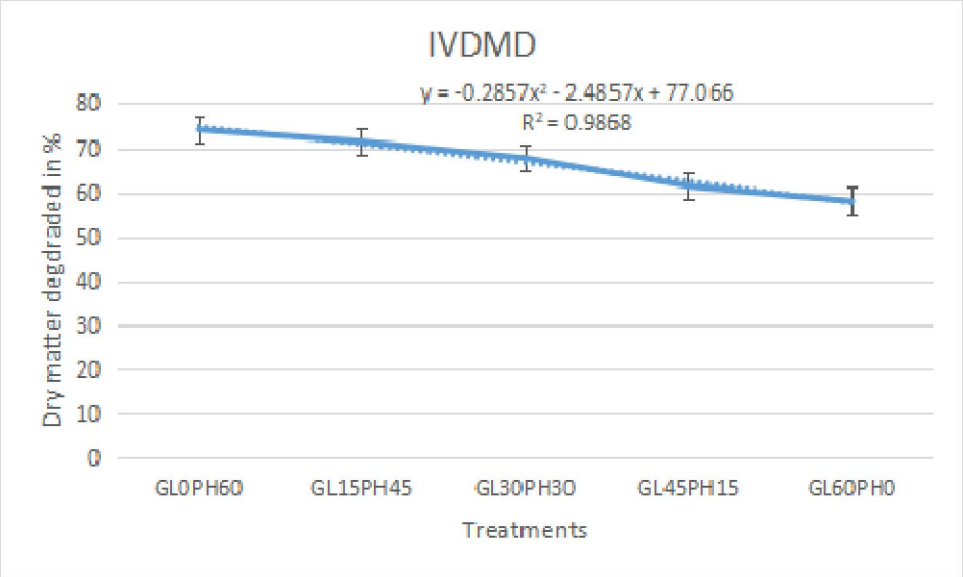

Figure 1: *In vitro* dry matter digestibility of guava leaves supplemented diets

CONCLUSION

It can be concluded from this study that guava leaves may be included up to 30% in the diet of West African dwarf goats for effective methane reduction with minimal effect on dry matter degradation

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