POTENTIALS OF PROANTHOCYANIDINS FROM TEMPERATE FORAGE LEGUMES AT DIFFERENT CONCENTRATION LEVELS TO REDUCE ENTERIC METHANE PRODUCTION USING *IN- VITRO* TECHNIQUES

[1]Akpensuen, T.T*. [2]Verma, S., [2]Taube, F. and [3]Malisch, C.S.
[1]Department of Animal Production, Faculty of Agriculture, University of Jos, Nigeria
[2]Institute of Crop Production and Plant Breeding, Grass and Forage Science/Organic Agriculture, Kiel University (CAU), Kiel, Germany
[3]Department of Agroecology, Faculty of Technical Sciences Aarhus University, Denmark,
*Corresponding author's email: theophilusa@unijos.edu.ng +2348058828892

ABSTRACT

A study was conducted to assess the effect of proanthocyanidins (PAs) concentration at different levels from temperate forage legumes to reduce enteric methane emission in- vitro. The study was carried out at Institutes of Animal Nutrition and Physiology as well as Grass and Forage Science/Organic Agriculture Laboratories, Kiel University (CAU), Germany. PAs were extracted from leaves of four tanniferous forages; Sulla, birdsfoot trefoil, big trefoil and salad burnet during vegetative stage of growth using acetone/H_2O (80:20 v/v) concentration. **.** *The PAs were measured using the ultra-performance liquid chromatography–tandem mass spectrometer (UPLC-MS/MS) method. The experimental design was a 4 (four legumes) x 3 (three tannins concentrations) factorial with 3 replicates. Results shows that Salad burnet and Birdsfoot trefoil produced highest (P<0.002) total gas, while Big trefoil had lowest amount of methane (11.72 ml/200mg). The In-vitro organic matter digestibility (IVOMD) was also lowest in Big trefoil (83.49 %). Adding 1, 2, and 3 % concentration levels has no significant (P>0.01) on total gas produced and IVOMD. However, PAs included at 3 % concentration has lower (P<0.01) methane production compared to the other levels. It is concluded that tannins from Big trefoil had lowest methane among the legumes when included at 3 % concentration level to grass samples.*

Key words: Methane, proanthocyanidins, legumes, digestibility, concentartion

INTRODUCTION

Agriculture and land use contribute more than 25 % to the total greenhouse gass (GHGs) globally (Tubiello *et al*., 2014)and ruminant livestock animals are one of the largest contributors to these emissions, responsible for an estimated 14 % of GHGs worldwide (Roque *et al.*, 2021). Apart from the CH_4 produce from ruminants, rumen methanogenesis results in the loss of 6–10 % of gross energy intake, or 8–14 % of the digestible energy intake of ruminants in temperate environments (Okine *et al*., 2004) and average of 10–11 % of gross energy intake from cattle in the tropics (Moraes *et al.*, 2014). The inclusion of forages rich in plant polyphenols such as tannins, saponins, flavonoids, etc. in the animal diet can potentially reduce CH_4 emissions (Beauchemin *et al.*, 2009) as well as decrease bloat in ruminants (Jonker and Yu, 2017). Tannins can be broadly classified as proanthocyanidins (PAs; syn. condensed tannins) and hydrolysable tannins (HTs) in plants. However, the effects of tannins on animal performance such as; digestion, productivity, methane reduction, etc. are variable between studies. Some of the variations may be explained by the type, concentration and protein binding capacity of the tannins, the type of technique used to measure the tannin concentration and failure to distinguish between PAs and hydrolyzable tannins (Makkar, 2003). The aim of this study was to evaluate methane gas production *in-vitro* of extracted PAs at different concentrations from Sulla (*Hedysarum coronarium*), and birdsfoot trefoil (*Lotus corniculatus* L.), Big trefoil (*Lotus pedunculatus*) and salad burnet *(Sanguisorba minor*).

MATERIALS AND METHODS

Location of the study: The study was conducted at in July and August 2021 at Institute of Animal Nutrition and Physiology as well as Grass and Forage Science/Organic Agriculture Laboratories, Kiel University (CAU), Germany.

***Experimental material and design*:** ThePAs were extracted from leaves of four tanniferous forages; Sulla, Birdsfoot trefoil, big trefoil and salad burnet during vegetative stage of growth**.** The tannins were extracted with acetone/H_2O (80:20, v/v) concentration. The PAs were measured using the ultra-performance liquid chromatography–tandem mass spectrometer (UPLC-MS/MS) method. The extracted tannins were then included at 1, 2 and 3 % concentration to grass (perennial ryegrass) for *in-vitro* gas production assessment. The experimental design was a 4 (four legumes) x 3 (three tannins concentrations) factorial with 3 replicates.

***In-vitro methane production measurement*:** Hohenheim gas test (HGT) apparatus was used for incubation of samples according to Menke and Steingass (1988). Briefly, 200 mg DM of plant plus PAs extracts samples were incubated with 10 ml of ruminal fluid and 20 ml of HGT buffer solution. The rumen fluid was obtained from three rumen-cannulated, non-lactating Jersey × German Black Pied heifers, with an average body weight of 565 ± 29 kg, belonging to the Institute of Animal Nutrition and Physiology of Kiel University. Animals were fed a ration consisting of 3 kg of grass hay and 3 kg of concentrate divided into 2 meals (7 a.m. and 4 p.m.). The use of standards, preparation of incubation media, filling of syringes and other additional procedures were done according to the protocol of Menke and Steingass (1988). The plant samples were incubated in a Rotor at 39^{o}C in three replicates in glass syringe of 100 ml and measurement was done at 8 and 24 hrs. Total methane (%) was obtained by dividing Total Methane (ml/200mg) by Total gas produced (ml/200mg) multiply by 100. In-vitro organic matter digestibility (IVOMD) was calculated as using the equation below:

IVOMD (%) =14.88 + 8.893 gas production (ml) + 0.448 CP (%) + 0.651 total ash (%).

Where the gas production value obtained after 24 h incubation period was corrected for the values with the different controls, thus accounting for differences in composition and activity of the ruminal fluid among runs.

Statistical analysis: All variables will be analyzed using the R. software. Significance of means will be tested using the Tukey's test.

RESULTS AND DISCUSSION

Table 1 shows the effect of PAs extracted at 80% acetone and mixed with grass at different concentrations on *in-vitro* gas production and organic matter digestibility. Salad burnet and Birdsfoot trefoil produced highest (P<0.002) total gas, while big trefoil had the lowest value (58.47 ml/200mg). Total methane produced was higher (P<0.0013) in Birdsfoots trefoil (12.77 ml/200mg). However, salad burnet (18.13 %) had lowest percent of methane from the total gas produced. Highest (P<0.0001) IVOMD was in Birdsfoot trefoil (89.05%). The differences in methane reduction among the legumes may depend on PAs concentration, the source and structural features (Hatew *et al*., 2015). The CH_4 percentage in the total gas can be used as an indicator of the potential CH_4 emissions per unit of organic matter degraded (Supriya *et al*., 2021). The PAs concentration in grass has no significant (P>0.01) effect on total gas produced and IVOMD. But, adding tannins at 3 % to grass sample produced lowest methane (11.58 ml/200mg) compared to the other two concentration levels. This result agreed with the report of Makkar (2003) that concentration of tannins could have effect on methane production. The interaction of PAs from legumes and concentration levels on methane gas production and is presented in Figure 1. There was reduction in methane produced from 1 to 3 % in all the forage legumes. The highest value was in Big trefoil (12.98 ml/200mg), while sulla produced the lowest value (10.59 ml/200mg). The decrease in methane emission with increase in proanthocyanidin concentration might be due to a reduction in the amount of forage substrate fermented in the rumen (reduced digestion), a shift in fermentation end-products (reduced H+ -producing acetate and to a lesser extent butyrate, and more H+ -utilizing propionate and valerate), and/or direct inhibition of the growth of methanogenic bacteria, as well as a decrease in symbiotic-associated protozoa numbers or a shift in microbial community composition(Janssen, 2010).

Table 1: Effect of proanthocyanidins concentrations extracted at 80% acetone in forage legumes mixed with grass on *in-vitro* gas production and organic matter digestibility

	Total Gas Produced (ml/200mg)	Total Methane (ml/200mg)	Total Methane (%)	IVOMD (%)
Grass and Legume (GL)				
Grass+ Sulla (HC)	62.68b	11.62c	18.75b	86.78b
Grass + Salad burnet (SM)	67.09a	12.18b	18.13c	85.21b
Grass + Birdsfoot trefoil (LC)	64.32a	12.77a	19.88a	89.05a
Grass + Big trefoil (LP)	58.47c	11.71c	20.09a	83.49c
SEM	1.43	0.19	0.22	1.27
P.Value	0.002	0.0013	0.0001	0.03
Tannin concentration (TC) (%)				
1	64.41	12.50a	19.44	87.26
2	63.60	12.19b	19.16	86.54
3	61.42	11.58c	19.05	84.60
SEM	1.24	0.003		1.10
P.value	0.23	0.16	0.37	0.23
GL X TC	0.73	0.041	0.92	0.73

Means with different alphabets differ significantly (P<0.01)

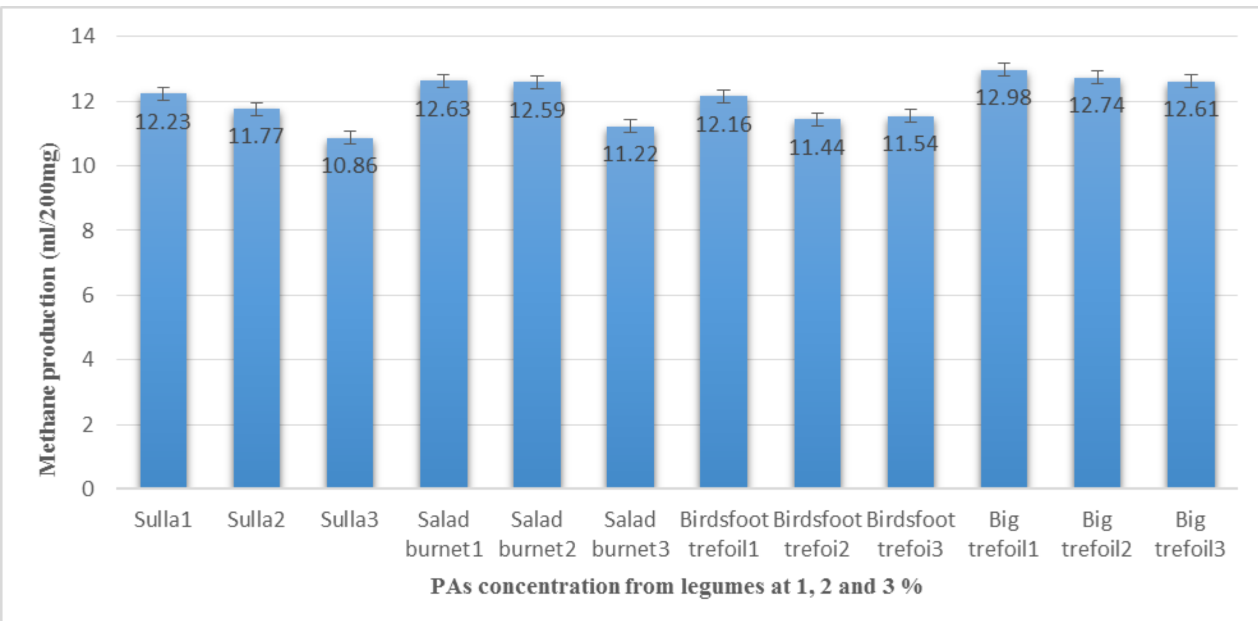

Fig.1: Interaction of legumes and three PAs concentration levels on methane gas production

CONCLUSION AND RECOMMENDATIONS

From the result, it was concluded that differences exist between the forage legumes in PAs and the ability of the plants to reduce methane emission. Big trefoil had the lowest value of methane emission. Also, inclusions of PAs in grass samples at 3 % produced lowest methane emission and Big trefoil produced the lowest methane emission. Studies needs to carry out to determine the optimum level of inclusion of PAs and other polyphenols in feeds of animal to reduce enteric methane emission with compromising the health status of the animals.

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