Check for updates

In vitro nutrient degradability and methane production of two dairy diets as affected by nutraceutical plants inclusion levels

Peter A. Dele, Oluteru E. Orimaye, Michael Wauku, Kelechi A. Ike, Rosetta M. Brice, Lydia K. Olagunju, Yasmine A. Shaw and Uchenna Y. Anele

Department of Animal Sciences, North Carolina Agricultural and Technical State University, Greensboro, NC, USA

Corresponding author email - delepa@funaab.edu.ng

Abstract The present study investigated the effects of two nutraceutical plants – *Petiveria alliacea* and *Waltheria indica* on *in vitro* gas production, dry matter degradability (DMD) and methane production of two dairy cow diets (high forage [HF] and high concentrate [HC]). A factorial arrangement of two diets and two nutraceutical plants with four levels of inclusion (2.5, 5.0, 7.5 and 10.0%) was used. Results demonstrate substrate x inclusion level interactions were significant ($P<0.05$) for gas volume, DMD and methane production. *P. alliacea* at 2.5 and 5.0% suppressed gas production in high forage (HF) by 7.5 and 7.9%, respectively and *W. indica* inclusion levels of 2.5 and 5.0% increased gas production. All inclusion levels of *P. alliacea* suppressed gas production in high concentrate (HC) diet and the lowest gas volume was noted in the 10.0% treatment. Contrary to this observation, 2.5% inclusion level of *W. indica* had the lowest gas volume in the HC diet. Compared with the control, DMD of HF diet was improved with the inclusion of both plants except for *P. alliacea* at 2.5 and 5.0%. There was a significant decrease in methane production across all levels of inclusion for both medicinal plants and substrates; *P. alliacea* at 10.0% decreased methane by 31.2%. Substrate x inclusion level interactions ($P<0.001$) were noted for neutral detergent fiber degradability (NDFD) and acid detergent digestibility (ADFD). *P. alliacea* at 2.5% and 10% inclusion levels reduced NDFD in HF and HC diets, respectively. A similar trend was noted with *P. alliacea* inclusion. Based on the present results, inclusion of *P. alliacea* above 5.0% and *W. indica* at all inclusion levels were able to improve DMD. Both medicinal plants reduced methane concentration irrespective of inclusion level.

Keywords:

Batch culture, dry matter, feed, methane emission, plant nutraceuticals

G. Uzochukwu et al. (eds.), *Proceedings of the National Conference on Next-Generation Sustainable Technologies for Small-Scale Producers (NGST 2022)*,
Advances in Biological Sciences Research 34,
https://doi.org/10.2991/978-94-6463-282-8_17

Introduction

Ruminants are major contributors to enteric methane production (Bodas et al. 2008). According to the IPCC (2007), ruminant animals produce 10-12% of the world's anthropogenic greenhouse gas emissions in carbon dioxide equivalents, and 2-15% of feed gross energy is lost in the form of methane (Johnson and Johnson 1995). The world population is expected to reach 8.5 billion by 2030, and this will invariably lead to increased demand for ruminant animal products - in the form of milk or meat (United Nations 2022). Recent research efforts have focused on improving dietary feed efficiency through inhibition of methane production.

In the recent past, concerns have been on how to effectively increase animal production with a corresponding reduction in greenhouse gas emissions (Hristov et al. 2013; Brice et al. 2022). The addition of plants rich in secondary metabolites in the feeds of ruminants have been suggested as a strategic nutritional-based approach to methane mitigation (Rira et al. 2015; Meljekal et al. 2017). Some of these plants are referred to as nutraceutical plants because they confer both nutritional and health benefits (Lopreiato et al. 2020; Torres-Fajardo et al., 2021; Uushona et al. 2021) to the animals.

Many of these nutraceutical plants have been known to have antimicrobial properties (Cowan 1999; Egamberdieva et al. 2017; Hossan et al. 2018). Nutraceutical plants have been reported as mitigators of enteric methane (Rira et al. 2015). Nutraceutical plants are known to improve ruminal fermentation efficiency (Khiaosa-ard and Zebeli, 2013) and are rumen modifiers (Khattab et al. 2020). *Petiveria alliacea* and *Waltheria indica* are commonly found in the tropical and subtropical climates of the world (Wagner et al. 1990) and are widely established in Florida USA (Wunderlin et al. 2022). They are widely known for their medicinal values and secondary metabolites such as tannin, saponins and polyphenols (Zongo et al. 2013; San Andres Larrea et al. 2014). There is limited information on the anti-methanogenic properties and feed efficiency potential of these two plants. Hence, the aim of this study was to evaluate the effects of inclusion levels of *P. alliacea* and *W. indica* in two dairy diets on *in vitro* gas production, nutrient degradability and methane production.

Methodology

The two nutraceutical plants used in the present study (*P. alliacea* and *W. indica*) were selected based on their known medicinal properties and location in the tropical climate. Plant materials (i.e., leaves) were air-dried at room temperature and ground to pass through a 1 mm sieve. Samples were preserved in tightly closed Ziploc bag and stored in a refrigerator maintained at 2°C.

The two dietary substrates were high concentrate (HC) made up of grain products, processed grains byproducts, plant protein products, molasses products, multi vitamins and mineral supplements and high forage (HF, corn silage). The samples were collected from North Carolina Agricultural and Technical State University Farm. The dietary substrates were dried in a forced air oven at 55°C to constant weight and milled through a 1 mm sieve and used for the *in vitro* batch culture.

The *in vitro* batch culture study was done to evaluate the effects of different inclusion levels of the two nutraceutical plants on *in vitro* gas production, methane, dry matter and fiber digestibility of the two dietary substrates (HC and HF). The study was arranged as a 2 × 9 factorial design to evaluate the effects of the two nutraceutical plants at four inclusion levels (2.5, 5, 7.5 and 10%) as well as the control (i.e., no additive) on the two dietary substrates (HF and HC). The *in vitro* incubation procedure was based on the method described by Anele et al. (2014). The Ankom bags used for this study were washed in acetone, dried and labeled and weighed. Approximately 0.5 ± 0.05 g of the substrates were measured into their respective bottles. Artificial saliva preparation was based on McDougall's recipe and maintained in a water bath at 39°C under continuous flushing with CO_2 until dispensed into the serum bottles. Ruminal fluid was collected 3 h after feeding from two ruminally cannulated dairy cows that were fed 18% protein grain, corn silage, and alfalfa hay. The batch culture media was dispensed into the 100 mL glass serum bottles in a 3:1 of artificial saliva-rumen fluid under constant flushing with carbon dioxide at 39°C. The bottles were capped with a rubber stopper and crimped with an aluminum seal cap. The serum bottles were incubated for 24 h at 39°C and agitated at a speed of 125 rpm using a orbital shaker. At 24 h post incubation, gas production was measured using a manometer.

Methane concentration was estimated using a portable gas analyzer (Biogas 5000, Landtec, Dexter, MI, USA). Immediately after gas reading and methane determination, the Ankom bags were removed from the serum bottles, rinsed, and dried in a 55°C oven for 48 h and calculated for *in vitro* dry matter digestibility according to Brice *et al.* (2022). After incubation, the contents of the bags were digested using Ankom 200 Fiber Analyzer (Ankom, Macedon, NY, USA) to estimate neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations. The NDF content was determined as described by Van Soest et al. (1991) using heat stable α-amylase with sodium sulfite. Acid detergent fiber was determined according to Association of Official Analytical Chemists [AOAC] (1995). Acid detergent lignin (ADL) was determined by soaking in concentrated sulfuric acid based on ANKOM Technologies analytical methods.

Data generated were analyzed using the GLM procedure of SAS 9.4 (SAS Inst., Inc., Cary, NC, USA) in a 2 × 9 factorial arrangement. The means were separated using Duncan's multiple comparisons test at $P < 0.05$.

Results

The current study was aimed to determine the effects of *P. alliacea* and *W. indica* leaves on dry matter degradability (DMD), total gas and methane production of two dairy diets using the *in vitro* gas production technique. The effects of inclusion levels of *P. alliacea* and *W. indica* leaves on *in vitro* gas production, digestibility, and methane concentration of the two diets is shown in Table 1.

Substrate x inclusion level interactions were significant ($P<0.05$) for total gas production, methane concentration and nutrient degradability except for ADLD. Total gas production of HF was reduced by 7.5% and 7.9% with *P. alliacea* inclusion at 2.5% and 5.0%, respectively. Contrary to this observation, *W. indica* inclusion increased gas production in HF except for 10.0% inclusion level. For the HC, all inclusion levels of *P. alliacea* reduced total gas production and only 2.5% inclusion level reduced total gas production in *W. indica*. The increase in total gas production with the inclusion of *W. indica* in both substrates is consistent with previous studies (Goel et al. 2008; Dey et al. 2014; Sarkar et al. 2018). Higher gas production could be as a result of improved DMD as more gas is released with increasing nutrient digestibility.

Higher DMD with the inclusion of the nutraceutical plants is consistent with previous reports by Goel et al. (2008) and Sarkar et al. (2018) which showed that the inclusion levels of the nutraceuticals encouraged the population and activities of microbes associated with DM degradation.

The inclusion levels of both nutraceutical plants reduced methane in both substrates with *P. alliacea* at 2.5% in HF and *P. alliacea* at 10.0% in HC, reducing methane by 23.8 and 31.2%, respectively. Consistent with results in the present study, Bhatta et al. (2015) reported a reduction in methane production of some tropical tree leaves using in vitro rumen fermentation.

Although there is limited information on the methane reduction potential of the nutraceutical plants in the current study, there are documented reports that they are rich in secondary metabolites such as tannins (Zongo et al. 2013; San Andres Larrea et al. 2014). Tannins are known to reduce total gas and methane production (Bhatta et al. 2009). The reduction in methane production with the inclusion of both nutraceuticals could help reduce energy loss in form of methane in ruminants.

The NDFD of HF was suppressed by the inclusion levels of the nutraceutical plants except for *W. indica* at 2.5 which improved NDFD by 5.1%. For the HC diet, only *P. alliacea* at 10.0% reduced NDFD. The inclusion of *W. indica* at 5.0% improved NDFD of HC the most by 11%. The inclusion of *P. alliacea* at 7.5 and 10.0% and *W. indica* at 5.0 and 7.5% improved ADFD of HF. For HC, the inclusion of *P. alliacea* at 10.0% and *W. indica* at 5.0% improved ADFD by 2 and 10.5%, respectively. The improvement of NDFD of HF by the inclusion of *W. indica* at 2.5 and 10.0% is consistent with a previous report by Tekipp et al. (2012) where different plants of North American origin (*Artemisia absinthium*, *A. atra* and *A. annua*) were evaluated as additives in a batch culture. Consistent with results noted for HC in the present study, Bhat et al. (2018) and Ishtiyak et al. (2010) reported an increase in NDFD when *A. absinthium* and *Trigonella foenumgraeccum* were used as feed additives. Current results showed that the nutraceutical plants improved the activities and/or population of fiber degrading bacteria and that they might be dose or inclusion level dependent. For HC, NDFD was reduced at 10.0% inclusion of *P. alliacea* which could suggest a suppression of the activities of the fiber degrading bacteria and that inclusion level above 7.5% might not be useful.

Conclusions

From this study, it can be concluded that the inclusion levels of *P. alliacea* above 5.0% and *W. indica* at all levels of inclusion were able to improve DMD and reduce methane production.

Table 1. Effect of nutraceutical plant inclusion levels on total gas, *in vitro* nutrient degradability and methane production of two diary dietary substrates

	Gas		DMD		Methane		NDFD		ADFD		ADLD	
NPIL (%)	HF	HC	HF	HC	HF	HC	HF	HC	HF	HC	HF	HC
Control	92.8^{g}	108^{c}	38.7^{e}	47.4^{c}	4.70^{b}	5.29^{a}	63.1^{b}	47.3^{g}	55.0^{b}	48.2^{d}	13.8	14.0
PA2.5	85.8^{i}	103^{d}	38.8^{e}	47.4^{c}	3.58^{e}	4.57^{b}	60.2^{d}	48.4^{f}	55.1^{b}	46.9^{e}	9.68	13.5
PA5.0	85.5^{i}	102^{d}	39.0^{e}	50.4^{b}	3.61^{e}	4.47^{bc}	61.1^{c}	49.5^{f}	55.2^{b}	47.1^{e}	10.2	13.3
PA7.5	95.3^{f}	101^{d}	42.1^{cd}	50.7^{b}	4.09^{cd}	3.85^{d}	63.9^{b}	49.2^{f}	56.7^{ab}	48.4^{d}	10.9	14.5
PA10.0	94.5^{f}	87.0^{h}	43.8^{cd}	43.6^{cd}	4.07^{cd}	3.64^{e}	62.6^{bc}	44.3^{h}	58.0^{a}	49.1^{d}	10.3	14.0
WI2.5	102^{d}	102^{d}	43.3^{cd}	50.2^{b}	4.27^{c}	3.93^{d}	66.3^{a}	49.3^{f}	55.1^{b}	48.2^{d}	9.17	11.4
WI5.0	98.6^{e}	115^{a}	40.9^{d}	52.4^{ab}	3.94^{d}	4.65^{b}	62.8^{bc}	52.5^{e}	56.0^{ab}	52.3^{c}	10.5	14.2
WI7.5	94.0^{f}	116^{a}	40.8^{d}	54.5^{a}	3.80^{d}	4.73^{b}	62.6^{bc}	51.9^{e}	57.2^{ab}	46.6^{e}	11.1	12.3
WI10.0	92.3^{g}	112^{b}	40.5^{d}	53.8^{a}	3.66^{e}	4.42^{bc}	64.7^{ab}	51.1^{e}	54.8^{bc}	46.1^{e}	18.6	12.2
SEM	1.8023	1.8023	0.7799	0.7799	0.1011	0.1011	0.9936	0.9936	0.5646	0.5646	0.5257	0.5257
P-value												
Substrate	0.0012	0.0012	<.0001	<.0001	0.0254	0.0254	<.0001	<.0001	<.0001	<.0001	0.1215	0.1215
NPIL	0.3742	0.3742	0.0748	0.0748	0.0373	0.0373	0.0502	0.0502	0.0117	0.0117	0.5228	0.5228
Substrate*NPIL	0.0017	0.0017	<.0001	<.0001	0.0392	0.0392	0.0001	0.0001	0.0001	0.0001	0.4381	0.4381

NPIL: Nutraceutical plant inclusion level; PA: P. alliacea; WI: W. indica; SEM: Standard error of means; HF: High forage; HC: High concentrate

$^{a–I}$:Means with different superscripts within the same column differ, $p < 0.05$

References

Anele, U.Y., Refat, B., Swift, M., He, Z., Zhao, Y., McAllister, T., & Yang, W.Z. (2014). Effects of bulk density, precision processing and processing index on *in vitro* ruminal fermentation of dry-rolled barley grain. *Animal Feed Science and Technology, 195*, 28–37.

AOAC. (1995). *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Washington, DC, USA.

Bhat, A.R., Ganai, A.M., Ishfaq, A., Beigh, Y. A., Sheikh, G.G., & Masood, D. (2018). *In vitro* effect of *Artemisia absinthium* (Titween) on digestibility and rumen parameters of small ruminants. *Indian Journal of Animal Research, 52* (4), 579-582

Bhatta, R., Saravanan, M., Baruah. L., & Prasad, C.S. (2015). Effects of graded levels of tannin-containing tropical tree leaves on *in vitro* rumen fermentation, total protozoa and methane production. *Journal of Applied Microbiology, 118*, 557–564

Bhatta, R., Uyeno, Y., Tajima, K., Takenaka, A., Yabumoto, Y., Nonaka, I., Enishi, O., & Kurihara, M. (2009). Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production, and methanogenic archaea and protozoal populations. *Journal of Dairy Science, 92*, 5512–5522.

Bodas, R., López, S., Fernández, M., García-González, R., Rodríguez, A.B., Wallace, R.J., & González, J.S. (2008). *In vitro* screening of the potential of numerous plant species as antimethanogenic feed additives for ruminants. *Animal Feed Science and Technology, 145*, 245–258.

Brice, R.M., Dele, P.A., Ike, K.A., Shaw, Y.A., Olagunju, L.K., Orimaye, O.E., Subedi, K., & Anele, U.Y. (2022). Effects of essential oil blends on *in vitro* apparent and truly degradable dry matter, efficiency of microbial production, total short-chain fatty acids and greenhouse gas emissions of two dairy cow diets. *Animals, 12*, 2185. https://doi.org/10.3390/ani12172185

Cowan, M.M. (1999) Plant products as antimicrobial agents. *Clinical Microbiology Review, 12*(4), 564-82. doi: 10.1128/CMR.12.4.564. PMID: 10515903; PMCID: PMC88925.

Dey, A., Paul, S.S., Pandey, P., & Rathore R. (2014). Potential of Moringa oleifera leaves in modulating *in vitro* methanogenesis and fermentation of wheat straw in buffalo. *Indian Journal of Animal Science, 84*, 533–538

Egamberdieva, D., Wirth, S., Behrendt, U., Ahmad, P., & Berg, G. (2017). Antimicrobial Activity of Medicinal Plants Correlates with the Proportion of Antagonistic Endophytes. *Frontier in Microbiology, 8*,199. doi: 10.3389/fmicb.2017.00199

Goel, G., Makkar, H.P.S., & Becker, K. (2008). Changes in microbial community structure, methanogenesis and rumen fermentation in response to saponin-rich fractions from different plant materials. *Journal of Applied Microbiology 105*, 770–777

Hossan, Md.S., Jindal, H., Maisha, S., Samudi Raju, C., Devi Sekaran, S., Nissapatorn, V., Kaharudin, F., Su Yi, L., Khoo, T.J., Rahmatullah, M., & Wiart, C. (2018). Antibacterial effects of 18 medicinal plants used by the Khyang tribe in Bangladesh. *Pharmaceutical Biology, 56*(1), 201–208. doi:10.1080/13880209.2018.1446030

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.S., Adesogan, A.T., Yang, W., Lee, C., Gerber, P.J., Henderson, B., & Tricarico, J.M. (2013). Special topics-mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *Journal of Animal Science, 91*(11), 5045–5069

IPCC (Intergovernmental Panel on Climate Change) (2007) *Climate change 2007*: the physical science basis. Cambridge University Press, Cambridge

Ishtiyak, A.M., Kumar, R., Sharma, R.K., & Barman, K. (2010). Effect of herbs on *in vitro* digestibility of feed with rumen liquor of goat. *Indian Journal of Veterinary Research, 19*, 13-18.

Johnson, K.A., & Johnson, D.E. (1995). Methane emissions from cattle. *Journal of Animal Science, 73*(8), 2483–2492

Khattab, M. S. A., Abd El Tawab, A. M., Hadhoud, F. A., & Shaaban, M. M. (2020). Utilizing of celery and thyme as ruminal fermentation and digestibility modifier and reducing gas production. *International Journal of Dairy Sciences, 15*(1), 22-27

Khiaosa-ard, R., & Zebeli, Q. (2013). Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants. *Journal of Animal Science, 91*(4),1819–1830.

Lopreiato, V., Mezzetti, M., Cattaneo, L., Ferronato, G., Minuti, A., & Trevisi, E. (2020). Role of nutraceuticals during the transition period of dairy cows: a review. *Journal of Animal Science and Biotechnology, 11*(1), 96–. doi:10.1186/s40104-020-00501-x

Medjekal, S., Bodas, R., Bousseboua, H., & López, S. (2017). Evaluation of three medicinal plants for methane production potential, fiber digestion and rumen fermentation *in vitro. Energy Procedia, 119*, 632–641. doi:10.1016/j.egypro.2017.07.089

Rira, M., Chentli, A., Boufenerab, S., & Boussebouaa, H. (2015). Effects of plants containing secondary metabolites on ruminale methanogenesis of sheep *in vitro*. *Energy Procedia, 74*, 15 – 24.

San Andrés Larrea, M.I., San Andrés Larrea, M.D., & Rodriguez Fernández, C. (2014). *Plants, poisonous (animals)*. In: Wexler P, editor. Encyclopedia of Toxicology. 3rd ed. Oxford: Academic Press 960–*969.* doi:10.1016/B978-0-12-386454-3.00462-0

Sarkar, S., Mohini, M., Mondal, G., Pandita, S., Nampoothiri Vinu, M., & Gautam, M. (2018). Effect of supplementing *Aegle marmelos* leaves on *in vitro* rumen fermentation and methanogenesis of diets varying in roughage to concentrate ratio**.** *Indian Journal of Animal Research, 52*(8), 1180-1184

Tekippe, J. A., Hristov, A. N., Heyler, K. S., Zheljazkov, V. D., Ferreira, J. F. S., Cantrell, C. L., & Varga, G. A. (2012). Effects of plants and essential oils on ruminal *in vitro* batch culture methane production and fermentation. *Canadian Journal of Animal Science, 92*, 395408.

Torres-Fajardo, R.A., González-Pech, P.G., Torres-Acosta, J.F.d.J., & Sandoval-Castro, C.A. (2021). Nutraceutical Potential of the Low Deciduous Forest to Improve Small Ruminant Nutrition and Health: A Systematic Review. *Agronomy, 11*, 1403. https://doi.org/10.3390/agronomy11071403

United Nations (2022). *World Population Prospects 2022: Summary of Results.* United Nations Department of Economic and Social Affairs, Population Division. UN DESA/POP/2022/TR/NO. 3.

Uushona, T., Chikwanha, O.C., Tayengwa, T., Katiyatiya, C.L.F., Strydom, P.E., & Mapiye, C. (2021). Nutraceutical and preservative potential of Acacia mearnsii and Acacia dealbata leaves for ruminant production and product quality enhancement. *The Journal of Agricultural Science, 159*, 743–756. https://doi.org/10.1017/ S0021859621001015

Van Soest, P.J., Robertson, J.B., & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597.

Wagner, W.L., Darrel, R.H., & Sohmer, S.H. (1990). *Manual of the flowering plants of Hawai'i.* 2 vols., Bishop Museum Special Publication 83. Honolulu: University of Hawaii Press and Bishop Museum Press. p. 1280.

Wunderlin, R.P., Hansen, B.F., Franck, A.R., & Essig, F.B. (2022). *Atlas of Florida Plants* (http://florida.plantatlas.usf.edu/). [S. M. Landry and K. N. Campbell (application development), USF Water Institute.] Institute for Systematic Botany, University of South Florida, Tampa

Zongo, F., Ribuot, C., Boumendjel, A., & Guissou, I. (2013). Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica L. (syn. Waltheria americana)*: A review. *Journal of Ethnopharmacology, 148*(1), 14–26. doi:10.1016/j.jep.2013.03.080