Communication

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Reduced Rumen Methane Eructation in Smallholder Cattle and Buffalo by Dietary Supplementation with a Plant Tannins and Citral Extract

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Simple Summary: Addressing the global climate change emergency requires reductions in greenhouse gas emissions (GHGe) from farmed large ruminants, particularly in developing countries with inefficient large ruminant production systems, are addressed. Recent studies in Laos found that emissions control molasses blocks (ECB) achieved a change in emission intensity reducing enteric emissions by 470 kg CO2e per block consumed by large ruminants. To enhance GHGe mitigation, dietary supplementation with a potential methane-reducing feed mix supplement was conducted with large ruminants in Laos. Dry cows (n=11) and lactating buffalo (n=7) were offered a chopped feed mix supplement containing plant tannins and citral extract for 4 weeks, with average methane concentration per animal (AMP) measured in the nasa-oral breath by a hand-held methane measuring device. Suppression of AMP by 36% in the dairy buffalo on an adequate forage-based diet and 18% in the beef cattle on a grazing pasture declining in quantity and quality from severely dry weather conditions was recorded. It was concluded that reductions in rumen methane excretion of up to 36% may be achievable by dietary supplementation, with work on incorporation of the mix into ECBs in progress.

Abstract: Large ruminant production is associated with high greenhouse gas emissions (GHGe) intensity, with the highest in southeast Asia being Laos at 102.9 CO2eq/kg meat produced, compared with average global meat emissions intensity of 33 CO2eq/kg. As recent studies in Laos identified emissions control molasses blocks (ECBs) achieved abatement of 470kg CO2eq/20kg block consumed, a study was conducted with a supplement mix with reported methane reducing properties, containing plant tannins and citral extract, salt, molasses and water. Groups of housed dairy buffalo (n=7) and pasture-fed grazing beef cows (n=11) were randomly selected, body condition scores estimated (BCS:1-5) and baseline nasa-oral methane eructation measured daily for 2 weeks. All animals were offered 300-400g/day of the mix for a month, with buffalo accessing abundant fresh cut forages whereas the grazing cows accessing rice straw and pasture declining in quality and quantity due to severe drought. The buffalo consumed all the mix and retained their BCS of 3, whereas the cattle consumed between 200-300g/day of the mix and their BCS declined from 1.5 to 1.0. Analysis of data points (buffalo n=309; cattle n=378) found average methane concentration (AMP) per animal of both cohorts increased over the 2 week baseline period, then declined during the supplementation period by 36% in the buffalo and 18% in cattle cohorts.

Keywords: greenhouse gas emissions; methane abatement; Asiatic swamp buffalo (*Bubalus bubalis*); dairy; beef; concentrate supplement; tannins

1. Introduction

Large ruminant production exploits the unique advantage of these animals being able to consume forages and graze lands not suitable for arable cropping, providing high value products for human consumption, with projections that global meat and milk production will increase by another 19% and 33% by 2030, respectively [1]. Yet, subsistence livestock production systems comprising smallholder farmers in developing countries, are inefficient and have been slow to transition to more modern nutritional and health practices to meet this rapidly rising demand for milk and meat in countries where historically, there has been very limited access to protein-rich animal-sourced foods (ASF) [1–3]. Achieving such gains requires adoption of emerging 'best practice' interventions, including improvements in feed resources, preventive health strategies and biosecurity, optimal manure management, food safe processing (e.g., risk based meat safety assurance), animal husbandry and product marketing, animal welfare and most recently, climate smart innovations to address the issue that ruminant production is associated with generation of greenhouse gas emissions (GHGe) [3–6].

Although meat and milk from ruminants provide an important source of protein and other nutrients for human consumption, 2% to 12% of the gross energy consumed is converted to enteric methane during ruminal digestion, contributing approximately 6% of global anthropogenic GHGe. Recent estimates are that improved livestock production efficiencies could potentially assist the global livestock sector to reduce GHGe by as much as 30% [1]. However, achieving livestock production efficiencies requires much improved nutritional and disease management and more effective strategies for managing the impacts of both increasing transboundary and emerging infectious disease risk, and climate variability. This includes improved preparedness for droughts, fires, storms, floods and other environmental impacts in addition to reduced GHGe per unit of meat or milk produced [7,8]. This is particularly important in Laos with an GHGe intensity for meat from cattle of 102.9 CO2eq/kg product, compared with average Global, Southern and Southeast Asia GHGe intensities for meat from cattle of 33, 63.2 and 53.1 CO2eq/kg product respectively [9].

Ruminants have the advantage of suitability for grazing and browsing on lands unsuitable for arable cropping. However, 2% to 12% of the gross energy consumed is converted to enteric methane (CH4) during ruminal digestion, contributing ~6% of global anthropogenic greenhouse gas emissions [10]. That large ruminant producers need to find methods of reducing GHGe whilst meeting consumer demand for ASF of improved quality, is challenging for the future of agriculture. Provision of cost-effective options for decreasing CH4 emissions is considered increasingly critical for the sustainability of large ruminant livestock production, despite the limitations of approaches currently available [10]. Although CH4 abatement may be achieved through advances in animal genetics, development of vaccines, early life programming, use of alternative hydrogen sinks, chemical inhibitors, fermentation modifiers through dietary formulation, it is likely that a combination of strategies will be required to attain the degree of reductions in CH4 required [10].

A multi-intervention livestock development strategy involving a combination of health and nutritional interventions including provision of high-quality molasses nutrient blocks (MNB) has been proposed as a scale-out strategy to assist smallholder large ruminant livestock farming efficiency in developing countries [3]. The strategy includes the combination of establishing forage plantations and improvements in feeding systems including MNBs, with multiple health interventions involving efficacious vaccination, biosecurity, and parasite management programs. Although MNBs are simple to deploy in the field and are widely accepted by farmers, historically, variations in the content and the quality of molasses blocks used created variable results, raising concerns of the validity of this approach. This problem requires establishment of a a robust manufacturing method that yields a consistently produced, high quality MNB, suitable for tropical conditions with sufficient hardness in the block necessary to assist in controlling consumption, with potential for addition of CH4 reducing agents to help mitigate GHGe.

A number of published trials undertaken by this research group in Lao PDR and reviewed recently [3], established that constraints with MNBs can be managed and health interventions readily delivered by incorporation into the formulation [11–13], Further, addition of methane reducing agents in the formulation enabled creation of emissions control blocks (ECBs) that also achieved high rates of acceptance by farmers, improved herd health and performance, and reduced GHGe from smallholder large ruminant farms by an impressive abatement in GHGe of 470kg CO2e per 20kg block consumed [3]. ECBs contribute to improved rumen function, increased lactation yields and enhanced large ruminant productivity in addition to reduced GHGe [3,5,7].

Further research is continuing to establish the potential merits of additional CH4 reducing components in the formulation of ECBs. The potential of the addition of red seaweed *Asparagopsis spp.* as a bovine dietary CH4 inhibitor were considered [3], although in a land-locked country such as Laos, attention naturally has turned to more locally available substrates. Of interest was the question, could the provision of a concentrate supplement (CS) containing locally derived plant tannins and citral extract, reduce CH4 excretion during rumen fermentation?

Dietary fermentation modifiers using feed with a high content of secondary plant ingredients (e.g., tannins, saponins) are of increasing relevance to CH4 abatement. Saponins form complexes in cell membranes causing the death of ruminal protozoa. Tannins can be either hydrolysing, forming complexes with proteins that inhibits the growth of methane-forming microorganisms, or condensed tannins that reduce the degradability of fiber components of the feed [10,14]. A diet with 1.5% extract containing a mixture of hydrolyzed and condensed tannins was found to have a significant methane-reducing effect, as was the feeding of lemongrass with 60g condensed tannins/kg DM to cattle in 2 trials [15,16]. Following a recent study from Austria [10] on the effect of feeding of 100g lemongrass as feed supplement on CH4 concentration in the respiratory air of beef cattle, indicating reductions in expired CH4 of 14.6% (range 7.8 to 23.4%), interest arose on the offering of a chopped feed mix supplement containing plant tannins and citral extract (CSE) [17].

This communication presents findings from preliminary trials with provision of *ad-libitum* access to the CSE supplement to a housed dairy buffalo cohort fed a forages-based diet and a beef cattle cohort fed rice straw and grazing native pasture declining in availability and quality due to extremely hot and dry weather. In this study, ruminal CH4 eructation was measured daily by a hand-held Laser methane mini™ device directed in vicinity of the nose enabling measurement of methane excreted during digestion. This occurred for a period of 2 weeks to provide baseline data, followed by a period of 4 weeks of feeding the supplement mix, when significant declines in eructed CH4 were measured that were considered attributable to the dietary modifications.

2. Materials and methods

Groups of intensively housed dairy buffalo in late lactation (n=7) and pasture-fed grazing dry beef cows (n=11) were randomly selected for the trials. Individual body condition score (BCS:1-5) estimates and baseline nasa-oral CH4 eructation measures were collected daily for a period of 2 weeks. CH4 was measured using a hand-held Laser methane mini™ device (LM), with the beam carefully directed at the philtrum of the nasal mucosa from a distance of 1.7 meters, as previously described [14]. All animals were then offered *ad-libitum* access to 300-400g/day of the CSE supplement mixture, containing plant tannins and citral extract, salt, molasses and water, fed through a rail (buffalo) or in a shallow trough (cattle). Dairy buffalo had access to fresh cut forages (30kg Napier grass) whereas the grazing cows had access to rice straw and unimproved pasture that was declining in quality and quantity due to severe drought conditions. The feeding time, amount of supplement consumed, CH4 reading time and level and other observations (e.g. BCS) were recorded for each animal.

Individual data points (median concentration of a two minute measurement period) for buffalo (n=309) and cattle (n=378) were subjected to tests for normality (Kolmogorov-Smirnov test), with outliers determined using a generalised ESD test (cattle outliers 6 pre-feeding, 9 supplemented; buffalo outliers 2 pre-feeding and 5 supplemented). The cleaned data was then examined using Wilcoxon Pair analysis.

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3. Results

Findings after statistical analysis of the data were that the mean Average Methane Production (AMP) per animal of the buffalo cohort increased over a 2-week period to 213ppm, then declined to 137ppm at trial completion after 4wks supplementation (Table 1). Increasing mean AMP was also observed in the cattle mob, achieving 200ppm prior the introduction of the CSE mix, then declining to lesser degree compared to the buffalo, with a mean AMP of 163ppm. These findings establish that the CSE supplement suppressed CH4 in the buffalo cohort by almost 36% but only by 18% in the cattle cohort (Table 1).

The average body condition score (BCS) of the buffalo cohort was maintained at an average of 3, whereas a decline in the average BCS of the cattle from 1.5 to 1.0 was observed during the study.

Table 1. Summary of naso-oral CH4 excretion data (ppm) from dairy buffalo and beef cattle.

Animal	Dairy buffalo	n=7	Beef cattle	n=11 (6*)
Cohort	Control	Concentrate fed	Control	Concentrate fed
	154.4	267.2	156.5	194.8
	228.5	208.6	109.4	229.3
	137.8	130.6	207.9	191.8
	205.9	138.1	326.3	213.4
	204.4	185.9	199.8	166.3
	236.2	89.9	337.9	110.8
	225.3	92.1	262.6	116.6
	256.2	71.3	156.5	84.5
	269.2	103.4		
Mean	213.1	136.7	200.1	163.4
% difference		35.8		18.3
Probability		P<0.01		P<0.025

Note: Analysis used Wilcoxon Pair test, with outliers removed by the Kolmogorov test*.

4. Discussion

These preliminary observations suggest that dietary supplementation for 4wks with a supplement containing plant tannins and citral extract reduces ruminal CH4 excretion of between 18% to 36% in grazing cattle and dairy buffalo, respectively. This likely reflects that the buffalo were on a much higher plane of nutrition, having been fed forages, whereas the CSE was less efficient at reducing average methane production in cattle on a diet declining in quality and quantity due to extreme weather events of March-April 2023 in Laos and neighbouring countries.

Earth is warming, with more GHGe and moisture in the atmosphere, causing record-breaking extreme weather events. Anthropogenic activities and particularly the burning of fossil fuels, deforestation and other land-use changes, have driven the climate crisis by releasing increased GHGe into the atmosphere, trapping heat. This warming of the planet disrupts crop production, destroys pastures, reduces water availability, causes societal distress and increasingly severe welfare impacts on animals as they attempt to cope with extremes beyond their thermoneutral zones, requiring additional energy for homeostasis, in addition to drought, fire and flood risks [7].

The emerging climate crisis requires many strategies to urgently reduce atmospheric GHGe. Ruminant livestock producers require cost-effective methods to reduce GHGe, particularly CH4, whilst attempting to meet the increasing global consumer demands for ASF of higher quality. Both decreases in emission intensity (g CH4/animal product) and absolute emissions (g CH4/day) are likely needed if the ruminant industries are to continue to grow sustainably, with further research required to determine the combinations of anti-methanogenic strategies that will have the most consistent additive effects in GHGe abatement [10,18]. Major constraints discussed for decreasing global enteric CH4 emissions from ruminants include: continued expansion of the ASF industries, increasing costs of mitigation; difficulties in applying mitigation strategies to grazing ruminants; inconsistent effects on animal performance; and the paucity of information on animal health, reproduction, product quality, cost-benefit, safety and consumer acceptance [10]. These constraints are particularly relevant to improving livestock production in a developing country context, where both the concepts and practices of improved animal nutrition and health are slow to gain traction in many regions and production losses in the dry season and during lactation currently remain largely unmanaged.

In developing countries, identifying motivations for farmers to adopt sustainable livestock production practice changes is exceptionally challenging. However, examination of recently published studies from Laos documenting the efficacy of a farmer-applied MNB and now ECB strategy, suggests an increasing willingness and capacity of producers to address nutritional and health deficit concerns in a developing country with the highest of regional GHGe intensities in the region [3]. With the recent opening of the AgCoTech Laos factory for the local manufacture and distribution of ECB and development of a dietary modifier that significantly reduces CH4 eructation from large ruminants, Laos has become the first country in Southeast Asia to actively participate in the global methane pledge. This initiative provides a practical pathway for other countries in the region to reduce CH4 from large ruminant production, whilst efforts progress to increase beef and dairy production efficiency at levels that can combat current and future food security issues [19].

Although slow or delayed methane action has been suggested as reasonable given that current climate policies heavily emphasise actions on decarbonisation and reaching net-zero emissions, with CH4 emitted over the next couple of decades having a limited role in long-term warming, fast methane action may considerably limit climate damages in the near-term [19]. ‘Scale up’ efforts in reducing CH4 emissions takes advantage of an achievable and affordable opportunity to simultaneously reduce GHGe and is aligned with Laos National Agricultural policy and of relevance to progressing the Sustainable Development Goals (SDGs) and improving EcoSystem Health in the region [3]. The findings reported here may offer a more receptive environment for the urgent change management required in sustainably progressing animal production, health, welfare and GHGe mitigation in livestock systems in other developing countries.

5. Conclusions

The finding that a dietary supplement containing plant tannins and citral extract may reduce rumen methane eructation by up to 36%, indicates that impressive abatement of GHGe is achievable in large ruminant smallholder production in a developing country. These and other nutritional management innovations including the feeding of ECBs, suggests that improved global food production system efficiencies are achievable, assisting the change management urgently required to reduce methane eructation from large ruminants, addressing global food security, one health, ecosystem health and climate crisis concerns.

Supplementary Materials: None necessary as all the work discussed is included.

Author Contributions: Julian Hill designed and analysed the study, with inputs from Peter Windsor and Susan Martin, with field work delivered by Daniel Olsson and Alex Cameron. The manuscript was written and prepared by Peter Windsor and Julian Hill.

Institutional Review Board Statement: The authors confirm that in addition to following current procedures on animal ethics processes in Laos, they complied with the Universities Australia Australian Code for the

Responsible Conduct of Research. This included ensuring that participants provided verbal informed consent for the collection of animal data.

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References

1. FAO. Mapping Supply and Demand for Animal-Source Foods to 2030. In Animal Production and Health Working Paper 2; FAO: Rome, Italy, **2018**; Available online: https://www.fao.org/3/i2425e/i2425e00.htm (accessed on 20 October 2020).
2. FAO. World Livestock: Transforming the Livestock Sector through the Sustainable Development Goals; FAO: Rome, Italy, **2011**; pp. xvi–xxxi. Available online: http://www.fao.org/3/CA1201EN/ca1201en.pdf (accessed on 20 October 2020).
3. Windsor, P.A.; Hill, J. Provision of High-Quality Molasses Blocks to Improve Productivity and Address Greenhouse Gas Emissions from Smallholder Cattle and Buffalo: Studies from Lao PDR. Animals 2022, 12,3319. https://doi.org/10.3390/ani12233319
4. Nampanya, S., Khounsy, S., Young, J., Naspasirth, V., Bush, R., Windsor, P. Smallholder large ruminant health and production in Lao PDR: challenges and opportunities for improving domestic and regional beef supply. *Animal Prod Sci*, **2016,** 57, 1001-1006. https://doi.org/10.1071/AN16023
5. Windsor, P. Multiple benefits of molasses nutrient blocks for cattle: Helping the fight against climate change. Research Outreach, 2023, 135. 10.32907/RO-135-4262384862 (Accessed 2023/04/23)
6. Windsor, P.A. Molasses block factory opened in Laos. https://mekonglivestock.wordpress.com/2023/05/08/molasses-block-factory-opened-in-laos/
7. Windsor, P., Martin, S., Khounsy, S., Young, J., Thomson, P., Bush, R. Improved Milk Production from Supplementation of Swamp Buffalo with Molasses Nutrient Blocks Containing 10% Urea. *Dairy* **2021**, *2*, 90–103. https://doi.org/10.3390/dairy2010009
8. Windsor, P.A. Progress with Livestock Welfare in Extensive Production Systems: Lessons From Australia. *Front. Vet. Sc.* **2021**, *8*:l, 674482. https//doi.org/10.3389/fvets.2021.674482
9. FAOSTAT. https://www.fao.org/faostat/en/#data/EI (accessed on 17 May, 2023).
10. Beauchemin, K.A., Ungerfeld, E.M., Eckard, R.J., Wang, M. Review: Fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation. *Animal*. **2020,** 14(S1):s2-s16. https://doi.org/10.1017/S1751731119003100
11. Windsor, P.A., Nampanya, S., Kinnavong, B., Phommasone, P., Bush, R.D., Khounsy, S. Do triclabendazole medicated molasses blocks have a role in control of *Fasciola gigantica* in smallholder cattle production in Lao PDR? *Anim. Prod. Sci.* **2019**, *59*, 787–793. https://www.publish.csiro.au/an/AN17255
12. Olmo, L., Nampanya, S., Nemanic, T., Selwood, N., Khounsy, S., Young, J.R., Thomson, P.C., Bush, R.D., Windsor, P.A. Can fenbendazole medicated molasses blocks control *Toxocara vitulorum* in smallholder cattle and buffalo calves in developing countries? Studies from upland Laos. *Anim. Prod. Sci.* **2020**, *60*, 2031–2043. https://doi.org/10.1071/AN19248
13. Windsor, P.A., Nampanya, S., Olmo, L., Khounsy, S., Phengsavanh, P., Bush, R.D. Provision of urea-molasses blocks to improve smallholder cattle weight gain during the late dry season in tropical developing countries. Studies from Lao PDR. *Anim. Prod. Sci.* **2020**, *61*, 503-513. https://doi.org/10.1071/AN20517
14. Goel, G., Makkar, H.P.S. Methane mitigation from ruminants using tannins and saponins. *Trop. Anim. Health Prod*. **2012**, *44*, 729–739. https://doi.org/10.1007/s11250-011-9966-2
15. Aboagye, I.A., M. Oba, M., Castillo, A.R., Koenig, K.M., Iwaasa, A.D., Beauchemin, A.D. Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high-forage diet. *J. Anim. Sci.* **2018.** *96*, 5276-5286. https://doi.org/10.1093/jas/sky352
16. Vázquez-Carillo, M.F., Montelongo-Pérez, H.D., González-Ronquillo, M., Castillo-Gallegos, E. Effects of three herbs on methane emissions from beef cattle. *Animals* **2020**, *10*, 1671. https://doi.org/10.3390/ani10091671
17. Guggenberger, T. Final Report. Lemongrass. AREC Raumberg-Gumpenstein. https://s26.q4cdn.com/317237604/files/doc_downloads/2021/04/Final-report-Lemongrass.pdf
18. Ocko, I.B., Tianyi Sun, T., Shindell, D., et al. Acting rapidly to deploy readily available methane mitigation measures by sector can immediately slow global warming. *Environ. Res. Lett.* **2021**, 16,054042. https://iopscience.iop.org/article/10.1088/1748-9326/abf9c8

 doi:10.20944/preprints202305.1495.v1

19. IEA. The Global Methane Pledge. https://www.iea.org/reports/global-methane-tracker-2022/the-global-methane-pledge (accessed May 17th, 2023)