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Dietary supplementation of medicinal herbs with total mixed ration to mitigate enteric methane emission in sheep

M Al-Mamun[1*], MRA Redoy[1], AAS Shuvo[1], MA Rahaman[1], MA Alam[1], MJ Khan[1] and AKMA Kabir[2]

[1]Feed Safety and Phyto Nutrition Lab, Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh; [2]Professor, Department of Animal Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

Abstract

The purpose of this study was to determine the effects of pineapple wastes (*Ananas comosus*), garlic leaves (*Allium sativum*), moringa leaves (*Moringa oleifera*) and their combination on growth, plasma metabolites, meat characteristics and enteric methane emission in sheep. A total of 15 sheep (initial BW: 8.3±0.2 kg; age: ~ 1 year; non-descript indigenous to Bangladesh) were randomly assigned to one of five dietary treatments with three sheep per treatment in a completely randomized design. Dietary treatments were: (1) CL-diet: a total mixed ration (TMR) pellet based on roadside grass and concentrates with a CP–16.72% and ME–10.7 MJ/kg; (2) PW-diet: CL-diet + 10g DM of pineapple waste (peels, cores, tops, and leaves)/day; (3) GL-diet: CL-diet + 10g DM of garlic leaves/day; (4) ML-diet: CL-diet + 10g DM of moringa leaves /day; (5) HM-diet: CL-diet + 10g DM of herbal mixture (3g pineapples wastes + 3g garlic leaves + 4g moringa leaves)/day. Weekly live weight gain and plasma metabolites did not show any significant variation among the treatments. Compared to the CL-diet group, the herbal supplemented group had 18–34% lower abdominal fat content, and the lowest value was found in the GL-diet group. Furthermore, the methane emission (g/day/sheep) was reduced by 5–13% in herb supplemented groups compared to the CL-diet group. Additionally, the lamb fed GL-diet emitted the lowest enteric methane (21.26 vs 24.07 g/kg DMI) compared to other experimental groups. Overall, garlic and moringa leaves could be added to TMR to minimize fat accumulation and enteric methane emission from sheep.

Keywords: medicinal herbs, bioactive components, enteric methane, plasma metabolites, sheep

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Introduction

Concerns regarding the environmental impacts of ruminants farming are emergent. After CO_2, CH_4 is the second most abundant greenhouse gas, accounting for 16% of worldwide greenhouse gas emissions (Edenhofer, 2014). As part of their normal digestion processes, livestock emits CO_2 and CH_4. CO_2 generated by livestock is not considered a net contribution to climate change since ruminants consume plants that utilize CO_2 during photosynthesis (Steinfeld *et al*., 2006). Moreover, CH_4, once released into the atmosphere, will endure from 9-15 years and trap 25 times more radiation than CO_2 (Ma *et al*., 2019). Livestock husbandry is an important contributor of CH_4 in the atmosphere. Around 95.5% of CH_4 production in ruminants comes from feed fermentation in the rumen, which results in a loss of 2.3-10.8% of feed energy depending on the food and animal (Guyader *et al*., 2014; Ma *et al*., 2019). Sheep generate an average daily 22.15 g CH_4, with around 7-8% of total CH_4 production coming from microorganisms in the rumen and gut fermenting carbohydrates (McGinn *et al*., 2006 and Nguyen *et al*., 2018).

Researchers seek ways to reduce enteric methane emissions from sheep while also improving mutton quality through dietary manipulation. Medicinal herbs like hop (Humulus lupulus) having polyphenolic compounds could enhance rumen propionate production (Al-Mamun *et al*., 2011) and thereby mitigate methane production in ruminants (Narvaez *et al*., 2011). From economic, safety, and value addition to mutton aspects, phytochemicals from phytogenic feed additives are more sustainable in reducing enteric methane emission than other dietary strategies implications (Beauchemin *et al*., 2008, Rahman *et al*., 2021). Tannin as a phytochemical inhibits the growth and development of rumen methanogenic and protozoa which helps to reduce methane production up to 55% (Bodas *et al.,* 2012). Saponin, a bioactive compound found

***Corresponding author**: mamamun@bau.edu.bd

in herbs, has the potentiality to reduce the multiplication of ruminal bacterial and fungal species and diminish the H2 production for methanogenesis in the rumen, thus mitigating CH_4 production (Bodas *et al*., 2012; Patra *et al*., 2009). Pineapple waste (PW) consists of the residual pulp, peels, stem and leaves used as a small and large ruminant feed additive, respectively (Schieber *et al*., 2001; Sruamisri, 2007). PW is an excellent source of phytochemicals that have the ability to represent a strong antioxidant (Oliveira *et al*., 2009). Moringa (*Moringa oleifera*) leaves are widely found in tropical and subtropical regions across the globe. They are utilized as supplements in animal feed because of their greater protein, vitamin, and mineral contents (Zhang *et al*., 2018). It also contains saponins, tannins, and polysaccharides, which have anti-inflammatory, antioxidant, and antibacterial properties (Mendieta-Araica *et al*., 2011; Azzaz *et al*., 2016). Garlic (*Allium sativum*) leaves also consist of greater amounts of flavonoid and polyphenolic components (Chung, 2006). Simultaneously, it includes a number of sulphur compounds (Martins *et al*., 2016) that influence rumen microbial fermentation and increase sheep performance (Panthee *et al*., 2017). Redoy *et al*. (2020) illustrated that sheep receiving 10g DM from garlic leaves significantly improved the antioxidants, immune status and reduced abdominal fat retained. Garlic enriched with 4.8% tannins and 4.3% saponins, which has the potentiality to reduce the enteric methane emission in sheep (Ali and Ebrahim, 2019) So, the present study was designed to assess the supplementation of pineapple waste, moringa, and garlic leaves and their combination in the total mixed ration (TMR) on growth performance, plasma metabolites, meat characteristics and enteric methane emission in sheep.

Materials and Methods

Animals, diets and sampling

Fifteen non-descript sheep (indigenous to Bangladesh) having initial body weight 8.3±0.2 kg were randomly allocated five dietary treatment groups (3 sheep in each dietary group) in a complete randomized design. Dietary treatments were: (1) CL-diet: a TMR pellet based on roadside grass and concentrates with a CP–16.72% and ME–10.7 MJ/kg; (2) PW-diet: CL-diet + 10g DM of pineapple waste (peels, cores, tops, and leaves)/day; (3) GL-diet: CL-diet + 10g DM of garlic leaves/day; (4) ML-diet: CL-diet + 10g DM of moringa leaves /day; (5) HM-diet: CL-diet + 10g DM of herbal mixture (3g pineapples wastes + 3g garlic leaves + 4g moringa leaves)/day. Feed was offered once daily at 09:00h, and fresh drinking water was available *ad libitum*. Methane production was measured *in vitro* after adaptation to these rations. Rumen pH was measured continuously with the view to prevent acidosis. The total experimental period was 60 days, of which the sampling was performed during days 54-60.

Table 1: Ingredients and nutritive value of ration, pineapple (*Ananas comosus*), garlic (*Allium sativum*), and moringa (*Moringa oleifera*)

Ingredients	**g/kg**			
Local roadside grass[1]	647			
Broken maize	89			
Wheat bran	193			
Mustard oil cake	55			
Salt	16			
Nutritive value (g/kg)	Ration	Pineapple	Garlic	Moringa
Dry Matter	216	159	105	145
Crude Protein	162	65	248	237
Crude Fibre	225	220	187	118
Ether Extract	32	35	45	38
Ash	93	45	155	90
In vitro organic matter digestibility	597	603	663	684

[1]*road side grass predominating Axonopus compressus, Panicum repens, Imperata cylindrica, Cynodon dactylon, Cynodon dactylon, Cyperus rotundus species.*

Sampling and chemical analysis

Feed samples and leftovers, if any, were collected every seven days interval throughout the experiment. Proximate components of ration, pineapple waste, garlic and moringa leaves were measured in accordance with the guideline of AOAC, (2005). Following proper thawing, plasma was analysed for glucose, triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL-C) using different enzymatic kits (HUMAN, China) in an Urit 810 bio-analyser (URIT Medical Electronic Group Co., Ltd., China) according to the manufacturer's guidelines. Plasma urea was quantified using a urea kit (HUMAN, China) in kinetic mode on an Urit 810 bio-analyser. The methane emission was measured according to the equation developed by Matt Bell *et al*. (2016) using digestible organic matter (DOMD), ether extract (EE) (both g/kg DM) and feeding level above maintenance intake:

CH_4 (g/kg DM intake) = 0.046 (±0.001) × DOMD - 0.113 (±0.023) × EE - 2.47 (±0.29) × (feeding level - 1), with concordance correlation coefficient (CCC) = 0.655 and RMSPE = 14.0%.

Statistical analysis

All raw data were arranged in Microsoft Excel software and analyzed in IBM SPSS 20 (USA) statistical tools using one way ANOVA. The significance of differences among means was determined using the Duncan`s Multiple Range Test, and the differences at $P < 0.05$ were considered statistically significant. The following model was used in this study.

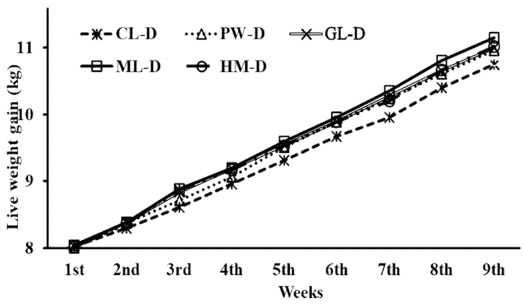
$Y_{ij} = \alpha + T_i + +\varepsilon_{ij}$

Where, Yij, all observed parameters are the dependent variables and Ti, the influence of dietary treatments (pineapple waste, garlic, moringa leaves and their combination) are the independent variable. εij is the error to the jth replication of the ith treatments and normally distributed with zero mean and constant variance.

Results

Growth performance

Lamb fed local roadside grass and concentrate based control diet (CL-diet) supplemented with 10g moringa leaves DM/d/lamb (ML-diet) had maximum growth performance compared to other supplemented groups (Figure 1). Growth performance of CL-diet group had the lowest ($P < 0.05$) throughout the experimental period. The growth of lamb fed a PW-, GL-, or HM-diets was comparable to each other's, but was lower than that of the ML-diet group.



CL-D, local roadside grass and concentrate mixture diet; PW-D, CL-D +10 g pineapple peels leaves and nonedible part diet; GL-D, CL-D + 10 g garlic leaves DM/d/sheep, ML-D: CL-diet + 10 g moringa leaves DM /d/sheep, HM: CL-D + herbs mixture/d/sheep (3.0 g pineapple + 3.0 g garlic leaves + 4.0 g moringa leaves DM)

Figure 1: Effect of different dietary forage herbs supplementation on growth performance of sheep

Plasma metabolites

The concentration of blood metabolites of lamb was shown in Table 2. However, no significant differences were observed in glucose, TG, cholesterol, HDL, LDL, urea, BUN values of lamb in all dietary supplemented groups. But numerically, the concentration of TG, cholesterol and LDL of lamb was the lowest in GL-diet group than control and other herb supplemented group where HDL concentration was maximum in the lamb of garlic leaves supplemented group (GL-diet). But blood urea level was the highest in CL-diet group and the lowest in GL-diet. Cholesterol and TG concentrations were 11% and 19% lower in GL-diet group than CL-diet, respectively. About 20% low LDL was observed in the blood of lamb where garlic leaves were supplemented group (GL-diet).

Meat characteristics

Significant effect of dietary supplementation of medicinal herbs was not present in the percentage of meat and bone but in case of fat percent, which was ($P < 0.05$) maximum 0.55 in lamb of CL-diet group. The lowest fat percent was observed in GL-diet group (0.36) which was about 35% lower than the CL-diet group. In ML-diet and HM-diet groups value for fat percent was close and was more or less 18% lower than control group.

Table 2: Effect of different dietary forage herbs supplementation on plasma metabolites in sheep

Parameters	Treatments					SEM	*P* value
	CL- diet	PW-diet	GL-diet	ML-diet	HM-diet		
Glucose (mg/dL)	73.46	63.14	62.91	63.09	63.07	3.22	0.06
Urea (mg/dL)	8.87	6.17	6.13	6.14	6.18	1.04	0.17
BUN (mg/dL)	20.6	21.89	22.01	21.83	21.93	1.33	0.74
TG (mg/dL)	70.21	59.15	57.03	59.01	59.11	2.18	0.14
Cholesterol (mg/dL)	82.12	74.78	73.08	74.12	74.21	2.41	0.08
HDL (mg/dL)	49.47	53.49	54.04	54.01	53.95	1.88	0.29
LDL (mg/dL)	20.21	16.81	15.98	15.02	15.61	1.80	0.36

***BUN:** blood urea nitrogen; **TG:** triglycerides; **HDL:** high density lipoprotein; **LDL:** low density lipoprotein.**CL-diet**: local roadside grass and concentrate mixture with a CP–16.72% and ME–10.7 MJ/kg; **PW-diet**: CL-diet +10g pineapple peels, leaves and nonedible part DM/d/sheep; **GL-diet:** CL-diet + 10g garlic leaves DM/d/sheep; **ML-diet:** CL-diet + 10g moringa leaves DM/d/sheep; **HM-diet:** CL-diet + 10g herbs mixture DM/d/sheep (3.0g DM pineapple + 3.0g DM garlic leaves + 4.0g DM moringa leaves); **SEM**: standard error of means, [a,b,c] Means in the same row with no common superscript differ significantly (P < 0.05).*

Methane emission

There was significant variation in methane emission rate in dietary supplemented group. Methane emission (g/kg DMI) was 21.26 in GL-diet and 21.43 ML-diet which was 11.67%, 10.96% lower than CL-diet group, respectively (Table 4). Methane emission on the basis of DMI was also 22.92 g/kg DMI in PW-diet and 22.01 g/kg DMI which value was close to GL-diet and ML-diet group. Individual methane emission per day of lamb was maximum ($p < 0.05$) in CL-diet group (8.19 g/day/lamb). 4.76%, 12.57%, 13.19%, 11.84% lower methane emission (g/day/lamb) was noticed in PW-diet, GL-diet, ML-diet and HM-diet group than CL-diet group.

Discussion

Growth performance

It is generally agreed that herbs can improve energy utilization, resulting in increased growth, by: i) improving fibre digestion; ii) reducing methanogenesis and (iii) type, level and nature of plant secondary metabolites (Cieslak *et al*., 2014). Both moringa and garlic leaf supplemented lamb demonstrated a similar response in terms of enteric methane emission (Table 4), implying that both groups might exhibit comparable growth performance. However, the lamb showed a 5% greater growth response in the ML-diet group than in the GL-diet group, indicating that some other potential factors may have an effect. The predominance of tannin in both herbs had a distinct molecular weight and chemical structure that might not matter to methanogenic bacteria but may matter to fibrinolytic bacteria (Animut *et al*., 2008; Wang *et al*., 2009). The findings of Cieslak *et al*. (2014) and Khiaosa-Ard *et al*. (2009) in sheep, *in vivo* and *in vitro* experiments, respectively, support this argument. In addition, moringa leaf's flavonoid profile is superior to those of other herbs supplemented in this experiment (Akbarpour *et al*., 2021; Siskawardani *et al*., 2021; Lourenço *et al*., 2021). The flavonoid component, a natural antioxidant, may trap a greater number of free radicals in lamb fed the ML-diet than others, resulting in less stress in the former groups and promoting growth (Sahoo *et al*., 2021; Zhang *et al*., 2021).

Plasma metabolites

Herbal supplementation in lamb yielded inconsistent findings in plasma metabolites (Redoy *et al*., 2020; Reza *et al*., 2021; Chaves *et al*., 2008; Shuvo *et al*., 2017). In this experiment, pineapple waste, garlic and moringa leaves supplementation had no effect on plasma glucose concentration, which is consistent with the findings of Redoy *et al*. (2020), Panthee *et al*. (2017) and Akanmu *et al*., (2020). However, existing research findings has demonstrated that pineapple waste, garlic and moringa have a considerable effect on the plasma lipid profile; however, our trial failed to reflect this (Ososanya *et al*., 2015; Redoy *et al*., 2020; Akanmu *et al*., 2020).

Table 3: Effect of different dietary forage herbs supplementation on meat characteristics of lambs

Parameters (g/100g)	Treatments					SEM	*P* value
	CL-diet	PW-diet	GL-diet	ML-diet	HM-diet		
Meat	12.44	13.08	13.09	12.89	12.99	0.89	0.73
Bone	18.70	19.47	19.43	19.01	19.02	0.55	0.50
Fat	0.55^{a}	0.39^{bc}	0.36^{c}	0.43^{b}	0.45^{b}	0.05	0.02

CL-diet: local roadside grass and concentrate mixture with a CP–16.72% and ME–10.7 MJ/kg; **PW-diet**: CL-diet +10g pineapple peels, leaves and nonedible part DM/d/sheep; **GL-diet:** CL-diet + 10g garlic leaves DM/d/sheep; **ML-diet:** CL-diet + 10g moringa leaves DM/d/sheep; **HM-diet:** CL-diet + 10g herbs mixture DM/d/sheep (3.0g DM pineapple + 3.0g DM garlic leaves + 4.0g DM moringa leaves); **SEM**: standard error of means, ^{a,b,c} Means in the same row with no common superscript differ significantly ($P < 0.05$).

Table 4: Effect of different dietary forage herbs supplementation on methane emission from sheep

Parameters	Dietary treatments					SEM	*P* value
	CL-diet	PW-diet	GL-diet	ML-diet	HM-diet		
Methane emission (g/kg DMI)	24.07^{a}	22.92^{b}	21.26^{c}	21.43^{c}	22.01^{c}	0.761	0.037
Methane emission (g/day/lamb)	8.19^{a}	7.80^{b}	7.16^{c}	7.11^{c}	7.22^{c}	0.246	0.037

CL-diet: local roadside grass and concentrate mixture with a CP–16.72% and ME–10.7 MJ/kg; **PW-diet**: CL-diet +10g pineapple peels, leaves and nonedible part DM/d/sheep; **GL-diet:** CL-diet + 10g garlic leaves DM/d/sheep; **ML-diet:** CL-diet + 10g moringa leaves DM/d/sheep; **HM-diet:** CL-diet + 10g herbs mixture DM/d/sheep (3.0g DM pineapple + 3.0g DM garlic leaves + 4.0g DM moringa leaves); **SEM**: standard error of means, ^{a,b,c} Means in the same row with no common superscript differ significantly ($P < 0.05$).

Though the herbal supplemented group's cholesterol content was substantial lower (P=0.08) than the control group. This finding can be justified by the fact that all three herbs possess a diverse phytochemical profile that has been shown to influence ruminal ecology, so enhancing propionic acid production and thereby promoting gluconeogenesis and insulin secretion (Oliveira *et al*., 2009; Martins *et al*., 2016; Al-Mamun *et al*., 2017; Zhang *et al*., 2018). Additionally, this increased gluconeogenesis might result in a positive energy balance in the experimental animal, resulting in decreased hepatic conversion to triglycerides and ketones in herbal supplemented group.

Meat characteristics

The qualities of lamb meat are determined by both genetic and non-genetic factors. Among non-genetic factors, nutrition has the greatest influence on carcass morphology, physicochemical properties, and meat quality (Odhaib *et al*., 2021). Additionally, herb supplementation has a significant effect on numerous tissue components, resulting in alterations in the flavor, mutton fat, and fatty acid composition (Odhaib *et al*., 2021). The lower fat percentage in the herb supplemented groups was consistent with the findings of Redoy *et al*. (2020) and Qwele *et al*. (2013), who reported that supplementing lamb and goat with garlic and moringa leaves dramatically reduced the meat fat percentage. Allicin in garlic leaves inhibited adipogenesis and promoted lipolysis in mice (Shi *et al*., 2019). Additionally, this component stimulates white adipocyte browning and inhibits the Krüppel-like factor 15 signaling cascade, which results in decreased fat accumulation (Shi *et al*., 2019). Additionally, the high flavonoid content of moringa leaf stimulates leptin expression, which alters cholesterol catabolism and results in decreased fatty acid synthesis and increased fatty acid -oxidation in mice (Bais *et al*., 2014), which may account for the lower fat percentages in the GL- and ML-diet groups.

Methane emission

During this experiment, lamb supplemented with herbs exhaled 9% less methane per kg DMI than lamb without herbs. The influence of fresh herbs, extracts, or herbal preparations on enteric methane emission in small ruminants, on the other hand, yielded inconclusive findings (Ahmed *et al*., 2021; Abarghuei *et al*., 2021; Diaz-Medina *et al*., 2021; Faniyi *et al*., 2021; Loza *et al*., 2021). These discrepancies could be explained by the variation of secondary metabolites in herbs, more especially the tannin content, which has a direct effect on reducing enteric methane production (Loza *et al*., 2021). The increased tannin content (twofold) of moringa and garlic leaves compared to pineapple waste resulted in a 7 percent reduction in lamb methane production per kg DMI in this experiment (Muhammad *et al*., 2019; Rabiu *et al*., 2018; Sultana *et al*., 2014). Additionally, Faniyi *et al*. (2021) observed that moringa supplementation significantly decreased methane production as a percentage of total gas production in sheep when compared to control. Additionally, Torres-Fraga *et al*. (2020) reported the enhanced anti-methanogenic activity of garlic leaf compared to alfalfa hay as solely or partly replacement in beef cattle. All the above-mentioned explanations are in favour with our current experimental findings. The increased tannin content of moringa and garlic leaves might result in a decrease in the overall number of protozoa, entodiniomorphs, and holotrichs in supplemented lamb, resulting in decreased methane production (Cieslak *et al*., 2014). Additionally, these herbs vastly enhance propionic acid production during ruminal fermentation, which may lead to a decrease in the H_2 sink for methane formation (Abdel-Raheem *et al*., 2021; Kewan *et al*., 2021)

Conclusion

To begin, none of the three herbs individually or in combination had a significant influence on growth performance or plasma metabolites under the present experimental conditions. Second, when compared to control, herbal supplementation decreased fat accumulation in sheep. Thirdly, and perhaps most significantly, garlic and moringa leaves significantly decreased enteric methane emission from sheep, which is critical for climate-smart sheep production. Finally, based on the results of this feeding trial, it could be concluded that garlic and moringa leaves can be added to TMR diet with the goal of reducing enteric methane emission from sheep and increasing lean mutton production.

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Conflict of interest

The authors would like to declare that there is no conflict of interest.

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