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Enteric methane production of sheep fed on grass hay alone or grass hay and legume mixtures under feedlot system

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Abstract
Indigenous range grasses are becoming an important feed resource for ruminant production in the rangeland ecosystems of Kenya. This is attributed to their high adaptability to the changing climate. However, empirical evidence reveal that limited research has been conducted to quantify the enteric methane production of animals consuming these grasses. A study was conducted to assess and quantify the enteric methane production of sheep fed on the following diets; *Cenchrus ciliaris* + *Lucerne*, *Enteropogon macrostachyus* + Lucerne, *Enteropogon macrostachyus* + *Desmodium*, *Cenchrus ciliaris* + *Desmodium*. Yearling dorper sheep (*n*:24, initial weight 20.62 ± 2.101 kg (mean ± s.m.e) were allocated to one of the six diets in a completely randomised design and fed *ad libitum* for a period of 91 days. Intake, live weights and enteric methane production were assessed. Mean voluntary dry matter intake (DMI) increased with inclusion of legume in diet. On the other hand, methane production and methane yield decreased with inclusion of legume in diet. Suitable feeding practices such as protein supplementation need to be promoted to enhance ruminant production and reduce enteric methane production for sustainable livestock production.

Keywords: Digestibility, enteric methane, grass, legume, rangeland, sheep

Introduction

Livestock production forms a major part of agriculture system in Sub-Saharan Africa. This is because livestock production and in particular, ruminant livestock supports the livelihoods of millions of people (Herrero *et al.*, 2010) [10]. Small ruminants (Sheep and goats) contribute significantly to meat production and this is supported by their short production cycle. Yet, Ironically, ruminants are both great contributors to and are affected by the increasing impact of climate change as a consequence of anthropogenic greenhouse gas (GHG) emissions (Smith *et al.*, 2014; FAO 2016) [16, 8].

Livestock are estimated to contribute 70% or more of African agricultural GHG emissions and this is dominated by methane (CH_4) from enteric fermentation. However, increased human population and demand for animal protein is putting pressure on land to support higher animal production despite the adverse effect of climate change on its production and increased competition for land (Thornton *et al.*, 2010) [10]. Higher and intensification of animal production to cater for food security and livelihood is likely to increase Greenhouse gas emission.

Ruminant livestock produce enteric methane during normal ruminal fermentation of feed in the rumen and this is affected by feed composition and quality (Lenka *et al.*, 2015) [13]. The emitted methane represents a significance loss in animal gross energy intake which could have been used in production. Moreover, the productivity of livestock is low due to low nutritional value of available forages due to their highly lignified cell walls, low digestibility and poor nitrogen content (Lee *et al.*, 2017) [12], thus supplementation with protein is an alternative (Cooke *et al.*, 2020) [7]. In addition, Inclusion of legume forages will have an effect on CH_4 production, as ruminants fed legumes may have a lower proportion of energy lost as methane than those fed grasses due to difference in digestibility and Kinetics (Kasuya and Takahashi,

2010) [19]. High levels of methane production increases as the fibre content increases and decreases as the protein content decreases (Johnson and Johnson 1995) [11]. Existing studies have rarely considered sheep and goats in estimating GHG emissions from livestock. Nevertheless, estimates of methane production in sub Saharan Africa are associated with high uncertainty due to limited availability of data (Tallec *et al.*, 2012) [17]. The main objective of the present study was to evaluate and estimate the enteric methane production in small ruminants (sheep) fed *ad libitum* on grass or legume and grass mixed diets (six diet) using Tier II methodology in order to increase the accuracy of assessments given the context of local livestock production system.

Materials and Methods

Study site

The study was carried out at Kipeto in Kajiado county. The area is characterised by tropical wet and dry climate or savannah climate with annual average temperature of 21.43ºc. The area typically receives about 101.01 millimetres (3.98 inches) of precipitation and has 188.92 rainy days (51.76% of the time) annually.

Experimental animal

Twenty-four (24) heads of sheep, aged 12-15 months with average live weight of 20.62 ± 2.101 kg (mean ± s.m.e) were used in this experiment. The sheep were housed in individual (4m by 3m) stall in a feed lot system. Daily feed offered and refusal of each animal was recorded in order to determine actual feed intake. Animal identification was done using ear tags with unique numbers. Age was determined by farmers' recall and dentition (Cash burn 2016) [6].

Feeding and data collection

The sheep were housed and fed individually twice a day at 0800hrs and 1400 hrs with half the day ration each time and watered *ad libtum*. After 14-day adaptation period all the sheep were fully adapted to confinement and routine procedures. Six diets were offered (*Cenchrus ciliaris* + Lucerne, *Cenchrus Ciliaris* + *Desmodium*, *Enteropogon Macrostachyus* + Lucerne, *Enteropogon Macrostachyus* + *Desmodium*, *Cenchrus ciliaris*, *Enteropogon Macrostachyus*. Feed intake (*ad libitum* access) and refusal were recorded daily before the morning meal. Initial weight was taken at the start of the experiment using digital portable weigh scale followed by subsequent weekly weighing for 91 days.

Diet nutritive analysis

Nutritive analysis of diets was performed by wet chemistry for dry matter (DM) (AOAC internationals 2005 (Method 930.15), total N (AOAC method 990.03), organic matter, neutral detergent fibre and acid detergent fibre (ADF; AOAC method 973.18). Gross energy (GE) was computed using the equation below

$$GE = 0.0226CP + 0.0407EE + 0.0192CF + 0.0177NFE \quad \text{(i)}$$

Where
CP =Crude protein
EE= Ether extract
CF= Crude fibre
NFE = neutral free extract

Calculation of daily methane emission

IPCC, 2019 equation shown below was used in computation for enteric methane emission

$$DMP\left(\frac{g}{d}\right) = (GE(MJ) * \frac{Ym(\%)}{100})/0.05565 \quad \text{(ii)}$$

Where
DMP is daily methane production in grams per day
Ym is the methane conversion factor, a factor of 6.7% was used, which represents the % of gross energy in feed converted to CH_4 according to IPCC, 2006,
GE is the gross energy intake of the diet
Methane yield which represented methane produced per dry matter intake was computed
Following the equation below

$$Methane\ yield\left(\frac{g}{kgDMI}\right) = \frac{DMP}{DMI} \quad \text{(iii)}$$

Where
DMP is daily methane production
DMI is the dry matter intake

Statistical analysis

The effect of diet on intake and methane production was analyzed using general linear model, with diet as fixed effect, using Genstat 14th edition. The means were separated with turkey HSD at 5% significant level. The relationship between DMI and CH_4 emissions per day and per kg DMI was determined using regression analysis.

Results

The daily methane production (DMP) and methane yield of sheep in Kipeto fed on *C. ciliaris; C. ciliaris* + Lucerne; *C. Ciliaris* + *Desmodium*; *E. macrostachyus*; *E. macrostachyus* + Lucerne; *E. macrostachyus* + *Desmodium* diets for a period of 91 days in a feedlot system is presented in Table 1. DMP (g CH_4/day) ranged from 14.02 to 19.36 g CH_4/day. DMP of *C. ciliaris* reduced by 24% (18.38 vs 14.02 g/day $p<0.05$) and 3% (18.38 vs 17.71 g /day) when mixed with *Desmodium* and lucerne respectively. On the other hand, lucerne and *Desmodium* reduced the DMP of *E. macrostachyus* by 22% (19.36 vs 15.15 g CH4/day) and 12% (19.36 vs 16.94 g CH4/day) respectively.

Methane yields obtained ranged from 20.75 -21.21 g/kg DMI. Methane yield per DMI decreased on inclusion of *Desmodium* to *C. ciliaris* and *E. macrostachyus* (21.21 vs 20.75 $p<0.05$) and (20. 99 vs 20.81 g CH4/kg DMI $p<0.05$) respectively.

Table 1: Daily methane production and methane yield of sheep fed on *C. ciliaris; C. ciliaris* + Lucerne; *C. Ciliaris* + *Desmodium*; *E. macrostachyus*; *E. macrostachyus* + Lucerne; *E. macrostachyus* + *Desmodium* ration on *adlibitum* basis over a period of 91 days in a feedlot system

Parameter	*C. ciliaris*	*C. ciliaris* + Lucerne	*C. ciliaris* + *Desmodium*	*E. macrostachyus* + Lucerne	*E. macrostachyus* + *Desmodium*.	*E. macrostachyus*	SEM	P value
DMI kg/day	0.866^{c}	0.836^{c}	0.675^{a}	0.714^{b}	0.814^{bc}	0.922^{c}	0.0370	**
Methane production (DMP g/day)	18.38^{c}	17.71^{c}	14.02^{a}	15.15^{ab}	16.94^{b}	19.36^{c}	0.812	**
CH_4 yeld (g CH_4/kg DMI)	21.21^{c}	21.19^{bc}	20.75^{a}	21.19^{bc}	20.81^{ab}	20.99^{abc}	0.127	**

a,b,c Mean values within a row with different superscript letter differ significantly at $p<0.05$

The nutritional composition of the feed basket is presented in Table 2, inclusion of legume in the feed improved the feed quality. For example the CP content increased with addition of a legume (*C. ciliaris* 43.8 vs *C. ciliaris* + Lucerne 120.7) while NDF content decreased

Table 2: Chemical composition of diets used in the experiment

Component	Chemical composition (g/kg/DM)				
	DM	CP	ADF	NDF	GE (MJ/kg/DM)
C. ciliaris	92.0	43.8^{ab}	553.7^{ab}	773.1^{ab}	17.61^{a}
C. ciliaris + Lucerne	96.7	120.7^{b}	435.3^{b}	690.6^{b}	17.60^{a}
C. ciliaris + *Desmodium*	92.0	70.9^{a}	497.0^{a}	714.0^{a}	17.24^{a}
E. macrostachyus + Lucerne	91.7	81.2^{bc}	456.7^{bc}	672.5^{bc}	17.6 ^{a}
E. macrostachyus + *Desmodium.*	93.9	117.9^{c}	399.9^{c}	706.1^{c}	17.2 ^{a}
E. macrostachyus	93.8	54.9^{ac}	493.8^{ac}	751.7^{ac}	17.43^{a}

^{a,b,c} Mean values within a row with different superscript letter differ significantly at $p<0.05$

CP = crude protein; ADF = acid detergent fibre; DM = dry matter; GE = gross energy; NDF = neutral detergent fibre

A significant ($p<0.001$) positive relationship between methane emission per day and dry matter intake was recorded. This accounted for 99% of the variation in methane emission per day (fig 1).

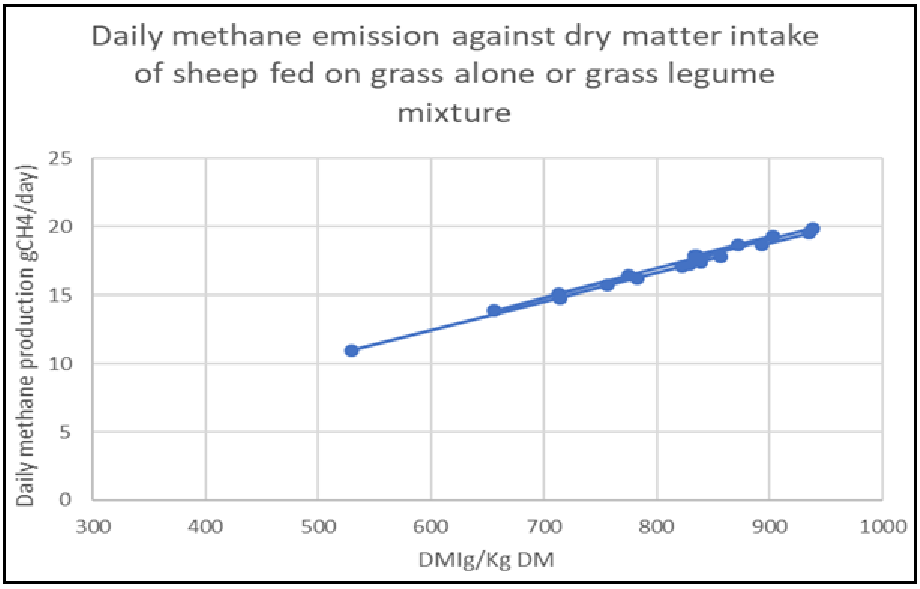

Fig 1: Linear relationship between methane emission (g/day) and DMI (g/kg DM) in sheep fed on grass and grass legume mixtures

Discussion

The decreased methane emission on inclusion of legume is tied to the fact that increased non fiber sugar content in ruminant feeds lead to a more propionate-based fermentation pattern which in turn decreased the amount of hydrogen produced (Archimède *et al*., 2014) [3] and consequently lead to lower methane emissions. Methane yields obtained were within the range found by other studies in tropical environment with tropical low quality forages (Archimede *et al*., 2018, Gera *et al.*, 2022) [4, 20]. For example, Archimede *et al*., 2018 [4] reported methane yield in range of 15-29 g/kg DMI from sheep fed on low quality C4 grasses; Gera *et al*., 2022 [20] reported higher CH_4 yield of 31 g/kg DMI from sheep fed on Rhodes grass hay while Savia *et al*., 2014 on their study on grazing sheep reported daily methane production (DMP) in the range of 12.2 to 37.3 g/day. Nevertheless, much lower CH_4 yields were recorded with other studies with sheep (Amaral *et al.*, 2016; Lima *et al.*, 2019; Fernandes *et al*., 2022) [1, 14, 9]. For example, the study of Amaral *et al*., 2016 [1] reported CH_4 yield of 13 g/kg DMI from sheep fed on high quality (N fertilized) C4 grasses. In the present study, the sheep were fed on low quality indigenous grasses with high fibre but low crude protein content hence the reason for higher emissions. Inclusion of a legume in tne diet was found to lower the emission.

The diet quality improved with the inclusion of legume which inturn increased the dry matter intake. The high dry matter intake is tied to the fact that legumes in the diet increases the essential nutrients to rumen microbes and increases the rate of passage of particulate and liquid matter hence reduction in amount of methane production per kilogram of DMI (Brask *et al*., 2013) [5]. Finishing sheep on high quality forages, methane emission per day would almost exclusively be explained by dry matter intake because increasing dietary CP concentration is associated with increase in feed intake and a reduction in methane yield (g/Kg DM). The direct significant relationship between Dry matter intake and daily methane emission was in agreement with the research by Tao *et al*., 2019 [21]; Yan *et al*., 2010 [18] who reported a direct relationship between DMI, gross energy intake (GEI) and methane. The nutritional quality of the diet offered to sheep directly influenced the voluntary intake and CH_4 emissions generated. The results implys that inclusion of legume in low quality grass pastures is a promising CH_4 mitigation strategy and good option for achieving the emission reduction goals for sustainable sheep production in the rangeland ecosystems.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential Conflict of interest.

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Conclusion
In conclusion, the study demonstrates the impact of various forage combinations on daily methane production (DMP) and methane yield in sheep. Incorporating legumes like lucerne and *Desmodium* into diets containing grasses such as C. ciliaris and E. macrostachyus resulted in notable reductions in methane emissions. This reduction was attributed to changes in fermentation patterns leading to decreased hydrogen production. The improved nutritional quality of diets containing legumes also contributed to higher dry matter intake, further reducing methane emissions per kilogram of dry matter intake. These findings suggest that incorporating legumes into low-quality grass pastures holds promise as an effective methane mitigation strategy in sheep production systems, supporting sustainability goals in rangeland ecosystems. The study underscores the importance of diet composition in influencing methane emissions and highlights the potential for targeted dietary interventions to mitigate greenhouse gas emissions in livestock production. Further research in this area can offer valuable insights into optimizing dietary strategies for sustainable sheep production while minimizing environmental impact.

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