#### **ORIGINAL ARTICLE**



# *In-vitro* digestibility and methane gas emission of indigenous and introduced grasses in the rangeland ecosystems of south eastern Kenya

Annastacia Nduku Maweu<sup>1,2</sup> · Bockline Omedo Bebe<sup>1</sup> · Simon Gichuru Kuria<sup>2</sup> · Olivier Basole Kashongwe<sup>1,3</sup>

Received: 18 March 2022 / Accepted: 3 December 2023 / Published online: 21 December 2023 © The Author(s) 2023

#### Abstract

Various grass species with high biomass yield and low moisture demand have been introduced in the rangelands of Kenya to realize increased ruminant productivity that could not be achieved with the low quality of the indigenous grasses. However, this intervention ignores the different methane emission of the indigenous and introduced grasses, a necessary consideration for realizing increased productivity while minimizing greenhouse gas emissions. This study determined in-vitro digestibility and methane emission of three indigenous grasses: Eragrostis superba (E. superba), Cenchrus ciliaris (C. ciliaris), Enteropogon macrostachyus (E. macrostachyus) and two introduced grasses (two varieties of Chloris gayana; Boma rhodes and Extozi rhodes. Samples of these five grasses (whole plant above ground) were collected from established pasture plots in South Eastern rangelands of Kenya. The grass samples were collected at bloom stage using one-meter square quadrats for proximate analysis and determination of neutral detergent fiber (NDF), acid detergent lignin (ADL) and acid detergent fiber (ADF) using AOAC (1990) methods. On average, relative to the indigenous grasses, the introduced grasses were higher in crude protein (74.05 g Kg-1 dry matter (DM) vs. 52.11 g Kg-1 DM), organic matter digestibility 62.7% vs 53.6%) and in NDF (712.7 g Kg-1 DM vs. 708.0 g Kg-1 DM), metabolizable energy (16.35 vs 12.90 MJ/kg DM), methane emission (25.61 ml vs 15.93 ml) but with lower in-vitro-dry matter digestibility 54.24% vs 58.12%. Methane production positively correlated with crude protein, NDF, metabolizable energy, ADF and *in-vitro* organic matter digestibility. Hence, utilizing the introduced grasses to boost cattle production would achieve increased productivity but a point of concern are the higher methane emissions, not to mention the ecosystem change caused by the introduction of new species, which should affect the sustainability of the rangeland ecosystem.

Keywords Rangelands grasses · Methane emission · In-vitro dry digestibility · Chemical composition · Sustainability

Communicated by Abubakari Ahmed and accepted by Topical Collection Chief Editor Christopher Reyer

Annastacia Nduku Maweu annmaweu5@gmail.com; Annastacia.Nduku@Kalro.org

- Olivier Basole Kashongwe Okashongwe@atb-potsdam.de; okashongwe@gmail.com
- <sup>1</sup> Department of Animal Sciences, Egerton University, Box 536-20115, Egerton, Kenya
- <sup>2</sup> Kenya Agricultural Livestock Research Organization, Arid and Research Institute, P.O Box 12-90138, Makindu, Kenya
- <sup>3</sup> Department of Sensors and Modeling, Leibniz Institute for Agricultural Engineering and Bioeconomy e.V. (ATB), 14469, Potsdam, Germany

# Introduction

Rangeland ecosystems support the largest proportion of ruminant production, but the predominant and adaptable indigenous grasses cannot support higher ruminant populations. These grasses are low in biomass yield and poor in quality, which has led to introduction of non-native grasses with higher biomass yield, better quality and low moisture demand to increase ruminant productivity. Though the indigenous grasses are highly adaptable and are the basal diet for grazing ruminants, their high lignification directly depress their quality and digestibility, which is likely to be associated with high emissions of the grazing cattle (Berndt and Tomkins 2013).Improving pasture management, whether indigenous or introduced, is therefore a necessary intervention in the rangelands to meet the rising demand for ruminant feedstock (Mganga et al. 2015) and to adapt the negative impacts of the climate change on livestock production.

Some indigenous perennial grasses including C. cilliaris, E. superba and E. macrostachyus predominate grazed pastures in the rangelands of Kenya (Ndathi et al. 2011). However, they are low in protein quality and high in fiber content, and are vulnerable to climate variability and increased land use pressure (Mganga et al. 2015). When grazed they are likely to result in higher methane  $(CH_4)$  from enteric fermentation relative to temperate grasses, which have a low methane emission value (0.49 g  $CH_4/Kg LW$  vs. 0.61 g  $CH_4/$ Kg LW), as observed by some authors (Archimède et al. 2018; Berndt and Tomkins 2013) when comparing ryegrass and Rhodes grass. Tropical grasses improved for high biomass yield and quality like Chloris gayana have been introduced from high agricultural potential areas into low potential rangelands to supplement the feed base (Shrestha et al. 2013). However, their response to high temperatures and low rainfall is poor compared to the indigenous grass pastures, because introduced grasses exhibit inability to cope high moisture stress. Maybe as kind of transition is needed. If they grow well, however, the introduced grasses are of high-quality with higher amounts of easily fermentable carbohydrates and less NDF, which can lead to increased feed intake, higher digestibility and passage rate and subsequently minimize  $CH_4$  production (Waghorn et al. 2002).

Poor quality grass pastures on the other hand when consumed by ruminants emit higher amount of  $CH_4$  as a byproduct of anaerobic fermentation in the rumen. The digestibility of pasture depends on its stage of growth with more mature pastures having higher fibre content and increased carbon to nitrogen ratio, which decrease digestibility hence inducing a higher  $CH_4$  yield. It is therefore important to prioritize not only feed quantity but also quality, methanogenic and carbon sequestration potentials of grass pastures in the rangeland ecosystems for sustainable ruminant production.

Ruminant livestock is the largest contributor to CH<sub>4</sub> emission in the agricultural sector (O'Mara 2011) through enteric fermentation of feed by the methanogenic archaea in the rumen. Ruminants accounts for 18% of the global CH4 emission and 3.3% of greenhouse gas (GHG) (Patra 2016). This also represents loss of 5 to 10% of animal Gross Energy intake depending on diet composition and intake level (Johnson and Johnson 1995; Haque et al. 2014) which represents a loss of dietary nutrients that would otherwise have been used for production of meat and milk (Eckard et al. 2010). Most of enteric CH<sub>4</sub> emission from livestock comes from large ruminants (Moss et al. 2000) due to their large rumen and it is influenced by quality and digestibility of the feed consumed (Doreau et al. 2016; Archimede et al. 2011). The highly digestible feed will have an increased feed intake and reduced enteric methane emission. Rumen microbes degrade structural plant fiber under anaerobic conditions to volatile fatty acids (VFA), CO<sub>2</sub> and H<sub>2</sub>.

Among the products,  $H_2$  is reduced using  $CO_2$  with the help of methanogenic archaea in the rumen to form  $CH_4$ .

 $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$  (Moss et al. 2000)

Dietary manipulation involving for example improving feed resource base to utilize grass pastures with higher nutritive quality, high carbon sequestration and low CH<sub>4</sub> production would mitigate enteric CH4 emission from extensive ruminant production systems. However, evidence is scanty on the characterization of CH<sub>4</sub> production potential of locally available grasses when fed to ruminant animals (Bezabih et al. 2014). Ruminants in- vivo studies are expensive, time consuming and require specialized facilities and resources. For this reason, researchers show interest on the use of in -vitro techniques to simulate the in- vivo process (Melesse et al. 2013). The in -vitro technique can study large numbers of species within a short time and at a low cost. This study tested the hypothesis that digestibility and methane emission differs significantly between indigenous grasses (E. superba, C. ciliaris and E. macrostachyus) and introduced grasses (Chloris gayana var Boma rhodes and Extozi rhodes) under Makueni rangeland ecosystems in South Eastern Kenya.

#### Materials and methods

#### Study site

The study was conducted in Arid and Rangelands Research Institute (ARLRI) of the Kenya Agriculture and Livestock Research Organization (KALRO) (Appendix 1), where grass samples were collected from established pasture plots. The station is located at Kiboko in Makindu Sub County of Makueni County, which is in the rangelands found in the South Eastern of Kenya. The area is in Agro Ecological Zone V at an elevation of 975 m above sea level and lies within latitude 2° 10' and 2° South and longitude 37° 40' and 37° 55' East (CIMMYT 2013). The precipitation in the area follows bimodal distribution, with long rainy season from March to May and short rainy season from October to December. The area receives mean annual rainfall of 600 mm and mean annual temperature of 23 °C (Mutiso et al. 2018). The plots where grass samples were obtained had Ferralsols soils ranging from sandy clay to loamy sand and were low in organic matter and highly vulnerable to erosion and biological degradation.

#### Sampling of the grass species

The studied grass samples were of three indigenous (*E. superba, C. ciliaris* and *E. macrostachyus*) and one introduced (*C. gayana* var. Boma rhodes and Extozi rhodes) grass species that were ratoon grasses in seven years old established rain fed five pasture plots (Fig. 1). In each plot, a line transects of 20.62 m was set and three selected sub sites along the transect were taken according to Maweu et al. (2022). One-meter square quadrat was used and all the above ground vegetation within the quadrat was clipped to ground level when the pastures were at bloom stage (the assumed grazing optimal stage of pasture growth). The grasses were kept under a shaded area until transported to the Laboratory of Arid and rangelands research institute (ARLRI), where they were oven-dried at 65°c for 48 h and ground to pass through a 1-mm sieve in a mill. The ground samples were taken for in vitro fermentation, methane gas production and chemical composition analyses.

#### **Chemical analyses**

The ground samples of each grass species were collected for nutritive content analysis, to determine: True DM (at 105°C for 24 h) in an air-forced oven (Genlab Oven, Genlab Ltd, UK.); Ash content by combustion in a muffle furnace at 550 °C for 4 h (HeraeusM110 muffle furnace, Heraeus Holding GmbH, Hanau, Germany) according to AOAC method (AOAC 1990 method no.924.05). Total nitrogen (N) content was determined following the micro Kjeldahl procedure (AOAC 1990, method no. 988.05) using selenium catalyst tablets. The crude protein content was estimated by multiplying total N by a factor of 6.25. Further, samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to AOAC (1990) method number 6.5.1 and 6.5.2 respectively, using an Ankom 200 fiber analyzer (Ankom Technology cooperation, Fairport, USA). In addition, Acid detergent lignin



A = C. ciliaris, B = E. macrostachyus, C = E. superba, D = ExtoziRhodes, E = Boma rhodes

Fig. 1 Sampling design for the experiment in the five pasture plots

(ADL) and Ether extract (EE) were analyzed using AOAC 1990 method number 973.18 and 14.018 respectively.

#### In-vitro gas production

In-vitro digestibility and gas production for each of the five grass species collected were determined according to the method of Menke and Steingas (1988) described by (Abdulrazak and Fujihara 1999). Rumen fluid was collected in the morning before feeding from one fisitulated Zebu steer which was fed on mixed grass hay for 7 days and watered ad libitum in a volumetric flask, then taken to the laboratory where it was strained through a double layer of cheese cloth to remove large particles. Strained rumen fluid was then mixed with buffer prepared at ratio of 3:1 to simulate action of saliva. One gram of the five grass samples was inoculated using 50 ml of the mixture in 100 ml gas tight graduated glass syringe barrel in triplicate. The syringe pistons were lubricated with petroleum jelly to ease movement and prevent escape of gas. Syringes were pre warmed at 39 °C prior to inoculation of buffer mixture and incubated in water bath maintained at 39 °C swirled gently at each reading and gas volume recorded at 3, 6, 9, 12, 24, 48 and 72 h of incubation.

The samples and blank (rumen fluid + buffer) were also run in triplicates to determine gas produced due to endogenous substrates. Net gas produced was computed from the total increase in volume minus the mean blank value from the recorded gas production of all samples. From the computed gas production values, the model of Ørskov and McDonald (1979) was applied to determine the kinetics of gas production of the grass samples:

$$Y = a + b\left(1 - e^{-ct}\right) \tag{1}$$

where,

- Y is the volume of gas produced with time (t)
- a is the initial gas production,
- b is the gas produced during incubation,
- c is the constant gas production rate constant (fraction/hour),
- t is the time of fermentation.

In this case, (a + b) represents the potential extent of the gas production.

The sample *in-vitro* organic matter digestibility (OMD %), Metabolizable energy (ME, Mj/Kg Dm) and dry matter digestibility (DMD%) content was estimated based on 24-h gas production (GP, ml/200 g DM), crude protein (CP) content and ash content using equation by Menke et al. 1979 as;

OMD (%) = 14.88 + 0.889GP + 0.448CP + 0.0651Ash (2)

where,

CP is the Crude Protein (% DM),GP is the 24 h gas production (ml/200 mg DM)Ash is the Ash content (%DM)

ME(Mj/Kg DM) = 2.20 + 0.136GP + 0.057CP + 0.0002859CF2(3)

where,

ME	Metabolizable energy (Mj/Kg DM)
GP	24 hr gas production (ml/200 mg Dm)
СР	Crude protein (%)
CF	Crude fat (%)

where,

DMD (%)dry matter digestibility (%)ADFAcid detergent fibre

DMD(%) = 88.9 - (0.779 \* ADF%)

# **Determination of methane emission**

Gas production was determined according to the procedure of Menke & Staingass (1988) and Bhatta et al. (2007). Gas samples were collected after every 3 h of incubation at 3, 6, 9, 12, 24, 48 and 72 h from gas tight ground glass syringe barrel headspace to fill a 60 mL syringe, and then transferred to 10 ml glass vials according to Pellikaan et al (2011). The collected gas samples were analyzed for CH<sub>4</sub> gas concentration using a gas chromatograph (model 8610C; SRI at the International Livestock Research Institute, Nairobi) fitted with a methanizer on the Flame ionization detector (FID). The gas chromatograph was operated with Hayesep D packed columns (3 m, 1/8"), an oven temperature at 70 °C and FID temperature of 350 °C. Nitrogen  $(N_2)$  was used as carrier gas at a flow rate of 25 ml min<sup>-1</sup>. An auto sampler (Model HT200H; Hta) was used to inject 5 ml of gas sample into the gas chromatograph (GC) system. The sample was temporary stored in a 1 ml sample loop then carried to the separation column by high purity nitrogen (carrier gas).

The detector output was in the form of peak areas with milli -volts as the units. The peak area and retention time of  $CH_4$  was measured, calculated and reported by digital processor which was then transferred to an excel work sheet for processing. The retention time for  $CH_4$  was then compared to the known standard in the literature. The peak areas were then converted into concentration using a calibration curve generated using gases of known concentrations.

#### **Statistical analysis**

This study employed a Completely Randomized Design. The grass species were the treatments, while the observation on digestibility, chemical composition, gas production and methane production were treated as dependent variables. Data was subjected to General Linear Model procedure using Statistical Package for Social Sciences (SPSS version 22). The level of significance was set at p < 0.05 for detection of grass effect on chemical composition, digestibility, gas production and methane production. The means were separated using Tukey HSD for multiple mean comparison. A Pearson correlation analysis was also performed to determine the association of methane production with chemical composition, fermentation characteristics and fibre constituents. The level of significance was also set at p < 0.05.

The model fitted was as follows;

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \tag{5}$$

where,

(4)

 $Y_{ij}$  observation on chemical composition, digestibility, gas production kinetics, methane production of i<sup>th</sup> grass species on j<sup>th</sup> replication

 $\mu$  overall mean,

- $T_i$  fixed effect of the grass species i
- $\varepsilon_{ij}$  residual error

# Results

### **Chemical composition**

Table 1 shows the chemical composition results for the sampled three indigenous and the introduced grass species. The significant differences in chemical composition between the introduced and indigenous grasses were observed for CP, ADF and EE. The introduced grasses (*C. gayana* var *Extozi rhodes*) had on average significantly higher CP (74.05 vs 52), ADF (444.9 vs 395.1 g/kg DM.), and EE contents (29.40 vs 22.99 g/kg DM), relative to indigenous grasses (*C. ciliaris, E. macrostachyus and E. superba*). The DM, NDF, OM, Ash and ADL recorded no significant difference between the introduced and indigenous grasses.

# In vitro gas production and fermentation Kinetics of the introduced and indigenous grass pastures studied

The in vitro cumulative gas production and the fermentation characteristics of the studied grasses are summarized in Table 2. There were significant differences in the cumulative gas production and fermentation kinetics among the studied grasses. The introduced grasses produced the highest gas at 72 h of incubation relative to the indigenous grasses (61.8 vs 42.3 ml). In general, the introduced grasses, tended to produce more gas and had more potential gas production rate than the indigenous grasses. The rate of gas production was highest (0.9 ml/hr) for introduced grasses and lowest (0.6 ml/hr) for indigenous grasses.

# Total *in-vitro* gas (GP), methane production, dry matter digestibility, organic matter digestibility and Metabolizable energy (ME) for the studied indigenous and introduced grass pastures

Table 3 presents the total *in-vitro* gas production (GP) and methane gas production expressed in ml/g DM at the end of 72 h incubation period, Metabolizable energy, Dry

matter digestibility and organic matter digestibility for the sampled three indigenous and two introduced grass species. Significant differences were noted in ME, DMD, OMD, CH4 and in total gas produced among the introduced and indigenous grasses. The highest values were obtained in introduced grasses relative to indigenous grass for (ME 16.35 vs 12.90, OMD 62.7 vs 53.6, CH4 25.61 vs 15 0.93 and GP 61.75vs 42.28) except for DMD which was highest in indigenous grasses relative to introduced (58.12 vs 54.24).

The cumulative gas and methane production pattern from the in vitro fermentation of the grass species is given in Fig. 2. The total volume and pattern of gas production varied among introduced and indigenous grasses. The introduced grasses produced the highest volume of gas/methane over all incubation times. The lowest gas/methane production was measured for *C. ciliaris* at 24 h, and *E. superba* during 72 h (Fig. 2).

Table 1Chemical composition(g.kg<sup>-1</sup> DM) of indigenous andintroduced grass species

Grass	Species	DM	СР	NDF	ADF	ADL	EE	ОМ	Ash
Indigenous	C. ciliaris	97.64 <sup>b</sup>	48.97 <sup>b</sup>	670.3 <sup>a</sup>	395.8 <sup>a,b</sup>	69.93 <sup>a</sup>	25.13 <sup>ab</sup>	914.9 <sup>a</sup>	85.1 <sup>c</sup>
	E. superba	96.86 <sup>a</sup>	63.13 <sup>c</sup>	703.7 <sup>b</sup>	401.0 <sup>b</sup>	65.40 <sup>a</sup>	21.97 <sup>a</sup>	923.3 <sup>bc</sup>	76.7 <sup>ab</sup>
	E. macrostachyus	97.91 <sup>b</sup>	44.23 <sup>a</sup>	749.9 <sup>d</sup>	388.6 <sup>a</sup>	85.27 <sup>b</sup>	21.87 <sup>a</sup>	915.6 <sup>ab</sup>	84.4 <sup>bc</sup>
	Average	97.47	52.11	708.0	395.1	73.50	22.99	917.9	82.1
Introduced	C. gayana var Extozi	97.63 <sup>b</sup>	80.97 <sup>e</sup>	702.5 <sup>b</sup>	440.7 <sup>c</sup>	63.00 <sup>a</sup>	28.60 <sup>bc</sup>	914.2 <sup>a</sup>	85.8 <sup>c</sup>
	C. gayana var Boma	96.98 <sup>a</sup>	67.13 <sup>d</sup>	722.9 <sup>c</sup>	449.2 <sup>c</sup>	78.00 <sup>b</sup>	30.20 <sup>c</sup>	925.4 <sup>c</sup>	74.6 <sup>a</sup>
	Average	97.30	74.05	712.7	444.9	70.50	29.40	919.8	80.2
	SEM	0.101	0.761	1.25	3.79	2.50	0.978	2.31	1.63
Grass effect		NS	**	NS	**	NS	**	NS	NS

DM Dry matter, CP Crude protein, NDF Neutral detergent fibre, ADF Acid detergent fibre, EE Ether extract, OM organic matter, ADL Acid detergent lignin

<sup>a-e</sup> Means within a column without a common letter superscript differ at p < 0.05. Grass effect not significant (NS) or significant at p < 0.05 (\*\*)

Category	Species	24 h	48 h	72 h	a+b	С
Indigenous	C. ciliaris	36.3 <sup>ab</sup>	39.0 <sup>b</sup>	42.8 <sup>b</sup>	42.8 <sup>b</sup>	0.6 <sup>b</sup>
	E.macrostachyus	41.7 <sup>bc</sup>	44.0 <sup>c</sup>	48.0 <sup>c</sup>	48.0 <sup>c</sup>	0.7 <sup>c</sup>
	E. superba	29.0 <sup>a</sup>	31.7 <sup>a</sup>	36.0 <sup>a</sup>	36.0 <sup>a</sup>	0.5 <sup>a</sup>
	Avarage	35.6	38.2	42.3	42.3	0.6
Introduced	C. gayana var Boma rhodes	50.0 <sup>c</sup>	69.0 <sup>e</sup>	63.0 <sup>e</sup>	63.0 <sup>e</sup>	0.9 <sup>e</sup>
	C. gayana var Extozi rhodes	50.2 <sup>c</sup>	56.8 <sup>d</sup>	60.5 <sup>d</sup>	60.5 <sup>d</sup>	0.8 <sup>d</sup>
	Avarage	50.1	62.9	61.8	61.8	0.9
	SEM	1.70	0.42	0.47	0.47	0.01
Grass effect		**	**	**	**	**

SEM is the Standard error of the means

<sup>a-e</sup> Means within a column without a common letter superscript differ at p < 0.05. Grass effect not significant (NS) or significant at p < 0.05 (\*\*); a, b and c are constants in the equation  $Y = a + b(1 - e^{-ct})$ .

a is the initial gas production, b is the gas produced during incubation, c is the constant gas production rate constant (fraction/hour), t is the time of fermentation, In this case, (a+b) represents the potential extent of the gas production.

Table 2In-vitro cumulative gasproduction (gas  $ml.g^{-1} DM$ )and fermentation kinetics /hour

characteristics

# Correlation between *in-vitro* methane (CH<sub>4</sub>) production and chemical composition

Methane production was negatively correlated with OM (-0.024), ADL (-0.023), DMD ( $-0.880^{**}$ ) of the grass species (Table 4, appendix). A significant positive correlation was noted between methane production and ADF ( $0.880^{**}$ ), CP ( $0.264^{**}$ ), EE ( $0.694^{**}$ ), ME ( $0.668^{**}$ ), OMD ( $0.568^{**}$ ) and Gas production ( $0.877^{**}$ ).

# Discussion

This study was conducted with the objective of comparing the digestibility and methane emission of introduced grass species (Chloris gayana var Boma rhodes and Extozi Rhodes) with the indigenous ones (E. superba, C. ciliaris and E. macrostachyus) in the rangelands ecosystem of Makueni County, Kenya. We evaluated the chemical composition, gas production and fermentation kinetics, total gas and methane production and the correlation between the measured parameters. The findings show that nutritive composition of the indigenous and introduced grasses in the present study were comparable with those of earlier reports in the same South Eastern rangelands of Kenya, from the studies by Ndathi et al., (2011) and Koech et al. (2016). The CP content of the indigenous grasses was below the 70 g.kg<sup>-1</sup> DM minimum requirement for optimal growth of rumen microbiota (Van Soest 1994), necessary to breakdown of cell wall content of forages. This suggests that utilization of these grasses for ruminants would supply sub-optimal nitrogen levels in the rumen, restricting microbial growth and activity, consequently hindering effective ruminal fermentation and limiting feed intake (Hariadi and Santoso 2010; NRC 2000).

The cell-wall contents (NDF, ADF and ADL) observed for both the indigenous and introduced grass species were

above the critical value for tropical grasses. For instance, the NDF levels for indigenous grasses in the range of 670.3 to 749.9 g.kg<sup>-1</sup> DM is above the critical value for tropical grasses of 600 g.kg<sup>-1</sup> to 650 g.kg<sup>-1</sup> DM (Van soest et al. 1991). In feeds, NDF value beyond the critical value is associated with low digestibility, prolonged digesta retention time in the rumen, which reduces the fermentation rate and increases methane production (Doreau et al. 2016). These authors found NDF to have significantly more influence on methane production than digestibility, implying a positive relationship between methane production and NDF content. Therefore, ruminants reared on these indigenous grasses may not achieve higher productivity due to nutritional limitations such as the high NDF that results also in increased methane production. NDF is a key driver to hydrogen production from carbohydrate fermentation in the rumen (Doreau et al. 2016) through the production of more acetate pathways and less propionate. The higher ME levels for introduced grasses may be due to higher CP values, which is supported by the fact that ME is directly proportional to CP content and inversely proportional to fibre constituents.

The present study recorded higher rate and extent of fermentation for introduced grasses compared to the indigenous ones. This could be related with their higher CP and overall lower content of NDF and ADF (Chino Velasquez et al. 2022). The introduced grass species (*Chloris gayana* var *Boma* and *Extozi rhodes*) produced also higher volumes of methane gas when compared to the indigenous grasses (*C. ciliaris, E. macrostachyus* and *E. superba*). A possible explanation could be the high fibre content particularly NDF, which is highly influential in hydrogen production from carbohydrate fermentation (Archimède et al. 2011; Doreau et al. 2016; Chino Velasquez et al. 2022). In addition, other fibre constituents like ADF, ADL, cellulose and hemicellulose which are important fibre fractions influencing CH<sub>4</sub> production in the rumen could also have

**Table 3** Total Gas and Methaneproduction (at 72 h *in-vitro*incubation) and calculatedMetabolizable energy (MJ/kgDM), Dry matter digestibility,Organic matter digestibilityfor three indigenous and twointroduced grasses studied inthe South Eastern rangelandsof Kenya

Grass	Species	Total gas (ml/gDM)	CH <sub>4</sub> (ml/g DM)	ME (MJ/kg DM)	DMD (%)	OMD (%)
Indigenous	C. ciliaris	42.83 <sup>b</sup>	17.19 <sup>a</sup>	11.46 <sup>a</sup>	58.07 <sup>b</sup>	46.08 <sup>a</sup>
	E. superba	36.00 <sup>a</sup>	14.30 <sup>a</sup>	14.37 <sup>c</sup>	57.66 <sup>b</sup>	56.45 <sup>b</sup>
	E. macrostachyus	48.00 <sup>c</sup>	16.29 <sup>a</sup>	12.89 <sup>b</sup>	58.63 <sup>b</sup>	58.31 <sup>c</sup>
	Average	42.28	15.93	12.90	58.12	53.6
Introduced	C. gayana var Extozi	60.50 <sup>d</sup>	25.64 <sup>b</sup>	16.83 <sup>e</sup>	54.57 <sup>a</sup>	61.75 <sup>d</sup>
	C. gayana var Boma	63.00 <sup>e</sup>	25.57 <sup>b</sup>	15.86 <sup>d</sup>	53.91 <sup>a</sup>	63.73 <sup>e</sup>
	Average	61.75	25.61	16.35	54.24	62.7
	SEM	0.471	0.977	0.125	0.295	0.058
Grass effect		**	**	**	**	**

DMD Dry matter digestibility, OMD Organic matter digestibility, CH<sub>4</sub> methane gas produced

<sup>a-e</sup> Means within a column without a common letter superscript differ at p < 0.05. Grass effect not significant (NS) or significant at p < 0.05 (\*\*)



Fig. 2 In-vitro Methane and total cumulative gas production trend for the indigenous and introduced grass species incubated for 72 h

been contributing factors in high methane production in the introduced grass pastures. The high NDF value modifies short fatty chain fraction towards acetate producing more hydrogen which is a major determinant in carbohydrate fermentations as observed by Migwi et al. (2013). These authors reported that intake of high fibre forages leads to a significant loss of energy from feeds to form CH<sub>4</sub> gas in ruminants. The findings of the present study were also consistent with those of Melesse et al. (2017) who observed a positive correlation between fibre constituents and CH<sub>4</sub> production, which was evident in our study for ADF not for NDF. Even though the authors in the latter study observed higher CH<sub>4</sub> production than in the present study (20.9 - 30.80 ml/ g DM), it should be noted that their diets had higher in-vitro OMD than the levels obtained in the present study.

The lower *in-vitro* organic matter digestibility of the indigenous grass species (*E. macrostachyus*) could be associated with high NDF and low CP levels. The low CP level could have supplied insufficient nitrogen for the proliferation of rumen microorganisms and hence lower fermentation levels compared to the other grasses. Additionally, the high NDF content in the grass could also mean a low supply of readily available energy to nourish the microbes hence further suppressing their activity to result in lower digestibility of the grass (NRC 2000).

We found a strong positive and significant correlation of ADF with  $CH_4$ , while NDF had a weak, though positive correlation with  $CH_4$  that was not significant. The correlation between carbohydrate fractions and cell wall constituents and methane production are also reported by Moss et al. (2000); Singh et al. (2012); Gemeda and Hassen (2014); Doreau et al. (2016). This makes carbohydrate fractions and cell wall constituents are better methane predicators compared to feed components. The positive correlation of CH<sub>4</sub> and CP constituents, although weak, was in agreement with the report by Kulivand and Kafilzadeh (2015). Studies report reduction of methane emission when CP increases but this is only the case for high CP feedstuffs (Melesse et al. 2017). This could be because CP contents above a critical threshold of 70 g/kg enhanced rumen microbial activity quickens fermentation and reduces retention time of digesta, resulting in lower methane production. The opposite is observed with CP values below this threshold as we observed in our study where all grass species had CP values slightly higher or below the threshold of 70 g/ kg (Hariadi and Santoso 2010). Both the indigenous and introduced grasses were high in fibre constituent (ADL, ADF, NDF), a major limitation to digestibility. The sample indigenous grass species in this study were of poorer nutritional value but lower methane gas production, relative to the introduced grass species. Indigenous grasses produced less CH<sub>4</sub> per gram dry matter of unit feed compared to the introduced grass pastures. Producing ruminants on the indigenous grasses thus would need nitrogen supplementation either inform of protein concentrates or leguminous fodder as recommended by other authors for such feeds (Korir et al. 2016; Sampaio et al. 2010).

# Conclusion

Introduced grass varieties had higher nutritive values than indigenous grasses which has prompted discussion about using them in Kenyan rangelands. However, this requires careful consideration, since the higher nutritional value of introduced grasses is still too low to support high livestock productivity and the methane emission of introduced grasses are higher than of indigenous grasses. Indigenous grasses, however, despite their lower methane emission are inadequate in quality to support higher ruminant productivity. We therefore conclude that alternative feeding strategies for rangelands need to be developed.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10113-023-02164-6.

Acknowledgements This research work was Funded by Africa Center of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM), a program at Egerton university funded by the World Bank, for which the authors are highly grateful. We acknowledge the Arid and rangeland research institute (ARLRI) Kiboko for allowing the collection of samples used in this study, international livestock research institute, Mazingira center in Nairobi for offering laboratory and technical support on Methane gas analysis using Gas chromatograph and University of Nairobi Animal production laboratory staff for technical advice and support in nutritive analysis and gas collection .Authors also acknowledge the support of the German Academic Exchange Service (DAAD) for covering the publication costs.

Funding Open Access funding enabled and organized by Projekt DEAL.

**Data Availability** Not applicable. Relevant data provided in supplementary file.

### Declarations

Conflict of interest The authors declare no conflict of interest.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

# References

- Abdulrazak SA, Fujihara T (1999) Animal nutrition: a laboratory manual. laboratory of animal science. faculty of life and environmental science. Shimane University Japan 1:24–28
- AOAC (1990) Official method of analysis (15th edn), Association of official analytical chemists, Washington D.C, U.S.A, pp 85–89

- Archimede H, Eugène M, Magdeleine CM, Boval M, Martin C et al (2011) Comparison of methane production between C3 and C4 grasses and legumes. Anim Feed Sci Technol 166:59–64. https:// doi.org/10.1016/j.anifeedsci.2011.04.003
- Archimède H, Rira M, Eugène M, Fleury J, Lastel ML et al (2018) Intake, total-tract digestibility and methane emissions of Texel and Blackbelly sheep fed C4 and C3 grasses tested simultaneously in a temperate and a tropical area. J Clean Prod 185:455–463. https:// doi.org/10.1016/j.jclepro.2018.03.059
- Berndt A, Tomkins N (2013) Measurement and mitigation of methane emissions from beef cattle in tropical grazing systems: a perspective from Australia and Brazil. Animal 7:363–372. https://doi.org/ 10.1017/S1751731113000670
- Bezabih M, Pellikaan WF, Tolera A, Khan NA, Hendriks WH (2014) Chemical composition and *in vitro* total gas and methane production of forage species from the Mid Rift Valley grasslands of Ethiopia. Grass Forage Sci 69(4):635–643. https://doi.org/10. 1111/gfs.12091
- Bhatta R, Tajima K, Takusari N, Higuchi K, Enishi O et al (2007) Comparison of *in vivo* and *in vitro* techniques for methane production from ruminant diets. Asian Australas J Anim Sci 20(7):1049– 1056. https://doi.org/10.5713/ajas.2007.1049
- Chino Velasquez LB, Molina-Botero IC, Moscoso Muñoz JE, Gómez Bravo C (2022) Relationship between Chemical Composition and *In Vitro* Methane Production of High Andean Grasses. Animals 12(18):2348. https://doi.org/10.3390/ani12182348
- CIMMYT (2013) CIMMYT Kiboko Crops Research Station: A Brief and Visitors' Guide. (Field Visitors Manual). CIMMYT, Nairobi, Kenya (2013)
- Doreau M, Benhissi H, Thior YE, Bois B, Leydet C et al (2016) Methanogenic potential of forages consumed throughout the year by cattle in a Sahelian pastoral area. Anim Prod Sci 56(3):613618. https://doi.org/10.1071/AN15487
- Eckard RJ, Grainger C, De Klein CAM (2010) Options for the abatement of methane and nitrous oxide from ruminant production: a review. Livest Sci 130(1–3):47–56. https://doi.org/10.1016/j. livsci.2010.02.010
- Gemeda BS, Hassen A (2014) In vitro fermentation, digestibility and methane production of tropical perennial grass species. Crop Pasture Sci 65(5):479–488. https://doi.org/10.1071/CP13450
- Haque MN, Cornou C, Madsen J (2014) Estimation of methane emission using the CO2 method from dairy cows fed concentrate with different carbohydrate compositions in automatic milking system. Livest Sci 164:57–66. https://doi.org/10.1016/j.livsci.2014.03.004
- Hariadi BT, Santoso B (2010) Evaluation of tropical plants containing tannin on in vitro methanogenesis and fermentation parameters using rumen fluid. J Sci Food Agric 90(3):456–461. https://doi. org/10.1002/jsfa.3839
- Johnson KA, Johnson DE (1995) Methane emissions from cattle. J Anim Sci 73(8):2483–2492. https://doi.org/10.2527/1995.73824 83x
- Koech OK, Kinuthia RN, Karuku GN, Mureithi SM, Wanjogu R (2016) Field curing methods and storage duration affect the quality of hay from six rangeland grass species in Kenya. Ecol Process 5(1):1–6. https://doi.org/10.1186/s13717-016-0048-2
- Korir D, Goopy JP, Gachuiri C, Butterbach-Bahl K (2016) Supplementation with Calliandra calothyrsus improves nitrogen retention in cattle fed low-protein diets. Anim Prod Sci 56(3):619–626. https:// doi.org/10.1071/AN15569
- Kulivand M, Kafilzadeh F (2015) Correlation between chemical composition, kinetics of fermentation and methane production of eight pasture grasses. Acta Sci Anim Sci 37:914. https://doi.org/ 10.4025/actascianimsci.v37i1.24336
- Maweu AN, Bebe BO, Kuria SG, Kashongwe OB (2022) Dry matter production and carbon sequestration potential of selected indigenous and introduced grasses under rangeland ecosystems of South

Eastern Kenya. Earth Environ Sci Res Rev 5(3):61–67. https://doi. org/10.33140/EESRR.05.03.04

- Melesse A, Steingass H, Boguhn J, Rodehutscord M (2013) In vitro fermentation characteristics and effective utilisable crude protein in leaves and green pods of Moringa stenopetala and Moringa oleifera cultivated at low and mid-altitudes. J Anim Physiol Anim Nutr 97(3):537–546. https://doi.org/10.1111/j.14390396.2012.01294.x
- Melesse A, Steingass H, Schollenberger M, Rodehutscord M (2017) Screening of common tropical grass and legume forages in Ethiopia for their nutrient composition and methane production profile in vitro. Trop Grassl-Forrajes Trop 5(3):163–175. https://doi.org/10.17138/tgft(5)163–175
- Menke KH, Steingas HH (1988) Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim Res Dev 28:7–55
- Menke KH, Raab L, Salewski A, Steingass H, Fritz D et al (1979) The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor. J Agric Sci 93:217–222. https://doi.org/10.1017/S0021859600086305
- Mganga KZ, Musimba NKR, Nyariki DM, Nyangito MM, Mwang'ombe AW (2015) The choice of grass species to combat desertification in semi-arid Kenyan rangelands is greatly influenced by their forage value for livestock. Grass Forage Sci 70(1):161–167. https://doi.org/10.1111/gfs.12089
- Migwi PK, Bebe BO, Gachuiri CK, Godwin I, Nolan JV (2013) Options for efficient utilisation of high fibre feed resources in low input ruminant production systems in a changing climate: A review. University of Nairobi. https://hdl.handle.net/11295/85114
- Moss AR, JouanyJ P, Newbold J (2000) Methane production by ruminants: Its contribution to global warming. Annales de Zootechnie 49:231–253. https://doi.org/10.1051/animres: 2000119
- Mutiso PM, Kinama JM, Onyango C (2018) Effect of in situ moisture conservation techniques on yield and water use efficiency of pearl millet in Makueni, Kenya. Int J Agron Agric Res.12(6):186–196. http://erepository.uonbi.ac.ke/handle/11295/155209
- Ndathi AJ, Nyangito MM, Musimba NK, Mitaru BN (2011) Climate variability and dry season ruminant livestock feeding strategies in Southeastern Kenya. Livest Res Rural Dev 23(9). http://www.lrrd. org/lrrd23/9/ndat23199.htm. Accessed 8 Dec 2022
- NRC (2000) Nutrient Requirements of Beef Cattle: 7th Revised Edition: Washington, DC: The National Academies Press. https://doi. org/10.17226/9791
- O'Mara FP (2011) The significance of livestock as a contributor to global greenhouse gas emissions today and in the near future.

Anim Feed Sci Technol 166:715. https://doi.org/10.1016/j.anife edsci.2011.04.074

- Ørskov ER, McDonald I (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric Sci 92(2):499–503. https://doi.org/10. 1017/S0021859600063048
- Patra AK (2016) Recent advances in measurement and dietary mitigation of enteric methane emissions in ruminants. Front Vet Sci 3:39. https://doi.org/10.3389/fvets.2016.00039
- Pellikaan WF, Hendriks WH, Uwimana G, Bongers LJGM, Becker PM et al (2011) A novel method to determine simultaneously methane production during in vitro gas production using fully automated equipment. Anim Feed Sci Technol 168(3–4):196–205. https:// doi.org/10.1016/j.anifeedsci.2011.04.096
- Sampaio CB, Detmann E, Paulino MF (2010) Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. Trop Anim Health Prod 42:1471–1479. https://doi.org/10.1007/s11250-010-9581-7
- Shrestha S, Ciaian P, Himics M, Van Doorslaer B (2013) Impacts of climate change on EU agriculture. Rev Agric Appl Econ (RAAE) 16(395-2016–24317):24–39. https://doi.org/10.22004/ag.econ. 158096
- Singh S, Kushwaha BP, Nag SK, Mishra AK, Singh A et al (2012) In vitro ruminal fermentation, protein and carbohydrate fractionation, methane production and prediction of twelve commonly used Indian green forages. Anim Feed Sci Technol 178(1–2):2–11. https://doi.org/10.1016/j.anifeedsci.2012.08.019
- Van Soest PJ (1994) Nutritional ecology of the ruminant. Cornell University Press, Ithaca, p 476
- Van soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597. https:// doi.org/10.3168/jds.S0022-0302(91)78551-2
- Waghorn GC, Tavendale MH, Woodfield DR (2002) Methanogens from forages fed to sheep. In Proceedings of the New Zealand Grassland Association (pp. 167171). https://doi.org/10.33584/ jnzg.2002.64.2462

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.