

Research

Impacts of herbage distribution, botanical composition, and rumen inoculum source on in vitro fermentation kinetics

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Received: 27 September 2024 / Accepted: 16 January 2025

Published online: 20 March 2025

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Abstract

The random sourcing of rumen liquor from abattoir-slaughtered cattle may influence in vitro gas production results due to variations in diet composition. This study evaluated the effects of herbage distribution, botanical composition, and rumen liquor source on rumen in vitro fermentation kinetics. Three districts in Uganda's cattle corridor with diverse grazing systems and vegetation types were purposively selected: Mbarara (improved mixed pastures), Nakasongola (woody shrublands), and Napak (grasslands). Karamoja had the highest herbage diversity ($n = 40$), followed by Mbarara ($n = 32$) and Nakasongola ($n = 19$), with grasses comprising over 75% of samples from Nakasongola and Mbarara, and 53% from Karamoja. Karamoja herbage exhibited significantly higher crude protein (132.6 g/kg DM). Gas production varied ($P < 0.001$) across herbage sources during incubation. Fermentation kinetics were influenced by both the rumen liquor and herbage source ($P < 0.001$), while gas production rate from the insoluble fraction (b) was only affected by herbage source ($P < 0.001$). In vitro organic matter digestibility (IVOMD), metabolizable energy, and short-chain fatty acids were also significantly influenced by herbage source. Herbage composition had a greater impact on fermentation kinetics and IVOMD than the source of rumen liquor. These results suggest that rumen liquor from cattle raised under similar grazing systems yields consistent in vitro gas production results. Consequently, these results demonstrate that rumen fluid can be sourced from animals slaughtered in abattoirs if precaution is taken to use rumen fluid from animals on a comparable diet.

Keywords Rumen fluid · Cattle corridor · In vitro technique · Pasture quality

1 Introduction

Ruminants efficiently utilize plant-based nutrients through microbial fermentation in the rumen [1, 2]. The diversity of rumen microbes and the quality of feed significantly influence nutrient extraction efficiency [2–4]. During rumen fermentation in cattle, methane represents a significant loss of energy from feed intake [5]. Factors such as feed intake, nutrient

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s44338-025-00049-y>.

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composition, and digestibility directly affect methane emissions [6, 7]. Cattle grazing on improved pastures emit less methane than those grazing on poor-quality tropical pastures. Additionally, diverse pastures enhance milk solids and nitrogen utilization while reducing methane emissions and nitrogen excretion [8].

Uganda's cattle corridor, stretches from the southwest to the northeast parts of the country, spanning varying agro-ecological zones that influence vegetation's botanical composition [9, 10]. The diversity in grasses, legumes, and forbs affects forage digestibility and methane yield [11]. This botanical variation also impacts rumen passage kinetics and the composition of rumen microbiota [12, 13], which can influence in vitro gas analysis results. This technique, commonly used in Uganda, is a low-cost alternative to screening ruminant feed resources with rumen-fistulated animals.

Rumen inoculum for in vitro gas assays is typically sourced from abattoir-slaughtered cattle aggregated from various regions, often without knowledge of their production systems or feed sources. This variability in cattle origin and feed resources may impact the accuracy of nutritional assessments. In Uganda the main cattle producing districts are found in the cattle corridor. In the central region, Nakasongola is the largest source beef cattle, Mbarara districts represents cattle production systems with improved pasture availability using rotational grazing, while Karamoja is the main source of cattle from nomadic pastoral system. Consequently, these districts were purposively selected to capture the biodiversity in forage availability as influenced by climatic condition and rangeland management practices, as well as the diversity in cattle management systems along the corridor. The study aimed to determine the effects of spatial herbage distribution, botanical composition, and rumen inoculum source on in vitro gas fermentation kinetics. We hypothesized that fermentation kinetics vary depending on herbage sampling location, botanical composition, and the rumen inoculum source.

2 Materials and methods

2.1 Description of study sites used as sources rumen liquor and herbage

Herbage samples and rumen liquor for this study were sourced from three districts in Uganda's cattle corridor: Nakasongola, Mbarara, and Napak (Karamoja) (Fig. 1). These districts were selected to represent variations in open grazing systems across three agro-ecological zones (AEZs). Nakasongola, located at an elevation of 1000–1400 m in the pastoral rangelands AEZ, receives annual rainfall of 1000–1600 mm across two distinct rainy seasons. Temperatures range from 15 to 33.7 °C [14]. The dominant cattle production system is open grazing on woody shrublands. Mbarara, situated in the southwestern farmlands AEZ, receives around 1200 mm of annual rainfall, with temperatures between 17 and 30 °C. Open grazing in ranches with improved mixed pastures is the predominant cattle production system. Napak, referred to as Karamoja in this study, is located at an elevation of 1356–1524 m in the northeastern drylands AEZ. It experiences an average annual rainfall of 800 mm, with a range of 300–1200 mm, and temperatures between 28 and 33 °C [15]. The primary cattle production system in Napak is open grazing on grasslands. These districts provide diverse environmental conditions and grazing systems, offering a representative sample for studying the effects of spatial herbage distribution and rumen inoculum on fermentation kinetics.

3 Data collection

3.1 Grazed pasture composition and sample collection

A GPS receiver was used to record the coordinates of the study area. The botanical composition of pastures was assessed through visual appraisal of all herbage in 1 m² quadrat. The grazing field was divided into 1 km grids, with 3 randomly selected for study. In each grid, three parallel transect lines, spaced 100 m apart and running 1000 m from south to north, were established. Along each transect, 10 quadrats (1 m²) were placed 50 m apart on alternating sides, starting 5 m away from a trail to avoid edge effects. In total, 30 quadrats were sampled per grid, with 2 grids sampled per field. The forage samples were collected during the rainy season of in the month of May when there is sufficient forage growth across the districts of the cattle corridor.

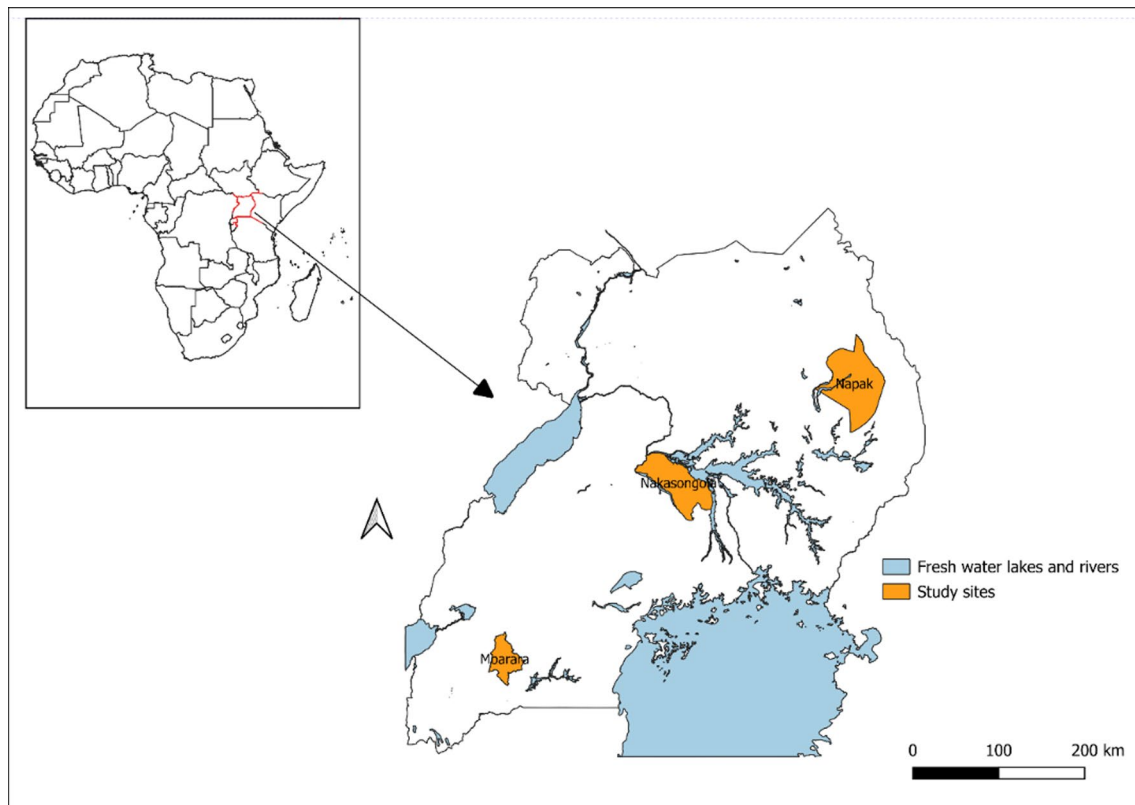


Fig. 1 Map of Uganda showing the location of the study sites, created using QGIS software by Muhamad Kiggundu

3.2 Collection of feed samples

At each study farm, 10 random forage quadrats (1 × 1 m) were sampled from the main grazing field by walking in a “W” pattern. A botanist assisted with visual identification of all plant species within each quadrat, which were then recorded. After identification, the vegetation in each quadrat was clipped at ground level, bagged, and weighed fresh before being transported to the lab for chemical analysis. In addition to the herbage samples from the three study sites, *Chloris gayana* hay was included as the fourth test feed resource.

3.3 Sources of rumen inoculum

Rumen fluid was collected from six mature donor bulls (two from each of the three selected districts representing the open grazing system), since majority of the animals slaughtered at the regional abattoirs are mostly bull culls from cattle raised from the cattle corridor. The bulls were humanely transported by truck to a central slaughterhouse in Kampala, near Makerere University’s Animal Science Laboratory, after being fasted for at least 8 h. Handling and care of the animals before and during slaughter complied with Uganda’s Animal (Prevention of Cruelty) Act of 2000 [16].

3.4 Chemical analysis

The Representative forage samples designated for chemical composition analysis were ground to pass through a 1 mm screen. The ground samples were analyzed for CP and EE AOAC [17]. Neutral detergent fiber exclusive of residue ash (NDF) and determined without the addition of alpha-amylase or sodium sulfite, acid detergent fiber (ADF), and ADL were determined according to [18]. The forages were oven-dried at 60 °C for 48 h for dry matter (DM) determination. The oven-dried samples were then bulked (a random of 3 and 2 forage quadrants) into two composite samples, and ground through a 1-mm screen. The ground samples were then analyzed for crude protein (CP), ether extract (EE), and Ash according to [19], as well as neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) according to [20] Gross energy (GE) content was determined using a bomb calorimeter (Gallenkamp auto bomb, UK).

3.5 In vitro fermentation and gas production

In vitro gas production analysis was conducted at the Animal Science Laboratory of Makerere University's College of Agriculture and Environmental Sciences, following the protocol by Menke and Steingass [21]. Rumen fluid was collected from two indigenous mature bulls (3 – 4 years of age) from each selected district, which were randomly chosen and fasted for at least 12 h before slaughter. The age of the animals was estimated based on dentition. Both the liquid and particulate fractions of the rumen fluid from each animal were placed in pre-warmed insulated thermos flasks, immediately sealed, and transported to the laboratory. At the lab, the rumen contents from the two bulls from each district were pooled within district and homogenized using a kitchen blender (PHILLIPS) while continuously flushing with carbon dioxide to obtain representative rumen fluid samples as earlier described by Spanghero et al. [22]. The blended rumen fluid was then filtered through four layers of cheesecloth under constant CO₂ flushing [23]. The strained rumen fluid was mixed with a buffer solution (artificial saliva) in a 1:2 ratio, stirred with a magnetic stirrer, and maintained at 39 °C in a water bath. For each test feed, approximately 200 mg (0.2 g) of samples, ground to pass through a 1-mm sieve, were weighed and placed into 100 ml graduated gas syringes. To each syringe, 30 ml of the rumen fluid-buffer mixture was added. The syringes were then incubated in a water bath at 39 °C and gently shaken for 30 min after incubation began. Three blank syringes containing only the rumen fluid-buffer mixture were incubated alongside the test samples. Gas production was measured at 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, and 120-h post-incubation. Net gas production for each feed sample was calculated by subtracting the average gas volume from the blanks from the total gas produced by the test samples.

3.6 In vitro organic matter digestibility, energy values and yield of short-chain fatty acids

The model [24] was fitted to the net gas volume data using the PROC NLIN of SAS [28]. The fermentation curves in the model were described by the exponential relation below.

$$Y = a + b(1 - e^{(-c(t-L))}) \quad (1)$$

where y = total gas production at time t; a = gas production from the readily soluble fraction (the intercept of the gas production curve); b = gas production from the insoluble fraction; a + b = the potential gas production (the asymptote of the gas production curve); c = the rate constant for the gas production from the insoluble fraction (b), t = incubation time. The in vitro organic matter digestibility (IVOMD) and metabolizable energy (ME) contents of samples were calculated based on the net 24 h gas volume, CP, EE and ash contents [22] according to the following equations:

$$\text{IVOMD (g/kg DM)} = 14.88 + 0.889 \times (\text{ml gas produced at 24 h}) + 0.0448 \times (\text{CP g/kg DM}) + 0.0651 \times (\text{Ash g/kg DM}) \quad (2)$$

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \times (\text{ml gas produced at 24 h}) + 0.057 \times (\text{CP g/kg DM}) + 0.00029 \times (\text{EE g/kg DM})^2 \quad (3)$$

In vitro yield of short-chain fatty acids was estimated according to Makkar [26]

$$\text{SCFA (mmol)} = 0.0222 \times (\text{ml gas produced at 24 h}) - 0.00452 \quad (4)$$

3.7 Statistical analysis

Initially, data normality checks were performed on each variable using PROC UNIVARIATE and the distribution pattern confirmed using the Shapiro–Wilk test due to the small sample size used in the study. A p-value higher than 0.05 was used as confirmation of the normality of the data. Data on the chemical composition of samples were analyzed using the PROC GLM procedure of SAS using the following model. In vitro gas production, IVOMD and ME with feed as the main factor using the PROC MIXED procedure with the model;

$$Y_{ij} = \mu + F_i + e_{ij}$$

where Y_{ij} is the independent variable, μ is the overall mean effect, F_i is the effect of source of test feed, and e_{ij} is the random error.

On the other hand, data on gas production (GP), gas kinetics, IVOMD, ME and yield of SCFA were analyzed using the PROC MIXED procedure of SAS [25] with the model;

$$Y_{ijk} = \mu + S_i + F_j + (SF)_{ij} + e_{ijk}$$

where Y_{ij} is the independent variable, μ is the overall mean effect, S_i is the effect of the source of rumen liquor, F_j is the effect of source of feed, $(SF)_{ij}$ is the interaction between source of rumen liquor and the source of feed and e_{ijk} is the random error. The LSMEANS statement was used to separate the least square means using the PDIF option SAS at $P < 0.05$ level of significance.

4 Results

4.1 Botanical composition of the herbage

Animals grazed on a mix of pasture species across all three study sites, as detailed in Supplementary Table S1. The ten predominant plant species and their proportions at each site are presented in Table 1. Karamoja featured a diverse blend of grasses, legumes, and herbs, while Mbarara and Nakasongola primarily consisted of grasses and legumes, with grasses being the most prevalent. The three dominant species in Karamoja were *Dactyloctenium aegyptium*, *Urochloa setigera*, and *Cynodon dactylon*. In Mbarara, the key species included *Brachiaria decumbens*, *Sporobolus pyramidalis*, and *Digitaria abyssinica*. Nakasongola's dominant species were *Hyparrhenia filipendula*, *Sporobolus pyramidalis*,

Table 1 Percentage contribution of the ten predominant forage species in each of the study sites

Species	Family name	Lifeform	Study sampling site		
			Karamoja	Mbarara	Nakasongola
<i>Dactyloctenium aegyptium</i> (L.) Willd	Poaceae	Grass	10.3	–	–
<i>Urochloa setigera</i>	Poaceae	Grass	9.2	–	–
<i>Cynodon dactylon</i>	Poaceae	Grass	8	6.7	–
<i>Oxygonum sinuatum</i>	Polygonaceae	Herb	7.5	–	–
<i>Digitaria longiflora</i>	Poaceae	Grass	5.7	–	–
<i>Setaria verticillata</i>	Poaceae	Grass	5.7	–	–
<i>Commelina benghalensis</i>	Commelinaceae	Herb	4.6	–	–
<i>Justicia exigua</i>	Acanthaceae	Herb	3.4	–	–
<i>Tephrosia pumila</i>	Papilionaceae	Legume	2.9	–	–
<i>Indigofera hochstetteri</i>	Papilionaceae	Legume	2.9	–	–
<i>Brachiaria decumbens</i>	Poaceae	Grass	–	16	11
<i>Sporobolus pyramidalis</i>	Poaceae	Grass	–	14.7	11.9
<i>Digitaria abyssinica</i>	Poaceae	Grass	–	6.7	–
<i>Chloris gayana</i>	Poaceae	Grass	–	5.5	–
<i>Hyparrhenia filipendula</i>	Poaceae	Grass	–	4.3	18.6
<i>Panicum maximum</i>	Poaceae	Grass	–	4.3	–
<i>Loudetia kagerensis</i>	Poaceae	Grass	–	4.3	–
<i>Neonotonia wightii</i>	Papilionaceae	Legume	–	3.7	–
<i>Bothriochloa insculpta</i>	Poaceae	Grass	–	3.7	–
<i>Brachiaria comata</i>	Poaceae	Grass	–	–	11.9
<i>Sporobolus festivus</i>	Poaceae	Grass	–	–	10.2
<i>Asystasia mysorensis</i>	Acanthaceae	Herb	–	–	7.6
<i>Paspalum scrobiculatum</i>	Poaceae	Grass	–	–	3.4
<i>Themeda triandra</i>	Poaceae	Grass	–	–	2.5
<i>Kyllinga bulbosa</i>	Cyperaceae	Sedge	–	–	2.5
<i>Kyllinga colorata</i>	Cyperaceae	Sedge	–	–	2.5

Table 2 Percentage composition of different plant species in herbage collected from the study districts

	Source of herbage		
	Karamoja	Nakasongola	Mbarara
Grasses	53.1	76.9	79.5
Forbs	33.9	6.0	7.6
Legumes	8.7	12.9	5.8
Others	4.3	1.6	9.1

Table 3 Chemical composition (g/kg DM) of *Chloris gayana* and forage samples collected from different districts from Uganda's cattle corridor

	Source of herbage				
	Karamoja	Mbarara	Nakasongola	Chloris	SEM
Crude protein (CP)	132.6 ^a	74.8 ^c	71.5 ^c	102.8 ^b	1.35
Ether extracts (EE)	13.8 ^b	13.8 ^b	14.8 ^a	14.6 ^{ab}	0.24
Crude fiber (CF)	234.4 ^c	326.7 ^a	306.7 ^b	317.6 ^{ab}	3.95
Neutral detergent fiber (NDF)	480.0 ^c	630.0 ^a	590.0 ^b	610.0 ^{ab}	8.66
Acid detergent fiber (ADF)	200.0 ^c	310.0 ^{ab}	270.0 ^b	340.0 ^a	12.25
Non fiber carbohydrates (NFC)	234.8 ^a	194.2 ^b	205.9 ^{ab}	157.3 ^c	8.71
Nitrogen free extracts (NFE)	480.4 ^b	497.5 ^a	489.2 ^{ab}	449.7 ^c	4.01
Ash	138.8 ^a	87.3 ^c	117.8 ^b	115.4 ^b	1.98

Means within rows with different superscripts differ significantly at $P < 0.05$, SEM=standard error of the mean

and *Brachiaria comata*. Only *C. dactylon* was common between Karamoja and Mbarara, while *B. decumbens*, *S. pyramidalis*, and *H. filipendula* were shared between Mbarara and Nakasongola.

Table 2 summarizes the aggregated lifeforms of forage species available to grazing animals across the study sites. In Karamoja, grasses constituted 53% of the sampled herbage, followed by forbs. In contrast, nearly 80% of the herbage sampled in Nakasongola and Mbarara districts was comprised of grasses. Notably, herbage from Nakasongola contained a higher proportion of legumes compared to the other two districts.

4.2 Herbage chemical composition

The chemical composition of *Chloris gayana* and herbage collected from different districts along Uganda's cattle corridor as well as the *Chloris gayana* hay used for the in vitro gas trial is presented in Table 3.

The CP content ranged from 132.6—71.5 g/kg DM. Forage samples from Karamoja had significantly higher CP, while forages collected from Nakasongola and Mbarara had the least CP content. On the other hand, the *Chloris gayana* and herbage from Nakasongola ether extracts but higher than ether extracts in herbage from Karamoja and Mbarara. Generally, herbage from Mbarara had significantly higher CF and NDF contents compared with *Chloris gayana* and herbage from the other districts. CF and NDF content of the herbage ranged from 326.7 – 234.4 g/kg DM and 630 – 480 g/kg DM respectively.

Cumulative gas production from incubated herbage samples, regardless of rumen liquor source, is shown in Fig. 2. Samples from Karamoja produced the most gas, while Mbarara pastures produced the least.

Figure 3 illustrates gas production when pastures from different districts were incubated with rumen liquor from the selected districts. Consistently, Karamoja pastures yielded the highest gas volume, while Mbarara pastures produced the least, regardless of rumen liquor source.

In the first 2 h of incubation, gas production differences among pasture samples were minimal (Fig. 4), but significant differences emerged after 4 h of incubation.

Fig. 2 Gas production profiles for different herbage incubated with rumen liquor collected from different locations

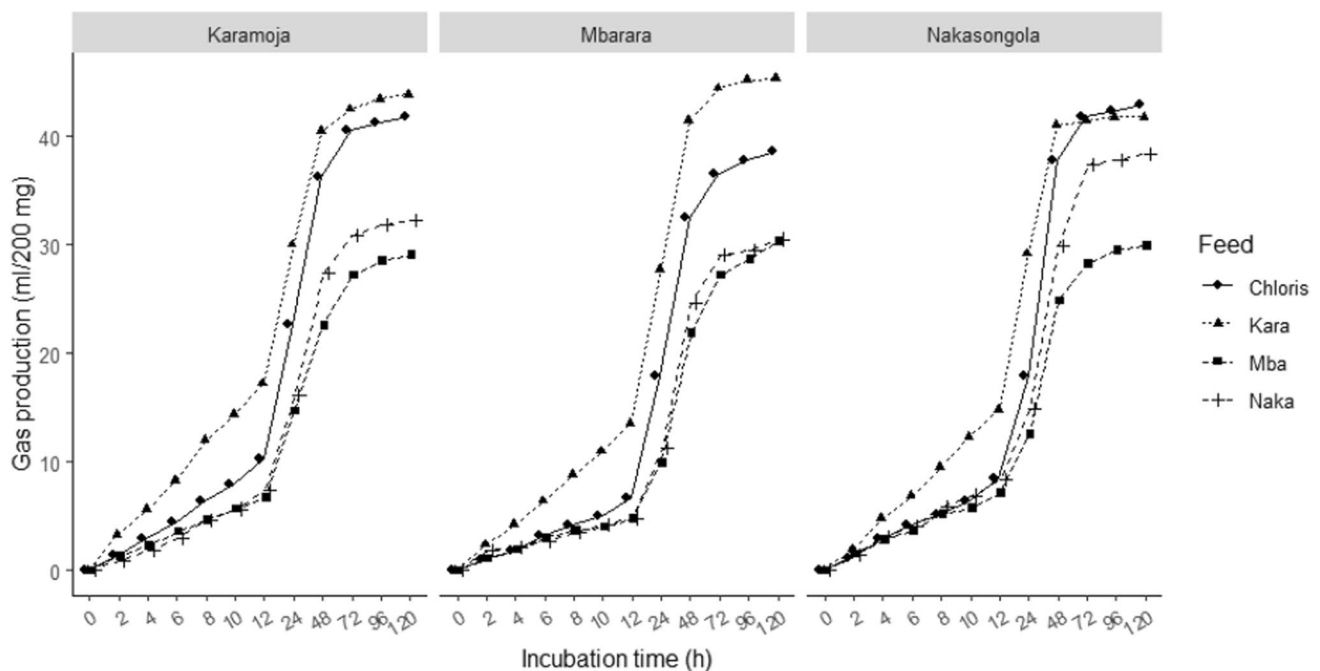
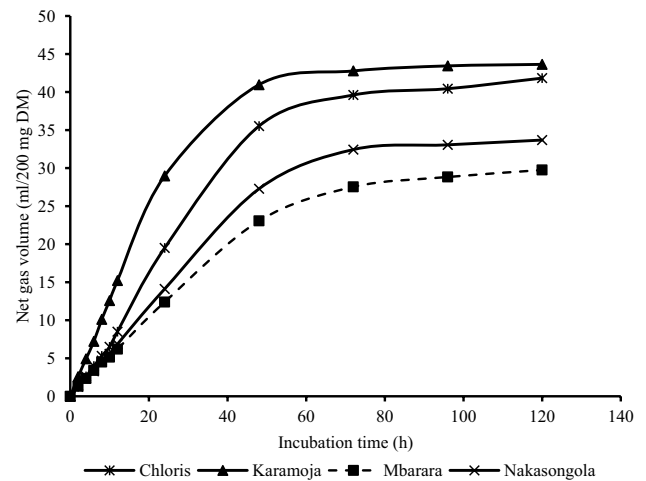


Fig. 3 Cumulative gas production from incubated herbage as affected by source of rumen liquor

4.3 In vitro gas fermentation characteristics

The in vitro net gas volumes from herbage samples varied significantly ($P < 0.001$) based on the source of herbage during incubation (Table 4), with no significant interaction between the source of rumen liquor and herbage source. The highest net gas production at 24 h was from Karamoja herbage (29.0 ml), while the lowest was from Mbarara (12.4 ml), a trend consistent at 48 and 72 h.

In vitro gas kinetics were significantly influenced ($P < 0.001$) by both the sources of rumen liquor and herbage (Table 4). Gas production from the readily soluble fraction (a) was unaffected by the rumen liquor source ($P > 0.05$), with Karamoja herbage yielding the highest gas. Gas production from the insoluble fraction (b) was not influenced by rumen liquor ($P > 0.05$) but was significantly affected ($P < 0.001$) by herbage source, with Mbarara yielding the highest rate (0.110 ml/h) and Karamoja the lowest when incubated with its own rumen liquor. Both the gas production constant from the insoluble fraction (c) and lag time (L) were influenced ($P < 0.05$) by the sources of rumen liquor and herbage. In vitro organic matter digestibility (IVOMD) estimated metabolizable energy (ME), and short-chain

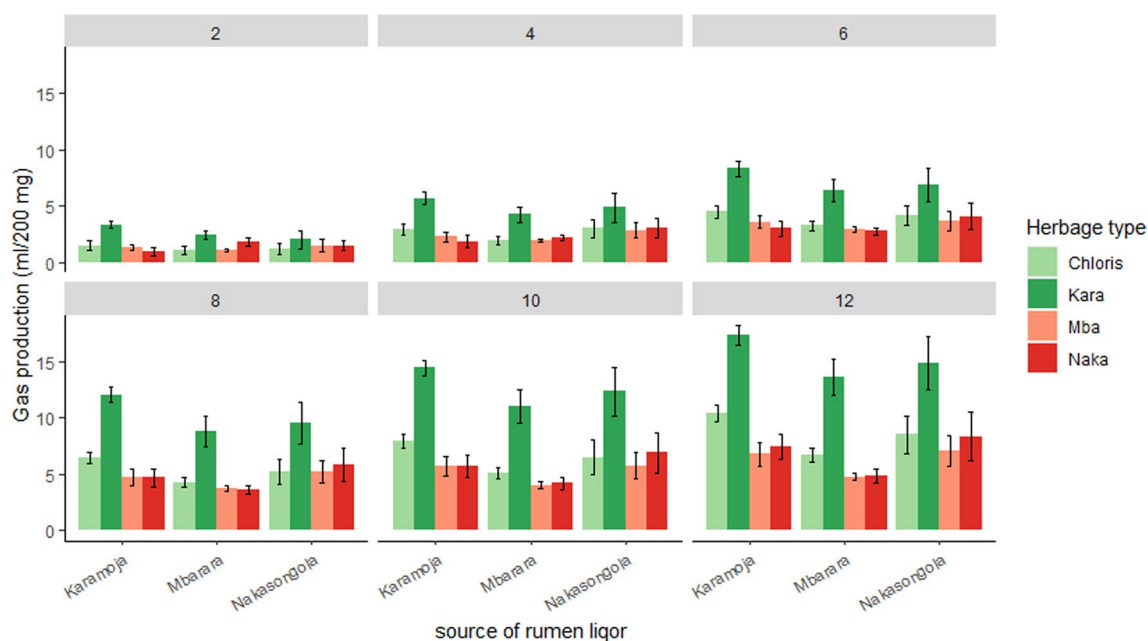


Fig. 4 Gas production from incubated herbage as affected by source of rumen liquor

fatty acids (SCFA) were significantly ($P < 0.001$) affected by herbage source, with the highest IVOMD and ME recorded when using rumen liquor from Karamoja incubated with herbage from the same region. Karamoja also produced the highest SCFA yield, while Mbarara herbage yielded the lowest SCFA estimate.

5 Discussion

Karamoja exhibited greater pasture species richness and diversity compared to Nakasongola and Mbarara. This aligns with previous findings highlighting Karamoja's diverse natural pastures [9]. Unlike the other districts, Karamoja's grazing grounds are communally managed [27], where livestock graze exclusively on natural, unimproved pastures. Being a communal resource, pasture improvement has barely been practiced or seldom adopted. In contrast, Nakasongola and Mbarara have benefited from pasture rehabilitation programs [28], which introduced monocrops of improved species through reseeding and oversowing. These interventions, while enhancing productivity, have reduced species richness in favor of dominant, high-yield species. This likely explains the higher species diversity observed in Karamoja compared to the other districts.

In Nakasongola and Mbarara, farmers have cleared brush and thickets to expand grazing areas, favoring grass species over other vegetation types. This study's findings of grasses comprising over 78% of sampled species, align with this trend. Additionally, the historical use of fire as a management tool in Uganda's rangelands has promoted the rapid regeneration of herbaceous grasses over woody species [29]. The dominant grass species in Nakasongola and Mbarara were similar but differed substantially from those in Karamoja. This difference may result from varying ecological drivers, such as rainfall patterns and human activities. In both Nakasongola and Mbarara, *Brachiaria* and *Sporobolus* grasses predominated, consistent with previous reports [30].

Forage samples from Karamoja had significantly higher crude protein (CP) content (132.6 g/kg DM) compared to those from Nakasongola and Mbarara. This difference may be attributed to the greater botanical diversity in Karamoja's pastures. Diverse forage species often offer superior nutrient profiles, which explains the higher CP levels. In contrast, the dominant species in Nakasongola and Mbarara, such as *Sporobolus*, tend to become fibrous quickly, leading to lower CP concentrations in the sampled biomass. Grass-dominated swards typically have higher neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractions, which contribute to lower CP content compared to swards with a higher proportion of forbs and legumes [31]. The average forage composition from Karamoja in this study exceeded values reported by Atuhaire et al. [32]. This was likely due to differences in sampling times and the

Table 4 Net cumulative gas production volumes (ml/200 mg DM), gas production kinetics and estimated nutritional parameters based on 24 h GP as affected by the source of rumen liquor (S) and feed source (F)

	Source of rumen fluid												Significance			
	Karamoja						Mbarara						Nakasongola			
	Chlo		Mba		Naka		Chlo		Kara		Mba		Naka		Chlo	
Incubation time (h)																
24	22.7	30.0	14.7	16.1	16.1	17.9	17.9	27.8	27.8	9.9	11.3	17.9	29.2	12.6	14.9	4.04
48	36.3	40.5	22.5	27.3	27.3	32.5	32.5	41.4	41.4	21.8	24.6	37.8	41.0	24.8	29.9	4.97
72	40.6	42.5	27.2	30.8	30.8	36.5	36.5	44.4	44.4	27.2	29.1	41.8	41.4	28.3	37.4	4.66
Gas production kinetics [†]																
a (ml)	16.5	17.6	10.3	13.1	13.1	14.1	14.1	19.3	19.3	10.4	11.0	16.1	18.3	11.6	14.4	2.30
b (ml)	16.3	27.0	12.6	11.9	11.9	14.8	14.8	17.9	17.9	10.6	11.1	16.5	21.3	11.0	14.2	2.44
c (ml h ⁻¹)	0.110	0.045	0.099	0.099	0.099	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.079	0.110	0.110	0.006
L (h)	9.0	12.8	8.9	12.4	12.4	9.0	9.0	9.0	9.0	9.0	9.0	9.0	11.0	9.0	9.0	0.57
Estimated parameters [‡]																
IVOMD (g/kg DM)	471.5 ^{ab}	565.3 ^a	369.5 ^{cd}	400.6 ^{bcd}	400.6 ^{bcd}	429.3 ^{bc}	429.3 ^{bc}	545.3 ^a	545.3 ^a	327.3 ^d	358.3	429.3 ^{bc}	557.9 ^a	351.0 ^{cd}	390.2 ^{bcd}	35.91
ME (MJ/kg DM)	5.9	7.1	4.7	4.9	4.9	5.3	5.3	6.8	6.8	4.0	4.2	5.3	7.0	4.4	4.7	0.55
SCFA (mmol)	0.50	0.66	0.32	0.35	0.35	0.39	0.39	0.61	0.61	0.22	0.25	0.39	0.64	0.28	0.33	0.090

^{abcd} Means within the same row with different superscripts are significantly different (P < 0.05); ns means not significant; * means P < 0.05; *** means P < 0.001

Chlo Chloris, Kara Karamoja, Mba Mbarara, Naka Nakasongola. S source of rumen fluid, F feed, S × F interaction between source of rumen liquor and feed type to assess whether fermentation kinetics whether the influence of liquor sources changes with feed type, a gas production from readily digestible feed fraction, b gas production from the insoluble fraction, c rate constant of gas production from the insoluble fraction, L lag time, IVOMD in vitro organic matter digestibility, ME metabolizable energy, SCFA short chain fatty acids

landscape's temporal and spatial heterogeneity, which affects plant phenology across Karamoja [33, 34]. Similar variability in plant phenology has been documented in other parts of Uganda's cattle corridor [35].

Cumulative 24-h gas production was influenced by the feed source, not the source of rumen liquor, consistent with findings by Cone et al. [36]. Karamoja forage samples produced the highest gas volumes at 24 h, indicating better degradation and likely reflecting their superior nutrient composition compared to those from other districts. This is further supported by the higher in vitro organic matter digestibility (IVOMD) estimates for Karamoja forages. These findings suggest that natural pastures, when harvested at the appropriate growth stage, can offer higher nutrient quality than some commercially available hays.

Since in vitro gas production is primarily driven by readily digestible and partially insoluble feed fractions, this study reinforces the idea that gas production is more influenced by the type of feed than by the source of rumen liquor, as reported by Aguiar et al. [37] and Cone et al. [36]. This finding suggests that researchers in low-income countries can use rumen fluid from abattoir-slaughtered animals for in vitro rumen fermentation trials. This can substitute the need for maintaining fistulated animals, and invasive techniques like esophageal tubing which are costly and challenging in the countries. Using abattoir-sourced rumen fluid allows cost-effective screening of potential ruminant feeds, if the feed base for donor animals is similar. This approach provides reliable, comparable results across trials while significantly reducing research costs. Karamoja forages were also estimated to have the highest metabolizable energy content, further indicating their superior quality compared to *Chloris gayana* hay and forages from Nakasongola and Mbarara.

IVOMD estimates were similarly unaffected by the source of rumen liquor, but strongly influenced by the forage source. This finding aligns with earlier research by Martínez et al. [38], which showed that the type of forage in donor animals' diets had a significant impact on in vitro methane production.

6 Conclusion

In conclusion, Karamoja's grazing pastures exhibited higher botanical diversity, which likely contributed to the superior nutrient quality observed in this study. The results suggest that the type of diet or feed resource exerts a greater influence on in vitro gas kinetics, metabolizable energy estimates, and IVOMD than the rumen liquor source. Additionally, the study highlights that using rumen liquor from animals raised in different parts of Uganda, if they are managed under open grazing systems, may not significantly affect in vitro assessment outcomes. This underscores the critical role of forage diversity and composition in influencing nutrient availability and digestion. Furthermore, these results demonstrate that in many low-income countries where the cost maintaining rumen fistulated animals is prohibitive, that the use of in vitro fermentation remains a viable option for screening ruminant feed resources. However, researchers should be cautious to ensure that rumen fluid is collected from animals fed on a comparable diet in terms of forage species diversity if results from in vitro fermentation trials must be compared. While this study was conducted with a limited number of animals grazing natural pastures, a controlled feeding trial with at least four animals per treatment is recommended to address these limitations and further validate the findings.

Acknowledgements We acknowledge the Norwegian Programme for Capacity Building in Higher Education and Research for Development (NORHED) project (UGA-13/0019) under the NORAD program for funding this study. We are grateful for the support of the technician in the animal science lab at Makerere University. Special thanks to Nabuin Zonal Agricultural Research and Development Institute staff, especially Dr. Boma Paul, Mbarara Zonal Agricultural Research and Development Institute and Staff of Veterinary and Production department Nakasongola district.

Author contributions N. K. D: Idea development, data collection, manuscript writing K. M: Idea development, Data analysis, manuscript writing E. J: Idea development; Manuscript writing M. B. V: Idea development, Manuscript writing N. A: Data collection, manuscript writing M. E: Data collection, manuscript writing T. J. R. S: PI to the project that provided funding, idea development, manuscript writing.

Funding We acknowledge the Norwegian Programme for Capacity Building in Higher Education and Research for Development (NORHED) project (UGA-13/0019) under the NORAD program for funding this study.

Data availability Data will be made available on request. Data will be provided by Dr. Nampanzira Dorothy (Corresponding author) on email: dnampanzira@gmail.com.

Declarations

Ethics approval and consent to participate All methods were carried out in accordance with relevant guidelines and regulations. The protocol was approved by Makerere University School of Veterinary Medicine Animal Resources (SVAR) higher degrees, SVAR research and ethics committees (SVAR-IACUC /90/2021) and certify that the study was performed in accordance with the ethical standard as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable standards.

Animal rights The animal handling during transportation and during slaughter was undertaken in compliance with statutory animal care, welfare and prevention of cruelty under the Animal Act of Uganda 2000 [16].

Competing interests The authors declare no competing interests.

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