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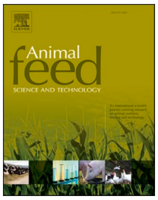

Methane production from locally available ruminant feedstuffs in Ethiopia – An *in vitro* study

Wondimagegne Bekele [a,c], Pekka Huhtanen [b], Abiy Zegeye [c], Addis Simachew [c], Abu Bakar Siddique [d], Benedicte Riber Albrectsen [d], Mohammad Ramin [a,*]

[a] *Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden*
[b] *Production Systems, Natural Resources Institute Finland (LUKE), Jokioinen FI-31600, Finland*
[c] *Institute of Biotechnology, Addis Ababa University, Addis Ababa, Ethiopia*
[d] *Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå SE-901 87, Sweden*

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ABSTRACT

Achieving optimal nutrient composition in locally sourced ruminant feeds is important, but can be challenging in resource-limited production systems. For example, improving the composition of available local feed resources is a key obstacle to efficiently mitigating enteric methane (CH_4) emissions in ruminants. This study characterized the nutritional content and *in vitro* methane (CH_4) yield of ruminant feedstuffs accessible in Ethiopia. A survey of 60 experienced farmers in two representative districts in Amhara region, Ethiopia, provided 33 feed samples, which were classified into four ruminant feed categories: Grasses (n=10); indigenous plants (trees, shrubs, herbaceous plants) (n=13); crop residues (n=5); and agro-industrial by-products (n=5). Nutritional composition was assessed by proximate and detergent methods. Methane yield (g CH_4/kg feed dry matter (DM)) and total gas yield (L/kg DM) were evaluated using a fully automated *in vitro* gas production system. A colorimetric assay was conducted to measure condensed tannin content (CT, mg/g) in relevant feeds. Lower crude protein (CP) values were observed for the grass (mean 65.2 g/kg DM) and crop residues (mean 54.5 g/kg DM) categories. Agro-industrial by-products had the highest CP (mean 260 g/kg DM), while indigenous plants exhibited intermediate levels (163 g/kg DM). There was significant variation in CH_4 yield ($P<0.01$) between grasses (12.4–24.7 g/kg DM) indigenous plants (1.8–19.3 g/kg DM), and agro-industrial by-products (8.1–26.9 g/kg DM). The indigenous plant *Trifolium acaule* gave the lowest *in vitro* CH_4 yield (1.8 g/kg DM). A positive relationship was observed between *in vitro* dry matter digestibility (IVDMD), CH_4, and total gas yield. Percentage of CH_4 in total gas production varied with feed category (grasses 14.5–19.6%; indigenous plants 3.1–16.9%; crop residues 15.8–20.6%; agro-industrial by-products 12.8–18.7%), and within category, e.g., *Trifolium acaule* (3.1%), *Acacia nilotica L.* (7.1%), *Ziziphus spina-christi* (9.9%), brewer's spent grains (BSG) (12.8%), local liquor (*areki*) residues (14.1%), and local beer (*tella*) residues (15.1%). A negative relationship was observed between CT content and *in vitro* CH_4 yield, with a stronger ($P<0.05$) correlation for soluble CTs ($R^2 = 0.46$) than cell-bound CTs ($R^2 = 0.25$) and total CTs ($R^2 = 0.29$). Based on

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Abbreviations: AIC, Akaike information criterion; ANDF, neutral detergent fiber assayed with a heat-stable amylase and expressed inclusive of residual ash; BSG, brewer's spent grains; CH_4, methane; CO_2, carbon dioxide; CP, crude protein, CT, condensed tannins; DM, dry matter; DMI, dry matter intake; GHGs, greenhouse gases; IVDMD, in vitro dry matter digestibility; INDF, indigestible neutral detergent fiber; M.a.s.l., meters above sea level; OM, organic matter; PA, Peasant Association; PdOM, potentially digestible organic matter; VFA, volatile fatty acid.

[*] Corresponding author.

E-mail address: Mohammad.Ramin@slu.se (M. Ramin).

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methanogenic properties and effects of CTs on *in vitro* CH_4 yield, indigenous plants should be prioritized in ruminant rations in Ethiopia. Making nutritional composition and CH_4 data publicly available could help develop environmentally sound, cost-effective rations for ruminant livestock, benefiting local farmers and leading to more sustainable and efficient livestock production in Ethiopia.

1. Introduction

Globally, emissions originating from livestock production systems, including feed production, enteric fermentation, and animal waste, are the primary sources of greenhouse gases (GHGs), contributing 37% of all human-induced methane (CH_4) emissions (Opio et al., 2013). Key factors driving GHGs emissions from livestock include feed quality, particularly in relation to enteric CH_4 emissions (Jouany, 2008; Sejian and Naqvi, 2012). With estimated 8–12% energy wastage in feeds and its implications for global warming, enteric CH_4 has become a significant concern for animal nutritionists and environmentalists (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995; Ramin and Huhtanen, 2013; Soren et al., 2017). Notably, CH_4 is a much more potent GHG than carbon dioxide (CO_2) (Pinares-Patiño et al., 2007).

Improving feed quality by dietary manipulation is a relatively simple and pragmatic approach for increasing animal productivity and reducing CH_4 emissions per unit of product (Haque, 2018). Depending on the degree of change and type of intervention, dietary manipulation can lower total CH_4 yield by up to 40% (Benchaar et al., 2001).

Besides providing a good source of nutrients, locally available feeds can be nutritionally customized for specific needs. Identifying locally sourced feedstuffs that reduce CH_4 emissions while maintaining the required nutrients is important for smallholder livestock production systems in Ethiopia, and can help to mitigate climate change (Berhanu et al., 2019). Locally available feeds that meet ruminant nutrient requirements can enhance productivity and fertility (Gerber et al., 2013).

To formulate a suitable diet for livestock, it is essential to have accurate knowledge of the nutrient composition of feedstuffs (Tran et al., 2020). Knowledge of the CH_4 yield from such feedstuffs can help to ensure that environmentally friendly feed is used in ration formulation. By making nutritional composition and CH_4 yield data publicly available, the research community can contribute to the design of scientifically based, environmentally proven, and cost-effective rations for meat, dairy, and draught animals used by small-scale African farmers. This will help promote sustainable and efficient farming practices in the region (ILRI, 2021).

Any approach used to evaluate potential feeds must consider animal preferences and the nutritional content of the feed (Mtengeti and Mhelela, 2006). Proximate analyses give reasonably accurate estimates of the nutritional potentials of feedstuffs, but the results may not be a realistic representation of the nutritive value. However, they provide a starting point for further *in vitro* or *in vivo* research (D'Mello, 1992; Fadiyimu et al., 2011). Additionally, the biological impacts of secondary plant metabolites are not captured by

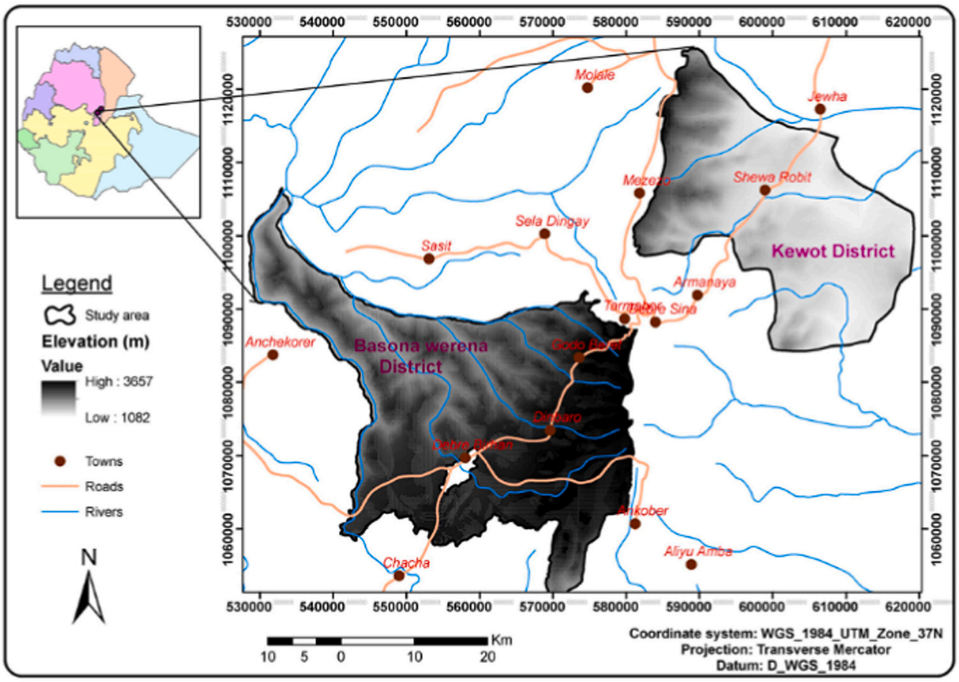

Fig. 1. Map of the study area in central Ethiopia. The dark and light grey areas represent the two districts in the highlands and lowlands, respectively, targeted in this study.

proximate chemical analyses, and hence *in vitro* techniques are needed to gain a better understanding of the nutritive value of different feedstuffs (Nsahlai et al., 1994; Kassi et al., 2000). Gas production is a valid method for evaluating the nutritive worth of feeds and is of particular importance for feeds that contain anti-nutritive substances (Siaw et al., 1993; Okunade et al., 2014). However, little information is available regarding CH_4 emissions from tropical forage species and feedstuffs (Koura et al., 2021). The objective of this study was thus to determine the *in vitro* CH_4 yield potential of locally available ruminant feeds in Ethiopia.

2. Materials and methods

2.1. Survey procedure

Exploratory questionnaires were prepared to identify available feed sources for ruminants. Surveys were conducted in the *Basona werena* (1500 to 3400 m.a.s.l.) and *Kewot* (1205 to 2500 m.a.s.l.) districts in the north Showa administrative zone of Amhara region, Ethiopia (Fig. 1). These two districts reflect differences between Ethiopia's highland and lowland agro-ecology. Three peasant associations (PA, locally known as *kebele*, the smallest administrative unit in Ethiopia) were selected in each district, based on accessibility and level of reliance on livestock production. Ten households were purposely selected for interview from each PA (in total 60 households), based on their livestock-keeping experience, willingness to participate in interviews, and familiarity with available local feed resources.

Through interviews and focus group discussions, the range of local feedstuffs to be investigated was narrowed to 33 samples, which were divided into four categories: 1) Grass (n=10); 2) indigenous plants (trees, shrubs, herbaceous plants) (n=13); 3) crop residues (n=5); and 4) agro-industrial by-products (n=5).

2.2. Feed sample collection and preparation

Each sample comprised around 1 kg of fresh material. Forage plants were collected and submitted for species identification at the Herbarium Center, College of Natural and Computational Sciences, Addis Ababa University. Agro-industrial feeds were purchased from the nearest dealer. All samples were air-dried, cut into pieces, and packed in labeled airtight bags for storage and transportation to Sweden for analysis.

2.3. Chemical analysis

At the laboratory, samples were ground and sieved through a 2-mm sieve for indigestible neutral detergent fiber (iNDF) studies of *in situ* parameters, and through a 1-mm sieve for studies of chemical composition (including analysis of condensed tannins, CTs), and *in vitro* incubation.

Nutrient content, i.e., organic matter (OM, method 942.05), dry matter (DM, method 930.15), nitrogen concentration (method 978.04), and total ash (method 942.05), was determined according to the method described by Horwitz and Latimer (2005). Total crude protein (CP) was estimated by multiplying the nitrogen concentration obtained by 6.25. Neutral detergent fiber (aNDF) was quantified by adding sodium sulfite and heat-stable α-amylase, according to the method of Van Soest et al. (1991).

In situ determination of iNDF was conducted based on methodology outlined by Krizsan et al. (2015). Samples weighing 2.1–2.2 g were placed in polyester bags with 11.5 μm pore size and pore area equivalent to 5% of the total surface area. The bags were incubated within the rumen of three fistulated Nordic Red cows, each fed an *ad libitum* diet of 600 g/kg DM grass silage and 400 g/kg DM concentrate. One empty bag was also incubated in each cow, as a control. The experiment took place at Röbäcksdalen research farm (Umeå, 63°45'N, 20°17'E), which is part of the Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences. Following the 12-day incubation period, the bags were removed and rinsed in cold water to halt enzyme activity. A further rinsing was carried out in a household washing machine (Electrolux Wascator W75MP, AB Electrolux, Stockholm, Sweden), using the rinse part of the wool wash cycle. The bags were dried for 24 h at 60°C and then boiled in neutral detergent solution containing a sodium sulfite concentration of 0.5 (±0.1) g per bag for one hour. Finally, the bags were dried again at 60°C for 24 h and weighed. The reported iNDF concentration includes the ash. Potentially digestible organic matter (pdOM) was determined by subtracting iNDF from OM.

Analysis of condensed tannins (CT) took place at the Umeå Plant Science Centre, Umeå University, Sweden. The acid-butanol method outlined by Porter et al. (1985) and reported by Bandau et al. (2015) was employed to evaluate soluble and cell-bound CTs. In a standard procedure, 1 mL of acetone solution (comprising 70% acetone, 30% Milli-Q water, and 0.01% of 10 mM ascorbic acid) was combined with 10±2 mg of sample powder and 2–3 tungsten beads in an Eppendorf tube. These samples were bead-milled for 10 min (at a frequency of 27) and centrifuged for 5 min (at 15,000 rcf). A 50-μL aliquot of supernatant were removed for analysis of soluble CTs, while the cell tissues were dried to form a pellet that was stored for subsequent extraction of cell-bound CTs.

The sample supernatant was combined with 600 μL of acid-butanol containing 20 μL of iron reagent in Eppendorf tubes to analyze soluble CT. This mixture was quickly vortexed and then incubated at 100ºC for 1 h before cooling to room temperature and centrifugation. A 50-μL portion of sample was transferred to a 96-well plate for analysis. Standard concentrations of procyanidin B2 (C30H26012, Sigma-Aldrich, St. Louis, MO, US) were prepared for the quantification process. Absorbance at 550 nm was measured using a spectrophotometer (Hitachi U-5100 UV/VIS, Hitachi High-Technologies, Tokyo, Japan), and the concentration of soluble CTs was determined as mg/g dry weight of the samples based on the absorbance readings and the standard curve. For assessment of cell-bound CTs, the pellet resulting from the initial extraction was dried using a vacuum centrifuge for 10 min and 50 μL of acetone were added, followed by brief vortexing. As for soluble CT, the concentration of cell-bound CTs was measured using the absorbance method.

2.4. *In vitro technique*

All procedures involving animals for *in vitro* incubation adhered to the guidelines established by the Swedish Ethics Committee on Animal Research (Dnr A 32–16), overseen by the Court of Appeal for Northern Norrland in Umeå, Sweden. The donor cows were fed a total mixed ration (TMR) composed of grass silage and concentrate (600:400 g/kg DM). The concentrate consisted of rolled barley and rapeseed meal (800:200 g/kg DM).

The rumen fluid used in all three *in vitro* runs was obtained from two Nordic Red cows. Rumen fluid from each cow was collected separately, strained through two layers of cheesecloth, and stored in pre-heated, CO_2-flushed steel thermos flasks. Dried and ground feed samples weighing 1 g were placed directly into 250 mL serum bottles (Schott, Mainz, Germany) and incubated in 60 mL of buffered rumen fluid for 72 h. Each run consisted of 33 samples and three blanks, with each blank containing only buffered rumen fluid. The *in vitro* incubation flasks received each feed sample randomly, while the same flask was never used for the same feed over more than one run. Continuous shaking was applied to the flasks during incubation at 39℃ (Ramin and Huhtanen, 2012).

2.5. *Gas sampling*

Gas production from each bottle of sample was withdrawn at 2, 4, 8, 24, 48, and 72 h of incubation using a gas-tight syringe (Hamilton, Bonaduz, Switzerland) for CH_4 estimates. The value obtained was subtracted from the blank mean gas production within a run to calculate sample gas production.

Methane yield (g CH_4/kg DM) was derived from the *in vitro* system. Initially predicted as CH_4 production in (mL), it was transformed into the kinetic parameter of CH_4 or gas (mL CH_4/g DM). Finally, it was expressed in g CH_4/kg DM, calculated as:

$$CH_4\ (\text{g/kg DM}) = CH_4\ (\text{mL/g DM})/22.4\ (\text{L/mol}) \times 16.04\ (\text{g/mol}) \quad (1)$$

The detailed kinetic parameter procedure followed the methodology outlined by Ramin and Huhtanen (2012).

Table 1
Chemical composition of grasses and indigenous trees, shrubs and herbaceous plants subjected to *in vitro* analysis in this study.

Vernacular name	Scientific name	DM, g/kg	Chemical composition (g/kg DM)					
			Ash	CP	aNDF	iNDF	pdOM	iNDF/aNDF
Grasses								
Lisha sar	*Sehima nervosum (Rottler) Stapf*	949	182	41	617	309	510	0.50
Serdo	*Pennisetum villosum Fresen.*	955	143	66	638	274	583	0.42
Asendabo	*Phalaris paradoxa L.*	938	119	109	447	69	812	0.15
Gaz'e sar	*Andropogon amethystinus Steud.*	944	84	46	652	232	684	0.35
Qumtie	*Pennisetum sp.*	946	82	50	630	175	743	0.27
Wogel sber	*Hyparrhenia hirta (L.) Stapf*	949	110	34	651	248	642	0.38
Wura arem	*Cynodon sp.*	949	98	67	599	201	701	0.33
Tasari	*Echinochloa colona (L.) Link*	949	140	110	543	120	741	0.22
Nech sar (1)	*Aristida adscensionis L.*	954	120	70	668	194	686	0.29
Desho grasses	*Pennisetum pedicellatum*	950	139	59	603	143	718	0.23
	Mean	948	121	65.2	605	197	682	0.31
	Standard deviation	4.9	30.6	26.1	65.8	73.1	86.1	0.10
Tree, shrubs, and herbaceous plants								
Kesseley	*Acacia nilotica (L.)*	946	60	204	142	96	844	0.67
Kurkura	*Ziziphus spina-christi*	927	82	158	254	93	825	0.36
Mezazign	*Acacia brevispica Harms*	937	82	160	262	251	668	0.95
Treelucern	*Chamaecytisus palmensis*	942	58	216	259	100	843	0.38
Alfalf	*Medicago sativa*	937	152	251	219	60	789	0.27
Anfar	*Buddleja polystachy*	937	73	178	276	192	736	0.69
Tosign	*Thymus vulgaris*	920	113	86	405	339	548	0.83
Ameja	*Trifolium acaule*	937	60	136	161	123	818	0.76
Washatema	*Trifolium sp.*	936	86	151	513	318	596	0.61
Telich (tedicha)	*Acacia tortilis (Forssk.) Hayne*	941	73	232	211	153	774	0.72
Magete	*Tembien clover (Trifolium tembense)*	939	112	93	519	199	689	0.38
Nechilo	*Conyza hypoleuca A. Rich.*	946	81	150	219	86	833	0.39
Enbacho	*Rumex nervosus Vahl*	945	108	106	387	231	661	0.59
	Mean	938	87.7	163	294	172	740	0.59
	Standard deviation	7.4	26.9	51.9	123.4	91.3	99.4	0.21

aNDF: NDF assayed with a heat-stable amylase and expressed inclusive of residual ash; CP: crude protein; DM: dry matter; iNDF: indigestible neutral detergent fiber; pdOM: potentially digestible organic matter.

2.6. Statistical analysis

Data were analyzed in R version 4.2.0 (R Core Team, 2023). Statistical analyses were performed to identify differences between feed categories. The variables CH_4 g/kg DM and gas yield L/kg DM were analyzed with ANOVA, using the 'aov' function in the 'agricolae' package and the following model:

$$Y_{ij} = \mu + T_i + R_j + e_{ij} \tag{2}$$

where Y_{ij} is an observed variable for the i^{th} feeds, j^{th} run, μ is the overall mean, T_i is i^{th} feed, R_j is run j, and e_{ij} is residual error.

Differences were considered statistically significant at $P<0.05$. Fisher's least significant difference (LSD) method was employed for *post hoc* analysis.

To model potential casual relationships between CH_4 yield and feed chemical composition, stepwise Akaike information criterion (AIC) regression was conducted in R using the "ols_step_both_aic" function, incorporating forward and backward selection methods, with multicollinearity considered using the variance inflation factor. The correlation between CH_4 yield and chemical composition data was also assessed using the 'tab_corr' function within the 'sjPlot' package. Simple linear regression analysis was performed with CH_4 yield as the dependent variable and soluble and cell-bound CT and IVDMD as independent variables, using the "ggscatter" function from the "ggpubr" package. Moreover, group comparisons between feed categories were carried out using the "ggstatsplot" package and the "ggbetweenstats" function (Patil, 2021).

3. Results

3.1. Chemical composition

Chemical composition of all 33 feed samples analyzed is presented in Tables 1 and 2. Samples in the grass category had average CP content of 65.2 g/kg DM (range 41–110 g/kg DM), and mean fiber content was 605 g/kg DM for aNDF and 197 g/kg DM for iNDF. Among the grasses analyzed, *Phalaris paradoxa L.* had the lowest iNDF and optimal CP.

Most feeds in the indigenous plants category, excluding *Thymus vulgaris* and *Trifolium tembense* (Tembien clover), contained over 106 g CP/kg DM. *Medicago sativa* had the highest CP content (251 g/kg DM), followed by *Acacia tortilis* (232 g/kg DM) and *Chamaecytisus palmensis* (216 g/kg DM). The aNDF content varied between 142 and 519 g/kg DM, and the iNDF content between 60 and 339 g/kg DM. Notably, the pdOM values within this category ranged from 548 to 844 g/kg DM.

The crop residues group had the lowest CP values (range 26–74 g/kg DM). Among the agro-industrial by-products analyzed, *Guizotia abyssinica* had the highest CP and the lowest aNDF content. A correlation matrix for chemical composition (Table 3) revealed a marked negative association between iNDF/aNDF and CP and pdOM.

Cell-bound CT content was higher than soluble CT content in almost all feedstuffs analyzed (Table 4), but soluble CTs had a greater effect on *in vitro* CH_4 yield.

3.2. Total gas and CH_4 yield

The data obtained for *in vitro* CH_4 (g/kg DM) and total gas (L/kg DM) yield from the 33 feed samples are presented in Tables 5–7. There were significant differences ($P<0.01$) in CH_4 and gas yield within each feedstuff category, excluding the crop residues category.

Table 2
Chemical composition of crop residues and agro-industrial by-products subjected to *in vitro* analysis in this study.

Vernacular name	Common name	DM, g/kg	Chemical composition (g/kg DM)					
			Ash	CP	aNDF	iNDF	pdOM	iNDF/aNDF
Crop residue								
Sinar/gerima	Forage oat seed with kernel	940	50	63	423	103	848	0.24
Yesendai geleba	Wheat straw	950	65	26	700	321	614	0.45
Yemashila girinbite	Striped leaf sorghum	945	183	66	572	168	649	0.29
Masho geleba	Mung bean straw	923	126	74	468	289	585	0.61
Yetef chid	*Teff* straw	954	52	43	668	256	692	0.38
	Mean	943	95.2	54.5	566	227	678	0.39
	Standard deviation	11.5	58.2	19.3	120.8	89.7	103.3	0.14
Agro-industrial by-product /brewery waste								
Yebeera teref mirt	Brewer's spent grain	934	44	253	519	193	764	0.37
Yesindai furishka	Wheat bran	923	58	166	380	78	864	0.20
Fagulo	Niger seed cake	940	77	442	191	101	823	0.52
Ye arek atella	Liquor residues-*areki*	902	56	255	473	143	801	0.30
Ye tela atella	Beer residues-*tella*	881	42	183	420	345	613	0.82
	Mean	916	55.2	260	396	172	773	0.44
	Standard deviation	24.4	14.0	109.5	126.6	106.1	96.5	0.24

aNDF: NDF assayed with a heat-stable amylase and expressed inclusive of residual ash; CP: crude protein; DM: dry matter; iNDF: indigestible neutral detergent fiber; pdOM: potentially digestible organic matter.

Table 3
Correlation matrix of feed chemical composition.

	DM	Ash	CP	aNDF	iNDF	pdOM
DM						
Ash	0.362					
	(.039)					
CP	-0.364	-0.366				
	(.037)	(.036)				
aNDF	0.278	0.325	-0.704			
	(.117)	(.065)	(<.001)			
iNDF	-0.142	0.103	-0.432	0.519		
	(.431)	(.569)	(.012)	(.002)		
pdOM	-0.016	-0.482	0.524	-0.586	-0.921	
	(.929)	(.005)	(.002)	(<.001)	(<.001)	

aNDF: NDF assayed with a heat-stable amylase and expressed inclusive of residual ash; CP: crude protein; DM: dry matter; iNDF: indigestible neutral detergent fiber; pdOM: potentially digestible organic matter;

Table 4
Condensed tannin (CT) content (mg/g) in local plant species relevant as ruminant feedstuffs in Ethiopia.

Plant species	Soluble	Cell-bound	Total CT
Acacia nilotica (L.)	7.5	7.8	15.3
Ziziphus spina-christi	46.3	294	341
Acacia brevispica Harms	40.3	26	66.3
Chamaecytisuspalmensis	5.0	14.7	19.6
Buddleja polystachy	5.3	27.8	33.1
Thymus vulgaris	4.9	16.7	21.6
Trifolium acaule	68.4	429	498
Acacia tortilis (Forssk.) Hayne	29.9	295	325
Conyza hypoleuca A. Rich.	5.2	12.9	18.1
Rumex nervosus Vahl	44.1	95	139

CT: Condensed tannins

Table 5
In vitro methane (CH_4) and gas yield of locally available grasses in Ethiopia.

Scientific name	**Gas**		**CH_4**					
	Gas yield L/ kg DM	Asymptotic gas yield L\kg DM	CH_4 yield, g/ kg DM	Asymptotic CH_4 yield, g/kg DM	IVDMD	Rate, 1/h	CH_4/total gas, (%)	CH_4/ pdOM
Sehima nervosum (Rottler) Stapf	96.8	156	12.8	18.9	0.63	0.027	18.8	0.025
Pennisetum villosum Fresen.	123	181	14.3	20.5	0.62	0.023	16.2	0.024
Phalaris paradoxa L.	213	282	24.7	36.5	0.70	0.033	16.2	0.03
Andropogon amethystinus Steud.	123	211	13.3	23.0	0.60	0.027	15.1	0.019
Pennisetum sp.	188	215	21.8	28.7	0.72	0.037	16.2	0.029
Hyparrhenia hirta (L.) Stapf	134	153	18.1	24.0	0.65	0.027	19.6	0.028
Cynodon sp.	168	250	19.1	30.6	0.65	0.030	15.9	0.027
Echinochloa colona (L.) Link	178	206	20.3	27.6	0.66	0.030	15.9	0.027
Aristida adscensionis L.	124	210	12.4	23.6	0.65	0.030	14.5	0.018
Pennisetum pedicellatum	173	241	21.7	33.0	0.67	0.030	17.5	0.03
Mean	152	211	17.8	26.6	0.66	0.029	16.6	0.025
CV	16.1	29.6	13.3	20.2	6.9	18.6	11.6	13.2
SEM	191.3	1233	1.7	9.2	0.0006	9.4E-06	1.0	3.7E-06
P	0.001	0.31	0.001	0.01	0.1	0.24	0.06	0.002
LSD	41.8	106.3	4.0	9.1	0.07	0.009	3.1	0.005

CV: Coefficient of variation; IVDMD: in vitro dry matter digestibility; LSD: least significant difference; pdOM: potentially digestible organic matter; P: probability; SEM: standard error of mean.

Table 6

In vitro methane (CH_4) and gas yield of locally available indigenous trees, shrubs, and herbaceous plants in Ethiopia.

Scientific name	Gas		CH_4					
	Gas yield L/kg DM	Asymptotic gas yield L\kg DM	CH_4 yield, g/kg DM	Asymptotic CH_4 yield, g/kg DM	IVDMD	Rate, 1/h	CH_4/total gas, (%)	CH_4/pdOM
Acacia nilotica (L.)	138	178	6.6	11.2	0.69	0.033	7.1	0.007
Ziziphus spina-christi	110	153	7.8	10.8	0.66	0.027	9.9	0.009
Acacia brevispica Harms	80.8	118	8.5	10.0	0.74	0.040	14.8	0.012
Chamaecytisuspalmensis	191	199	16.5	27.4	0.72	0.036	12.1	0.019
Medicago sativa	167	211	19.3	28.4	0.71	0.033	16.2	0.024
Buddleja polystachy	136	168	13.4	18.9	0.73	0.040	13.7	0.018
Thymus vulgaris	118	152	11.2	16.5	0.71	0.036	13.5	0.02
Trifolium acaule	89.7	117	1.8	2.8	0.64	0.030	3.1	0.002
Trifolium sp.	127	159	15.3	21.8	0.73	0.040	16.9	0.025
Acacia tortilis (Forssk.) Hayne	91.4	132	9.9	13.2	0.68	0.033	15.3	0.012
Tembien clover (Trifolium embense)	157	221	13.4	24.0	0.68	0.033	12.1	0.019
Conyza hypoleuca A. Rich.	80.7	102	6.4	9.1	0.74	0.040	11.1	0.007
Rumex nervosus Vahl	71.0	112	5.1	7.2	0.58	0.023	10.0	0.007
Mean	119.8	155.7	10.4	15.5	0.69	0.034	12.0	0.014
CV	12.9	17.7	12.6	19.9	7.5	3.8	12.8	12.6
SEM	56.4	212.4	0.48	2.6	0.0007	4.7E-07	0.66	9.3E-07
P	0.001	0.001	0.001	0.001	0.03	0.009	0.001	0.001
LSD	25.9	46.4	2.2	5.1	0.08	0.002	2.6	0.003

CV: Coefficient of variation; IVDMD: in vitro dry matter digestibility; LSD: least significant difference; pdOM: potentially digestible organic matter; P: probability; SEM: standard error of mean.

Table 7

In vitro methane (CH_4) and gas yield of locally available crop residues and agro-industrial by-products in Ethiopia.

Common name	Gas		CH_4					
	Gas yield L/kg DM	Asymptotic gas yield L\kg DM	CH_4 yield, g/kg DM	Asymptotic CH_4 yield, g/kg DM	IVDMD	Rate,1/h	CH_4/total gas, (%)	CH_4/pdOM
Crop residue								
Forage oat seed with kernel	159	215	23.2	31.5	0.77	0.04	20.6	0.027
Wheat straw	150	219	19.4	26.6	0.67	0.027	16.5	0.032
Sorghum leaf strips	136	197	16.9	25.5	0.69	0.03	17.5	0.026
Mung bean straw	167	200	20.2	27.6	0.76	0.043	16.8	0.035
Teff straw	167	252	18.8	29.7	0.66	0.03	15.8	0.027
Mean	156	216	19.7	28.2	0.71	0.034	17.4	0.029
CV	11.5	10.4	11.7	11.9	3.8	10.7	12.2	11.7
SEM	144	229	2.4	5.1	0.0003	5.9E-06	2.0	5.4E-06
P	0.2	0.08	0.07	0.2	0.001	0.001	0.1	0.05
LSD	32.6	41.1	4.2	6.1	0.05	0.006	3.8	0.006
Agro industrial by-products/brewery waste								
Brewer's spent grain	89.6	116	8.1	11.9	0.72	0.037	12.8	0.01
Wheat bran	224	216	26.9	31.3	0.81	0.05	16.8	0.03
Guizotia abyssinica	168	187	22.4	28.9	0.81	0.053	18.7	0.027
Liquor residue-areki	110	144	11.1	15.7	0.74	0.04	14.1	0.013
Beer residue-tella	177	182	19.2	26.3	0.77	0.043	15.1	0.031
Mean	153.8	168.8	17.5	22.8	0.77	0.045	15.5	0.022
CV	7.4	22.0	7.3	14.0	2.7	10.0	6.1	7.5
SEM	59.1	621.7	0.74	4.6	0.0001	8.9E-06	0.4	2.9e-06
P	0.001	0.05	0.001	0.001	0.001	0.005	0.001	0.001
LSD	20.9	67.8	2.3	5.8	0.038	0.008	1.7	0.003

CV: Coefficient of variation; IVDMD: in vitro dry matter digestibility; LSD: least significant difference; pdOM: potentially digestible organic matter; P: probability; SEM: standard error of mean.

Among grass samples, *Phalaris paradoxa* showed the highest CH_4 yield and CH_4/pdOM, while *Aristida adscensionis L.* showed the lowest values for these parameters. Among indigenous plants, the herb *Trifolium acaule* had the lowest CH_4 yield of all feedstuffs analyzed. Within the crop residues category, striped leaf sorghum (*Sorghum bicolor*) displayed the lowest CH_4 yield, total gas yield, and CH_4/pdOM. In the agro-industrial by-products category, BSG and homemade liquor (*areki*) residues showed the lowest CH_4 yield (8.1 and 11.1 g/kg DM, respectively). The correlation matrix revealed significant positive associations ($P<0.05$) between CH_4 and gas

Table 8
Correlation matrix of methane (CH_4) and gas yield data obtained in *in vitro* analysis of 33 feedstuff samples collected in Ethiopia.

	Gas yield L/kg DM	Asymptotic gas yield L\kg DM	CH_4 yield, g/kg DM	Asymptotic CH_4 yield, g/kg DM	IVDMD	Rate,1/h	CH_4/total, (%)	CH_4/pdOM
Gas yield L/kg DM	1							
Asymptotic gas yield L\kg DM	0.735 (<.001)	1						
CH_4 yield, g/kg DM	0.862 (<.001)	0.679 (<.001)	1					
Asymptotic CH_4 yield, g/kg DM	0.804 (<.001)	0.747 (<.001)	0.902 (<.001)	1				
IVDMD	0.299 (.003)	-0.083 (.415)	0.303 (.002)	0.316 (.001)	1			
Rate,1/h	0.268 (.007)	-0.120 (.236)	0.217 (.031)	0.242 (.016)	0.929 (<.001)	1		
CH_4/total gas, %	0.306 (.002)	0.327 (.001)	0.694 (<.001)	0.598 (<.001)	0.138 (.175)	0.022 (.827)	1	
CH_4/pdOM	0.759 (<.001)	0.620 (<.001)	0.937 (<.001)	0.851 (<.001)	0.227 (.024)	0.143 (.159)	0.734 (<.001)	1

IVDMD: in vitro dry matter digestibility; pdOM: potentially digestible organic matter.

yield, IVDMD, Rate, CH_4/total gas, and CH_4/pdOM, and weak negative correlations between asymptotic gas yield and IVDMD (1/h) (Table 8).

3.3. *Group comparison among feedstuff categories*

Welch F-tests for unequal variances and sample sizes showed highly significant variation in CH_4 and gas yield among the feedstuff categories (Figs. 2 and 3). Indigenous trees, shrubs, and herbaceous plants generally had the lowest CH_4 and gas yields (mean 10.4 g/kg DM and 119.8 L/kg DM, respectively). This category of feedstuff is commonly associated with the presence of secondary plant compounds, such as tannins.

3.4. *Relationships between feed composition, IVDMD, and tannin content and in vitro CH_4 and total gas yields*

The relationship between CH_4 and gas yield and the chemical composition of feedstuffs is shown in Table 9. Indigestible NDF and CP were inversely related in the grass category, whereas aNDF, CP, and pdOM showed a positive relationship in the indigenous plant category. Dry matter showed an inverse relationship with CH_4 and gas yields for all feed categories (Table 9).

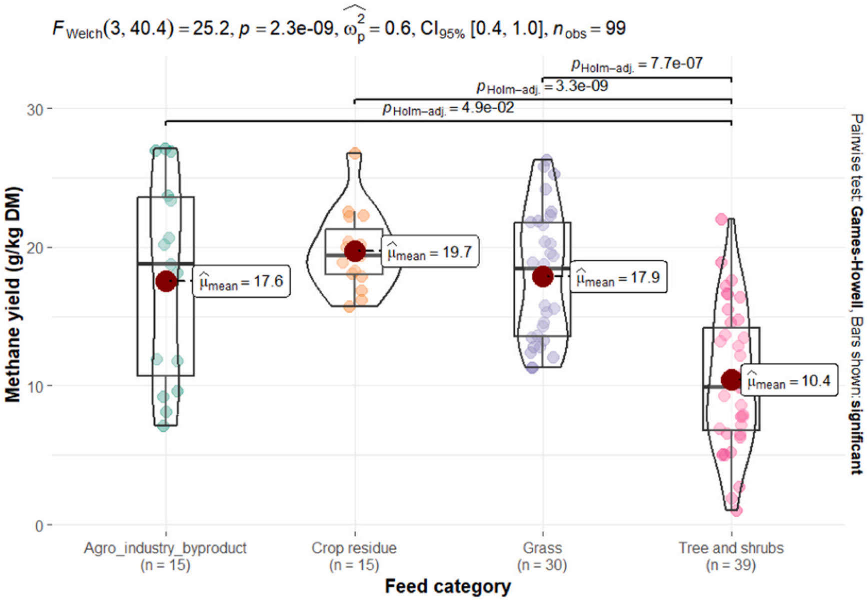

Fig. 2. Comparison of methane (CH_4) yield from four feedstuff categories (grasses, indigenous trees and shrubs, agro-industrial by-products, crop residues) typical for Ethiopian husbandry. Methane yield was measured *in vitro*; significant values are shown above significant comparisons between two feed categories connected by horizontal lines. n: number of observations.

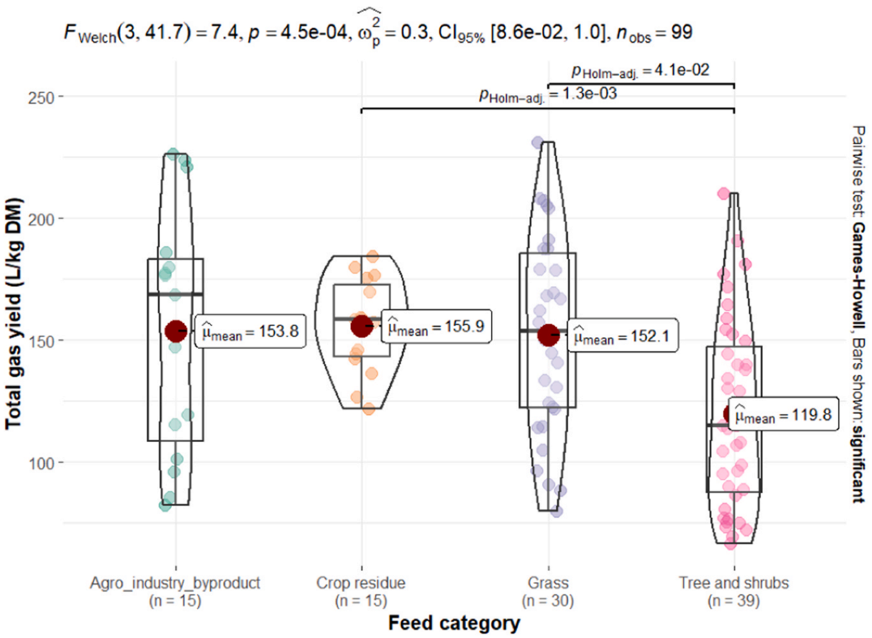

Fig. 3. Comparison of total gas yield from four feedstuff categories (grasses, indigenous trees and shrubs, agro-industrial by-products, crop residues) typical for Ethiopian husbandry. Gas yield was measured *in vitro*; significant values are shown between two feed categories connected by horizontal lines. n: number of observations.

Table 9
Regression models based on dietary chemical composition and *in vitro* methane (CH_4) and gas yield from feedstuffs collected in Ethiopia.

Feeds category	Model		RMSE	AIC	Adjusted R^2	P-value
Grass(n=10)	CH_4	Y=194.4 – 0.15DM - 0.06iNDF - 0.06CP –0.01OM	3.2	58.4	0.52	0.001
	Gas	Z=811.4 – 0.69DM – 0.44iNDF – 0.09CP + 0.1OM	26.6	195.9	0.58	0.001
Tree, shrubs and herbaceous plants (n=13)	CH_4	Y=176.7 – 0.18DM + 0.04aNDF + 0.09CP –0.03OM + 0.009pdOM	2.4	60.2	0.76	0.001
	Gas	Z=1392.1 – 1.7DM + 0.31aNDF + 0.49CP – 0.04OM + 0.28pdOM	29.4	261.9	0.4	0.001
Crop residue(n=5)	CH_4	Y=145.1–0.12DM – 0.03 Ash – 0.01iNDF	2.2	27.8	0.38	0.03
	Gas	Z=505.1 – 0.37DM + 0.006aNDF	20.5	90.5	-0.12	0.8
Agro-industrial by-products (n=5)	CH_4	Y=188.6 – 0.16DM – 0.02iNDF - 0.04aNDF	2.2	27.8	0.38	0.03
	Gas	Z=1559.5 – 1.3DM – 0.18iNDF – 0.27aNDF	42.6	77.1	0.29	0.07
total feeds samples(n=33)	CH_4	Y=88.9–0.12DM + 0.04Ash + 0.02aNDF + 0.03pdOM	5.4	341.2	0.25	0.001
	Gas	Z=911.3 – 0.8DM + 0.13aNDF –0.25OM + 0.27pdOM	37.4	716.4	0.20	0.001

AIC: Akaike information criterion; RMSE: root mean square error; R2: coefficient of determination; P-value: probability level; Y: CH4 yield (g/kg of DM); Z: Gas yield (L/kg of DM).

A scatter plot revealed a clear positive linear correlation between IVDMD and CH_4 yield, with the CH_4 yield increasing by 31–150 g/kg DM with each unit increase in IVDMD (Fig. 4). Coefficient of determination (R^2) was greatest for the agro-industrial by-product category ($R^2 = 0.75$), followed by crop residues ($R^2 = 0.44$) and grass ($R^2 = 0.38$).

The relationship between total gas yield and IVDMD displayed a similar positive trend, with each unit increase in IVDMD leading to an increase in total gas yield to 160–970 L/kg DM (Fig. 5). However, the ability of the model to predict total gas yield for the crop residue and indigenous plants categories was deemed non-significant ($P>0.05$) (Fig. 5).

Condensed tannins showed a moderate negative correlation with *in vitro* CH_4 yield, with a significantly ($P<0.05$) stronger correlation for soluble CT ($R^2=0.46$) compared with cell-bound CT ($R^2=0.25$) and total CT ($R^2=0.29$) (Fig. 6).

4. Discussion

This in vitro study provides the first comprehensive information on in vitro CH_4 yield and nutritional composition of different feedstuff categories available to smallholder farmers in Ethiopia. It also provides baseline iNDF data for Ethiopian feeds.

The results indicated that indigenous plant feedstuffs (trees, shrub, herbaceous plants) produced less CH_4 and gas than the other feedstuff categories analyzed. Non-conventional feed sources such as distillation residues and BSG also showed lower CH_4 and gas

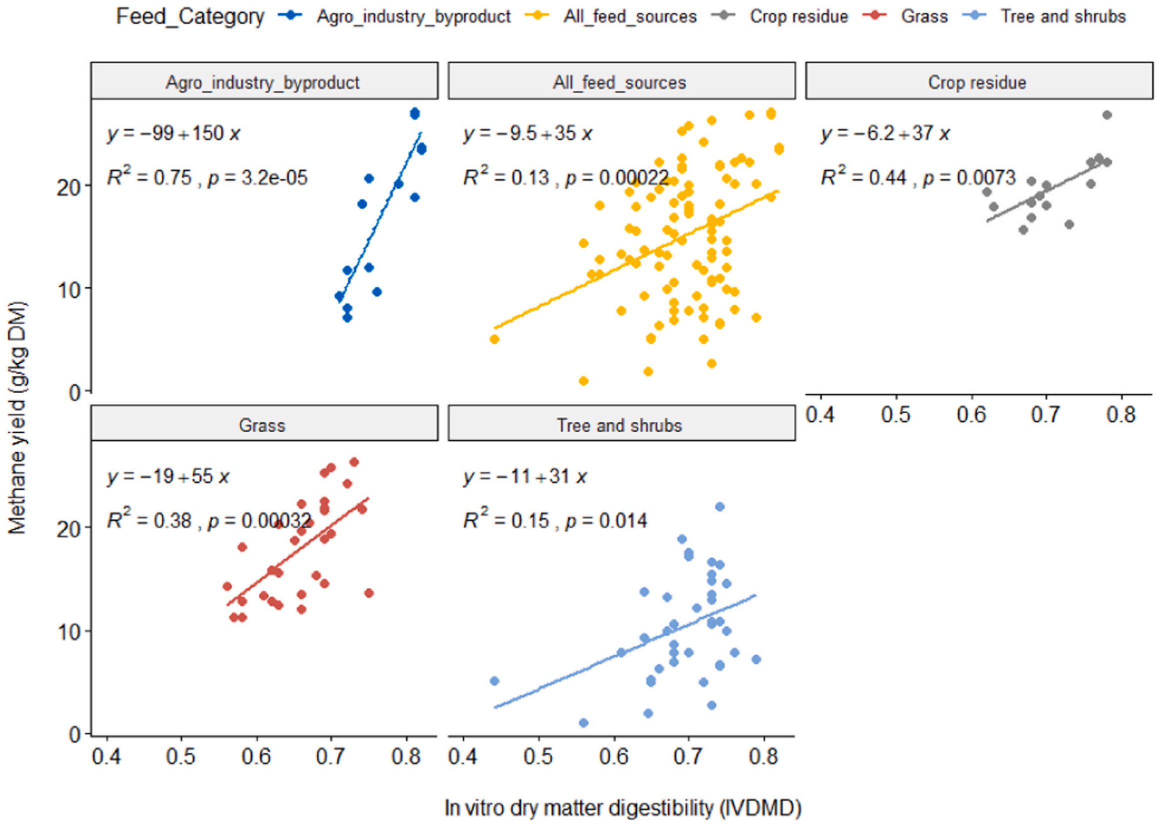

Fig. 4. Methane (CH_4) yield (g/kg DM) of four feedstuff categories (grasses, indigenous trees and shrubs, agro-industrial by-products, crop residues) typically used by Ethiopian farmers, as a function of *in vitro* dry matter digestibility (IVDMD, incubation for 72 h).

yields. A possible limitation of the study is that feedstuff collection was restricted to two geographical areas, although these represented lowland and highland areas of Ethiopia.

4.1. Chemical composition of feedstuffs

Only two of the grasses analyzed, *Phalaris paradoxa L.* (109 CP g/kg) and *Echinochloa colona (L.) Link* (110 g/kg), met the minimum protein requirement of 70 g/kg DM necessary for optimal rumen microbial activities (Van Soest, 1994; National Research Council (NRC), 2007). Of the 13 indigenous plants (trees, shrubs, herbaceous plants) analyzed, only *Thymus vulgaris* and Tembien clover contained less than 100 g CP/kg DM. In Ethiopia, tree and shrub foliage is commonly used as feed for ruminants, especially in the dry, semi-arid, and mountainous regions that are home to most of the country's livestock (Mengistu et al., 2017).

The crop residues analyzed generally exhibited low CP values, falling short of meeting minimal ruminant nutrition requirements. This agrees with findings of low CP values in other studies in Ethiopia (e.g., Tolera et al., 2012; Chalchissa et al., 2014; Bayissa et al., 2022). In the context of smallholder mixed crop-livestock systems in Sub-Saharan Africa, crop residues serve as a vital resource for livestock sustenance. They will play an increasingly significant role in livestock feed in future, due to expansion of arable land and associated decreases in grazing resources (Duncan et al., 2016).

Within the agro-industrial by-product category, BSG demonstrated the highest CP values, while liquor (*areke*) and beer (*tella*) residues showed promising CP values of 255 g/kg DM and 183 g/kg DM, respectively. *Areke* is a type of spirit that is usually distilled at home from corn, wheat, or barley, and consumed during various celebrations and events. *Tella* is a traditional homebrewed beer made from roasted corn paste, yeast, and gesho (*Rhamnus prinoides*). The by-products of *areke* and *tella* production, referred to as *atella*, are traditionally used as supplementary feed for livestock. Agro-industrial by-products play a significant role in feeding livestock in Ethiopia, especially in urban and peri-urban livestock production systems. They are beneficial when animals require a high nutrient supply to achieve their productive potential (Mengistu et al., 2017).

4.2. Total gas and CH_4 yield

In the grass category, significant variations ($P<0.05$) were observed in CH_4 yield (g/kg DM), gas yield (L/kg DM), asymptomatic CH_4 yield (g/kg DM), CH_4/pdOM, and CH_4/pdNDF. *Phalaris paradoxa L.* demonstrated the highest values, owing mainly to its superior

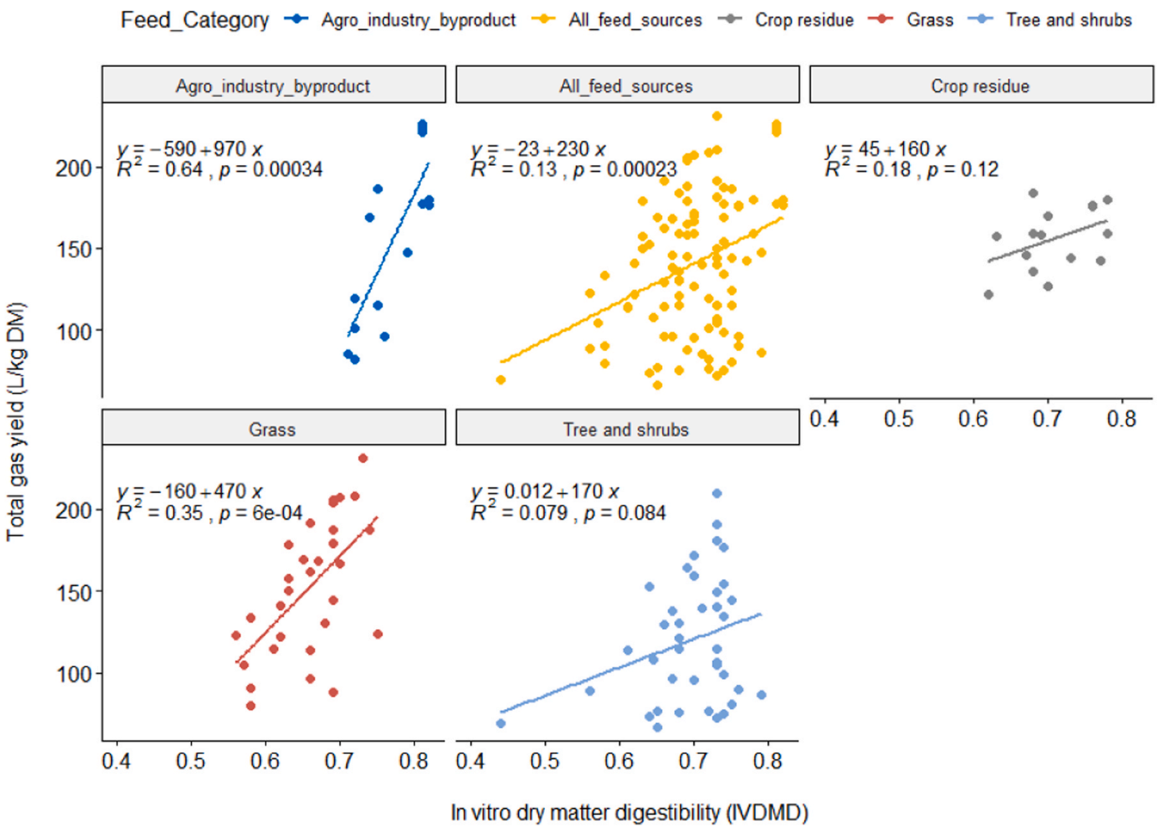

Fig. 5. Total gas yield (L/kg DM) of four feed categories (grasses, indigenous trees and shrubs, agro-industrial by-products, crop residues) typically used by Ethiopian farmers, as a function of *in vitro* dry matter digestibility (IVDMD, incubation for 72 h).

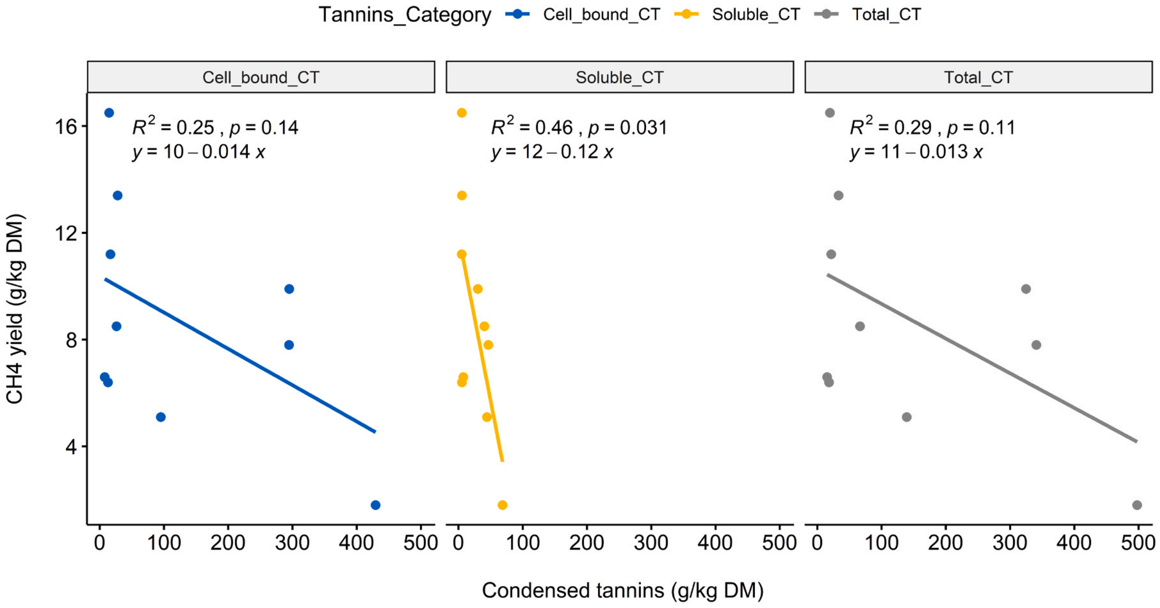

Fig. 6. Relationship between *in vitro* methane (CH_4) yield and content of soluble and cell-bound condensed tannins (g/kg DM).

quality as characterized by the lowest iNDF and intermediate CP (109 g/kg DM). Forages with enhanced quality often promote higher rumen microbial activity and associated gas production (Du Toit et al., 2018; Cardoso et al., 2023). In contrast, tropical grasses have low CP content and low nitrogen levels, which can restrict microbial activity within the rumen (McAllister and Newbold, 2008; de Moss et al., 2018). Fiber degradation and microbial protein synthesis also tend to be constrained by low ammonia levels (Bekele et al., 2009). Some studies report a decrease in CH_4 yield with increasing forage maturity and fiber content (Purcell et al., 2011; Molina-Botero et al., 2020). We found that CH_4 yield from grass was less than 24.1 g/kg DM, as reported previously for C4 grass (Archimède et al., 2011). However, our findings for *Phalaris paradoxa L.* align with findings by Ku-Vera et al. (2020) of CH_4 yield of 17.0 g/kg DMI in experiments involving cattle consuming low-quality tropical grass in respiration chambers in Mexico.

In the indigenous plant category (trees, shrubs, herbaceous plants), all CH_4 and gas variables exhibited significant differences ($P<0.05$). Remarkably, certain CH_4 values within this feed category aligned with suggested values for feed additives intended to mitigate enteric CH_4 emissions in ruminants (Chagas et al., 2019). *Trifolium acaule*, *Acacia nilotica (L.)*, and *Ziziphus spinachristi* demonstrated lower CH_4/total gas (%), CH_4/pdOM, aNDF, and higher pdOM. The potential for CH_4 production from feed varies due to chemical composition and plant metabolite content (Benchaar et al., 2001). Additionally, the quantity of gas produced from feeds relies strongly on their chemical composition and the rate and extent of degradability (Blümmel et al., 1999).

Feedstuffs in the crop residue category showed non-significant differences ($P>0.05$) in CH_4 and gas yield. However, Melesse et al. (2019) found that the CH_4 yield of four crop residues ranged from 14.4 to 17.2 g/kg DM, which was lower than in our study (16.9–23.2 g/kg DM). In general, straw tends to produce relatively more CH_4 than forage residues (Pal et al., 2015). In Ethiopia, crop residues from teff (*Eragrostis teff*) and wheat are among the most commonly used feedstuffs for livestock (Tolera et al., 2012).

In the agro-industrial by-product category, BSG demonstrated notably lower CH_4 and gas yield than other by-products ($P<0.001$). BSG, a vital by-product of the beer brewing industry, has significant nutritional value for animal production and has antioxidant and antimicrobial properties, and is widely used in the food and feed industries to prevent spoilage and deterioration (Socaci et al., 2018; Dai et al., 2022).

In this study, CH_4 as a percentage of total gas yield ranged from 14.5% to 19.6% for grasses, 3.1–16.9% for indigenous plants, 15.8–20.6% for crop residues, and 12.8–18.7% for agro-industrial by-products. There were marked differences ($P<0.01$) particularly within the indigenous plant and agro-industrial by-product categories. Percentage of CH_4 in total gas yield is a vital indicator of the methanogenic traits of feedstuffs (Okunade et al., 2014) and is pivotal in screening available feeds for creating low CH_4 emission ruminant diets (Bezabih et al., 2014). A lower percentage indicates reduced methanogenic potential of digestible feed components, resulting in lower CH_4 production per unit net gas volume output (Moss et al., 2000; Okunade et al., 2014). In this study, *Trifolium acaule* (3.1%), *Acacia nilotica* (L.) (7.1%), and *Ziziphus spina-christi* (9.9%), in the indigenous plants category, exhibited the lowest percentage of *in vitro* CH_4 in total gas. Therefore, these feedstuffs are suitable choices of low CH_4 emitting feed sources compared with those with higher ratios (Berhanu et al., 2019). However, feedstuffs with very high CT content should be avoided, as it can impair productivity in animals.

4.3. Relationships between feed composition, IVDMD, and tannins and in vitro CH_4 and gas yield

The model developed revealed increased iNDF and CP in the grass category, penalizing both CH_4 and gas yield (Table 9). This was possibly due to the grass feedstuffs analyzed in our study being less fermentable and more lignified, since fiber content can influence gas production rate (Pal et al., 2015). Production of CH_4 in an *in vitro* gas system is strongly associated with fermentation of structural carbohydrates (Marín et al., 2021).

In the indigenous plant category (trees, shrubs, herbaceous plants), the variables aNDF, CP, and pdOM were positively correlated with CH_4 and gas yield. This is consistent with findings by Uribe et al. (2022), who evaluated 11 forage types and found that those with higher NDF and CP contents gave increased gas and CH_4 yield. The model, which considered total feed samples, also demonstrated that higher levels of aNDF and pdOM led to higher CH_4 and gas yield. It is well-known that fermentation of fibrous carbohydrates leads to higher CH_4 production than non-fibrous carbohydrates (Johnson and Johnson, 1995; Ribeiro et al., 2014).

As seen in Fig. 4, there was a substantial increase in CH_4 yield with each unit increase in IVDMD. Previous studies, e.g., by Ramin and Huhtanen (2013), Fant et al. (2020), and Gaviria-Uribe et al. (2020), have reported positive correlations between diet true DMD and CH_4 production. The digestibility values of feedstuffs are intricately linked to their structural carbohydrate composition, including lignin concentration (Quintero-Anzueta et al., 2021) and the influence of various secondary metabolites (Lascano and Cárdenas, 2010). When IVDMD falls below 550 g/kg, this imposes physical limitations on feeding and digestion, leading to live weight loss (Ridgman, 1991; Okunade et al., 2014). A study by Molina-Botero et al. (2020) noted a declining trend in CH_4 production as DMD decreased.

Comparison of CH_4 and gas yield across various feed categories (Figs. 2 and 3) revealed an intriguing trend of notably lower CH_4 and gas yield for the indigenous plant category. Similarly, Berhane et al. (2006) observed reduced gas production from certain browsed plant species potentially containing phenolic compounds that hinder microbial fermentation. This is supported by our findings on the relationship between CH_4 yield and soluble and cell-bound CT, (Fig. 6).

Tannins and saponins have known potential to suppress CH_4 production during rumen fermentation (Hariadi and Santoso, 2010; Bezabih et al., 2014). Optimal tannin concentrations below 5% may be of benefit, while higher concentrations can impair nitrogen digestibility and intake (Mlambo et al., 2004; Min et al., 2005; Patra and Saxena, 2011). A previous study by our research group revealed a positive impact of including *Acacia nilotica* (rich in CT) on weight gain in sheep (Bekele et al., 2022). Data obtained in the present study also indicated a general decrease in CH_4 yield at higher CT levels, particularly at elevated soluble CT concentrations. *Trifolium acaule* had the highest soluble CT content, resulting in the lowest *in vitro* CH_4 yield, possibly due to inhibitory effects of CTs on protein degradation (Patra and Saxena, 2011).

Phenolic compounds are suggested to inhibit microbial gas production *in vitro* through bactericidal or bacteriostatic pathways (Kumar and Singh, 1984; Berhanu et al., 2006). Moreover, secondary phenolic compounds in legumes eliminate protozoa associated with methanogen production (Patra et al., 2017; Molina-Botero et al., 2020). Consequently, phenolic compound content should not be overlooked in feed evaluation, including their impact on digestibility and gas production in ruminants.

5. Conclusions

Methane yield (g/kg DM) profiles of locally available ruminant feed resources in central Ethiopia revealed that indigenous trees, shrubs, and herbaceous plants gave lower CH_4 and gas yields than grasses, crop residues, or agro-industrial products. There was a strong positive correlation between CH_4 yield and *in vitro* dry matter digestibility. Stepwise regression analysis showed that grasses had lower fermentability. Percentage of CH_4 in total gas yield was lowest for *Trifolium acaule*, *Acacia nilotica (L.)*, *Ziziphus spina-christi*, BSG, and local liquor-beer residues (*atella*), making them promising candidates for future evaluation of low enteric CH_4-producing diets. Approximately half of the tree and shrub material analyzed had total CT levels exceeding the limit of 5%, necessitating further *in vivo* research to confirm the potential of CTs in reducing CH_4 emissions. Overall, this study provides a comprehensive database on the nutrient composition and on *in vitro* CH_4 and gas yield of locally available ruminant feed sources in Ethiopia. This information can be valuable in formulating ruminant rations with reduced CH_4 emissions (considering the CT content of feedstuffs), contributing to sustainable livestock practices.

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CRediT authorship contribution statement

Wondimagegne Bekele: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Pekka Huhtanen:** Writing – review & editing, Validation, Supervision. **Abiy Zegeye:** Writing – review & editing. **Addis Simachew:** Writing – review & editing. **Abu Bakar Siddique:** Writing – review & editing, Methodology, Investigation. **Benedicte Albrectsen:** Writing – review & editing. **Mohammad Ramin:** Writing – review & editing, Validation, Supervision, Investigation.

Declaration of Competing Interest

The authors have no competing interests to declare.

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