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Research Article

Nutritional Value and In Vitro Volatile Fatty Acid Production of Forage Grasses Cultivated Using Farmyard Manure and *Desmodium intortum* Intercropping in the Upper Blue Nile Basin, Ethiopia

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Integrating farmyard manure (FYM) and legume intercropping improves soil chemical and microbial properties, thereby increasing forage productivity and nutritional value. The purpose of this study was to evaluate how fertilizer treatments affected the chemical composition, in vitro gas production and organic matter digestibility (IVOMD), and volatile fatty acid production (VFA) of Napier and desho grasses in the upper Blue Nile basin of Ethiopia. Apart from ether extract content, the chemical composition of the fertilizer treatments in the Aba Gerima watershed did not differ significantly, whereas significant differences were observed in acid detergent fiber and crude protein contents at the Guder watershed. In both watersheds, Napier grass had significantly higher crude protein levels than desho grass. In Aba Gerima, ash content was significantly higher in 2018 than in 2019, but not in Guder. For Napier and desho grasses, there were an increment in IVOMD (6.7 vs 4.7%), metabolizable energy (5.5 vs 4.5%), and VFA production (23.1 vs 3.0%) in the *Desmodium intortum* plus FYM treatment than in the control in Aba Gerima. In Guder, however, IVOMD (13.7 vs 4.6%), metabolizable energy (13.3 vs 3.3%), and VFA production (11.2 vs 5.6%) increased in the same treatment for Napier and desho grasses than in the control. This study suggests that the *Desmodium intortum* plus FYM improves the nutritional value, IVOMD, and VFA production of Napier and desho grasses in dryland areas of the upper Blue Nile basin, facilitating their growth as alternative livestock feed for better production in these areas.

1. Introduction

The Ethiopian livestock subsector contributes significantly to both household and national economies [1], even though animal productivity remains among the lowest in Africa. In most farming systems in Ethiopia, feed scarcity in both qualitative and quantitative dimensions is a major impediment to livestock production [2]. Thus, increased livestock production can only be realized through the cultivation of high-yielding quality forages in a variety of agroecological settings [3].

Grazing and crop residue provide more than 69.5% of the feed resources for ruminant animals in Ethiopia's highlands [4]. The nutritional value of such feed resources, on the other hand, is characterized by a high cell wall content and a low protein, energy, and mineral content [5]. Improved forages, on the other hand, provide a reasonable source of nutrients for animal production while conserving soil fertility, water resources, and air quality [6]. The most common forage grasses used by livestock producers in northwestern Ethiopia are Napier grass (*Pennisetum purpureum*) and desho grass (*Pennisetum pedicellatum*) [3, 7]. According to Zewdu et al. [3], at a 1-meter harvest, Napier grass has 65.5% in vitro organic matter digestibility (IVOMD) and 9.0 MJ kg^{-1} metabolizable energy (ME). Desho grass, on the other hand, has a mean IVOMD and ME value of 43.9% and 6.2 MJ kg^{-1}, respectively [7].

Forage grass chemical composition varies with soil type, landscape, management practice, and other environmental factors that influence forage yield and quality [8]. Manure improves the physical, chemical, and microbial properties of soil, increasing productivity and nutritional value while remaining environmentally sustainable [9]. Farmyard manure (FYM) has a high organic matter content and serves as a source of plant nutrients through decomposition and nutrient release. Intercropping compatible forage grasses with legumes like *Desmodium intortum* improves and maintains soil fertility via nitrogen fixation and increases productivity and profitability [10]. Thus, for improved ruminant livestock production, the effects of integrated FYM and *Desmodium* intercropping on the nutritional value and volatile fatty acid (VFA) production of forage grasses need further investigation.

Chemical composition, IVOMD, and VFA production are useful feed evaluation indicators for ruminant livestock production ration balancing. More degradable by-products have higher IVOMD values and a wider range of ruminal fermentability [11] for improved animal production. In this case, using in vitro gas production to evaluate forage digestibility is a quick and low-cost method of determining ruminant feed nutritive value [12]. It is easy to use, fast, and can handle a large number of samples at once. In tropical forage grasses, Bezabih et al. [13] discovered a link between forage chemical composition and in vitro fermentation parameters. In contrast, nitrogen (N) fertilization improved biomass yield, crude protein, cellulose, and lignin content by decreasing the energy content available for metabolism and slowing the fermentation rate as well as decreasing in vitro dry matter disappearance and cumulative gas production [14].

Previous research has found that applying FYM improves forage grass growth and biomass yield [11, 15]. However, there is little scientific evidence on the effects of integrated FYM and *Desmodium* intercropping as fertilizer treatments and season of production on forage grass nutritional value, IVOMD, VFA, and methane production. Thus, the objective of this study was to assess the effects of FYM and *Desmodium intortum* on the chemical composition, IVOMD, VFA, and methane production of Napier and desho grasses in midland and highland agroecologies in dryland areas of Ethiopia's upper Blue Nile basin.

2. Materials and Methods

2.1. Study Areas. The research was undertaken in two watersheds, Aba Gerima and Guder, of the upper Blue Nile basin, Amhara region, Ethiopia (Figure 1); these watersheds represent midland and highland agroecologies, respectively. The Aba Gerima watershed lies in the Bahir Dar Zuria district within the region 370 29' 24″ E to 370 32′ 24″ E latitude and 110 38′ 24″ N to 110 40′ 12″ N longitude at 1970 m above sea level. The mean annual rainfall is 1558 mm, with most rain occurring from June to September. In the two study years, the mean minimum and mean maximum annual temperature varied from 13.3 to 27.6°C. The main means of livelihood for farmers in the watershed are mixed crop cultivation and livestock rearing. The main livestock species are cattle (*Bos indicus*), sheep (*Ovis aries*), goat (*Capra hircus*), and donkey (*Equus africanus*) [16]. The Guder watershed is located in the Fagta Lekoma district 10°59′34″N to 11°01′01″N latitude and 36°54′09″E to 36°55′55″E longitude at 2577 m above sea level. It has higher annual rainfall (mean, 2370 mm) and lower mean annual temperatures than the Aba Gerima watershed: the data from a nearby meteorological station showed that the mean minimum and mean maximum annual temperature in the Guder watershed varied from 10.6 to 25.2°C. The main livestock species reared in the Guder watershed are cattle, sheep, and horse (*Equus caballus*) [16].

2.2. Experimental Design and Treatments. A randomized complete block design with a 2 × 4 × 2 factorial arrangement was used for the experiment for two consecutive years (2018 and 2019). The factors were the type of forage grass (two levels: Napier and desho grasses), the fertilizer treatments (four levels: no fertilizer [control], *Desmodium*, FYM, and a combination of *Desmodium* and FYM [hereafter, "*Desmodium* plus FYM"]), and the year (two levels: 2018 and 2019). The experiment was carried out in both watersheds under rain-fed conditions. The FYM used in the experiment was collected from the Bahir Dar University dairy farm, Zenzelma campus, Ethiopia, and consisted of cattle manure with a small amount of roughage feed residues and urine. One month before planting forage grasses, farmyard manure was applied at a rate of 12 *t* ha^{-1} Ayichew [17]. The FYM was 17.5% organic carbon, 1.5% total nitrogen, 11.7 : 1 carbon to nitrogen ratio, 32.8 mg kg^{-1} available phosphorous, and a pH of 7.4. Both forage grasses were planted in 2018 with healthy root splits obtained from a nearby research facility, and data were collected over the course of 2 years. Napier grass was planted with a 1-m inter-row spacing and a 50-cm intra-row spacing, whereas desho grass was planted with a 50-cm inter- and intra-row spacing. *Desmodium intortum* was sown between two rows of forage grass 1 month after the grass was planted for *Desmodium* intercropping. For better forage grass establishment and production, each plot was kept weed-free throughout the experiment.

2.3. Sample Collection and Preparation. Healthy root splits were used to plant the forage grasses. For better biomass yield and nutritional value, Napier grass was clipped at a

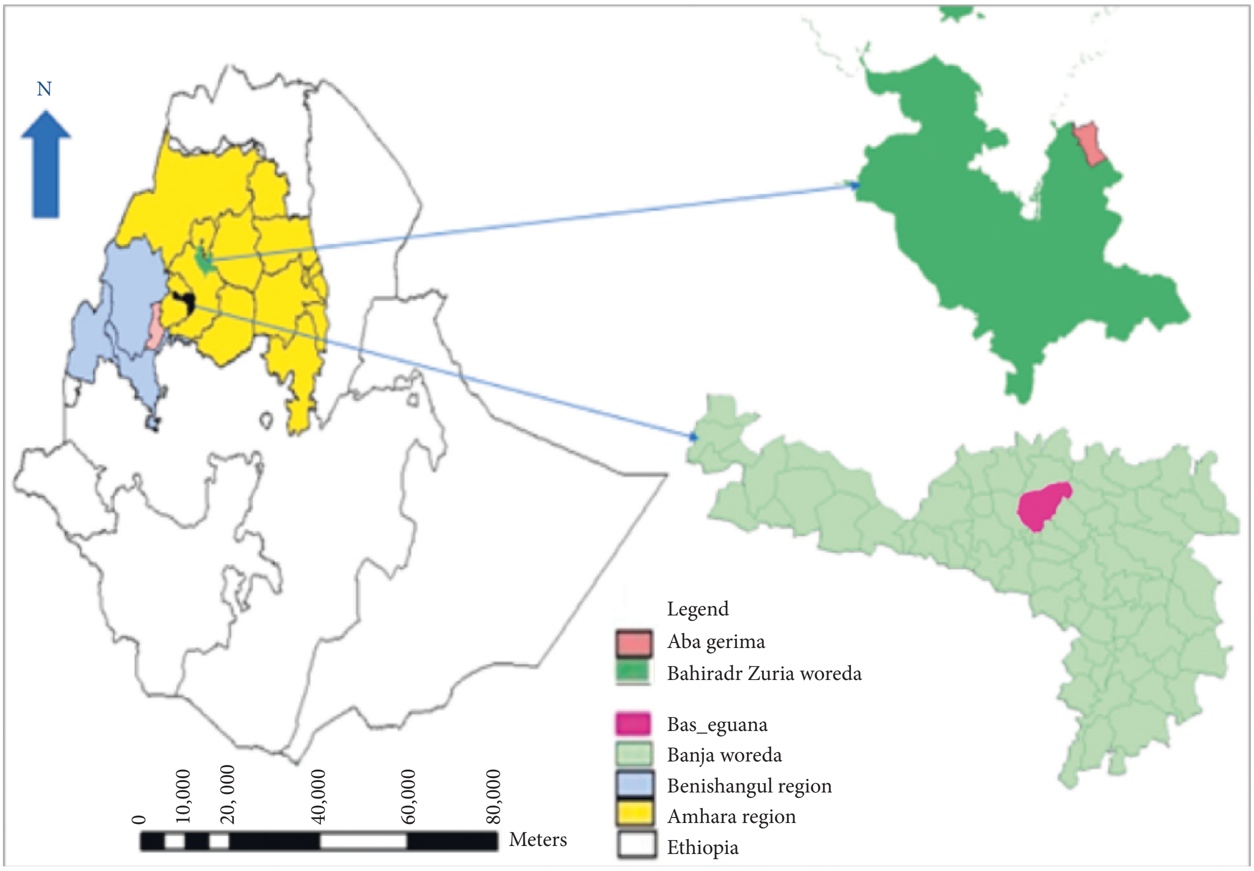

Figure 1: Map of study areas.

height of 1 m [3], while 50–60 cm was used for desho for each year [18]. Harvested fresh samples were immediately chopped manually into small pieces with a sickle, placed inside a plastic bag, and weighed. The samples were then transported from the field to the College of Agriculture and Environmental Sciences at Bahir Dar University. All harvested samples from each plot were thoroughly mixed, and a representative whole forage sample was taken for each watershed in each year. The samples were oven-dried at 60°C for 72 h to a constant weight. The dried samples were then ground to pass through a 1-mm sieve and placed in air-tight plastic bags to determine chemical composition, gas production, IVOMD, and VFA production.

2.4. Chemical Analysis. The determination of dry matter (DM), total ash, and crude protein (CP) followed the procedures of the Association of Official Analytical Chemists [19], while ether extract (EE) was analyzed with ethyl ether using gravimetrical methods in the same procedure. Van Soest et al. [20] determined the contents of the fiber fractions neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). Total ash content was determined by combusting the samples in a muffle furnace at 550°C for 3 h [19]. The samples were ignited, and the nitrogen content was determined with a carbon-nitrogen elemental analyzer (Shimadizu, Tottori, Japan). The CP content was calculated by multiplying the nitrogen content by a factor of 6.25.

2.5. In Vitro Gas Production, Organic Matter Digestibility, and Production of VFAs. The syringe method was used for in vitro fermentation to determine IVOMD of the forage grasses by Menke and Steingass [21]. Two Japanese Corriedale sheep were rumen-canulated to collect rumen fluid for in vitro fermentation. The sheep were fed fresh forage dominated by Timothy grass and were given free access to water and mineral licks. Samples of rumen fluid were obtained from both sheep at 3 h after the morning feed. The samples were flushed with CO_2 and strained through two layers of cheesecloth into a prewarmed, insulated thermos flask. The filtered rumen fluid was combined in a 1 : 2 ratio with a culture medium containing macrominerals, microminerals, and bicarbonate buffer reducing solution and kept at 39°C with a constant flow of CO_2 to maintain anaerobic conditions. Then, as Menke and Steingass [21] described, 0.2 g of DM forage samples was accurately weighed and inserted into 100-ml glass syringes; two syringes without substrate were incubated as controls, and 30 mL of rumen fluid + medium was added under a stream of CO_2, closed with rubber stoppers and crimp seal to prevent the accumulation of fermentation gases. The syringes were placed in a rotor inside an incubator set to 39°C and rotated once every

Table 1: Effects of fertilizer treatments on the chemical composition of forage grasses at the Aba Gerima watershed.

Variables	Ash	NDF	ADF	ADL	EE	CP	GE
	% Of DM					g kg^{-1} DM	
Fertilizer treatment (F)							
Control	6.1	70.9	39.1	11.1	2.4b	85.0	12.8
Desm	6.3	70.6	38.6	10.2	2.1c	78.6	13.0
FYM	6.0	71.3	42.0	11.3	2.4b	78.3	12.7
Desm plus FYM	6.1	72.0	42.6	11.7	2.6a	80.8	12.8
± SEM	0.08	0.86	1.44	0.55	0.03	3.07	0.10
Forage grass (FS)							
Desho grass	6.3a	69.9b	39.5	11.2	3.0a	70.0b	13.0a
Napier grass	6.0b	72.5a	41.7	10.9	1.8b	91.3a	12.7b
± SEM	0.06	0.61	1.01	0.39	0.02	2.17	0.07
Year (Y)							
2018	6.3a	69.2b	41.2	12.1a	2.3b	81.7	12.8
2019	5.9b	73.2a	40.0	10.0b	2.5a	79.6	12.9
± SEM	0.06	0.61	1.01	0.39	0.02	2.17	0.07
Overall mean	6.1	71.2	40.6	11.1	2.4	80.6	12.8
P-values							
F	0.2753	0.7236	0.1340	0.2925	<0.0001	0.3991	0.1994
FS	0.0003	0.0043	0.1347	0.4873	<0.0001	<0.0001	0.0109
Y	<0.0001	<0.0001	0.3738	0.0008	<0.0001	0.4844	0.5318
F*FS	0.6920	0.0045	0.8172	0.8329	<.0001	0.4012	0.0013
F*Y	0.3584	0.1512	0.2891	0.3197	0.9542	0.5698	1.0000
FS*Y	0.0881	0.1138	0.1506	0.0122	0.0810	0.4672	0.9947

Means with different superscripts in a column are significantly different ($P < 0.05$, Tukey's test); Control, no fertilizer treatment. ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; Desm, *Desmodium intortum*; DM, dry matter; EE, ether extract; FYM, farmyard manure; GE, gross energy; NDF, neutral detergent fiber.

minute. At Shimane University in Japan, cumulative gas volume measurements of samples were taken before incubation (0 h) and then after 3, 6, 12, 24, 48, 72, and 96 hours of incubation.

IVOMD was estimated based on 24-h gas production (GP24; ml 0.2 g^{-1} DM) and CP content (g kg^{-1} DM) by using (1), while metabolizable energy (ME) content (MJ kg^{-1} DM) was estimated using GP24, CP content, and EE content (g kg^{-1} DM) using (2) as described by [21].

$$\text{IVOMD}\,(\%) = 18.53 + 0.9239 \times GP24 + 0.0540 \times CP, \quad (1)$$

$$ME\left(MJkg^{-1}DM\right) = 2.43 + 0.1206 \times GP24 + 0.0069 \times CP + 0.0187 \times EE. \quad (2)$$

In the analysis of VFA production, 5 ml was collected at the end of in vitro gas production and centrifuged for 15 minutes at 4°C. Then the supernatant was transferred to a micro-centrifuge tube and stored at −20°C until further analysis. The frozen samples were defrosted and filtered through gauze to remove foreign materials before being centrifuged at 10,000 RPM for 30 minutes at 4°C. To make the internal standard, 0.5 mL of supernatant was mixed with 10 mmol L^{-1} crotonic acid. The external standard was made up of the same concentrations of acetic, propionic, n-butyric, isobutyric, and crotonic acids as the internal standard. The samples were injected into a gas chromatography machine (Shimadizu Corp., Kyoto, Japan) to determine VFA concentrations. After subtracting the value for the corresponding control, the final VFA concentrations were reported. The predicted methane (CH_4) emissions from the VFAs were calculated using (3), which was developed by Moss et al. [22].

$$CH4\left(\text{mmol}L^{-1}1\right) = 0.45 \times \text{acetate} - 0.275 \times \text{propionate} + 0.40 \times \text{butyrate}, \quad (3)$$

where acetate, propionate, and butyrate are the concentrations in mmol L^{-1}.

The gross energy of the forage grasses was determined using a bomb calorimeter (Shimadzu, Japan).

2.6. Statistical Analysis. Data on chemical composition, in vitro gas production, IVOMD, and VFA of forage grasses were analyzed using the general linear model procedure of Statistical Analysis Software SAS JMP13 [23] for each watershed separately. Tukey's honestly significant difference test was used to determine whether there were significant differences between treatment means.

3. Results

3.1. Forage Grass Chemical Composition under Various Fertilizer Treatments. The effects of fertilizer treatments on the chemical composition of the forage grasses are presented in Table 1 (Aba Gerima watershed) and Table 2 (Guder watershed). Fertilizer treatments had no effect on the chemical composition of the forage grasses in the Aba Gerima watershed, with the exception of EE ($P < 0.001$). EE values ranged from 2.1% in forage grasses cultivated with

Table 2: Chemical composition of forage grasses cultivated using various fertilizer treatments at the Guder watershed.

Variables	Ash	NDF	ADF	ADL	EE	CP	GE
	% Of DM					$g\ kg^{-1}$ DM	
Fertilizer treatment (F)							
Control	6.2	70.5	42.2a	12.0	2.2b	114.6b	12.7
Desm	6.3	69.1	38.4b	11.6	2.2b	107.8c	12.5
FYM	6.2	68.9	39.3ab	12.0	2.5a	118.9a	12.6
Desm plus FYM	6.2	67.3	38.6b	11.7	2.6a	118.3a	12.5
± SEM	0.10	0.80	0.85	0.52	0.03	2.90	0.14
Forage grass (FS)							
Desho grass	6.3a	68.1	39.4	11.0b	1.6b	98.2b	12.8a
Napier grass	6.1b	69.7	39.9	12.7a	3.1a	131.6a	12.3b
± SEM	0.07	0.57	0.60	0.37	0.02	2.05	0.10
Year (Y)							
2018	6.1	67.0b	40.2	13.1a	2.3b	114.0	12.6
2019	6.3	70.8a	39.1	10.5b	2.5a	115.8	12.6
± SEM	0.07	0.57	0.60	0.37	0.02	2.17	0.10
Overall mean	6.2	68.9	39.8	11.8	2.4	114.9	12.6
P-values							
F	0.8773	0.0608	0.0113	0.9128	<0.0001	0.0412	0.5453
FS	0.0109	0.0676	0.5427	0.0031	<0.0001	<0.0001	0.0011
Y	0.0890	<0.0001	0.1983	<0.0001	<0.0001	0.5497	0.6501
F*FS	0.3649	0.0208	0.3815	0.7172	0.0139	0.9550	0.3814
F*Y	0.5003	0.2143	0.2049	0.8463	0.7136	0.0045	1.0000
FS*Y	0.7808	0.2170	0.5806	0.0015	0.0973	<0.0001	0.9929

Means with different superscripts in a column are significantly different ($P < 0.05$; Tukey's test). Control, no fertilizer treatment.

Desmodium to 2.6% in those cultivated with *Desmodium* plus FYM. At the Aba Gerima watershed, the EE content of the FYM and *Desmodium* plus FYM fertilizer treatments increased in Napier grass (14.4–17.9%) than the control, whereas desho grass did not show an increase in the same fertilizer treatments (Table S3).

The CP and NDF contents of Napier grass were significantly higher than those of desho grass, while the ADF and ADL contents of the two forage grasses at the Aba Gerima watershed were comparable. In our study, the concentrations of NDF ranged from 69.9% in desho grass to 72.5% in Napier grass at Aba Gerima, while 68.1% to 69.7% for desho and Napier grasses, respectively, at the Guder watershed. Fertilizer treatments had no effect on the CP content of Napier or desho grass in Aba Gerima, but variation was observed in the Guder watershed. In the same watershed, the NDF content of FYM and *Desmodium* plus FYM fertilizer treatments was 6.5% higher in Napier grass, while desho grass decreased by 6.0% and 3.9%, respectively. Furthermore, the EE and GE contents of desho grass were significantly higher ($P < 0.001$) than those of Napier grass. In 2019, the NDF and EE contents were significantly higher ($P < 0.001$) than in 2018, while the ash content was lower. The other chemical composition variables were not significantly different between the two years. The GE content in Napier grass was 3.7% higher in the FYM fertilizer treatment than the control at the Aba Gerima watersheds, while it was 4.6% lower in the desho grass in the same fertilizer treatment (Table S3).

In the Guder watershed, fertilizer treatment had a significant ($P < 0.05$) effect on ADF, EE, and CP contents, but variation in ash, NDF, ADL, and GE was not observed in similar treatments. The fiber fraction values tended to be higher in the control treatment than in the other fertilizer treatments, but this difference was only significant for ADF (control versus FYM; control versus *Desmodium* plus FYM). The fiber fraction values of the two forage grasses did not differ significantly. Napier grass contained significantly more ADL, EE, and CP than desho grass, while the latter contained more ash and GE.

In comparison to the control, fertilizer treatments FYM and *Desmodium* plus FYM (2.6 and 1.4%) increased the CP content of Napier grass, while desho grass increased by 4.9–5.2% in the same fertilizer treatments. Under FYM and *Desmodium* plus FYM fertilizer treatments, the NDF content was 0.01–7.4% lower in Napier grass and 5.6–2.2% lower in desho grass than in control (Table S4). However, unlike in the Aba Gerima watershed, the NDF and EE contents were significantly higher in 2019 than in 2018. However, in the Aba Gerima watershed, the ADL content was significantly higher in 2018 than in 2019.

3.2. Effects of Fertilizer Treatment on GP, IVOMD, and VFAs. The effect of fertilizer treatment and year on GP24, IVOMD, ME, and VFAs of forage grasses is shown in Table 3 (Aba Gerima) and Table 4 (Guder). In both watersheds, the value for GP24 was significantly lower in the control and *Desmodium intortum* (*Desmodium*) treatments than in the FYM and *Desmodium* plus FYM treatments. Furthermore, in both watersheds, the IVOMD value increased in the order of control < *Desmodium* < FYM < *Desmodium* plus FYM (Tables S3 and S4). When compared to the control, the GP content of Napier and desho grasses increased by 3.5–12.4% and 2.9–8.6%, respectively, in the FYM and *Desmodium* plus FYM fertilizer treatments at the Aba Gerima watershed.

Table 3: Results of Tukey's tests exploring the interaction effects of forage grasses, fertilizer treatments, and year on GP24, IVOMD, and ME contents at the Aba Gerima watershed.

Factors			GP24	IVOMD	ME
Fertilizer sources	Forage grass	Year	ml 0.2 g^{-1} DM	%	MJ kg^{-1}
Control	Desho	2019	45.0^{ab}	54.1^{ab}	9.2^{a}
Desm	Desho	2019	47.0^{a}	65.3^{ab}	9.0^{ab}
FYM	Desho	2019	44.0^{b}	62.6^{b}	8.9^{ab}
Desm plus FYM	Desho	2019	47.3^{a}	66.0^{a}	8.7^{b}
Control	Desho	2018	30.1^{d}	50.3^{def}	7.2^{de}
Desm	Desho	2018	28.2^{de}	48.9^{efg}	6.8^{ef}
FYM	Desho	2018	33.3^{c}	53.1^{cd}	7.5^{cd}
Desm plus FYM	Desho	2018	34.8^{c}	54.1^{c}	7.6^{c}
Control	Napier	2019	29.0^{d}	50.5^{de}	6.9^{e}
Desm	Napier	2019	34.0^{c}	54.7^{c}	6.4^{g}
FYM	Napier	2019	30.0^{d}	51.1^{de}	6.9^{e}
Desm plus FYM	Napier	2019	34.0^{c}	55.0^{c}	7.4^{cd}
Control	Napier	2018	23.4^{f}	45.2^{h}	6.3^{g}
Desm	Napier	2018	25.1^{f}	46.3^{gh}	6.4^{g}
FYM	Napier	2018	24.3^{f}	45.4^{h}	6.3^{g}
Desm plus FYM	Napier	2018	25.8^{ef}	47.5^{fgh}	6.5^{fg}
Overall mean			33.5	53.8	7.43
SEM			0.565	0.397	0.075
P-values			0.0015	0.0134	0.0196
CV (%)			2.9	1.8	1.7

Means with different superscript letters in a column are significantly different ($P < 0.05$). Abbreviations: CV, coefficient of variation; Desm, *Desmodium intortum*; F, fertilizer treatments; FYM, farmyard manure; GP24, 24-h gas production; IVOMD, in vitro organic matter digestibility; ME, metabolizable energy; SEM, standard error of mean.

Table 4: Results of Tukey's tests exploring the interaction effects of forage grasses, fertilizer treatments, and year on GP24, IVOMD, and ME contents at the Guder watershed.

Factors			GP24	IVOMD	ME
Fertilizer sources	Forage grass	year	ml 0.2 g^{-1} DM	%	MJ kg^{-1}
Control	Desho	2019	44.6^{b}	63.5^{b}	8.6^{a}
Desm	Desho	2019	32.0^{f}	52.0^{d}	7.1^{c}
FYM	Desho	2019	44.0^{b}	64.7^{ab}	8.6^{a}
Desm plus FYM	Desho	2019	47.0^{a}	67.1^{a}	8.9^{a}
Control	Desho	2018	34.4^{def}	56.5^{c}	7.7^{b}
Desm	Desho	2018	36.9^{de}	59.4^{c}	8.1^{b}
FYM	Desho	2018	36.5^{de}	57.3^{c}	7.7^{b}
Desm plus FYM	Desho	2018	37.0^{d}	58.6^{c}	7.9^{b}
Control	Napier	2019	26.0^{g}	50.2^{de}	7.0^{c}
Desm	Napier	2019	28.0^{g}	51.5^{de}	7.2^{c}
FYM	Napier	2019	34.0^{ef}	58.0^{c}	8.0^{b}
Desm plus FYM	Napier	2019	41.0^{c}	64.7^{ab}	8.9^{a}
Control	Napier	2018	25.4^{g}	48.6^{e}	6.9^{c}
Desm	Napier	2018	26.7^{g}	49.7^{de}	7.1^{c}
FYM	Napier	2018	27.9^{g}	50.8de	7.1^{c}
Desm plus FYM	Napier	2018	27.2^{g}	49.9^{de}	7.1^{c}
Overall mean			34.2	56.4	7.8
SEM			0.564	0.580	0.077
P-value			<.0001	<.0001	<.0001
CV (%)			2.9	1.8	1.7

Means with different superscript letters in a column are significantly different ($P < 0.05$). GP24: 24-h gas production in ml 0.2 g-1 DM; IVOMD, in vitro organic matter digestibility in %, ME, metabolizable energy; NDF, neutral detergent fiber in %; TVFA, total volatile fatty acids in mmol L-1.

Furthermore, the value for IVOMD increased in Napier grass by 1.2–6.7% and desho grass by 1.0–4.7% in the same fertilizer treatment than the control (Table S5). The IVOMD value in the same watershed ranged from 45.2% (2018 production year) to 66.0% (2019 production year), with an overall mean of 53.8%; in Guder, it ranged from 48.6% (Napier grass) to 67.1% (desho grass), with an overall mean of 56.4%.

Desmodium plus FYM produced significantly higher IVOMD and ME values than all other treatments in both watersheds (Tables S3 and S4). Desho grass had significantly higher ($P < 0.001$) GP24, IVOMD, and ME than Napier grass in both watersheds: the mean GP24 values for desho and Napier grasses at the Aba Gerima watershed were 38.7 and 28.2 ml 0.2 g^{-1} DM, respectively, and 39.0 and 29.5 ml 0.2 g^{-1} DM, respectively, at the Guder watershed (Tables S3 and S4). In both watersheds, GP24, IVOMD, and ME were significantly higher ($P < 0.001$) in 2019 than in 2018.

There was a significant effect ($P < 0.001$) for the two-way interaction in forage grass × fertilizer treatment and forage grass × year in the case of GP24, IVOMD, and ME, as well as for the three-way interaction of forage grass × fertilizer treatment × year. In both watersheds, the three-way combination of forage grass = desho, fertilizer treatment = *Desmodium* plus FYM, and production year = 2019 resulted in significantly higher ($P < 0.001$) GP24, IVOMD, and ME values than the other combinations of these three variables (Tables 3 and 4).

In both watersheds, the forage grass × fertilizer interaction significantly ($P < 0.001$) influenced the concentrations of acetate (A), propionate (P), total VFA (TVFA), and methane (CH_4), as well as the A:P ratio. The proportions of A (1.4–8.7%), P (10.0–15%), and TVFA (4.2–11.2%) were higher in the FYM and *Desmodium* plus FYM treatments than in the control at the Guder watershed. In contrast, most VFA proportions for desho grass decreased under similar treatment conditions (Table S6). The A and P values in the control group were significantly lower than those in the other treatment groups in the same watershed, with the exception of A in the FYM group and P in the *Desmodium* group.

In the Aba Gerima watershed, the concentrations of A and P were significantly higher ($P < 0.001$) after the application of FYM compared to other treatments (Table 3); in addition, they were also significantly higher after the application of *Desmodium* plus FYM in the Guder watershed (Table 4). The lowest concentrations of A and P were observed in *Desmodium* treatment groups in the Aba Gerima watershed and in the control treatment at the Guder

Table 5: Correlation between chemical composition, in vitro organic matter digestibility, and VFA production of forage grasses. GP24 ($r = -0.569$, $P < 0.001$), IVOMD ($r = -0.378$, $P < 0.001$), and methane production ($r = -0.505$, $P < 0.05$).

	CPact	NDF	ADF	ADL	GP24	IVOMD	ME	TVFA	CH_4
CP	1	0.215^{ns}	0.119^{ns}	0.536^{ns}	-0.569^{**}	-0.378^{**}	-0.369^{ns}	-0.507^{ns}	-0.505^{*}
NDF		1	0.677^{ns}	0.225^{ns}	-0.384^{ns}	-0.327^{ns}	-0.327^{ns}	0.251^{ns}	0.534^{ns}
ADF			1	0.323^{ns}	-0.140^{ns}	-0.099^{ns}	-0.104^{ns}	0.256^{ns}	0.309^{ns}
ADL				1	-0.630^{ns}	-0.599^{ns}	-0.593^{ns}	-0.348^{*}	-0.640^{*}
GP24					1	0.974^{***}	0.973^{***}	$0.759*$	0.421^{ns}
IVOMD						1	0.999^{***}	0.741^{*}	0.397^{ns}
ME							1	0.741^{*}	0.390^{ns}
TVFA								1	0.707^{*}
CH_4									1

Values shown are Pearson's correlation coefficients; $^{*}P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

watershed; these results were statistically significant except for A in the control vs FYM group and P in the control vs *Desmodium* group in the Guder watershed.

Farmyard manure and *Desmodium* plus FYM increased the concentrations of most VFAs, whereas the concentrations of B and V did not differ significantly among fertilizer treatments in the Guder watershed. In both watersheds, the A:P ratio was significantly higher in *Desmodium* plus FYM than in the other treatment groups. Furthermore, methane production in FYM and *Desmodium* plus FYM was significantly higher ($P < 0.001$) than in other treatment groups in the Aba Gerima watershed (Table 3), while it was significantly lower ($P < 0.001$) in the FYM treatment group than in others in the Guder watershed (Table 4).

Acetate concentrations were significantly higher in Napier grass than in desho grass, and other VFA concentrations did not differ significantly by forage grass type. The A:P ratio for Napier grass was significantly higher in both watersheds than for desho grass (Tables 3 and 4). Acetate, TVFA, and methane production in both watersheds were significantly higher ($P < 0.001$) in 2019 than in 2018, whereas other VFAs did not differ significantly by production year. The A:P ratio at Aba Gerima was higher in 2019 than in 2018, but no variation was observed at the Guder watershed.

3.3. Correlation between Chemical Composition, IVOMD, and VFA Production. Table 5 shows the correlations between chemical composition, IVOMD, and VFA production of forage grasses under fertilizer treatments and production years. There were positive but nonsignificant correlations between CP content and NDF, ADF, and ADL contents. The content of CP was significantly inversely related to the content of

IVOMD ($r = 0.947$, $P < 0.001$) and ME ($r = 0.973$, $P < 0.001$) were significantly and strongly positively correlated with GP24, but not with methane production ($r = 0.421$, $P < 0.05$). Furthermore, IVOMD demonstrated a significant and strong positive correlation with ME ($r = 0.999$, $P < 0.001$) as well as a significant and moderate correlation with TVFA ($r = 0.741$, $P < 0.05$). Metabolizable energy and TVFA had a significant positive and moderate positive correlation ($r = 0.741; P < 0.05$), but there was no significant correlation between ME and methane production. Finally, a moderate and significant positive correlation ($r = 0.707$, $P < 0.05$) was discovered between TVFA and methane production.

4. Discussion

4.1. Chemical Composition of Forage Grasses. This study at the Aba Gerima watershed is contrary to the findings of Kering et al. [24] who reported that N fertilization increased CP content and decreased NDF and ADF contents in Bermuda grass (*Cynodon dactylon* L.). The discrepancy might be related to variation in the fertilizer sources used, climatic conditions, and forage grasses used. Our results agree with the findings of Meale et al. [25] who reported that concentrations of NDF ranged from 67.1 to 71.3% in comparable forage grasses in Ghana. The NDF contents in our study were above the critical level (60.0%) that adversely affects voluntary dry matter intake and feed conversion efficiency in ruminant animals [26]. This might be because both the forage grasses were tropical forages characterized by fibrous feed with high fiber content consisting of structural carbohydrates.

Conversely, the NDF values reported in our study were below the maximum cell wall concentration (70%–75%) that does not affect intake and animal performance in beef cattle [27]. However, the same author reported that high-yielding dairy cows need lower NDF content in feeds for adequate energy and fiber in the diets; for such animals, the forage grasses tested here would need additional supplemental quality feeds with lower fiber content for better feed intake and digestibility. The NDF values for Napier grass were higher than that in the previous report of Napier grass grown with *Desmodium* or the legume lablab (*Lablab purpureus*) in northwestern Ethiopia [28]. Here, the lower fiber content in desho compared with Napier grass in both watersheds might be related to the larger leaf to stem proportion in desho grass.

Unlike in our study, where fertilizer type did not significantly affect the total ash content of desho or Napier grass, a previous study also performed in northwestern Ethiopia found that total ash content was higher in manure-treated desho grass than that grown without fertilizer [29]. Moreover, Ayub et al. [30] reported that the total ash content of sorghum grass was significantly affected by the level of nitrogen fertilization in Pakistan. Conversely, Bayble et al. [28] reported lower ash content in Napier grass grown without fertilizer than in that grown with different fertilizer treatments in our study. In agreement with our study, Heseen et al. [31] reported that the application of organic and inorganic fertilizers did not affect the total ash content of sorghum cultivars at a site in Egypt.

In our study, the variation in CP content of forage grasses under various fertilizer treatments was not consistent across the two agroecologies. Similar to our results for the forage grasses at the Aba Gerima watershed, Yiberkew et al. [32] reported that the use of organic fertilizer, like manure, did not influence the CP content of *Brachiaria* Hybrid Mulato II grass, when grown in the lowlands of northwestern Ethiopia. In contrast, Bayble et al. [28] study found that integrating Napier grass with *Desmodium* and lablab improved the grass's CP content. The difference between our results and those in the previous reports might relate to differences in the soil types and climatic conditions in the various regions in northwestern Ethiopia and/or the types of forage grasses used. Our results from the Guder watershed are in line with the findings of various studies in which the CP content of forage grasses improved noticeably with the application of fertilizer [33–36].

The relatively high CP content achieved in the highlands of the Guder watershed than in the midlands of the Aba Gerima watershed might be associated with the Guder watershed's lower temperatures, higher rainfall, lower light intensity, and different soil properties. The result is in agreement with that of Rochana et al. [37] who found that forages in highland regions tend to have a better CP yield than those in midland regions, but conflicts with the reports of Asmare et al. [7] who found higher CP content in midland than highland agroecologies. Crude protein levels above 70 g kg^{-1} DM, such as those observed in our study, increase microbial multiplication, thus improving fermentation [38].

Our finding that the EE content of the forage grasses was higher with *Desmodium* plus FYM treatment than in the control in both watersheds suggests that such integrated treatment could provide ruminants with more energy content. Consistent with our results at the Guder watershed, but not at the Aba Gerima watershed, Jimoh et al. [36] reported a significant difference in EE content between fertilized (manure) and unfertilized treatments. The same authors reported that EE content of forage grasses differs with the animal origin of the manure: a higher value was reported for grasses grown using cattle and swine manure than poultry manure or no fertilizer. Ayub et al. [30] reported the EE content increased as the level of nitrogen fertilizer increased; the minimum concentration was recorded with no fertilizer, which is similar to our result for the Guder watershed.

4.2. In Vitro Gas Production, Organic Matter Digestibility, and Production of VFAs. The higher GP24 and VFA production in *Desmodium* plus FYM treatment group compared with control could be due to the better IVOMD and relatively low fiber contents of the former treatment group in both watersheds. Integrated use of FYM and *Desmodium* increased GP24, IVOMD, and ME of the forage grasses. This might be associated with the higher digestibility in organic matter and lower fiber concentration, indicated by lower ADF in forage grass grown with *Desmodium* plus FYM compared with control in the Guder watershed, although no such difference was observed in the Aba Gerima watershed. Conversely, Cui et al. [14] showed that the application of N fertilizer improved crude protein, cellulose, and lignin content by decreasing the energy content available for metabolism and slowing the fermentation rate as well as decreasing in vitro dry matter disappearance and cumulative gas production. According to Vandermeulen et al. [39], volatile fatty acids are the primary source of metabolizable energy for ruminants; a feed with lower fermentability could elucidate the lower VFA production observed in this study. Our results are in agreement with those of Heseen et al. [31] who reported that the use of compost alone or combined with mineral fertilizer gave higher GP24 and ME than the use of control for sorghum in the Guder watershed, but no similar finding in ME with Aba Gerima watershed. In addition, Utamy et al. [15] reported an increased digestibility due to increased manure application for Napier grass, which agrees with our study. Moreover, similar to our result, Bayble et al. [28] reported a higher IVOMD for Napier grown in association with either *Desmodium* or lablab. The IVOMD values in our study are within the range reported for tropical forages (50%–80%) [40]. The relatively low IVOMD in our Napier grass compared with the report of Zewdu et al. [3] might relate to our use of rumen fluid from Japanese Corriedale sheep for in vitro gas production, because ruminants may vary in their composition of rumen microbial inoculum [41].

The variations in GP24, IVOMD, and ME between fertilizer treatments, forage grasses, and years were consistent across the agroecologies in our study. The lower IVOMD value in the midland versus highland agroecology in our study agrees with the report by Van Soest [42] who report that high temperature initiates lignification of cell walls, thereby hindering the digestibility of plants. We found that IVOMD was higher with *Desmodium* plus FYM than FYM in both watersheds; in such case, the addition of FYM to *Desmodium* treatment increases the nitrogen supply to the plant by facilitating N_2 fixation by *Desmodium*. The result of our study is consistent with the report of Van Soest [42] who reported that an increased nitrogen supply to plants lowers structural carbohydrate content, which lowers the proportion of cell wall constituents and improves digestibility. In addition, desho grass grown with *Desmodium* plus FYM in the second year of production showed higher GP24 and IVOMD as compared to Napier grass in similar treatments in both watersheds (Tables 3 and 4). This might be associated with the lower fiber fraction contents in desho compared with Napier grass, since high fiber content hinders gas production and digestibility [43]. The same author indicated that the gas produced reflects the degradation of the sample, the rate and the maximum potential of gas production, and thereby the digestibility of the feed for evaluating the quality of forages which agree with our study.

The effects of the *Desmodium* plus FYM treatment on TVFAs were consistent across the two watersheds in our study. However, we observed variation in the concentration of TVFA due to the type of forage grass and year of production. Getachew et al. [43] and Khan et al. [44] reported that differences in VFA content between grasses present an opportunity for improving the nutritional value of forage. In a study of *Atriplex* (*Atriplex patula*), plantago (*Plantago lanceolata*), and alfalfa (*Medicago sativa*) in Turkey, Kara et al. [45] reported higher concentrations of VFAs, but a similar methane production, to that of Napier and desho grass used in our experiment. Such variation in VFA concentration among forages is consistent with earlier findings Khan et al. [44] and Dierking et al. [46]. The high A:P ratios observed here for the grasses are in line with the report of Makkar [47] who found that plants with slowly fermentable carbohydrates like tropical forage grasses yield higher A compared with P. Our observation that the fermentation of the Napier and desho grasses yielded a high A:P ratio of ~2 agrees with the report by Getachew et al. [43], which states that plants with higher levels of structural carbohydrates give higher A:P ratios. Consistent with our study, feedstuffs having higher GP24 and IVOMD tend to cause more methane production [38]. Conversely, the VFA and methane production are greatly influenced by the species of ruminant animal and the feeding level [48].

4.3. Correlation between Chemical Composition, IVOMD, and VFA Production. The IVOMD of forage grasses were positively affected by ME content, which could explain the significant and strong correlation between digestibility with ME and VFA production. According to the report of Bayble et al. [28], there was a negative correlation between IVOMD and the fiber fraction contents, which agrees with our result. Similar to our results, the negative correlation between NDF and GP24 might be related to high NDF and ADF contents, which could be considered to affect microbial activity through reduced availability of fermentable carbohydrates [38]. The relatively strong positive correlation between TVFA production and IVOMD in our study is similar to the finding of Medjekal et al. [49]; in our study, there was higher VFA with higher IVOMD under FYM as well as under *Desmodium* plus FYM compared with the control treatment. The reason for the high IVOMD might be that optimization of CP and ME contents of the forage grasses under the integrated treatment improved the production of VFA at the Guder watershed, and thereby improved the IVOMD of the feed stuffs. We found no significant correlation between IVOMD and methane production, which agree with the report of Robinson et al. [48] who reported that the weakness of the correlation between VFA concentration and methane production for grazing sheep might be related to changes in rumen volume increasing surface area and VFA absorption. In addition, similar to our result, Bezabih et al. [13] reported there were higher associations between IVOMD and ME content in mid-rift valley grasslands of Ethiopia. This study found a positive relationship between methane production and fiber fractions (NDF and ADF), which is consistent with previous finding [50]. On the other hand, Moss [22] discovered a negative relationship between CP and methane production, which is consistent with our findings.

5. Conclusions

The forage grasses studied under the various fertilizer treatments varied in IVOMD and VFA production. The chemical composition of forage grasses did not vary in the Aba Gerima watershed, whereas fertilizer treatments influenced CP in the Guder watershed. The CP content achieved in our experiments was higher than the minimum ($70\,g\,kg^{-1}$ DM) required for adequate microbial multiplication for proper rumen fermentation. *Desmodium* plus FYM increased the IVOMD, ME, and TVFA concentrations of Napier and desho grasses in both watersheds, and FYM increased the IVOMD concentration in both watersheds, implying that they are suitable as alternative livestock feeds in the study areas and similar agroecologies.

Abbreviations

A: Acetate
ADF: Acid detergent fiber
ADL: Acid detergent lignin
AOAC: Association of Official Analytical Chemists
A:P: Acetate to propionate ratio
B: Butyrate
CH_4: Methane
DM: Dry matter
FYM: Farmyard manure
GP24: 24-h gas production
ME: Metabolizable energy
TVFA: Total volatile fatty acids
V: Valerate
VFA: Volatile fatty acids.

Data Availability

The data analyzed for this study are available from the first author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Data Title. Nutritional value and in vitro volatile fatty acid production of forage grasses cultivated using farmyard manure and *Desmodium* intercropping in the upper Blue Nile basin, Ethiopia, Journal: Advances in Agriculture. Table S1. Effects of fertilizer treatment on in vitro organic matter digestibility and volatile fatty acid production at Aba Gerima under different fertilizer treatments. We have included this to support the main and interaction effects between forage grasses, fertilizer treatments and year on gas production, in vitro organic matter digestibility, and metabolizable energy. The result showed that *Desmodium* plus FYM and desho in 2019 production season was highly significant compared to 2018 in the same treatment combination for GP24 and IVOMD. Conversely, *Desmodium* plus FYM and Napier indicated lower GP24 and IVOMD but higher metabolizable energy in 2018 production year. Table S2. Effects of fertilizer treatments on in vitro organic matter digestibility and volatile fatty acid production at the Guder watershed. Table S2. Supports that the main and interaction effects of *Desmodium* plus FYM and desho in 2019 production season showed higher GP24, IVOMD, and ME at the Guder watershed. This showed that irrespective of agroecologies *Desmodium* plus FYM and desho in 2019 production season was better in GP24 and IVOMD, which is an important parameter for livestock production. In addition, from our result, we can say that within the same treatment interaction and agroecologies second production season was better in in vitro digestibility. This may be associated with as forage grasses are perennial, it may reach for harvest within a shorter time interval with relatively lower acid detergent lignin constitutes in the second year, which may improve the digestibility of forage grasses. Tables S3 and S4. Relative value on chemical composition under various fertilizer treatments at Aba Gerima and Guder watersheds. In both the tables, the percent increment or decrease in the chemical composition of forage grasses under various fertilizer treatments compared to forage grass alone at Aba Gerima and Guder watersheds is shown. Tables S5 and S6. Relative value on in vitro gas and volatile fatty acid production of forage grasses under various fertilizer treatments at Aba Gerima and Guder watersheds. In both the tables, the percent increase or decrease in in vitro gas and volatile fatty acid production of forage grasses under various fertilizer treatments compared to the control in the two watersheds is shown. The relative change indicates percent value increment or decrement in in vitro gas production, IVOMD, ME, and volatile fatty acid production of forage grasses. (*Supplementary Materials*)

References

[1] R. Behnke and F. Metaferia, *The Contribution of Livestock to the Ethiopian Economy" – Part II*, Policy Brief Series, Nirobi, Kenya, 2013.

[2] Food and Agriculture Organization of the United Nations (FAO) "Grassland Index, *A Searchable Catalogue of Grass and Forage Legumes*, Food and Agriculture Organization of the United Nations, Rome, Italy, 2010.

[3] T. Zewdu, R. M. T. Baars, and A. Yami, "Effect of plant height at cutting, source and level of fertiliser on yield and nutritional quality of Napier grass (*Pennisetum purpureum* (l.) Schumach.)," *African Journal of Range & Forage Science*, vol. 19, no. 2, pp. 123–128, 2002.

[4] Central Statistical Agency (Csa), *Agricultural Sample Survey, Report on Livestock and Livestock Characteristics of Private Peasant Holdings for the Year 2019/20, " Volume II*, Central Statistical Agency (Csa), Addis Ababa, Ethiopia, 2019.

[5] S. Melaku, "Feed intake, digestion kinetics and rumen volatile fatty acids in Menz rams supplemented with *Lablab purpureus* or graded levels of *Leucaena pallida* 14203 and *Sesbania sesban* 1198," *Animal Feed Science and Technology*, vol. 117, no. 1, pp. 61–73, 2004.

[6] A. S. Chaudhry, "Forage based animal production systems and sustainability, an invited keynote," *Revista Brasileira de Zootecnia*, vol. 37, pp. 78–84, 2008.

[7] B. Asmare, S. Demeke, T. Tolemariam, F. Tegegne, A. Haile, and J. Wamatu, "Effects of altitude and harvesting dates on morphological characteristics, yield and nutritive value of desho grass (*Pennisetum pedicellatum* Trin.) in Ethiopia," *Agriculture and Natural Resources*, vol. 51, no. 3, pp. 148–153, 2017.

[8] D. Gemiyo, A. Hassen, and T. Kocho, "Chemical composition and digestibility of major feed resources in mixed farming system of Southern Ethiopia," *World Applied Sciences Journal*, vol. 26, pp. 267–275, 2013.

[9] A. O. Abusuwar, "Effect of fermented manures on intercropped Sudan grass (*Sorghum sudanese* L.) and clitoria (*Clitoria ternate*) grown in an arid saline environment," *Journal of Animal and Plant Sciences*, vol. 29, pp. 269–277, 2019.

[10] E. Yildirim and I. Guvenc, "Intercropping based on cauliflower: more productive, profitable and highly sustainable," *European Journal of Agronomy*, vol. 22, no. 1, pp. 11–18, 2005.

[11] J. García-Rodríguez, M. J. Ranilla, J. France, H. Alaiz-Moreton, M. D. Carro, and S. Lopez, "Chemical composition, in vitro digestibility and rumen fermentation kinetics of agro-industrial by-products," *Animals*, vol. 9, no. 11, pp. 861–913, 2019.

[12] V. Fievez, O. J. Babayemi, and D. Demeyer, "Estimation of direct and indirect gas production in syringes: a tool to estimate short chain fatty acid production that requires minimal laboratory facilities," *Animal Feed Science and Technology*, vol. 123-124, pp. 197–210, 2005.

[13] M. Bezabih, W. F. Pellikaan, A. Tolera, N. A. Khan, and W. H. Hendriks, "Chemical composition and in vitro total gas and methane production of forage species from the Mid Rift Valley grasslands of Ethiopia," *Grass and Forage Science*, vol. 69, no. 4, pp. 635–643, 2014.

[14] J. H. Cui, H. J. Yang, C. Q. Yu et al., "Effect of urea fertilization on biomass yield, chemical composition, in vitro rumen digestibility and fermentation characteristics of forage oat straw

in Tibet of China," *Journal of Agricultural Science*, vol. 2016, pp. 1–14, 2016.

[15] R. F. Utamy, Y. Ishii, S. Idota, and L. Khairani, "Effect of repeated application of manure on herbage yield, quality and wintering ability during cropping of dwarf Napier grass with Italian ryegrass in hilly Southern Kyushu," *Japan," Agronomy*, vol. 8, 2018.

[16] K. Ebabu, A. Tsunekawa, N. Haregeweyn et al., "Effects of land use and sustainable land management practices on runoff and soil loss in the Upper Blue Nile basin, Ethiopia," *Science of the Total Environment*, vol. 648, pp. 1462–1475, 2019.

[17] D. Ayichew, "Effect of manure levels and alfalfa (*Medicago sativa*) cultivars intercropping on dry matter yield napier grass (*Pennisetum purpureum* L.) under irrigation in kobo district, Ethiopia," *Journal of Fisheries & Livestock Production*, vol. 05, no. 3, pp. 3–5, 2017.

[18] D. Bereket, B. Worku, T. Tewodros, and A. Tesfaye, *Best Fit Practice Manual for Desho Grass Production and Management in Southern Nations and Nationality Regional State*, Hawassa University, Hawassa, Ethiopia, 2016.

[19] Association of Official Analytical Chemists, *Official Methods of Analysis*, AOAC. Association of Official Analytical Chemists, Virginia, USA, 1990.

[20] P. J. Van Soest, J. B. Robertson, and B. A. Lewis, "Methods for dietary fiber, neutral detergent fiber, and nonstarch Polysaccharides in relation to animal nutrition," *Journal of Dairy Science*, vol. 74, no. 10, pp. 3583–3597, 1991.

[21] H. H. Menke and H. Steingass, "Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid," *Animal Research and Development*, vol. 28, pp. 7–55, 1988.

[22] A. R. Moss, J. P. Jouany, and J. Newbold, "Methane production by ruminants: its contribution to global warming," *Annales de Zootechnie*, vol. 49, no. 3, pp. 231–253, 2000.

[23] SAS Statistical analysis software, *JMPVR 13 Basic Analyses*, SAS. Statistical analysis software, Cary, USA, 2016.

[24] M. K. Kering, J. Guretzky, E. Funderburg, and J. Mosali, "Effect of nitrogen fertilizer rate and harvest season on forage yield, quality, and macronutrient concentrations in Midland Bermuda grass," *Communications in Soil Science and Plant Analysis*, vol. 42, no. 16, pp. 1958–1971, 2011.

[25] S. J. Meale, A. V. Chaves, J. Baah, and T. A. McAllister, "Methane production of different forages in in vitro ruminal fermentation," *Asian-Australasian Journal of Animal Sciences*, vol. 25, no. 1, pp. 86–91, 2011.

[26] H. H. Meissner, H. H. Köster, S. H. Nieuwoudt, and R. J. Coertze, "Effect of energy supplementation on intake and digestion of early and mid-season ryegrass and Panicum/Smuts finger hay, and on in sacco disappearance of various forage species," *South African Journal of Animal Science*, vol. 21, pp. 33–42, 1991.

[27] D. R. Buxton, "Quality-related characteristics of forages as influenced by plant environment and agronomic factors," *Animal Feed Science and Technology*, vol. 59, no. 1-3, pp. 37–49, 1996.

[28] T. Bayble, S. Melaku, and N. K. Prasad, "Effects of cutting dates on nutritive value of Napier (*Pennisetum purpureum*) grass planted sole and in association with *Desmodium* (*Desmodium intortum*) or Lablab (*Lablab purpureus*)," *Livestock Research for Rural Development*, vol. 19, pp. 1–11, 2007.

[29] B. Mihret, B. Asmare, and Y. Mekuriaw, "Effect of fertilizer type and plant spacing on plant morphological characteristics, yield and chemical composition of desho grass (*Pennisetum pedicellatum* Trin.) in Northwestern Ethiopia," *Agricultural Science and Technology*, vol. 10, pp. 107–114, 2018.

[30] M. Ayub, A. N. Muhammad, A. T. Tanveer Asif's, and A. H. Husnain Azhar's, "Effect of different levels of nitrogen and harvesting times on the growth, yield and quality of sorghum fodder," *Asian Journal of Plant Sciences*, vol. 1, no. 4, pp. 304–307, 2002.

[31] F. S. I. Fatma Sh Ismail, M. S. Hassan, A. M. M. Badr, F. S. Ismail, and F. A. Elsalam, "Effect of using different fertilizers sources on forage sorghum yield, digestibility and energy parameters by in vitro gas test production," *Annals of Agricultural Science, Moshtohor*, vol. 55, no. 4, pp. 805–816, 2017.

[32] N. Yiberkew, Y. Mekuriaw, and B. Asmare, "Effects of fertilizer types and Plant spacings on Plant morphology, biomass yield and chemical composition of Brachiaria Hybrid Mulato II Grass grown in Lowlands of Northwest Ethiopia," *Animal Science and Biotechnologies*, vol. 53, pp. 20–35, 2020.

[33] A. H. Ahmad, A. Wahid, F. Khalidg, N. Fiaz, and M. S. I. Zamir, "Impact of organic and inorganic sources of nitrogen and phosphorus fertilizers on growth, yield and quality of forage oat (*Avena sativa* l.)," *Cercetari Agronomice in Moldova*, vol. 44, no. 3, 2011.

[34] T. Kamal, A. Hashen, and N. R. Sarker, "Effect of organic manure on napier grass production," in *Proceeding of the International Book Market Service Ltd., Member of Omniscriptum Publishing Group*, LAP LAMBERT Academic Publishing, Beau Bassin, Mauritius, 2017.

[35] K. C. McRoberts, D. Parsons, Q. M. Ketterings et al., "Urea and composted cattle manure affect forage yield and nutritive value in sandy soils of south-central Vietnam," *Grass and Forage Science*, vol. 73, no. 1, pp. 132–145, 2018.

[36] S. O. Jimoh, A. A. Amisu, P. A. Dele, A. O. Victoria Olubunmi, T. A. Adeyemi, and J. A. Olanite, "Effects of animal manures and cutting height on the chemical composition of two *panicum maximum* varieties (local and Ntchisi) harvested at different stages of growth," *Pertanika Journal of Tropical Agricultural Science*, vol. 42, pp. 359–376, 2019.

[37] A. Rochana, N. P. Indriani, B. Ayuningsih et al., "Feed forage and nutrition value at altitudes during the dry season in the west java," *Animal Production*, vol. 18, no. 2, pp. 85–93, 2016.

[38] A. A. Njidda and A. Nasiru, "In vitro gas production and dry matter digestibility of tannin-containing forges of semi-arid region of north-eastern Nigeria," *Pakistan Journal of Nutrition*, vol. 9, no. 1, pp. 60–66, 2009.

[39] S. Vandermeulen, S. Singh, C. A. Ramírez-Restrepo et al., "In vitro assessment of ruminal fermentation, digestibility and methane production of three species of Desmanthus for application in northern Australian grazing systems," *Crop and Pasture Science*, vol. 69, no. 8, pp. 797–807, 2018.

[40] S. Adjolohoun, A. Buldgen, C. Adandedjan, V. Decruyenaere, and P. Dardenne, "Yield and nutritive value of herbaceous and browse forage legumes in the Borgou region of Benin," *Tropical Grasslands*, vol. 42, pp. 104–111, 2008.

[41] Z. Sileshi, E. Owen, M. K. Theodorou, M. S. Dhanod, and S. Bediye, "Factors affecting in vitro gas production from fermentation of forages as determined by pressure transducer techniques," pp. 250–263, ESAP Proceeding. ESAP, Addis Ababa, Ethiopia, 1998.

[42] P. J. Van Soest, *Nutritional Ecology of the Ruminants" 2nd*, Comstock Publishing Associates, Cornell University Press, Ithaca, NY, USA, 1994.

[43] G. Getachew, E. J. DePeters, and P. H. Robinson, “In vitro gas production provides effective method for assessing ruminant feeds,” *California Agriculture*, vol. 58, no. 1, pp. 54–58, 2004.

[44] N. A. Khan, M. W. Farooq, and M. Ali, “Effect of species and harvest maturity on the fatty acids profile of tropical forages,” *Journal of Animal and Plant Sciences*, vol. 25, pp. 739–746, 2015.

[45] K. Kara, E. Aktuğ, and S. Özkaya, “Ruminal digestibility, microbial count, volatile fatty acids and gas kinetics of alternative forage sources for arid and semi-arid areas as in vitro,” *Italian Journal of Animal Science*, vol. 15, no. 4, pp. 673–680, 2016.

[46] R. M. Dierking, R. L. Kallenbach, and C. A. Roberts, “Fatty acid profiles of Orchard grass, tall fescue, perennial ryegrass, and alfalfa,” *Crop Sci*, vol. 50, no. 1, pp. 391–402, 2010.

[47] H. P. S. Makkar, “Recent Advances in the in Vitro Gas Method for Evaluation of Nutritional Quality of Feed Resoures,” 2004, http://www.fao.org/DOCREP/ARTICLE/AGRIPPA/570_EN_htm.

[48] D. L. Robinson, J. P. Goopy, and R. S. Hegarty, “Can rumen methane production be predicted from volatile fatty acid concentrations,” *Animal Production Science*, vol. 50, no. 6, pp. 630–636, 2010.

[49] S. Medjekal, R. Bodas, H. Bousseboua, and S. López, “Evaluation of three medicinal plants for methane production potential, fiber digestion and rumen fermentation in vitro,” *Energy Procedia*, vol. 119, pp. 632–641, 2017.

[50] S. Singh, B. P. Kushwaha, S. Nag, A. Mishra, A. Singh, and U. Y. Anele, “In vitro ruminal fermentation, protein and carbohydrate fractionation, methane production and prediction of twelve commonly used Indian green forages,” *Animal Feed Science and Technology*, vol. 178, no. 1, pp. 2–11, 2012.