Agricultural Sciences, 2023, 14, 522-540
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Effects of *Stylosanthes scabra* Forage Supplementation on *in Vitro* Gas Production and Fiber Degradation of *Eragrostis* Grass Hay

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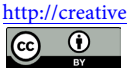
How to cite this paper: Mpanza, T.D.E. and Hassen, A. (2023) Effects of *Stylosanthes scabra* Forage Supplementation on *in Vitro* Gas Production and Fiber Degradation of *Eragrostis* Grass Hay. *Agricultural Sciences*, **14**, 522-540.
https://doi.org/10.4236/as.2023.144035

Received: January 4, 2023
Accepted: April 18, 2023
Published: April 21, 2023



Abstract

Natural pastures constitute a major component of ruminant livestock feed, and are the most cost-effective feed resource available for smallholder subsistence farmers. However, this feed resource does not meet animal nutritional requirement due to deficiency in nitrogen, energy and minerals. In addition, at maturity lignification is the major concern since it reduces digestibility and contributes to methane emission. Thus, the objective of this study was to evaluate the effect of supplementing low-quality *Eragrostis* grass hay with five (9281, 11,252, 11,255, 11,595 and 11,604) selected *Stylosanthes scabra* accessions on *in vitro* ruminal fermentation and neutral detergent fiber degradation. Therefore, *in vitro* study was conducted on grass hay, accessions and the mixture of grass hay with each accession included at two (15%, 30%) levels. The substrates (grass hay, accessions and the mixtures) were incubated in separate serum bottles for 72 h. Neutral detergent fiber (NDF) of the accessions ranged from 300 to 350 g/kg DM with crude protein (CP) value ranging from 177.5 to 184.1 g/kg DM. *Eragrostis* grass hay had NDF value of 813 g/kg DM, with CP value of 34.3 g/kg DM. Grass hay fermented slowly, it took 30 h for grass hay to produce gas volume above 50 mL, while *Stylosanthes scabra* accessions took 12 h. Supplementing grass hay with accessions significantly improved fermentation. However, it was observed that 15% inclusion took 30 h to produce gas volume above 50 mL, whereas at 30% inclusions it took 24 h for accession 9281, 11,595 and 11,604. Accession 11,604 improve grass fermentation by almost three times the value of grass hay in 2 h. Grass hay supplemented with accession 11,604 at 30% had a positive associative effect and significantly improved NDF degradability. In conclusion, accession 11,604 may be fed strategically as forage supplement to low-quality forage for ruminants.

Keywords

Low-Quality Forage, *In Vitro* Gas Production, Associative Effect, Fiber Degradation

1. Introduction

Livestock contributes substantially to the livelihood of smallholder farmers in Sub-Saharan Africa, which includes food and economic security [1]. According to FAO [2] livestock has the potential to reduce poverty in developing countries. Livestock production is reported to increase the availability of animal-derived food (*i.e.* meat and milk) as source of protein and contribute to income through selling of milk, meat and skin [3]. However, natural pastures play a significant role in livestock production [4]. This is because, livestock under smallholder farming system depends solely on natural pastures as the cost-effective feed resource available for ruminants feeding in sub-Saharan Africa [4] [5]. However, this feed resource is in adequate to supply the nutrient demand for livestock due to deficiencies in nitrogen, metabolizable energy and minerals [6] [7] [8]. Furthermore, during the dry season period forage quantity is not enough. Subsequently, their use as sole feed to livestock limit animal production performance [9] [10]. In the tropical and sub-tropical regions, grass grows faster due to the conducive climatic condition (*i.e.* soil temperature, rainfall and light intensity), hence it matures faster and that is coupled with the lignification due to increasing fibre content [11]. Lignification is attributed to poor digestibility of the forage, low digesta flow, and hence reduces the dry matter intake of the forage [12] [13]. Moreover, feeding fibrous forage may lead to extra baggage of enteric methane production [14] [15], which is the major concern due to its environmental effect. Methane emission represents about 12% of gross energy loss from animal [16], and that has an adverse effect on animal production performance [17]. Neutral detergent fiber (NDF) quantifies from most of the cell wall components of the forage [18]. Hence, it is used as common measure of fiber content required in animal feed [19]. Therefore, NDF level above 65% in the feed may reduce dry matter intake [20], and that may lead to an adverse effect on potential milk and meat production and quality [21]. According to Tirado-Estrada *et al.* [22], NDF proportion and degradability of the forage can be used to predict the dry matter intake and productive performance of the animal.

Therefore, research has been focusing on strategies on how to improve ruminal NDF degradability in order to increase dry matter intake while improving animal production and the quality of animal-derived food (*i.e.* meat and milk) [23]. This includes pre-digesting fibers with exogenous fibrolytic enzymes [24] and ruminal degradable protein [13]. Supplementation with rumen degradable nitrogen and energy is reported to improve forage quality, and rumen fermentation and thus enhance dry matter intake [13]. According to Hariadi and Santoso

[25], low (below 7%) protein content in forage restricts rumen microbial activity and thus reduces feed digestibility. Consequently, in order to improve the utilisation of low-quality forage with high NDF by the rumen microbes, concentrates have been used as supplement to improve protein content [26] [27] [28]. Nevertheless, concentrates are inaccessible to smallholder subsistence farmers owing to their high costs. Subsequently, leguminous trees, shrubs and herbaceous forages have been used as an alternative cost-effective protein source supplement [29] [30] [31] [32]. This is because foliage from leguminous plants contains high protein content even at maturity [33] [34].

In this study, *Stylosanthes scabra* cv. Shrubby stylo was strategically used as protein source for low-quality grass hay with high NDF content. *Stylosanthes scabra* is an erect shrubby perennial legume, that originates from South America, mainly Brazil, Colombia and Venezuela [35]. Shrubby stylo is a drought tolerant, hence it can survive semi-arid regions [36] [37]. Various accessions of *Stylosanthes scabra* were evaluated for adaptation in subtropical climate of Pretoria, South Africa under rain-fed condition and their biomass yield ranged from 3.5 to 5.6 t/ha DM over three years [34]. Forage of the adapted accessions was reported to contain more than 17% of crude protein with an insignificant level of tannins when grown in Pretoria, hence they can be utilised as supplementary forage [34]. Therefore, an *in vitro* study was conducted in order to evaluate the effects of *Stylosanthes scabra* forage on improving low-quality grass hay ruminal fermentation. We hypothesis that supplementing low-quality grass hay with forages of *Stylosanthes scabra* accessions will improve fermentation and fiber degradability thus enhancing utilisation efficiency of the forage. Thus, this study's objective was to determine the effects of supplementing up to 30% level of *Stylosanthes scabra* accession forages on *in vitro* ruminal fermentation and neutral detergent fibre degradation of low-quality *Eragrostis* grass hay.

2. Material and Methods

2.1. Study Site and Chemical Composition of the Substrates

This study was conducted at the University of Pretoria in the Department of Animal and Wildlife Sciences located at 25˚44'30"S, 28˚15'30". Details on climate condition of the study area are described on our previous study [34]. The substrates that were used for the experiment were *Eragrostis* grass hay (hereafter referred as grass hay), five (9281, 11,252, 11,255, 11,595 and 11,604) accessions of *Stylosanthes scabra* forages. The numbers assign to each accessions are according to International Livestock Research Institute (ILRI), Ethiopia who donated the seeds that were evaluation for adaptation [34]. Forages of *Stylosanthes scabra* accessions that were used in this study were produced during the evaluation study conducted in Pretoria [34]. Grass hay that was used in this study was purchased from the local suppliers. Forages from grass and *Stylosanthes scabra* accessions were oven dried at 60˚C for 72 h and ground to pass through 1 mm sieve and were used to determine chemical composition which includes; dry

matter, ash, crude protein as described by AOAC [38], neutral detergent fiber as described by Mertens [39], acid detergent fiber and acid detergent lignin as described by van Soest *et al.* [18]. Total tannins analysis was determined only on forages of *Stylosanthes scabra* accessions following the procedure of Makkar [40]. Hemicellulose (HC) and neutral detergent soluble (NDS) were calculated as HC g/kg DM = NDF − ADF and NDS% = 100% − NDF%, respectively.

2.2. Buffer Solution Preparation and Rumen Fluid Collection

Buffer (*i.e.* macro and micro mineral) solutions were prepared following the procedure of Goering and van Soest [41]. In order to reduce Sulphate (SO_4) in buffer solution, Magnesium Sulphate ($MgSO_4 \cdot 7H_2O$) was replaced by Magnesium Chloride ($MgCl_2 \cdot 6H_2O$) in the preparation of macro-mineral solution as proposed by Mould *et al.* [42]. For rumen fluid collection, two South African Merino sheep (males) fitted with a permanent rumen cannula were used as the rumen fluid donor. Donor animals were fed alfalfa hay as basal feed with free access to clean water. In each of the donor animals, an amount of 450 mL of rumen fluid was collected before morning feeding. The rumen fluids from the two donor sheep were mixed and strained through four layers of cheese-cloth into a pre-warmed thermos flask and immediately transported into the laboratory within 30 minutes. Upon arrival at the laboratory, the flask with rumen fluid was placed in the water bath set at 39˚C and was continuously purged with CO_2 gas to maintain anaerobic condition.

2.3. *In Vitro* Gas Production

In vitro gas production study was conducted following the procedure of Menke and Steingass [43]. Grass hay, five accessions of *Stylosanthes scabra* and the grass hay supplemented with two levels (15% and 30%) of each accession were used as treatment substrates for *in vitro* gas production. Four hundred mg of grass hay and accessions were weighed separately into serum bottles of 120 mL capacity each. For a 15% accession inclusion level, 340 mg grass hay was mixed with 60 mg of accession while for a 30% accession inclusion level 280 mg grass hay was mixed with 120 mg of accession to make 400 mg, respectively. This resulted into 16 treatments; one grass hay, five *Stylosanthes scabra* accessions and ten mixtures (grass hay mixed with each of the accession) at two levels. Substrates were weighed into serum bottes a day before incubation and kept in an incubator that has been set to 39˚C in order to keep the substrates warm. Each of the treatment was replicated three times per run and there were two runs conducted separately. After putting the samples into the serum bottles, 40 mL buffered rumen solution (consisting of 15 mL rumen fluid and 25 mL buffer solution) was dispensed per serum bottle and flushed with CO_2, thereafter sealed by a butyl rubber stopper and aluminium crimp sealer. In each run, three serum bottles with buffered rumen solution only were included. Bottles were incubated in a continuous shaking incubator (120 revolution per minute) set at 39˚C and

gas pressure was recorded in 2, 4, 8, 12, 24, 30, 48 and 72 h post incubation. Gas pressure recordings were done following the procedure described by Akanmu *et al.* [44]. The average gas pressure recorded from blanks (serum bottle with buffered rumen solution only) was subtracted from each gas pressure readings per treatment. Gas pressure readings were recorded in psi and converted to volume using Boyle's gas law equation as described by [45] Equation (1).

$$\text{Gas volume}(\text{mL}) = (Vh/Pa) \times Pt \quad (1)$$

where Vh is the volume of head space in the serum bottle (mL); Pa is the atmospheric pressure in Pretoria (psi); Pt is pressure transducer reading (psi).

Metabolizable energy (ME) was calculated as described in Menke *et al.* [46] Equation (2), while Organic matter digestibility (OMD) and short chain fatty acids (SCFA) were calculated as according to Menke and Steingass [43], Equations (3) and (4), respectively. Since the equations for calculating ME, OMD and SCFA were derived based on 200 mg incubated substrate while in this study we used 400 mg therefore gas volume at 24 h post incubation was adjusted to 200 mg.

$$\text{ME}(\text{MJ/kg DM}) = 2.2 + 0.136\text{GV} + 0.057\text{CP} + 0.002859\text{CP}^2 \quad (2)$$

$$\text{OMD}(\%) = 14.88 + 0.889\text{GV} + 0.45\text{CP} + 0.651\text{XA} \quad (3)$$

$$\text{SCFA}(\mu\text{mol/g DM}) = 0.0239\text{GV} - 0.601 \quad (4)$$

where GV is gas volume at 24 hours, CP is protein content, XA is ash.

2.4. Neutral Detergent Fiber Degradation

To determine degradability of neutral detergent fiber, a 30 hours *in vitro* incubation of substrates was conducted. Preparation of buffer solution and rumen collection was as described in Section 2.2 above. The same treatments that were used for *in vitro* gas production (Section 2.3) above were used for NDF degradability, except that an amount used was 500 mg for each treatment was weighed and dispensed into each of the serum bottle. Bottles with substrates were place in water bath set at 39˚C the day before incubation. There were two runs, and each runs had each sample in duplicates and two blanks (serum bottle with buffered rumen solution only) were included in each run. An amount of 40 mL of buffered rumen solution was dispensed in each of the serum bottle with substrate after which bottles were sealed with a lid that allows a buildup gas in the head space to escape without allowing air to enter. The bottle was than incubated for 30 h in a shaking water bath set at 39˚C. To prevent floating of serum bottle, each bottle was clamped to the surface of the water bath. After 30 h incubation bottles with samples were removed from the water bath and each bottle was vigorously shaken and the content was emptied into a 50 mL Gooch crucibles attached in a vacuum filter. After filtering the content was rinsed with NDF solution and emptied into 250 mL glass biker for NDF determination following the procedure of Mertens *et al.* [39]. The NDF degradability data was used to deter-

mine the associative effect of grass hay with *Stylosanthes scabra* accessions applied at two different levels as defined by Niderkorn *et al.* [47].

2.5. Statistical Analysis

All data were subjected into analysis of variance as a complete randomized design using the general linear model procedure of SAS (SAS institute Inc., Cary, NC united States). The *in vitro* gas and the NDF degradation data on each run was treated as experiment units. Significant effect was declared at $P \leq 0.05$. Whenever the significant differences occurred, the means were compared using Tukey's tests.

3. Results

The chemical composition of grass hay and *Stylosanthes scabra* accessions are shown in Table 1. Grass hay had a crude protein (CP) value of 34 g/kg DM, this value is 52.4% less the 70 g/kg required CP value for animal maintenance. On the other hand, the average CP value of *Stylosanthes scabra* accessions at maturity was 182 g/kg DM. Grass hay showed very high fibre (NDF 813 g/kg DM and ADF 476 g/kg DM) concentrations with hemicellulose levels that were five times the value of accession. There were no variations on fiber (NDF and ADF) content amongst accessions, and hemicellulose values were low as compared to grass hay. Neutral detergent soluble of the accessions was less than half the value recorded for grass hay. *Stylosanthes scabra* accessions total tannins contents observed in this study were in the range of 0.9 g/kg DM recorded for accession 11,604 and 1.6 g/kg DM recorded for accession 11,595. On grass hay, total tannins were not in the detectable level.

In vitro gas production for pure substrates (grass hay and *Stylosanthes scabra* accessions) is shown in Table 2. Results of this study showed that grass hay

Table 1. Chemical composition of *Eragorstis* grass hay and *Stylosanthes scabra* accessions.

Treatments	DM (%)	CP	NDF	ADF	ADL	HC	Ash	TT	NDS (%)
		g/kg DM							
Grass hay	93.7	34.3	813.4	475.8	65.4	335.6	33.1	nd	19
9281	92.9	184.1	330.8	282.9	42.4	47.9	96.9	1.22	67
11,252	92.2	177.5	349.7	300.6	42.6	49.1	93.6	1.02	65
11,255	92.3	182.8	349.7	292.9	43.5	56.8	80.1	1.14	65
11,595	92.4	185.4	300.3	273.9	63.4	26.3	94.4	1.64	70
11,604	92.5	181.7	347.1	316.4	41.3	30.7	102.7	0.87	65

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; HC, hemicellulose; NDS, neutral detergent soluble; TT, total tannins. nd, not detected.

Table 2. *In vitro* gas production (mL/400mg) of low-quality grass hay and *Stylosanthes scabra* accessions.

Treatments	Incubation period (hrs)							
	2 h	4 h	8 h	12 h	24 h	30 h	48 h	72 h
Grass hay	5.5^{d}	11.3^{c}	18.0^{b}	26.5^{b}	44.1^{b}	56.4^{b}	75.2^{b}	86.5^{b}
9281	19.8^{a}	34.9^{a}	49.5^{a}	60.5^{a}	74.2^{a}	83.6^{a}	94.4^{a}	100.5^{a}
11,252	17.0^{bc}	32.7^{b}	47.8^{a}	59.0^{a}	72.3^{a}	81.7^{a}	92.5^{a}	99.3^{a}
11,255	17.3^{bc}	33.7^{ab}	48.2^{a}	59.2^{a}	72.5^{a}	81.6^{a}	92.1^{a}	98.5^{a}
11,595	17.8^{b}	33.6^{ab}	49.6^{a}	60.6^{a}	73.5^{a}	82.7^{a}	93.5^{a}	100.3^{a}
11,604	16.9^{c}	32.2^{b}	48.3^{a}	59.7^{a}	73.3^{a}	82.1^{a}	92.3^{a}	98.4^{a}
SEM	0.28	0.49	0.76	0.87	0.82	0.97	1.04	1.22

^{a} - ^{d}Means within a column with different superscript differ significantly at $P < 0.05$. SEM, Standard error of the means.

was poorly fermented by rumen microbes as compared to forages of *Stylosanthes scabra* accession. This is elaborated by a significant ($P < 0.05$) high gas volume that was recorded for each accession than that was recorded for grass hay throughout the incubation period. The volume of gas production from the accessions in the first two hours of incubation was three times higher than that of grass hay, and the difference decreased as incubation times advanced. Grass hay needed more time of exposure into ruminal microbes before fermentation could take place. This is shown by the improvement on gas production as time advanced even though it was still significantly ($P < 0.05$) lower than that of the accessions. There was a significant ($P < 0.05$) difference in the volume of gas production among the accessions during the first four hours of incubation. However, as the incubation time advanced the difference was not significant ($P > 0.05$).

The results on the effects of *Stylosanthes scabra* accession supplementation to low-quality grass hay are shown in Table 3. A significantly ($P < 0.05$) improved fermentation was observed on grass hay that was supplemented with 15% forage of *Stylosanthes scabra* up to 30 h of incubation, except for accession 11,604. The similar trend of improved grass hay fermentation up to 30 h was also notice when supplementation level of each accession was increased to 30%. However, an exception was observed for accession 11,604 at 30% level of supplementation that significantly ($P < 0.05$) improved fermentation throughout the incubation period.

The organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acid (SCFA) of grass hay, *Stylosanthes scabra* accessions and supplemented grass hay are shown in Table 4. It was observed that grass hay had a significant ($P < 0.05$) low level of OMD, ME and SCFA, while accessions had the level almost double that of grass hay. However, it was observed that, supplementing low-quality grass hay with *Stylosanthes scabra* accessions significantly

Table 3. Effects of supplementing low-quality grass hay with different *Stylosanthes scabra* accession on *in vitro* gas production (mL/400mg).

Treatments	Incubation period (hrs)							
	2 h	4 h	8 h	12 h	24 h	30 h	48 h	72 h
	15% supplementation level							
Grass hay	5.5^{d}	11.3^{d}	18.0^{c}	26.5^{c}	44.1^{c}	56.4^{b}	75.2^{b}	86.5^{a}
Grass hay + 15% 9281	7.3^{bc}	14.6^{ab}	21.8^{a}	30.5^{a}	47.4^{ab}	59.0^{a}	76.7^{ab}	86.9^{a}
Grass hay + 15% 11,252	7.8^{ab}	14.8^{ab}	22.2^{a}	30.9^{a}	47.3^{ab}	58.9^{a}	76.9^{ab}	87.3^{a}
Grass hay + 15% 11,255	7.9^{a}	15.2^{a}	22.6^{a}	31.3^{a}	48.4^{a}	60.1^{a}	78.0^{a}	88.6^{a}
Grass hay + 15% 11,595	7.2^{c}	14.4^{b}	21.9^{a}	30.0^{a}	46.6^{b}	58.4^{a}	76.8^{ab}	87.5^{a}
Grass hay + 15% 11,604	5.5^{d}	12.8^{c}	19.9^{b}	28.1^{b}	44.6^{c}	56.4^{b}	74.8^{b}	85.6^{a}
SEM	0.17	0.20	0.34	0.53	0.41	0.56	0.77	1.01
	30% supplementation level							
Grass hay	5.5^{e}	11.3^{d}	18.0^{d}	26.5^{d}	44.1^{d}	56.4^{d}	75.2^{cd}	86.5^{b}
Grass hay + 30% 9281	9.9^{c}	18.2^{b}	26.4^{b}	34.9^{b}	50.8^{b}	61.9^{b}	77.5^{b}	87.1^{b}
Grass hay + 30% 11,252	8.8^{d}	16.2^{c}	23.7^{c}	31.9^{c}	48.5^{c}	58.7^{c}	73.7^{d}	83.5^{c}
Grass hay + 30% 11,255	9.3^{d}	16.8^{c}	24.3^{c}	32.6^{c}	48.7^{c}	58.9^{c}	75.3^{cd}	84.9^{bc}
Grass hay + 30% 11,595	10.7^{b}	18.3^{b}	25.6^{b}	34.2^{b}	51.1^{b}	61.3^{b}	77.2^{bc}	86.6^{b}
Grass hay + 30% 11,604	14.4^{a}	22.6^{a}	31.3^{a}	40.0^{a}	57.4^{a}	67.8^{a}	83.0^{a}	92.1^{a}
SEM	0.18	0.20	0.33	0.44	0.52	0.53	0.70	0.92

$^{a-e}$ Means within a column with different letters in superscript differ significantly at $P < 0.05$. SEM, Standard error of the means.

Table 4. Feeding values of grass hay, *Stylosanthes scabra* accessions and supplemented grass hay.

Treatments	OMD (% DM)	ME (MJ/kg DM)	SCFA (μmol/g DM)
Grass hay	38.2^{h}	5.4^{i}	0.46^{f}
9281	62.5^{a}	9.3^{a}	0.83^{a}
11,252	61.1^{b}	9.0^{c}	0.80^{a}
11,255	60.5^{b}	9.1^{bc}	0.81^{a}
11,595	62.0^{a}	9.2^{ab}	0.82^{a}
11,604	62.3^{a}	9.2^{ab}	0.82^{a}
Grass hay + 15% 9281	41.3^{f}	5.8^{g}	0.51^{de}
Grass hay + 15% 11,252	41.2^{f}	5.8^{g}	0.50^{de}
Grass hay + 15% 11,255	41.6^{f}	5.9^{g}	0.51^{de}
Grass hay + 15% 11,595	40.9^{f}	5.8^{g}	0.49^{e}

Continued

Grass hay + 15% 11,604	40.1^{g}	5.6^{h}	0.47^{f}
Grass hay + 30% 9281	44.4^{d}	6.3^{e}	0.54^{c}
Grass hay + 30% 11,252	43.3^{e}	6.1^{f}	0.51^{de}
Grass hay + 30% 11,255	43.2^{e}	6.1^{f}	0.52^{d}
Grass hay + 30% 11,595	44.5^{d}	6.3^{e}	0.55^{c}
Grass hay + 30% 11,604	47.5^{c}	6.7^{d}	0.63^{b}
SEM	0.28	0.04	0.007

$^{a-h}$ Means within a column with different letters in superscript differ significantly at $P < 0.05$. OMD, organic matter digestibility; ME, metabolizable energy; SCFA, short chain fatty acid; SEM, standard error of the means.

($P < 0.05$)) improved the OMD, ME and SCFA values as compared with that of grass hay alone. Furthermore, it worth noting that supplementing grass hay with accessions 11,604 at 30% level significantly ($P < 0.05$) improved the OMD, ME and SCFA above the grass hay supplement with other accessions at the same level. Generally, grass hay supplemented with 30% of accession 11,604 had OMD, ME and SCFA improved by 8.3%, 8.1% and 18.9%, respectively. Figure 1 below showed a strong positive relationship between short chain fatty acid and gas production.

Figure 2(a) shows the observed versus expected gas production of grass hay supplemented with 30% of *Stylosanthes scabra* accessions, while Figure 2(b) shows the associative effect between grass hay and *Stylosanthes scabra* accessions. The expected gas production values from grass hay supplemented with *Stylosanthes scabra* accession were above the observed values for most of the accessions. However, grass hay supplemented with accession 11,604, showed a different trend where observed values were higher than expected values throughout the incubation period. Subsequently, accession 11,604 was the only accession that showed a positive associative effect with grass hay throughout the incubation period (Figure 2(b)).

Figure 3 shows NDF degradability of grass hay, accessions and low-quality grass hay supplemented with accessions at 15% and 30% level of inclusion. Figure 3(a), shows that NDF degradability of lows-quality grass hay was significantly ($P < 0.05$) lower than that of *Stylosanthes scabra* accessions. However, it was observed that supplementing low-quality grass hay with accession 11,604 at 30% level of inclusion significantly ($P < 0.05$) improved NDF degradability of low-quality grass hay by 15% as compared with that of grass hay alone (Figure 3(b)).

4. Discussion

Chemical composition of grass hay used in the present study shows that it was of low-quality will lower digestibility due to high NDF level. This reflect the quality

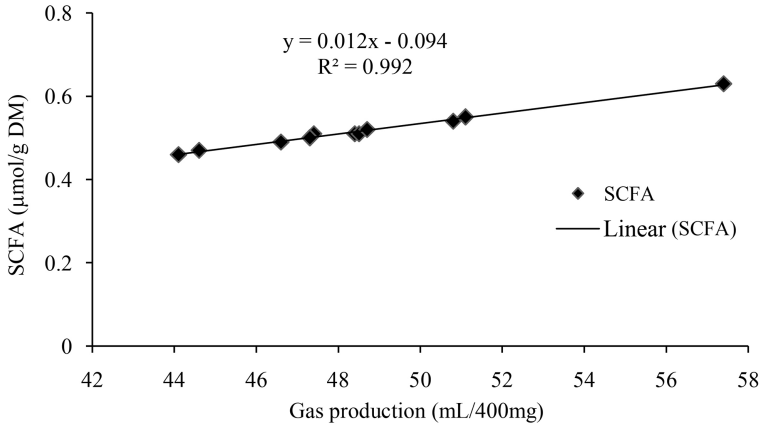
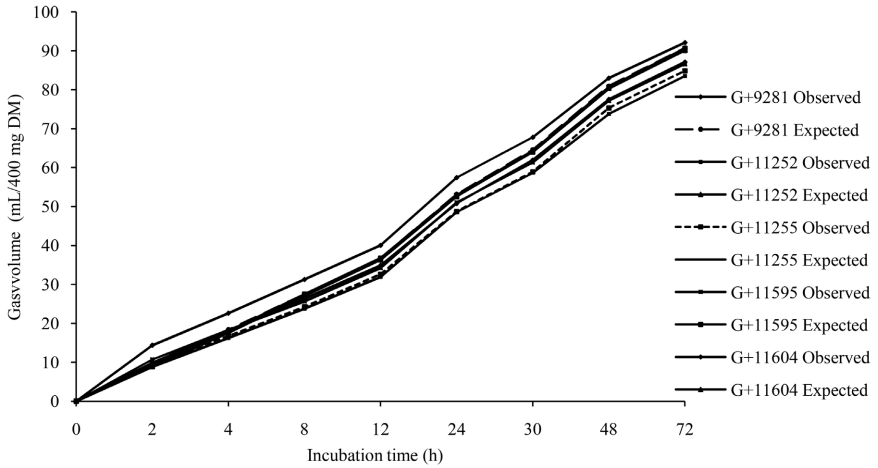
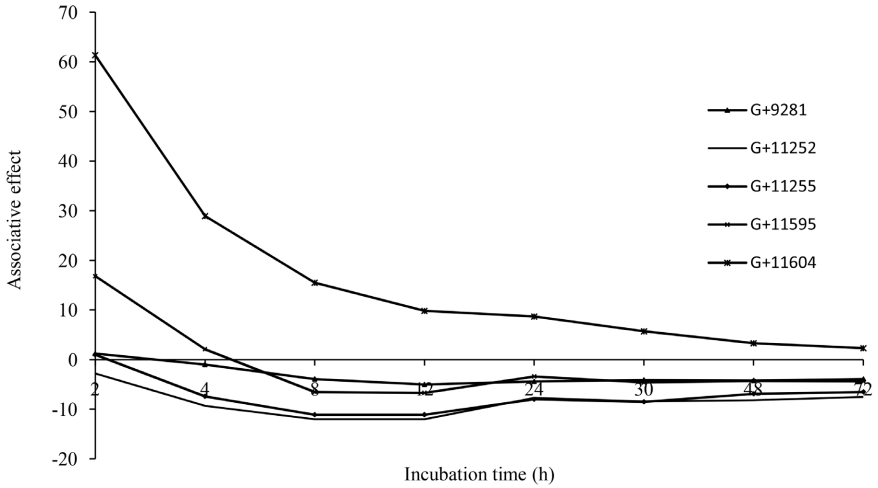


Figure 1. Relationship between *in vitro* gas production and short chain fatty acid content.



(a)



(b)

Figure 2. Gas production of grass hay supplemented with *Stylosanthes scabra* accessions at 30% of forage supplementation (a) expected vs observed gas production over 72 h incubation period (b) associative effect recorded over 72 h incubation period. G = grass hay.

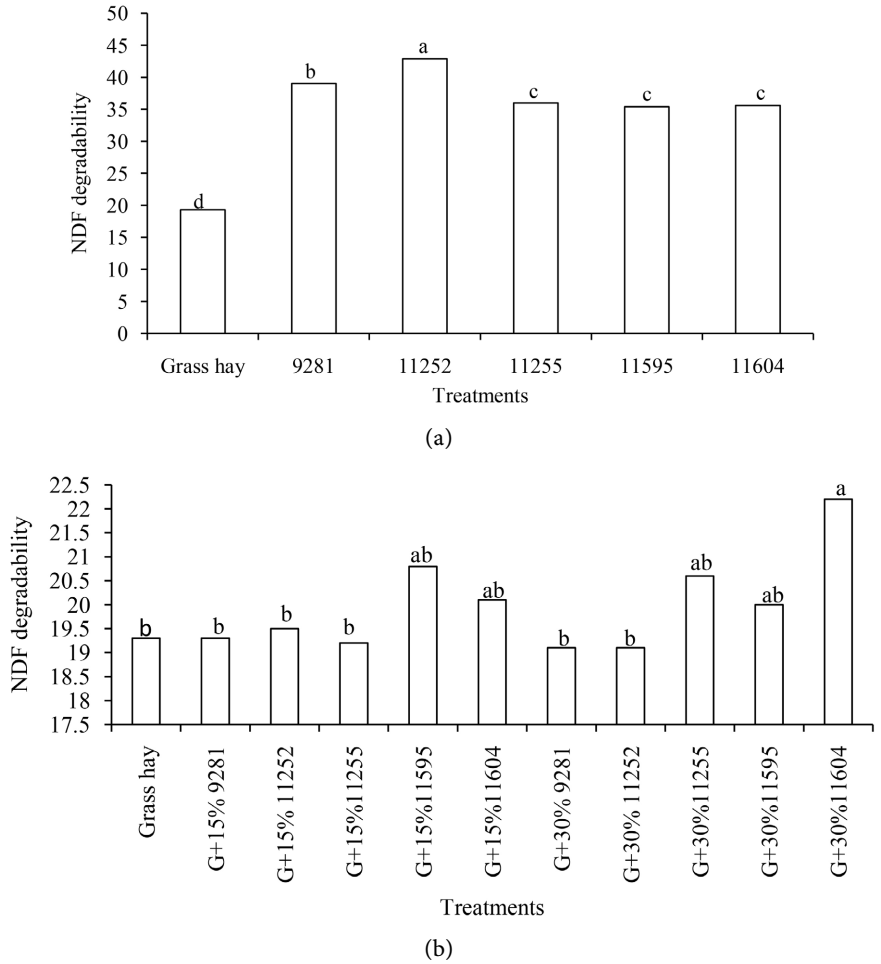


Figure 3. Thirty-hour *in vitro* neutral detergent fiber degradability of (a) grass hay vs *Stylosanthes scabra* accessions, (b) grass hay vs grass hay supplemented with *Stylosanthes scabra* accessions. G = grass hay.

of grass that is mostly available for livestock under smallholder farming system during the dry season period. Crude protein (CP) content recorded for grass hay was far below the value of 70 g/kg, the minimum CP value required for normal functioning of the rumen [48]. Furthermore, fiber (*i.e.* NDF and ADF) contents of grass hay were above the C-4 grass NDF and ADF range recorded by McDonald *et al.* [49] and Mertens [39]. The NDF level on C-4 forages is influenced by maturity stage, and growing environment (*i.e.* temperature, water/soil moisture and latitude) in tropical and subtropical regions [50]. Contrariwise, the CP value of *Stylosanthes scabra* accessions at maturity was above 170 g/kg, and that shows that *Stylosanthes scabra* forages can be used as protein source for low-quality forage. In fact, the CP value of the *Stylosanthes scabra* accessions was adequate for lactating dairy cows [51]. The NDF in the other hand was below the range of 60% to 65% that is reported to depress feed intake due to low digestibility [18] [50]. The ADF values for the accessions were lower than the range of values recorded by Mertens [39] for *Medicago sativa*. However, the *Stylosanthes scabra*

accessions used in this study contained tannins, but it was far below the threshold value of 20 g/kg DM which reported to reduce fermentation and hence low gas production [52].

In vitro study can be used with a reasonable accuracy to predict the digestibility of the forage and that may help in determining a strategy in which it can be used for an *in vivo* study. Therefore, this *in vitro* study showed that *Stylosanthes scabra* accessions were highly fermentable compared to grass hay (**Table 2**), and this is attributed to the chemical composition of the two substrates as shown in **Table 1** above. Even though forages of *Stylosanthes scabra* accessions contained tannins but ruminal fermentation was not reduced, since tannins were below the threshold level of 20 g/kg DM. This could basically mean that forages of *Stylosanthes scabra* accessions were readily available for microbes to ferment. In this study, supplementation of *Stylosanthes scabra* accessions to low-quality grass hay improved fermentation of grass hay (**Table 3**). This is because forage legume supplementation to low-quality forage provides additional nutrients and thus improves nutritional quality and the fermentation pattern of the forage [53]. According to Abegunde *et al.* [54], the rate at which feed substrate are degraded in the rumen is as important as the extent of digestion. This study showed that supplementing *Stylosanthes scabra* accessions forages to low-quality grass hay improved the feeding value (*i.e.* OMD, ME and SCFA) of grass hay. This could be associated with improved gas production as observed in **Table 3**. However, the ME values recorded in this study were below the value of 8.4 MJ/kg DM, which is recommended for dry ewes [55], except the ME for the accessions. The short chain fatty acid recorded in this study particularly for supplemented grass hay showed and improved energy contents in relation to pure grass hay. Short chain fatty acid contents in the feedstuff indicate the energy content of the feedstuff [56]. The significant high value for SCFA in relation to grass hay was observed when accession 11,604 was supplemented at 30% level of inclusion to grass hay. The same treatment was observed to have a significantly high gas production throughout the incubation period (see **Table 3**). This may be attributed to the increased levels of SCFA and OMD contents as shown in **Table 4**. Gas production was reported to proportional related with OMD and SCFA level of the feed [57] [58]. This is in agreement with the results of this study, where gas production had a strong ($r = 0.99$) positive relationship with SCFA (**Figure 1**). Similar positive relationship was reported by Andualem *et al.* [57] for *Urtica simensis* forage.

When different feed ingredients are mixed to form a diet to be fed to animal, during digestion those ingredients interact as one entity known as associative effect, they are not digested separately [59]. Associative effect occurs when feed mixture (from different ingredients) is digested as one feedstuff not independent of each another as separate ingredients that form the diet [60]. Likewise in the present study, the supplementation of low-quality grass hay with *Stylosanthes scabra* accessions is expected to have an associative effect during *in vitro* ruminal

fermentation. However, in this study the positive associative effect was only observed on grass hay supplemented by 30% of accession 11,604 (**Figure 2(b)**). Hence, this was the only accession at 30% inclusion level that significantly improved NDF degradability of low-quality grass hay (**Figure 3**). Fiber degradability particularly NDF in the forage determines the retention period of the forage in the rumen and that regulate the dry matter intake by the animal. Quick degradability of NDF by rumen microbes reduces retention period of forage [61] and that lowers the production of hydrogen (H_2) and carbon dioxide (CO_2) [62]. This could translate into low enteric methane because methanogens (archaea bacteria) use H_2 and CO_2 gases to produce enteric methane [63]. In addition, NDF degradability is regarded as a good indicator of dry matter disappearance [64]. Therefore, this study showed that increasing the inclusion level of the accession 11,604 to 30% improves the efficient utilization of low-quality grass hay by the rumen microbes. According to Ephrem *et al.* [65], NDF contents in forage is the major factor that affects feed intake and feed conversion efficiency by ruminant.

5. Conclusion

This study showed that supplementing low-quality grass hay with 30% forage of *Stylosanthes scabra* accessions improved ruminal fermentation. The low level of tannins in, *Stylosanthes scabra* forage indicates its potential to improve livestock production by controlling internal parasites, improving internal microbial ecosystem and enhancing gut health. However in this study, the supplementation by accession 11,604 at 30% further improved ruminal NDF degradability, and this may positively influence dry matter intake of the low-quality. Therefore, accession 11,604 is recommended for a further systematic evaluation to determine its effect on feed use efficiency of low-quality forage, ruminal fermentation profile and animal performance.

Acknowledgements

Authors are grateful to Gauteng Department of Agriculture and Rural Development (GDARD) and National Research Foundation (NRF) for the financial supports provided for this research.

Conflicts of Interest

Authors declare that there is no conflict of interest regarding the publication of this research work.

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