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GRC papers are treated in the same way as research papers, so should be structured accordingly, and are peer-reviewed. They are not meant to consist merely of data sets with limited description but should contain all relevant methodological details as in a Research Article and culminate in clear conclusions and suggestions.

Submissions for this paper category are welcome, even when they present data collected years ago.

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Research Paper

Improvement of plant regeneration and *Agrobacterium*-mediated genetic transformation of *Stylosanthes guianensis*

Mejoramiento de la regeneración de plantas y transformación genética mediada por Agrobacterium *en* Stylosanthes guianensis

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Abstract

As a pioneer tropical pasture legume, stylo (*Stylosanthes guianensis*) is well adapted to growth-limiting factors in acid soils. Considering the importance of stylo, there is a need to improve *Agrobacterium*-mediated genetic transformation to enable development of elite cultivars. In this study, *S. guianensis* cv. RY5 was used to systematically optimize *Agrobacterium*-mediated transformation based on its plant regeneration. Results showed that Murashige and Skoog (MS) medium containing 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 2 mg/L 6-benzylaminopurine (6-BA) was the optimal callus induction medium. MS medium supplemented with 2 mg/L 6-BA was suitable for shoot regeneration from cotyledon-derived calluses, and 0.5 mg/L indole-3-acetic acid (IAA) and 0.5 mg/L indole-3-butyric acid (IBA) applications were beneficial for rooting. The highest transformation efficiency (67%) was obtained at an *Agrobacterium* concentration of optical density = 0.6 combined with an infection time of 15 min and 3 days of co-cultivation. Furthermore, 200 mg/mL carbenicillin (Carb) and 0.6 mg/L Basta® supplements were effective in eliminating excess bacterial growth and selecting transgenic plants, respectively. Subsequent polymerase chain reaction (PCR) analysis confirmed that the β-glucuronidase (*GUS*) and *BAR* genes were successfully integrated into the stylo genome. Wider testing of this improved protocol as a means of enhancing genetic improvement and gene function analysis of stylo seems warranted.

Keywords: Genetic engineering, GUS staining, plant hormones, tissue culture, tropical legumes.

Resumen

Como leguminosa pionera para pasturas tropicales, stylo (*Stylosanthes guianensis*) es una especie bien adaptada a los factores limitantes del crecimiento en suelos ácidos. Considerando la importancia de stylo, existe la necesidad de mejorar la transformación genética mediada por *Agrobacterium* para permitir el desarrollo de cultivares élite. En este estudio, conducido en China tropical, se utilizó *S. guianensis* cv. RY5 para optimizar en forma sistemática la transformación mediada por *Agrobacterium* con base en la regeneración de plantas. Los resultados mostraron que el medio Murashige & Skoog (MS) que contenía 0.2 mg/L de ácido 2,4-diclorofenoxiacético (2,4-D) y 2 mg/L de 6-bencilaminopurina (6-BA) fue el medio óptimo para la inducción de tejido calloso. El medio MS suplementado con 2 mg/L de 6-BA fue adecuado para la regeneración de brotes a partir de callos derivados de cotiledones, y aplicaciones de 0.5 mg/L de ácido indol-3-acético (IAA) y 0.5 mg/L de ácido indol-3-butírico (IBA) fueron beneficiosas para la formación de raices. La mayor eficiencia de transformación (67%) se obtuvo a una concentración de *Agrobacterium* con densidad óptica de 0.6,

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combinada con un tiempo de infección de 15 min y 3 días de co-cultivo. Además, suplementos en forma de 200 mg/ml de carbenicilina (Carb) y 0.6 mg/L de Basta® fueron eficaces para eliminar el crecimiento bacteriano excesivo y seleccionar plantas transgénicas, respectivamente. El análisis posterior de PCR confirmó que los genes de la β-glucuronidasa (*GUS*) y *BAR* fueron exitosamente integrados en el genoma de stylo. Se concluye que pruebas más amplias con este protocolo mejorado, usándolo como una efectiva herramienta en el mejoramiento genético y análisis de la función de genes de *S. guianensis*, parecen justificadas.

Palabras clave: Cultivo de tejido, fitohormonas, ingeniería genética, leguminosas tropicales, tinción de GUS.

Introduction

Species of *Stylosanthes*, referred to as stylos, are important pasture legumes and widely distributed across the tropical and subtropical areas of the Americas, Africa and Asia. The genus includes more than 40 species, such as *S. guianensis* (common stylo), *S. hamata* (Caribbean stylo), *S. scabra* (shrubby stylo), *S. viscosa* (sticky stylo) and *S. seabrana* (Caatinga stylo) (Chandra 2013). Stylo is regarded as the 'tropical alfalfa' due to its multiple uses in tropical agricultural systems, including sown pastures, feed for livestock and improvement of soil properties, natural grassland and orchard mulching. Considering the importance of this pasture legume, it was introduced to China from Colombia and Australia in the 1960s (Tang et al. 2009). *Stylosanthes guianensis* cv. RY5, a single plant isolated from the population of its parent CIAT 184, exhibits the traits of earlier flowering, higher seed production, anthracnose resistance and cold tolerance compared with its parent, and is considered an elite stylo cultivar that is widely grown in South China (Liu et al. 2001; Tang et al. 2009).

With the development of technology, stylo has attracted large research interest in many aspects, including ecological studies (Vander Stappen et al. 1999; Sawkins et al. 2001), physiological and biochemical analyses (Zhou et al. 2005; Sun et al. 2014; Chen et al. 2015; Wang et al. 2017; Liu et al. 2018) and genetic diversity studies (Vander Stappen et al. 2000; Jiang et al. 2005; Ding et al. 2015). For example, stylo has been recognized as a pioneer tropical pasture with extensive adaptation to growth-limiting factors in acidic infertile soils, such as phosphorus (P) deficiency and aluminum (Al) and manganese (Mn) toxicity (Chen et al. 2015; Jiang et al. 2018; Liu et al. 2019). It has been demonstrated that malate synthesis in roots and exudation from roots of *S. guianensis* are the critical tolerance strategies for Al and Mn toxicity in acidic soils (Sun et al. 2014; Chen et al. 2015). Recently, a root-associated purple acid phosphatase, SgPAP23, was characterized as a primary mediator of extracellular phytate-P utilization in stylo (Liu et al. 2018).

Although much work has been conducted as described above, extensive studies of stylo are restricted by some challenges, such as limited available germplasm resources, narrow genetic variability, lack of genome information and susceptibility to chilling stress as well as anthracnose disease (Chandra 2013; Wang et al. 2017). To solve these issues, genetic improvement through biotechnological techniques is one of the most effective and useful approaches that will help to improve the desirable traits of plant adaptation to biotic or abiotic stresses, contributing to agricultural production (Mittler and Blumwald 2010).

Over the past decades, significant development in efficient genetic transformation methods has been made in many plants. Among them, *Agrobacterium*-mediated transformation based on plant tissue culture is the most useful method for genetic transformation in various crops, such as rice (*Oryza sativa*) (Ozawa 2012), maize (*Zea mays*) (Ishida et al. 2007), sorghum (*Sorghum bicolor*) (Gurel et al. 2012), soybean (*Glycine max*) (Wang et al. 2009) and alfalfa (*Medicago sativa*) (Tesfaye et al. 2001). Although great efforts have been made to develop an *Agrobacterium*-mediated genetic transformation system for the genus *Stylosanthes*, such as *S. hamata* (Iji et al. 1995; Kumar and Chandra 2010), *S. humilis* (Manners and Way 1989), *S. seabrana* (Kumar and Chandra 2009; 2010) and *S. guianensis* (Quecini et al. 2006; Wang et al. 2008; Yuan et al. 2011; Bao et al. 2016), the genetic transformation procedure has not been adequately improved. Furthermore, combination analyses of *Agrobacterium*-mediated transformation based on plant regeneration systems are rarely performed on stylo. Only a few studies have successfully developed transgenic stylo plants thus far (Wang et al. 2008; Bao et al. 2016; Chen et al. 2016). Therefore, optimization of plant regeneration and an *Agrobacterium*-mediated transformation system are critical for the genetic improvement of stylo cultivars and basic research.

The efficiency of *Agrobacterium*-mediated genetic transformation is influenced by many factors, such as plant species and explants, the process of callus induction and differentiation, and *Agrobacterium* infection (Cheng et al. 2004; Trivellini et al. 2015). In this regard, the *S. guianensis* cultivar RY5 was used to optimize the plant regeneration system in this study, including callus

induction, shoot regeneration and root induction. Subsequently, factors influencing the *Agrobacterium*-mediated transformation system, including bacteriostatic antibiotics, *Agrobacterium* concentrations, infection and co-cultivation duration and Basta® concentrations, were analyzed according to β-glucuronidase (GUS) staining analysis. Furthermore, polymerase chain reaction (PCR) analysis was performed to examine the putative transformed stylo plants.

Materials and Methods

Plant material and explant preparation

In this study, the stylo cultivar RY5 was used. Seeds of RY5 were soaked in hot water at 80 ℃ for 3 min, followed by surface sterilization using 75% ethanol (v/v) and 2% sodium hypochlorite (v/v), and were subsequently germinated in Petri dishes containing basal Murashige and Skoog (MS) solid medium containing 30 g/L sucrose and 7 g/L agar (Liu et al. 2018). The pH of the medium was adjusted to 5.8 using 1 mol/L KOH or H_2SO_4. After 6–8 days germination, explants of euphylla, cotyledon and hypocotyl, approximately 10 mm in length, were used for callus induction.

Callus induction and plant regeneration

For embryogenic callus induction, the different explants were inoculated on MS solid medium containing different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) (0–0.5 mg/L) and 6-benzylaminopurine (6-BA) (0–2 mg/L) supplemented alone or in combination. All treatments had 4 biological replicates, and each replicate included 10 explants. The explants were sub-cultured every 10 days on fresh MS medium. After 30–40 days of sub-culture, a granular callus approximately 5 mm in size was recorded for callus induction.

For shoot regeneration, the embryogenic callus from the explants of the cotyledon was selected and transferred onto MS medium supplemented with different concentrations of 6-BA (0–4 mg/L) for shoot regeneration. The cultures were incubated at 28 ℃ under an irradiance of 80 μmol/m/s (16 h of light:8 h of dark). All treatments had 3 biological replicates and each replicate included 10 calluses. After 8–10 weeks of culture, the shoots with 2–4 leaves that elongated from the callus were recorded.

For root induction, elongated shoots were excised from the callus and transferred onto different rooting media, including M1 (MS containing 20 g/L sucrose, 0.8 g/L activated charcoal and 7.0 g/L agar; pH 5.8), M2 [MS containing 0.5 mg/L indole-3-acetic acid (IAA), 0.5 mg/L indole-3-butyric acid (IBA), 20 g/L sucrose, 0.8 g/L activated charcoal and 7.0 g/L agar; pH 5.8] and M3 [MS containing 0.5 mg/L IAA, 0.5 mg/L α-naphthaleneacetic acid (NAA), 20 g/L sucrose, 0.8 g/L activated charcoal and 7.0 g/L agar; pH 5.8]. After 3 weeks of culture, the rooting efficiency was determined. All experiments had 4 biological replicates and each replicate included 10 shoot buds.

Carbenicillin (Carb) concentration determination

To detect the effects of Carb on the growth of *Agrobacterium tumefaciens* strain EHA105, *A. tumefaciens* with an initial absorbance of 0.02 at 600 nm was grown in YEB liquid medium (5 g/L tryptone, 1 g/L yeast extract, 5 g/L beef extract, 5 g/L sucrose, 0.5 g/L $MgSO_4 \cdot 7H_2O$) supplied with different concentrations (0–400 mg/mL) of Carb at 28 ℃. After 48 h of growth, the absorbance of *A. tumefaciens* was recorded. To detect the effects of Carb on callus induction, cotyledon explants were incubated in the optimal callus induction MS solid medium (MS medium supplied with 0.5 mg/L 2,4-D and 2.0 mg/L 6-BA) containing different concentrations (0–1,000 mg/mL) of Carb. The numbers of embryogenic calluses were recorded after 30–40 days of culture. All experiments had 5 biological replicates and each replicate included 100 explants.

Infection and co-culture assay

The binary vector pCAMBIA3301, containing the *GUS* reporter gene driven by the cauliflower mosaic virus 35S (CaMV 35S) promoter, was used as the transformation vector in this study. The *BAR* gene was used as a selection marker under the control of the CaMV 35S promoter. *Agrobacterium tumefaciens* strain EHA105 harboring pCAMBIA3301 was grown in 50 mL of YEB liquid medium containing 50 mg/L kanamycin and 50 mg/L rifampicin at 28 ℃ for 48 h. After centrifugation at 5,000 rpm for 3 min, the bacterial cells were harvested and further re-suspended in YEB liquid medium.

The cotyledon explants were incubated in 50 mL of *Agrobacterium* suspension cells harboring the pCAMBIA3301 vector with different absorbances at 600 nm (from 0.2 to 1.0), which contained 50 mg/L acetosyringone (AS). The mixtures were then cultured for 5–25 min with gentle shaking. Subsequently, the *Agrobacterium*-infected explants were blotted dry on sterile filter paper and were then transferred onto co-cultivation medium (MS medium containing 0.5 mg/L 2,4-D and 2.0 mg/L 6-BA) supplied with 50 mg/L AS for

1–5 days. After that, the explants were transferred onto the callus induction MS medium (MS medium supplied with 0.5 mg/L 2,4-D and 2.0 mg/L 6-BA) containing 200 mg/mL Carb and 0.2–0.8 mg/L Basta®. After 30–40 days of culture, the transformation efficiency was determined based on GUS staining analysis as described by Qin et al. (2012). Briefly, the putative transformed callus was incubated in the GUS staining solution containing 0.2 mol/L Na_2HPO_4-NaH_2PO_4 and 1 mmol/L 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (pH 7.0) at 37 ℃ for 12 h, followed by washing in 70% ethanol (v/v). All experiments had 3 biological replicates and each replicate included more than 40 explants.

Putative transformed plant generation

Under the optimal procedure of infection, co-culture and selection, the basta-resistant callus was transferred onto selection medium (MS medium containing 2.0 mg/L 6-BA, 0.6 mg/L Basta® and 200 mg/mL Carb) for shoot regeneration for 8–10 weeks. Subsequently, the regenerated plantlets were transferred onto the optimal rooting medium (MS medium containing 0.5 mg/L IAA, 0.5 mg/L IBA, 20 g/L sucrose and 0.8 g/L activated charcoal; pH 5.8) for 3 weeks of root growth. All of these cultures were incubated at 28 ℃ under an irradiance of 80 μmol/m/s (16 h of light:8 h of dark). Finally, the putative transformed plantlets with sufficient root systems were transplanted and cultured in a 1:1 soil:sand mix for normal growth in the greenhouse.

Transgenic plant determination

For polymerase chain reaction (PCR), total genomic DNA was isolated from leaves of putative transgenic stylo plants using the cetyltrimethyl ammonium bromide (CTAB) extraction method modified by Ding et al. (2015). The primers *BARf* (5'-GGTCTGCACCATCGTCAACC-3') and *BARr* (5'-CCCACGTCATGCCAGTTCC-3') were used to amplify the *BAR* gene, and the primers *GUSf* (5'-TCGCCGATGCAGATATTCGT-3') and *GUSr* (5'-CCCGCTAGTGCCTTGTCCA-3') were used to amplify the *GUS* gene. For semi-quantitative RT-PCR analysis, the total RNA from leaves was extracted using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. First-strand cDNA synthesis from 2 mg of DNase I-treated RNA was performed using PrimeScript reverse transcriptase (Takara, China). The RT-PCR reaction contained 1 μL of cDNA as a template. The PCR reaction was stopped after 28 cycles. The primers *SgEF-1af* (5'-GCACTGTCATTGATGCTCCC-3') and *SgEF-1ar* (5'-TGGCACAGTTCCAATACCAC-3') were used to amplify the housekeeping *SgEF-1a* gene as an internal control. The binary vector pCAMBIA3301 and wild-type stylo plant were used as positive and negative controls, respectively.

Statistical analysis

The callus induction rate (%) was calculated as the ratio of the numbers of induced calluses and the numbers of explants used. Shoot regeneration efficiency (%) was calculated as the ratio of the numbers of regenerated shoots and the numbers of calluses used. Rooting efficiency (%) was calculated as the ratio of the numbers of regenerated roots and the numbers of shoot buds used. The frequency of GUS-stained calluses (%) was calculated as the ratio of the numbers of GUS-stained calluses and the numbers of calluses used. All statistical analyses were performed by one-way ANOVA using the SPSS program (SPSS Institute, USA, v. 13.0).

Results

Optimization of the plant regeneration system of stylo

In this study, 3 explants, hypocotyl, cotyledon and euphylla were used to investigate the effects of different hormone supplements on embryogenic callus induction. The results (Table 1) showed that after 30–40 days of culture, callus induction rates were approximately 10% for the 3 tested explants under control treatment (without hormone addition), in which the callus displayed a pale yellow or white and watery texture. Callus induction rates were significantly increased by 2,4-D and 6-BA treatment, alone or in combination. The callus induction rates were 100, 87.5–90 and 95–100% for hypocotyl, cotyledon and euphylla, respectively, under 2,4-D treatment. Treatment with 6-BA enhanced the callus induction rates with more than 500, 500 and 380% increases in hypocotyl, cotyledon and euphylla, respectively, as compared with their respective controls. Furthermore, 2.0 mg/L 6-BA in combination with 0.2–0.5 mg/L 2,4-D supplement significantly increased the callus induction rates (approximately 100%), especially for cotyledon and euphylla, compared with their respective controls. However, no significant differences in callus induction were observed among the 3 tested explants under the same hormone treatment. Therefore, the optimal callus induction medium was MS medium containing 2 mg/L 6-BA and 0.2 mg/L 2,4-D, in which granular callus displayed a light green and compact texture (Table 1, Figures 1a–1d).

Table 1. Effects (mean ± s.e.) of different hormone treatments on callus induction in hypocotyl, cotyledon and euphylla explants of *Stylosanthes guianensis* after 30–40 days.

Hormone (mg/L)		No. of explants	Explant					
			Hypocotyl		Cotyledon		Euphylla	
2,4-D	6-BA		Mean of explants producing callus (%)	Color and texture of callus	Mean of explants producing callus (%)	Color and texture of callus	Mean of explants producing callus (%)	Color and texture of callus
0	0	40	12.5±2.5d[1]	Pale yellow/Watery	9.8±1.2c	White/Watery	10.0±1.8d	White/Watery
0	1	40	75.0±6.5c	Green/Compact	60.0±10.8b	Green tawny/Compact	47.5±7.5c	Green/Compact
0	2	40	87.5±7.5b	Dark green/Compact	60.0±9.1b	Dark green/Compact	70.0±7.1b	Dark green/Compact
0.2	0	40	100.0±0.0a	Pale yellow/Watery	87.5±7.5a	Tawny/Watery	95.0±5.0a	Tawny/Watery
0.2	1	40	100.0±0.0a	White green/Compact	95.0±5.0a	Yellow green/Compact	92.5±4.8a	Yellow green/Compact
0.2	2	40	100.0±0.0a	Light green/Compact	100.0±0.0a	Light green/Compact	100.0±0.0a	Light green/Compact
0.5	0	40	100.0±0.0a	White/Watery	90.0±4.1a	White/Watery	100.0±0.0a	White/Watery
0.5	1	40	100.0±0.0a	White green/Compact	97.5±2.5a	White green/Compact	92.5±4.8a	White green/Compact
0.5	2	40	95.0±2.9a	Green/Loosened	100.0±0.0a	Green/Loosened	100.0±0.0a	Green/Loosened

[1]Values within columns followed by different letters indicate significant differences at P<0.05.

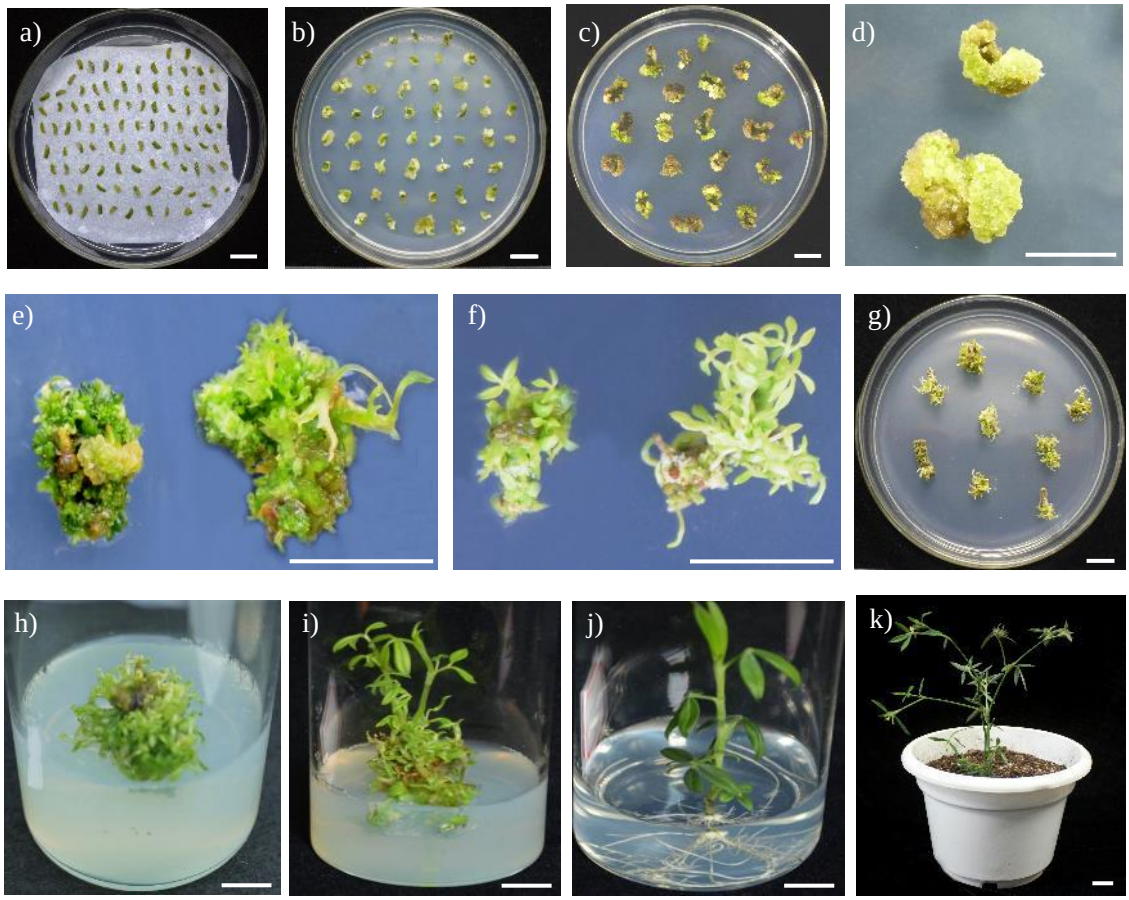

Figure 1. Plant regeneration from the cotyledon-derived callus and *Agrobacterium*-mediated transformation of *Stylosanthes guianensis*. a) Co-culture duration; b), c) and d) Callus induction; e) to i) Shoot regeneration; j) Rooting of plantlet; k) Transgenic stylo plant. Scale bar is 1 cm.

The light green and compact texture granular callus induced from the cotyledon became green and then produced a lot of tubers after cultured on the medium containing 6-BA for 50–70 days, whereas control calluses which appeared white, sticky and watery were unable to generate shoots on the differentiation medium (Figures 1e–1i). The effects of different levels of 6-BA on shoot differentiation from the cotyledon-derived callus of cv. RY5 were demonstrated as shoot regeneration rates and were significantly increased with increasing 6-BA concentrations; highest shoot regeneration rates (approximately 80.0%; P<0.05) were observed under 2 and 4 mg/L 6-BA supplements (Figure 2a). Subsequent rooting efficiency from the regenerated shoot after 3 weeks of root induction was higher in M2 and M3 media with different hormone supplements than in M1 medium without hormone addition (Figure 2b). The highest rooting efficiency (100%) was observed in M2 medium containing 0.5 mg/L IAA and 0.5 mg/L IBA (Figure 1j, Figure 2b).

Improvement of Agrobacterium*-mediated transformation*

Growth of *Agrobacterium* was significantly inhibited by increasing Carb concentrations compared with the control without Carb addition (Figure 3a) with decreases of 69.6 and 98.8% under 50 and 200 mg/mL Carb treatments, respectively, compared with the control. Callus induction rate was significantly (P<0.05) decreased when Carb concentrations were higher than 600 mg/mL with decreases of 29.2 and 66.6% under 800 and 1,000 mg/mL Carb treatments, respectively, compared with the control (Figure 3b).

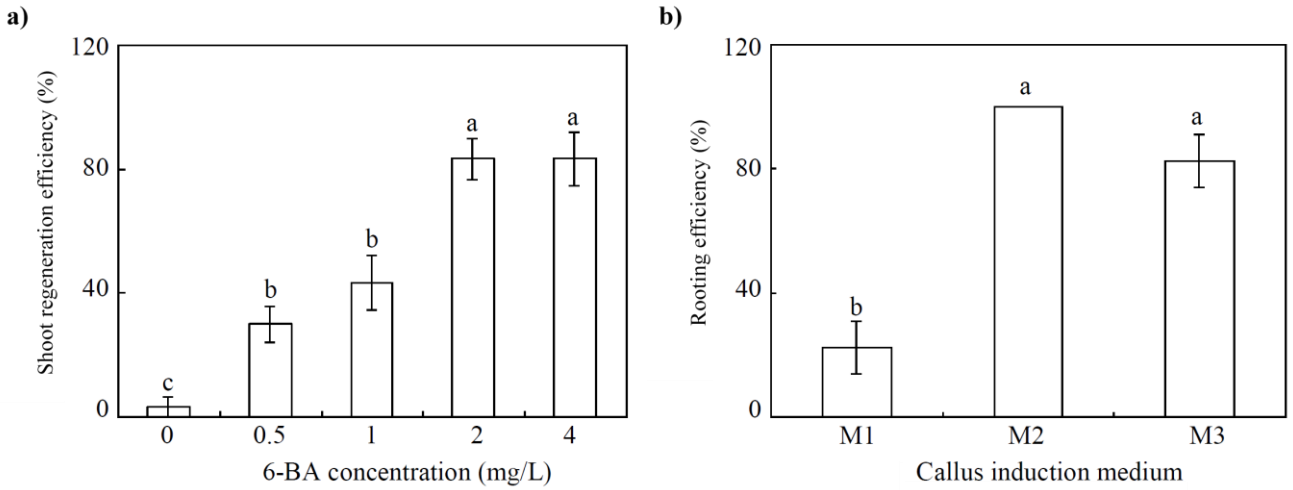

Figure 2. Effects of different hormone treatments on: a) Shoot regeneration efficiency; and b) Rooting efficiency of *Stylosanthes guianensis*. Vertical bars indicate the standard errors of means. Different letters on columns indicate significant differences at P<0.05.

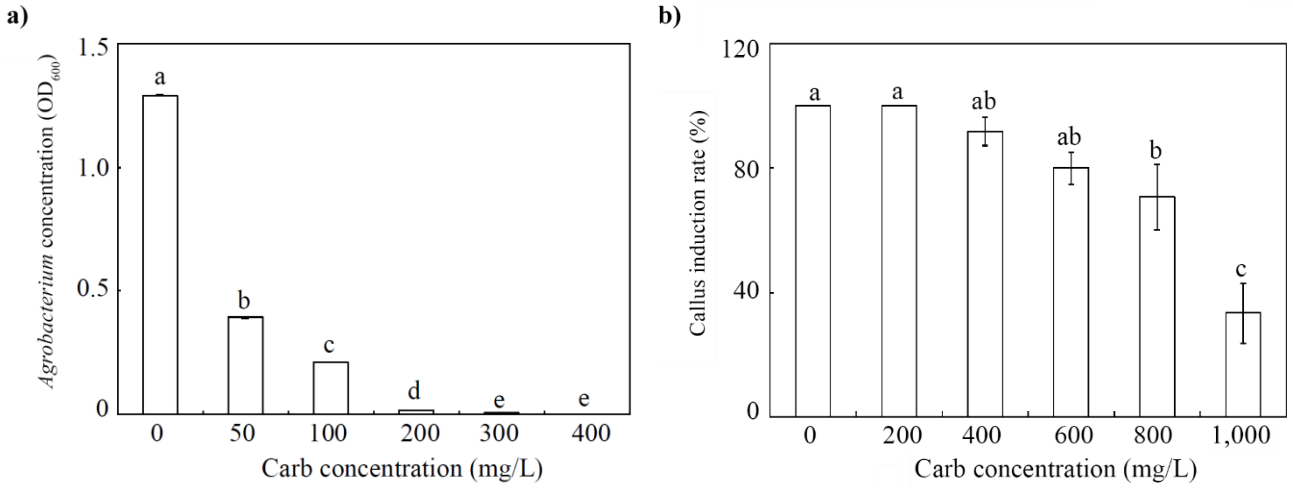

Figure 3. Effects of Carb on: a) *Agrobacterium* concentration; and b) Callus induction rate of *Stylosanthes guianensis*. Vertical bars indicate the standard error of the mean. Different letters on columns indicate significant differences at P<0.05.

Subsequently, the effects of *Agrobacterium* concentration, infection time and co-cultivation duration on callus transformation efficiency were evaluated based on GUS staining analysis (Table 2). A callus with blue coloration was considered a positive transformed callus after GUS staining. Highest transformation efficiency (67.5%) was observed at an *Agrobacterium* concentration of optical density (OD_{600}) of 0.6 and transformation efficiency decreased when the *Agrobacterium* concentration (OD_{600}) exceeded 0.6. Similarly, transformation efficiency increased with increasing *Agrobacterium* infection time from 5 to 20 min, and the highest transformation efficiency (59.3%; $P<0.05$) was achieved with infection times of 15 and 20 min. However, transformation efficiency was decreased significantly (by 93.8%) after 25 min of infection compared with that after 15 min of infection. The transformation efficiency was also affected by different co-cultivation durations. The maximum transformation efficiency was obtained under 3 days of co-cultivation, when it reached 69.4%.

Additionally, the effects of Basta® on transformed callus selection were also examined and showed that transformation efficiencies varied among different Basta® treatments (Table 2). Transformation efficiency increased with increasing Basta® concentrations, peaking at 0.6 mg/L at a value of 62.1%, but then decreased at Basta® concentrations greater than 0.6 mg/L. Therefore, 0.6 mg/L Basta® was chosen for appropriate callus selection.

Transformed plant production

Subsequently, cotyledon explants were used for transformation under the optimized procedure of an *Agrobacterium* concentration (OD_{600}) of 0.6 with 15 min of *Agrobacterium* infection and 3 days of co-cultivation (Figure 4). The infected explants were transferred onto MS medium containing 0.2 mg/L 2,4-D, 2.0 mg/L 6-BA and 200 mg/L Carb for 1 week of recovery growth. After that, the explants were transferred onto callus induction medium containing 0.2 mg/L 2,4-D, 2.0 mg/L 6-BA, 0.6 mg/L Basta® and 200 mg/mL Carb for 30–40 days. A mass of basta-resistant calluses was generated and then transferred onto shoot regeneration medium containing 2.0 mg/L 6-BA, 0.6 mg/L Basta® and 200 mg/mL Carb for 8–10 weeks. The regenerated plantlets were cut and further transferred onto rooting medium containing 0.5 mg/L IAA and 0.5 mg/L IBA for 3 weeks. Finally, the putative transformed plantlets with a sufficiently vigorous root system were transplanted and cultured into a soil and sand mixture (1:1) in a greenhouse for normal growth (Figure 4, Figure 1k).

Table 2. Different factors affecting the transformation efficiency of *Stylosanthes guianensis*.

Factor		No. of explants	No. of resistant calluses	GUS-stained rates (%)[1]
Agrobacterium concentration (OD_{600})	0.2	139	138	53.2±4.60ab[2]
	0.4	121	120	52.5±2.50ab
	0.6	136	134	67.5±8.26a
	0.8	122	122	36.1±1.95b
	1	153	150	38.5±8.74b
Infection time (min)	5	141	139	28.4±6.03b
	10	141	140	31.6±7.20b
	15	159	157	59.3±9.44a
	20	129	125	56.6±6.30a
	25	135	133	3.65±1.30c
Co-cultivation time (days)	1	121	120	29.2±5.07c
	2	125	121	32.3±4.51bc
	3	133	130	69.4±3.78a
	4	140	137	45.2±2.32b
	5	129	128	32.3±5.35bc
Basta® concentration (mg/L)	0.2	122	120	24.2±2.20c
	0.4	129	125	49.4±3.78b
	0.6	130	126	62.1±3.08a
	0.8	120	120	46.7±5.46b

[1]GUS-stained rate calculated from the ratio of the numbers of GUS-stained calluses and the numbers of resistant calluses for each treatment. Data are mean values of 3 replications with standard error. [2]Values within factors followed by different letters indicate significant differences at $P<0.05$.

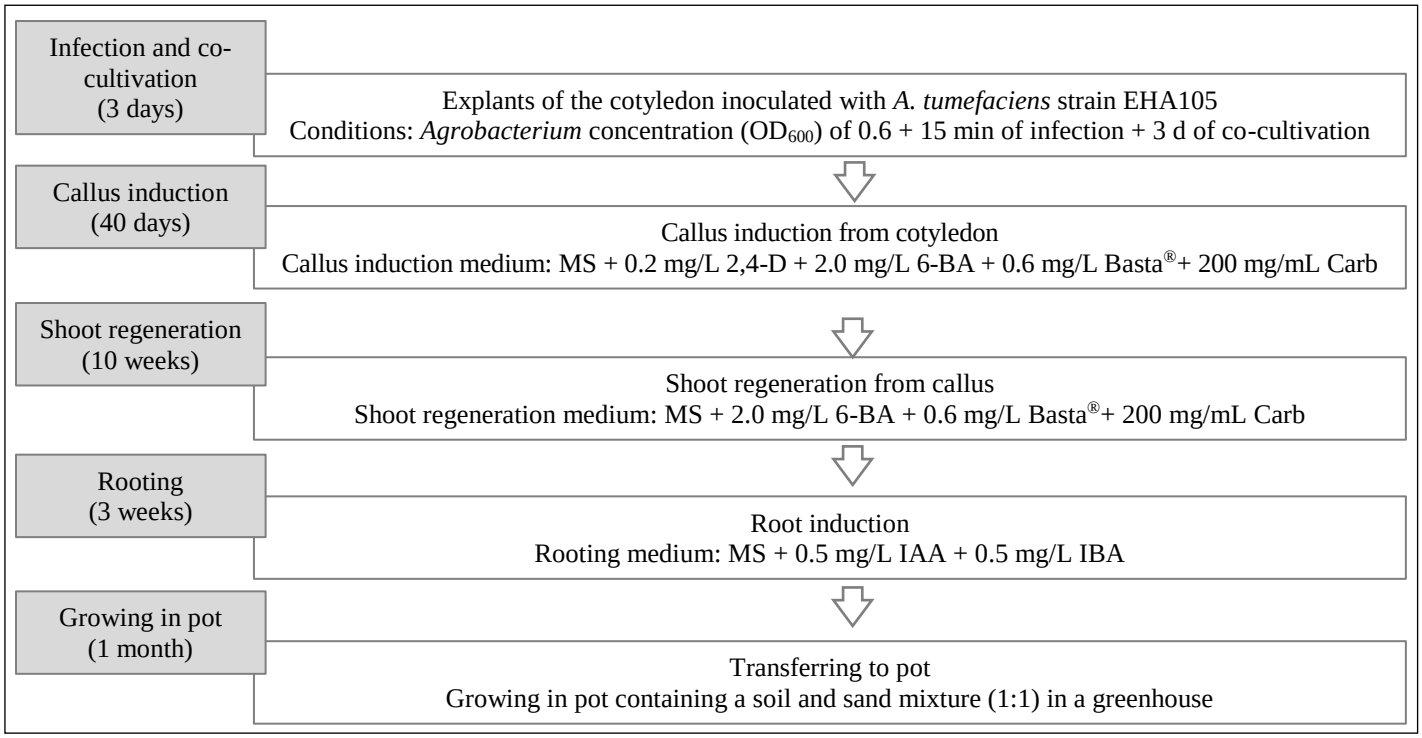

Figure 4. Optimal protocol for *Agrobacterium*-mediated transformation of *Stylosanthes guianensis*.

Determination of putative transgenic stylo plants

As shown in Figure 5, clear GUS staining was observed in callus, leaf, root and stem of transgenic plants, suggesting that *GUS* gene was successfully expressed in different tissues of stylo plants. In this study, a total of 850 explants were used for *Agrobacterium*-mediated transformation according to the optimized procedure, and 51 putative transformed lines were obtained. To further verify the integration of foreign genes into the stylo genome, genomic DNA extracted from leaves of 15 putative transformed lines was used for PCR analysis. The results showed that 426 and 533 bp of the expected DNA fragments of the respective *BAR* and *GUS* genes were successfully amplified from 9 out of 15 randomly selected lines (Figures 6a–6c), suggesting the successful integration of foreign

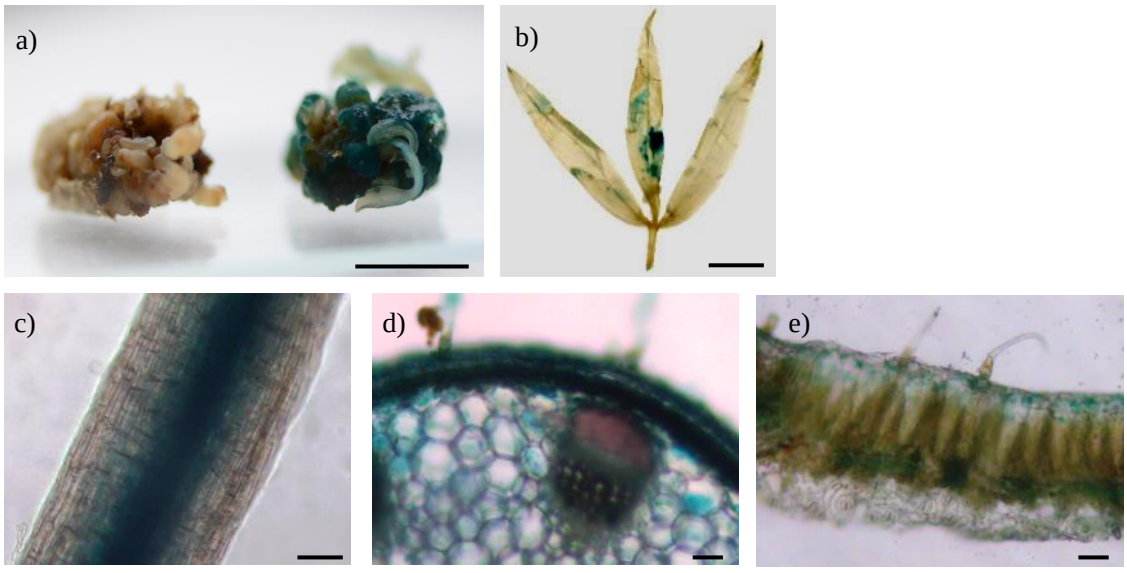

Figure 5. GUS staining of putative transformed *Stylosanthes guianensis* tissues: a) and b) GUS staining of callus and leaf, respectively; c) GUS staining of mature root; d) and e) Cross-section of stem and leaf. Scale bar in a) and b) is 1 cm. Scale bar in c), d) and e) is 100 µm.

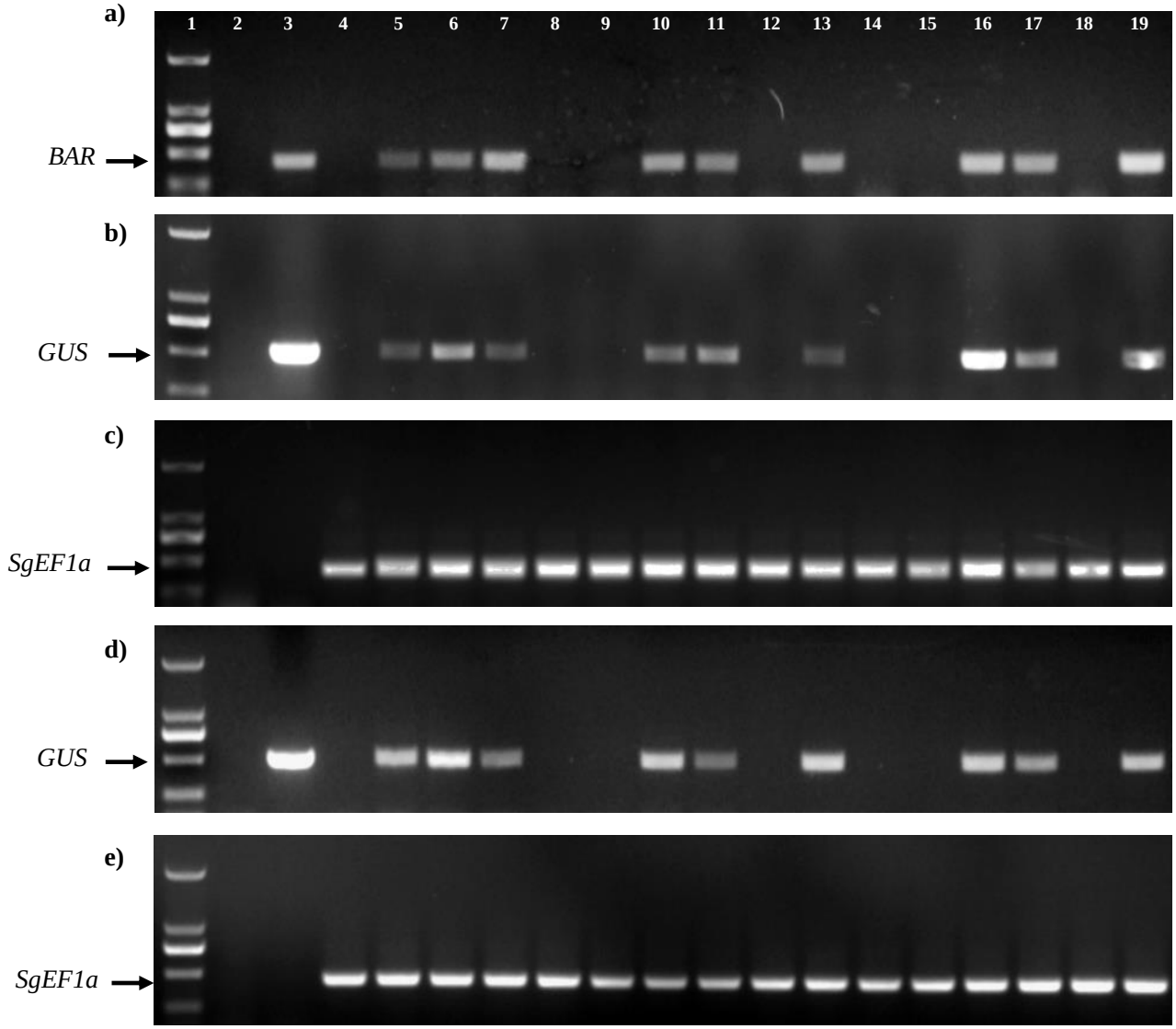

Figure 6. Molecular analysis of the putative transgenic *Stylosanthes guianensis* plants: a) Genomic PCR detection of the *BAR* gene; b) Genomic PCR detection of the *GUS* gene; c) Genomic PCR detection of the housekeeping *SgEF-1a* gene; d) Expression of the *GUS* gene via RT-PCR analysis; and e) RT-PCR analysis of the *SgEF-1a* gene. 1 = DL2000 DNA marker; 2 = negative control; 3 = positive control; 4 = wild type plant; and 5–19 = 15 putative transgenic lines.

genes into the stylo genome. Furthermore, the expression of the *GUS* gene in the leaves of the 15 tested putative transgenic stylo lines was also detected using RT-PCR analysis. The results showed that the foreign *GUS* gene was stably expressed in 9 out of 15 tested transgenic stylo lines (Figures 6d and 6e). In total, 33 out of 51 putative lines were proved to be positive transgenic lines, resulting in a transformation efficiency of 3.88%.

Discussion

The *Agrobacterium*-mediated genetic transformation approach is widely used for the development of transgenic plants (Cheng et al. 2004). Despite the importance of the *Stylosanthes* genus for pasture, only a few transgenic plants have been generated and used for gene function analysis of *Stylosanthes* species (Wang et al. 2008; Bao et al. 2016), suggesting that the existing protocols might not be efficient for the development of transgenic stylo plants. Therefore, it is essential to improve the *Agrobacterium*-mediated genetic transformation procedure for stylo based on its plant regeneration.

This study has shown that MS medium containing 0.2 mg/L 2,4-D and 2 mg/L 6-BA was the optimal callus induction medium, especially for cotyledon and euphylla explants (Table 1). As growth regulators, 2,4-D, 6-BA

and NAA have been generally used for callus induction of stylo (Wang et al. 2008; Yuan et al. 2011; Bao et al. 2016). Yuan et al. (2011) found that the optimal condition for callus induction from cotyledons of *S. guianensis* cv. Reyan 2 was MS medium containing 3.0 mg/L 2,4-D with highest rates of callus induction reaching 74%, which is lower than that measured in this study. On the other hand, Godwin et al. (1987) showed the optimal callus induction medium for *S. scabra* leaves was MS medium supplemented with both 2,4-D and 6-BAP. These variations in appropriate hormone application can be attributed to different stylo species, cultivars or genotypes. Similar variations have also been observed in other plant species. For instance, a combination of 0.5 mg/L 2,4-D and 2.0 mg/L 6-BA resulted in better callus induction from *Vitis vinifera* flowers (Dai et al. 2015), while 2.0–3.0 mg/L 2,4-D was the optimal concentration for promoting callus induction in leaves of *Urochloa* (Yaguinuma et al. 2018).

In addition to callus induction, the differentiation of shoots from the callus is also essential for plant regeneration. Shoot organogenesis induced from the callus can be improved by application of appropriate hormone (Cheng et al. 2004; Yaguinuma et al. 2018). In this study, although shoot differentiation from the cotyledon-derived callus of cv. RY5 was observed in hormone-free MS medium, the percentage of shoot regeneration was only 3.3% (Figure 2a). Our findings that MS medium with 2 mg/L 6-BA applications was most efficient for shoot regeneration (Figure 2a), producing a shoot regeneration rate of approximately 80%, is consistent with earlier findings that 2–4 mg/L 6-BA applications significantly increased the regeneration of shoots from the cotyledon-derived callus of cv. Reyan 2 (Yuan et al. 2011). Similarly, a high frequency of shoot regeneration was observed from the leaf-derived callus of *S. scabra* on MS medium containing 2.0 mg/L BAP (Godwin et al. 1987) and from the cotyledon-derived callus of *S. macrocephala* on MS medium supplemented with 0.1 mg/L NAA and 0.4 mg/L BAP (Vieira et al. 1990). However, other factors, such as callus derived from different types of explants and callus states (e.g. color and texture) at different hormone treatments, may influence the regeneration capacity of shoots in stylo, which merits further study.

The increases in rooting efficiency we observed with hormone applications support the increased rooting efficiencies of *S. seabrana* and *S. hamata* in response to the application of IAA and IBA alone or in combination compared with untreated controls (Kumar and Chandra 2010). MS medium containing NAA and IBA is beneficial for rooting of *S. guianensis* (Kelemu et al. 2005). In contrast, it has been reported that hormone-free medium is suitable for rooting of *S. scabra* and *S. guianensis* (Valarini et al. 1997; Quecini et al. 2006; Bao et al. 2016). We found that the appropriate rooting medium was MS medium supplemented with 0.5 mg/L IAA and 0.5 mg/L IBA, in which rooting efficiency was significantly higher than that of hormone-free MS medium (Figure 2b). Similar results have been found for other plants, such as white ash (*Fraxinus americana*) and grass pea (*Lathyrus sativus*), where IAA and IBA applications promoted rooting efficiency (Barpete et al. 2014; Palla and Pijut 2015). Taken together, the results indicate that stable plantlet regeneration was optimized in cv. RY5 using cotyledons as explants.

Carb is used for terminating rudimentary *Agrobacterium* growth at suitable doses after the co-culture period during T-DNA insertion into the plant chromosome (Li et al. 2015). In our study, a dose-response experiment showed that 200 mg/mL Carb efficiently inhibited excess bacterial growth but did not affect callus induction. Optimal Carb concentrations that inhibit excess *Agrobacterium* growth range from 200 to 1,000 mg/L (Figure 3). For example, it has been demonstrated that 200, 500 and 1,000 mg/L Carb are efficient for the elimination of *Agrobacterium* in grapes (*Vitis vinifera*) (Dai et al. 2015), loblolly pine (*Pinus taeda*) (Tang et al. 2004) and tobacco (*Nicotiana tabacum*) (Nauerby et al. 1997), respectively, during bacterial infection.

Agrobacterium concentration, infection and co-cultivation duration are important factors that affect transformation efficiency. An inappropriate concentration of *Agrobacterium* may produce toxic contamination events or may not be effective for the callus, and a long or short infection time may result in a low frequency of transformation (Cheng et al. 2004; Sharma et al. 2011; Trivellini et al. 2015). In this study, the highest transformation efficiency (67%) was obtained under the conditions of an *Agrobacterium* concentration of OD_{600} = 0.6, combined with an *Agrobacterium* infection time of 15 min and 3 days of co-cultivation based on GUS staining analysis (Table 2). This finding is similar to the *Agrobacterium* concentration of OD_{600} = 0.4–0.6, an infection time of 10 min and a co-cultivation duration of 2–3 days found to be optimal conditions for transformation of baby bamboo (*Pogonatherum paniceum*) (Li et al. 2015) and white ash (*Fraxinus americana*) (Palla and Pijut 2015). Furthermore, *Agrobacterium* infection for 2–10 min with 2–4 days of co-cultivation has been shown beneficial for increasing transformation efficiency in other stylo species, e.g. *S. humilis* and *S. guianensis* (Manners 1987; Manners and Way 1989; Bao et al. 2016; Chen et al. 2016).

Basta® is commonly used for the selection of transgenic plants that contain the herbicide-resistant selection marker *BAR* gene, which is resistant to Basta® (Lin et al. 2009; Mayavan et al. 2015). Optimization of the Basta® concentration for selection pressure is important for increasing transformation efficiency. In this study, 0.6 mg/L Basta® was found to be suitable for transformed callus selection (Table 2), which is similar to the level recommended by Bao et al. (2016). It is noteworthy that *Agrobacterium*-mediated genetic transformation efficiency is influenced by many factors, such as phytohormone, antibiotic, growth medium and bacterial infection process (Nandakumar et al. 2004; Sharma et al. 2011). Therefore, in this study, the transformation efficiency of stylo may have been potentially affected by different hormone supplements and culture media in the regeneration and rooting processes, which need to be studied further.

The total of 33 positive transgenic plants we generated from 850 explants of the cotyledon, representing a transformation efficiency of 3.88%, was higher than that observed in *S. humilis* cv. Paterson, where only 0.3% of explants generated transgenic plants (Manners 1988). However, it is similar to the transformation efficiency of 3.47% in *S. guianensis* cv. Mineirão using microparticle bombardment reported by Quecini et al. (2006). Besides, only 25% of the putative transformed *S. guianensis* cv. RY5 lines generated from hypocotyl-derived calluses by Chen et al. (2016) proved to be positive transgenic plants, which is lower than the frequency of 64.7% obtained from cotyledons herein (Figure 6). It appears that the transformation efficiency of stylo was improved using the optimal protocol in the current study.

In conclusion, this study has developed an optimal strategy for the *Agrobacterium*-mediated genetic transformation of cv. RY5 based on its plant regeneration system. This optimal procedure provides an appropriate platform to investigate the molecular mechanisms underlying stylo adaptation to environmental stresses. Furthermore, based on the optimized protocol, we can modify the specific traits of various stylo varieties by biotechnological and molecular approaches, which are beneficial for the genetic improvement of stylo.

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Research Paper

Whole corn grain addition in sugarcane silage avoids fermentative losses and improves in situ degradation of silage

La adición de granos enteros de maíz a ensilaje de caña de azúcar reduce pérdidas por fermentación y mejora la degradación in situ del ensilaje

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Abstract

Sugarcane silage (SS) is generally susceptible to yeast action, resulting in dry matter losses due to high soluble carbohydrate concentration. We evaluated the effects of adding corn grain and microbial inoculant at ensiling on fermentative profile, losses, chemical composition and degradation of silages. Forty experimental silos (PVC tubing) were assigned at random to a 5 × 2 factorial arrangement with: (1) 5 corn additions at ensiling: CONT - straight sugarcane silage; GC2 - sugarcane with ground corn (processed through a 2 mm sieve) added at ensiling; GC8 - sugarcane with ground corn (processed through an 8 mm sieve) added at ensiling; WC - sugarcane with whole corn grain added at ensiling; and RCS - rehydrated corn ensiled without sugarcane; and (2) 2 microbial inoculant additions at ensiling: 0 and 8 mg of commercial inoculant per kg of feed. Corn grain was added at the rate of 100 g per kg of fresh sugarcane. Adding corn grain to sugarcane at ensiling improved SS fermentation and silage chemical composition. There was no benefit from grinding the grain before adding it to sugarcane. Microbial inoculant had little effect on SS fermentation. Studies comparing corn grain with other energy sources, e.g. molasses or cassava, for addition at ensiling sugarcane seem warranted along with feeding studies with livestock to assess intake and subsequent performance. The overall benefits of adding the energy sources at ensiling versus feeding them directly to animals with untreated sugarcane silage should be determined.

Keywords: Corn processing, digestibility, grain kernels, microbial inoculant, water activity.

Resumen

El ensilaje de caña de azúcar es generalmente susceptible a la acción de levaduras resultando en pérdidas de materia seca (MS) debido a la alta concentración de carbohidratos solubles. En un estudio realizado en la Universidade Federal de São Carlos, Araras, Brazil, se evaluó el efecto de la adición, al momento de ensilar, de maíz en grano e inoculante microbiano en el perfil fermentativo, la pérdida de MS, la composición química y la degradación del ensilaje. Cuarenta silos experimentales (tubos de PVC) fueron distribuidos aleatoriamente en un diseño factorial 5 × 2. Se evaluaron: (1) cinco tratamientos de adición de maíz: CONT - ensilaje de caña de azúcar sin maíz; GC2 - ensilaje con maíz procesado por un tamiz de 2 mm; GC8 - ensilaje con maíz procesado por un tamiz de 8 mm; WC - ensilaje con granos enteros de

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maíz; y RCS - ensilaje de maíz rehidratado sin caña de azúcar; y (2) dos tratamientos de adición de inoculante microbiano: 0 y 8 mg de inoculante comercial por kg de material a ensilar. Se utilizaron 100 g de maíz por kg de caña de azúcar fresca. Los resultados mostraron que la adición de maíz a la caña de azúcar al momento de ensilar mejoró la fermentación y composición química del ensilaje. La molienda del grano antes de adicionarlo a la caña de azúcar no mostró beneficios en la calidad del producto final. El inoculante microbiano tuvo poco efecto sobre la fermentación. Estudios para comparar el maíz en grano con otras fuentes de energía, p.ej. adicionando melaza y yuca al momento de ensilar, parecen justificados, igual que estudios de alimentación del ganado para evaluar el consumo y la producción animal subsiguiente. También se debe determinar si el suministro de las fuentes de energía en forma de aditivos al ensilaje es más favorable que el suministro directo a los animales como complemento de ensilaje no tratado de caña de azúcar.

Palabras clave: Actividad de agua, digestibilidad, inoculante microbiano, procesamiento de maíz.

Introduction

In subtropical conditions, sugarcane generally produces higher dry matter (DM) yields per unit area and energy value at maturity than other tropical forages (Daniel et al. 2013) and fresh sugarcane is traditionally fed to cattle during periods of low pasture availability (Santos et al. 2010). Conserving sugarcane as silage would allow greater flexibility in feeding strategies. However, sugarcane silage (SS) is generally susceptible to the action of yeast fungi owing to high soluble carbohydrate concentration, producing a typical alcoholic fermentation of soluble carbohydrates into ethanol, CO_2 and water (Sá Neto et al. 2013) and, consequently, increased DM losses (Pedroso et al. 2008). Soluble carbohydrate concentration in the final product is lower and the level of fibrous components is higher than in the raw material, while ruminal degradation of the ensiled material is lower than that of fresh sugarcane.

One alternative to counteract the undesirable outcomes from natural fermentation in SS is the inclusion of corn grain and other additives at ensiling. Incorporating corn grain with fresh sugarcane when making SS could reduce ethanol production and DM losses (Gómez-Vázquez et al. 2011), while the use of other additives, e.g. inoculants, should help to inhibit epiphytic yeast populations and mitigate nutrient losses (Ávila et al. 2014).

Maize cultivars produced in Brazil have a vitreous endosperm, which limits starch digestibility in the gastrointestinal tract of animals. Rehydrated corn grain silage (RCS) has been used to improve starch digestibility of corn kernels in Brazilian production systems (Silva et al. 2018). Ethanol produced in SS can also solubilize proteins of the grain's endosperm (Zhang et al. 2011), improving starch availability. In addition, grinding of corn grain could affect starch digestibility by animals and starch solubilization in the silos. As suggested by Junges et al. (2017), bacterial activity is the most critical determinant of protein degradation and could be enhanced by increasing the soluble carbohydrate concentration in SS.

Among silage additives, microbial inoculants have been used to reduce the undesirable effects of SS fermentation (Carvalho et al. 2014; Santos et al. 2015; Jacovaci et al. 2017). Inoculants containing homolactic bacteria improve lactic acid production and reduce silage pH, without positively affecting alcohol fermentation (Pedroso et al. 2008; Santos et al. 2015). *Pediococcus acidilactici* establishes a low silage pH (Fitzsimons et al. 1992) and *Propionibacterium* spp. produce propionic acid from lactic acid (McDonald et al. 1991) with potential negative effects on yeast growth.

The present study aimed to evaluate any benefits from the inclusion of corn grain, processed at different particle sizes, and bacterial inoculants at ensiling on fermentation, chemical composition and in situ degradation of DM and neutral detergent fiber (NDF) of SS. We hypothesized that the addition of corn grain when ensiling sugarcane, regardless of the microbial inoculant supply, would reduce fermentative losses in SS and improve chemical composition and DM degradation relative to straight SS without corn or rehydrated corn grain silage (RCS).

Materials and Methods

The experiment was carried out at the Federal University of São Carlos, Araras, São Paulo, Brazil. Sugarcane (variety RB83-5054), at 8 months of growth (first cut) and 17.5% Brix, was used. Sugarcane from 5 locations/plots was manually harvested and chopped in a stationary cutter (Dedini®, Piracicaba, Brazil) to an ideal cut length of 10 mm.

Forty experimental silos (PVC tubes - 28 cm diameter and 25 cm long) were randomly assigned to a 5 × 2 factorial arrangement to evaluate: (A) 5 levels of corn addition: 1) control (CONT) - sugarcane ensiled without corn addition; 2) GC2 - SS with 100 g ground corn (2 mm sieve-processed)/kg fresh sugarcane added at ensiling; 3) GC8 - SS with 100 g 8 mm sieve-processed corn/kg fresh sugarcane; 4) WC - SS with 100 g whole corn grain/kg fresh sugarcane; and 5) RCS - rehydrated corn silage

(whole grain ensiled without sugarcane); and (B) 2 levels of microbial inoculant addition at ensiling: 0 and 8 mg commercial inoculant/kg total ensiled material. Each kg of inoculant contained 3.9×10^{10} colony-forming units (CFU)/g of *Pediococcus acidilactici* and 3.75×10^{10} CFU/g of *Propionibacterium acidipropionici*. Samples of fresh sugarcane and corn grain were collected for chemical analyses (Table 1).

Table 1. Chemical composition of sugarcane and corn grain before ensiling.

Item	Sugarcane[1]	Corn grain
Dry matter (DM) (g/kg)	257	870
Organic matter (OM) (g/kg DM)	961	986
Neutral detergent fiber (NDF) (g/kg DM)	527	139
Acid detergent fiber (ADF) (g/kg DM)	229	17.8
Non-fiber carbohydrates[2] (NFC) (g/kg DM)	396	753
Crude protein (CP) (g/kg DM)	27.0	74.3
Ether extract (EE) (g/kg DM)	10.8	17.4

[1]Sugarcane cultivar RB83-5054: 8 months of growth and Brix 17.5%.

[2]Calculated as: NFC (g/kg) = 1,000−(ash+CP+NDF+EE).

Ensiling was performed using PVC tubes equipped with Bunsen valves. Sand (2 kg) was placed in the bottom of the tubes and separated from the ensiled material by a nylon mesh screen to drain effluent. Inoculant and corn were added individually to the sugarcane and the total thoroughly mixed manually before being assigned to a tube. Microbial inoculant was diluted in water and sprayed onto the fresh sugarcane. Ensiled material was compacted manually (650 kg/m^3 for SS and 1,000 kg/m^3 for RCS) and tubes were sealed, weighed and stored at room temperature (about 25 °C) in a shed for 60 days. Immediately before opening, the silos were reweighed to determine DM and gas losses, expressed as a proportion of the DM ensiled.

Samples (500 g) from the center of the mass of those silos treated with whole corn grain (WC) were used for whole corn grain selection and recuperation calculation. Subsamples of corn grain from the WC treatment were called 'recovered' and used for chemical analysis and in situ degradation assay.

Fresh forage and silages were analyzed for DM concentration in a forced-air oven at 60 °C for 72 h, then ground through a 2 mm screen (SL-31, Solab Científica, Piracicaba, Brazil) and analyzed for ash, crude protein (CP) and ether extract (EE) according to AOAC (2000). Neutral detergent fiber (NDF, with heat-stable amylase, without sodium, and expressed inclusive of residual ash) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991). Two cannulated dairy cows previously adapted to a diet with 60:40 forage:concentrate ratio were used for in situ degradation assays. Ruminal incubation was performed for 96 hours, using 5 × 5 cm non-woven tissue bags (Casali et al. 2008). After removal, bags were washed in running water and evaluated for NDF concentration.

To identify and quantify yeasts and molds, 10 g silage was diluted with 90 mL sterilized peptone water (1%, w/v). Serial dilutions were pour-plated on Dichloran Rose Bengal Chloramphenicol Agar. Agar plates were incubated aerobically at 28 °C for 7 days. Colony-forming units were transformed into log10/g values (Downes and Ito 2001). Water activity (WA) was assessed using a benchtop water activity meter (Aqualab 4T, Decagon Devices Inc., Pullman, USA).

Another sample (500 g) of SS was used for silage juice extraction with a hydraulic press. The extract was filtered through cheesecloth and pH was determined immediately. The silage juice sample was centrifuged (500 × g for 15 min) and the supernatant was used for NH_3-N and organic acid evaluation. Ammonia nitrogen was determined by the colorimetric phenol-hypochlorite method (Broderick and Kang 1980). Concentrations of ethanol plus acetic, propionic and butyric acids were determined by gas chromatography (GC-2010 Plus chromatograph, Shimadzu, Barueri, Brazil), fitted with a flame-ionization detector and automatic sample injection. Lactic acid concentration in silage samples was assessed using the spectrophotometric method (Pryce 1969).

Gas and effluent losses were calculated according to the following 3 equations:

$$\text{GL (g/kg)} = \frac{\text{SWE (g)} - \text{SWO(g)}}{\text{EDM (kg)}}$$

where: GL is gaseous loss; SWE and SWO are the silo weights at ensiling and opening, respectively; and EDM is the ensiled dry matter.

$$\text{EL (g/kg)} = \frac{\text{ESWO (g)} - \text{ESWE (g)}}{\text{EDM (kg)}}$$

where: EL is effluent loss; ESWO and ESWE are the empty (but including the sand plus effluent) silo weights at opening and ensiling, respectively.

$$\text{DMR (g/kg)} = \frac{\text{DMO (g)}}{\text{DME (kg)}}$$

where: DMR is dry matter recovery; DMO is dry matter at silo opening; and DME is dry matter at ensiling.

Statistical analysis

For fermentative profile, losses and chemical composition, data from RCS were removed for statistical analysis. The PROC MIXED of SAS 9.3. (SAS Institute Inc., Cary, USA) was used, according to the following statistical model:

$$Y_{ijk} = \mu + C_i + I_j + C \times I_{ij} + e_{ijk}$$

with $e_{ijk} \approx N\left(0, \sigma_{ij}^2\right)$;

where: Y_{ijk} is the value of the dependent variable; μ is the overall mean; C_i is the fixed effect of corn (i = 1 to 4); I_j is the fixed effect of microbial inoculant (j = 1, 2); $C \times I_{ij}$ is an interaction term; e_{ijk} is the residual error; and N stands for Gaussian distribution.

The corn effect was separated into 3 orthogonal contrasts: C1: corn addition effect (CONT vs. GC2 + GC8 + WC); C2: corn grinding effect (GC2 + GC8 vs. WC); and C3: sieve size effect (GC2 vs. GC8). For recovered corn analysis, we used a similar model, changing corn by processing effect (P_i, i = 1 and 2).

Results

Corn addition decreased (P<0.01) acetic, propionic and butyric acid concentrations in SS and tended to decrease (P=0.07) ethanol concentration, while decreasing (P≤0.01) losses and increasing (P<0.01) DM recovery (Table 2). Microbial inoculant addition increased (P≤0.04) silage pH plus ethanol and butyric acid concentrations, but decreased (P≤0.04) acetic acid concentration, mold and yeast counts and DM recovery, and tended (P≤0.07) to increase lactic acid concentration and effluent losses. Compared with ground corn, WC tended (P≤0.07) to decrease mold and yeast counts and to increase DM recovery. The corn × microbial inoculant interaction was significant (P≤0.02) for NH_3-N and water activity (WA) plus gaseous and total losses. Corn addition decreased (P<0.01) NH_3-N only in silages treated with microbial inoculant (Figure 1). Ground corn tended to increase (P≤0.09) NH_3-N in silages relative to whole corn, regardless of microbial inoculant application (Figure 1) and the effect was stronger with finer grinding (P≤0.06).

Regardless of inoculation, corn addition decreased (P<0.01) gaseous and total fermentative losses of silage (Table 2; Figure 2). Whole corn decreased (P=0.02)

Table 2. Effects of addition of ground or whole corn grain and microbial inoculant at ensiling on fermentation, mold counts and fermentative losses in sugarcane silage.

Item	Corn addition[1]				INO[2]		s.e.m.	P[3]				
	CONT	GC2	GC8	WC	−	+		INO	CORN × INO	C1	C2	C3
pH	3.91	3.88	4.00	3.88	3.83	4.00	0.014	<0.01	0.38	0.78	0.15	0.15
NH_3-N (mg/dL)	1.06	1.33	0.74	0.75	1.01	0.93	0.034	0.35	0.01	0.11	0.02	0.77
Ethanol and organic acids (g/kg DM)												
Ethanol	24.9	20.5	14.4	14.0	12.6	24.3	1.37	0.02	0.51	0.07	0.32	0.38
Lactic acid	45.6	39.4	41.2	42.7	38.2	46.2	1.94	0.07	0.21	0.45	0.56	0.75
Acetic acid	15.3	10.5	11.2	10.5	14.6	9.17	0.32	<0.01	0.45	<0.01	0.51	0.46
Propionic acid	0.48	0.18	0.23	0.25	0.27	0.31	0.018	0.31	0.97	<0.01	0.40	0.43
Butyric acid	0.13	0.08	0.08	0.07	0.08	0.09	0.002	0.04	0.42	<0.01	0.36	0.83
Microbial evaluation												
Yeast and mold (log10/g as fed)	15.7	12.2	14.0	10.6	16.0	10.3	0.70	<0.01	0.19	0.20	0.07	0.10
Water activity	0.99	0.99	0.98	0.93	0.99	0.95	0.003	<0.01	<0.01	0.01	<0.01	0.04
Fermentative losses (g/kg DM)												
Gaseous	152	102	109	100	103	128	0.8	<0.01	<0.01	<0.01	0.05	0.01
Effluent	133	101	113	96.3	106	115	2.0	0.06	0.24	0.01	0.03	0.01
Total	284	203	222	196	209	244	2.0	<0.01	0.02	<0.01	0.01	<0.01
DM recovery	657	768	760	825	777	728	10.9	0.04	0.40	<0.01	0.06	0.82

[1]CONT = Sugarcane silage (SS) without corn; GC2 = SS with 2 mm sieve-processed corn; GC8 = SS with 8 mm sieve-processed corn; WC = SS with whole corn grain. [2]Microbial inoculant. [3]Probabilities: INO: microbial inoculant effect; CORN × INO: interaction between corn and inoculant effects; C1 = Control vs. corn addition; C2 = whole corn vs. ground corn; and C3 = 2 mm grind vs. 8 mm grind.

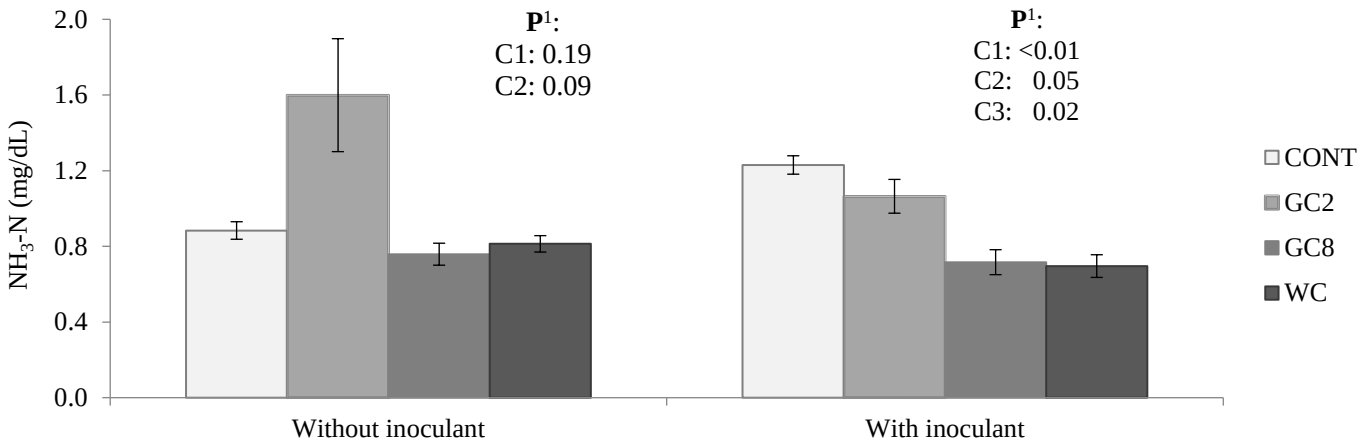

Figure 1. NH_3-N concentration in sugarcane silage with additions of corn grain and microbial inoculant. CONT = Sugarcane silage (SS) without corn; GC2 = SS with 2 mm sieve-processed corn; GC8 = SS with 8 mm sieve-processed corn; WC = SS with whole corn grain. [1]Probabilities (P): C1 = Control vs. corn addition; C2 = whole corn vs. ground corn; and C3 = 2 mm grind vs. 8 mm grind.

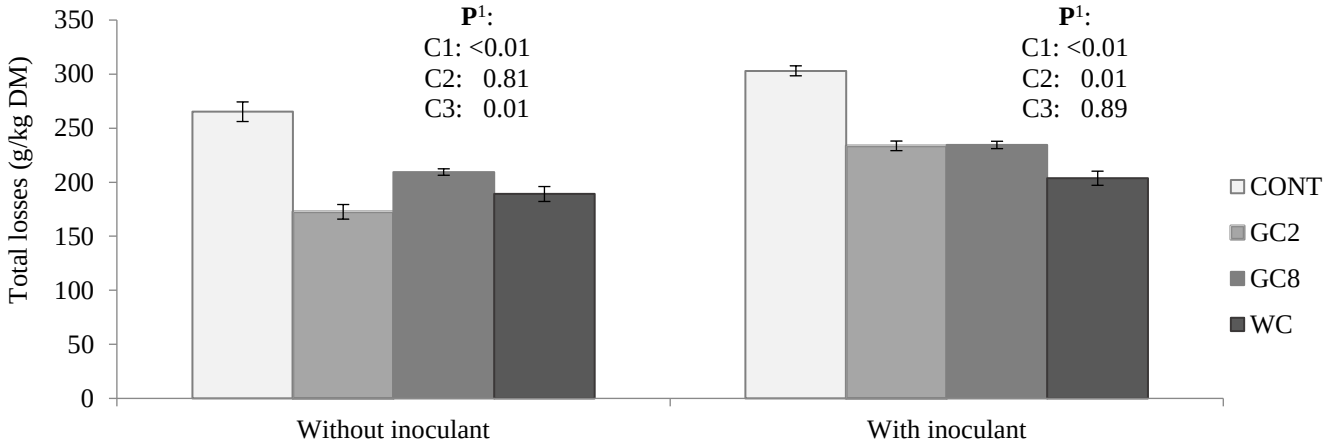

Figure 2. Total fermentative losses of sugarcane silage treated with corn grain and microbial inoculant. CONT = sugarcane silage (SS) without corn; GC2 = SS with 2 mm sieve-processed corn; GC8 = SS with 8 mm sieve-processed corn; WC = SS with whole corn grain. [1]Probabilities (P): C1 = Control vs. corn addition; C2 = whole corn vs. ground corn; and C3 = 2 mm grind vs. 8 mm grind.

gaseous and total losses relative to ground corn only in silage treated with inoculant. Finer grinding reduced gaseous and total fermentative losses relative to coarser grinding (P≤0.01) only without addition of microbial inoculant (Table 2; Figure 2).

Adding corn decreased water activity (WA) in inoculated silages (P=0.01). In addition, silage with WC showed lower WA if treated with microbial inoculant (P<0.01; Table 2) and a tendency for lower WA in non-inoculated silage (P=0.09). There was no corn addition × INO interaction effect on chemical composition and DM degradation of silage (P≥0.22; Table 3). Corn addition increased (P≤0.01) silage DM, OM, NFC, CP and EE concentrations and DM degradation and decreased (P<0.01) NDF and ADF concentrations.

Microbial inoculant addition decreased (P=0.03) silage OM concentration and increased (P=0.04) CP concentration (Table 3). Ground corn tended to decrease NFC and to increase NDF concentrations, in comparison with whole corn (P≤0.09). Particle size of ground corn had no effect (P≥0.13) on SS composition and DM degradation (Table 3).

Recovered corn from WC and RCS showed similar OM, NDF, ADF and NFC concentrations (P≥0.12; Table 4). However, recovered corn had lower (P≤0.01) DM and CP concentrations and higher (P<0.01) EE concentration than RCS. Additionally, recovered corn tended to have higher (P=0.09) DM degradation than RCS. Microbial inoculant had no effect on any aspects of corn grain chemical composition and DM degradation (P≥0.15).

Table 3. Effects of addition of microbial inoculant and corn grain at ensiling on chemical composition and in situ ruminal degradation of sugarcane silage.

Item	Corn addition[1]				INO[2]		s.e.m.	P[3]			
	CONT	GC2	GC8	WC	–	+		INO	C1	C2	C3
DM (g/kg)	182	261	260	280	252	240	3.6	0.10	<0.01	0.28	0.15
OM (g/kg DM)	959	970	970	972	969	966	0.5	0.03	<0.01	0.45	0.24
NDF	758	593	618	544	635	619	12.8	0.59	<0.01	0.09	0.61
ADF	438	261	264	243	307	296	4.8	0.35	<0.01	0.14	0.84
NFC	154	301	265	336	252	276	9.1	0.55	<0.01	0.06	0.27
CP	34.7	54.9	56.0	59.8	49.4	53.3	0.87	0.04	<0.01	0.57	0.13
EE	11.3	20.8	42.6	32.6	36.2	17.5	3.25	0.07	0.01	0.34	0.29
DM degradation	477	658	630	657	603	608	6.1	0.71	<0.01	0.19	0.97

[1]CONT = Sugarcane silage (SS) without corn; GC2 = SS with 2 mm sieve-processed corn; GC8 = SS with 8 mm sieve-processed corn; WC = SS with whole corn grain. [2]Microbial inoculant. [3]Probabilities: INO: microbial inoculant effect; CORN × INO: interaction between corn and inoculant effects P≥0.22; C1 = Control vs. corn addition; C2 = whole corn vs. ground corn; and C3 = 2 mm grind vs. 8 mm grind.

Table 4. Chemical composition and in situ ruminal degradation of rehydrated corn silage and recovered whole corn grain from sugarcane silage.

Item	−INO[1]		+INO		s.e.m.	P[4]	
	RCS[2]	RWCG[3]	RCS [2]	RWCG[3]		PROC	INO
DM (g/kg)	616	574	625	561	1.7	<0.01	0.50
OM (g/kg DM)	987	990	985	988	0.8	0.12	0.23
NDF	95.6	89	97.9	93	2.61	0.31	0.56
ADF	26.2	24.4	26.1	24.8	0.65	0.27	0.92
NFC	757	778	752	764	4.8	0.25	0.47
CP	95	74	95.7	71.2	1.66	<0.01	0.80
EE	39.3	49	39.1	61.5	2.15	<0.01	0.15
DM degradation	856	864	861	873	2.6	0.09	0.23

[1]Microbial inoculant. [2]Rehydrated corn silage. [3]Recovered whole corn grain from sugarcane silage with grain added at ensiling. [4]Probabilities: PROC - processing effect; INO - microbial inoculant effect; PROC × INO - interaction between processing and inoculant effects P≥0.14.

Discussion

Fresh sugarcane used in the present study averaged 17.5% Brix and 257 g DM/kg, which is lower than the recommended level for ensiling (300 g/kg; McDonald et al. 1991). However, these values are similar to those reported by other authors, e.g. Sá Neto et al. (2013) for fresh sugarcane. We chose this material to provide a higher challenge for evaluation of the treatments, as ensiling of sugarcane with low DM concentration could benefit more from corn addition.

As expected, corn addition to sugarcane at ensiling increased DM concentration of ensiled material and consequently improved fermentation conditions, decreasing the production of acids and WA, especially in inoculant-treated silos. According to Greenhill (1964), after the breakdown of the cell walls, WA depends mainly on the moisture content of the ensiled material. Material with high DM concentration shows decreased WA, lower bacterial counts and delayed growth of lactic acid bacteria (LAB) (Castro et al. 2006). Bernardes et al. (2007) showed that WA is directly associated with counts of mold and yeast. While corn addition decreased acid concentration and ethanol production in the present study, ground corn increased WA relative to whole corn.

Although concentrations of acids in corn-treated silages were lower than in straight sugarcane silage, in general, corn addition had no effect on pH of the silage. This result supports the findings of Andrade et al. (2001), who added ground corn ears to sugarcane with urea at ensiling and found no differences in silage pH. In the present study, the production of lactic acid, the primary acidogenic acid evaluated in the current trial, was not affected by corn addition at ensiling. Similarly, Bernardes et al. (2007) evaluated the addition of dehydrated corn grain with cob and straw to sugarcane at ensiling and also found no effects on silage pH, but chemical composition was improved.

Water may be produced during bacterial fermentation of sugars, mainly during the conversion of substrate to ethanol by yeasts (Pedroso et al. 2008). This water accumulation results in increased effluent losses and decreased silage DM concentration and DM recovery in silos without corn treatment. Additionally, WC decreased total losses and improved DM recovery, relative to GC, especially in inoculant-treated silos. Besides higher WA, GC2 addition resulted in lower effluent and gaseous losses, without affecting DM recovery, relative to GC8. Finely ground corn with smaller particle size can make compaction of material and expulsion of air from the ensiled material easier than whole or coarsely ground corn.

Furthermore, corn processing increases microbial adhesion to the endosperm and consequently increases NH_3-N concentration (Lee et al. 2002). The critical interaction between corn particle size and action of microbial inoculant was highlighted by the more evident corn effect in inoculated silos. As NH_3-N is produced from proteolysis (Albrecht and Muck 1991), decreased microbial action could negatively affect the NH_3-N level, as observed in corn-treated silos. Although Oliveira et al. (2017) found decreased NH_3-N concentration in silages treated with homofermentative LAB regardless of forage type, inoculant had no effect in the present study. Despite the presence of propionic acid bacteria in the evaluated inoculant, the addition of inoculants showed no effect on propionic acid concentration but increased ethanol concentration of the silage. Borreani et al. (2018) observed that yeast activity converted glucose to ethanol, resulting in high fermentative losses in silage. Both homofermentative and heterofermentative lactic acid bacteria potentially improve silage fermentation, but heterofermentative bacteria are more effective in inhibiting fungal growth (Filya 2003). Santos et al. (2015) observed that adding homofermentative LAB (*Propionibacterium acidipropionici*, *Lactobacillus plantarum* and *Enterococcus faecium*) to sugarcane at ensiling increased ethanol production and DM losses, even when heterofermentative LAB were included, whereas silage inoculated exclusively with hetero-fermentative LAB (*L. buchneri*) had reduced DM losses and alcoholic fermentation. In the present study, microbial inoculant decreased DM recovery and concentrations of DM and OM in silage.

In general, corn addition to SS reduced fermentative losses and, consequently, improved chemical composition of silage. Traditionally, in SS, high yeast populations (Ávila et al. 2010) convert most of the water-soluble carbohydrate, which is in the NFC component, into fermentation end-products, such as volatile organic compounds and mainly ethanol (Daniel et al. 2013). In keeping with these outcomes, corn addition reduced fermentative losses in SS and increased the concentration of NFC in our study, resulting in decreased fiber concentration via a dilution effect and improved DM degradation. The different responses in NDF and NFC as a result of the addition of ground and whole corn seem related to lower fermentative losses in silos containing WC grain.

In evaluating recovered whole corn grain (RWCG) relative to rehydrated corn silage (RCS), we found similar OM, NDF, ADF and NFC concentrations for both forms of corn, while RWCG had lower DM and CP concentrations and higher EE concentration than RCS. Although Junges et al. (2017) attributed 60% of protein degradation in RCS to bacterial activity, solubilization in fermentation end-products could increase protein degradation (Lawton 2002) and improve silage DM degradation. Lower CP concentration and increased DM degradation observed in RWCG relative to RCS are reflected in increased acid levels and bacterial activity in the silos. Higher ethanol concentration in SS could solubilize kernel protein (Zhang et al. 2011), which was not recovered in the silage samples.

At ensiling rehydrated corn contained 660 g DM/kg and DM recovery after ensiling was 926 g DM/kg silage, which is in accordance with average recovery levels reported by Kung Jr et al. (2004). On the other hand, RWCG showed a recovery rate of 966 g DM/kg ($P>0.05$). However, owing to the importance of this variable in silage making, more studies are necessary to evaluate the role of adding corn in improving DM recovery in sugarcane silage. Our results suggest that there is little merit in grinding the grain before adding it to the sugarcane at ensiling. In some situations, other energy sources like molasses or cassava may be a cheaper source than corn grain and studies are needed to test their efficacy relative to corn. In addition, feeding studies to evaluate feed intakes by livestock and subsequent performance using whole corn grain and other energy additives in SS at ensiling or fed directly with sugarcane silage are warranted.

Conclusions

Microbial inoculant containing mainly homolactic bacteria had little effect on fermentation of sugarcane silage and there appears little merit in adding it to fresh sugarcane at ensiling. The addition of corn grain at ensiling improved SS fermentation and silage composition but there seems little value in grinding the grain before adding it to the sugarcane. Further studies comparing other energy sources with corn as additives at the ensiling of sugarcane seem warranted as well as feeding studies to compare intakes of

the various products by livestock and subsequent performance. The alternative of feeding the energy sources with straight sugarcane silage as opposed to adding them at ensiling should be assessed.

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(Note of the editors: All hyperlinks were verified 7 November 2019.)

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Research Paper

Herbaceous plant species diversity in communal agro-pastoral and conservation areas in western Serengeti, Tanzania

Diversidad de especies herbáceas en áreas de uso agropastoril comunal y protegidas en Serengeti occidental, Tanzania

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Abstract

Agro-pastoralism involves the growing of crops and keeping of livestock as a livelihood strategy practiced by communities in rural areas in Africa and is highly dependent on environmental factors including rainfall, soil and vegetation. Agro-pastoral activities, e.g. livestock grazing and land clearing for crop cultivation, impact on environmental condition. This study evaluated the impacts of agro-pastoral activities on herbaceous plant species diversity and abundance in western Serengeti relative to conservation (protected) areas. A vegetation survey was conducted along the grazing gradients of ten 4 km transects from within village lands to protected areas. A total of 123 herbaceous species belonging to 20 families were identified. Higher herbaceous species diversity and richness were found in protected areas than in communal grazing lands. Similarly, the number of perennial herbaceous species was higher in the former than the latter, while occurrence of annuals was higher in the village areas. This observation indicates poor rangeland condition in village communal grazing lands as compared with protected areas. It is obvious that current agro-pastoral activities have contributed to a reduction in herbaceous species diversity in village lands in western Serengeti. However, the array of pasture species, especially desirable perennial species, still present in communal grazing areas, suggests that rejuvenation of these areas is possible. Resting of grazing land is recommended to reverse the trend towards diversity reduction and ensure future availability of feed resources for grazing animals in village lands.

Keywords: Ground cover, land use type, pasture condition, species composition.

Resumen

El sistema de uso agropastoril de la tierra se define como la combinación de cultivos con la producción de ganado y es una estrategia de producción y sustento practicada por las comunidades en las zonas rurales de África que depende, en gran medida, de factores ambientales como la precipitación, el tipo de suelo y la vegetación. Actividades agropastoriles, tales como el pastoreo de ganado y la preparación del suelo para cultivos, impactan en el medioambiente, sobre todo en la composición florística. En este estudio se evaluaron los impactos de las actividades agropastoriles en la diversidad y abundancia de especies de plantas herbáceas, en comparación con áreas de conservación (áreas protegidas), en la región del Serengeti occidental, Tanzania. Para el efecto se hizo un levantamiento de la vegetación a lo largo de gradientes de pastoreo en 10 transectos de 4 km cada uno, desde áreas de uso comunal hasta áreas protegidas. Se identificaron un total

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de 123 especies herbáceas pertenecientes a 20 familias. Se encontró mayor diversidad y riqueza de especies en áreas protegidas que en áreas de pastoreo comunal. Del mismo modo, el número de especies herbáceas perennes fue mayor en áreas protegidas que en áreas comunales, mientras que en estas últimas la presencia de plantas anuales fue mayor. Estos resultados indican un estado deteriorado de las áreas para pastoreo en las tierras comunales en comparación con las áreas protegidas. Es obvio que en el Serengeti occidental las actuales actividades agropastoriles han contribuido a una reducción de la diversidad de especies herbáceas en las áreas comunales. Sin embargo, la variedad de especies útiles para pastoreo, especialmente especies perennes deseables, todavía presentes en áreas de pastoreo comunales, indica que la rehabilitación de estas áreas es posible. Se sugiere permitir periodos de descanso adecuados en estas áreas con el fin de revertir la tendencia hacia la reducción de la diversidad de especies y asegurar la disponibilidad futura de recursos forrajeros para los animales en pastoreo en las tierras comunales.

Palabras clave: Cobertura del suelo, composición botánica, manejo de pastoreo, uso de tierra.

Introduction

Agro-pastoralism is a combination of cropping and keeping of livestock as a livelihood strategy practiced by communities in rural areas. Local communities perceive that their survival is dependent on having sufficient cropland and pastureland, while they derive no benefit from biodiversity conservation (Kaltenborn et al. 2003; Kideghesho 2008). Agricultural production involves land clearing, which impacts negatively on vegetation structure and species composition. Grace et al. (2010) found a strong interaction between agro-pastoralism and plant biodiversity showing that agro-pastoralism and biodiversity conservation have conflicting goals, which poses a challenge in managing plant resources in the ecosystem.

The success of agro-pastoralism in western Serengeti is heavily reliant on environmental factors including rainfall, soil and vegetation (Salami et al. 2010). Crops grown by agro-pastoralists during 4–8 years include food crops such as maize (*Zea mays*), cassava (*Manihot esculenta*), sorghum (*Sorghum vulgare*) and finger millet (*Eleusine coracana*); cotton (*Gossypium hirsutum*) as a cash crop; and other food crops such as sweet potatoes (*Ipomoea batatas*), beans (*Phaseolus vulgaris*) and a variety of vegetables (Mfunda and Røskaft 2011). Subsequently land is left fallow for 4–5 years. Livestock grazing is normally conducted in communal grazing lands and abandoned or fallow lands (Kavana et al. 2017).

Annual rainfall affects plant growth, vegetation type and hence the feed resource base for both wildlife and domestic animals in eastern and western Serengeti. Muchane et al. (2013) conducted a biodiversity study in 4 parts of north-eastern Serengeti (Ololosokwan, Loliondo, Machokwe and Nyansurura), aiming to identify optimal land use and management practices, which would favor biodiversity while still providing livelihoods for the pastoralists. Their results for plant diversity were based on a rapid vegetation survey conducted from July 2009 to December 2010 covering only a small patch of the ecosystem that differs from western Serengeti in terms of mean annual rainfall (550 vs. 1,050 mm, respectively). Generally, previous studies on agro-pastoralism have been limited in coverage and time. Here we conducted a study on the effects of agro-pastoralism on herbaceous plant diversity over a period of 2 years covering 2 wet seasons in western Serengeti to determine the influence of agro-pastoralism on herbaceous plant composition and diversity, especially the effects of livestock and wildlife grazing, following a grazing gradient in 3 areas: communal lands with livestock grazing; areas with mixed livestock and wildlife grazing; and protected areas with wildlife grazing only. In addition, fallow lands were included in the study to reflect the impact of cultivation and grazing as part of the combined effects of agro-pastoral activities on the diversity of herbaceous plant species.

Materials and Methods

Study sites

Western Serengeti is part of the Serengeti ecosystem and is wooded savanna grassland, which is situated in agro-ecological zone III characterized by intensive agriculture and the keeping of cattle, goats, sheep and poultry (NBS 2015). It encompasses Serengeti, Bunda, Busega, Magu, Meatu and Bariadi districts. While this zone has low agricultural potential and is only marginally suitable for arable agriculture, it is occupied by agro-pastoralists. Average annual rainfall ranges between 500 and 1,200 mm, declining towards the Serengeti National Park boundary and increasing towards Lake Victoria (Sinclair et al. 2000). The area is highly diverse in terms of ethnicity, including more than 20 ethnic groups, and is among the most densely settled parts of the Greater Serengeti ecosystem with population growth rates exceeding those to the north, east and south of the National Park (Kideghesho 2010).

The study was conducted in 4 districts with respective villages shown in brackets (Figure 1): Serengeti (Park Nyigoti), Bunda (Nyamatoke), Meatu (Makao) and Bariadi (Mwantimba and Mwashibaba).

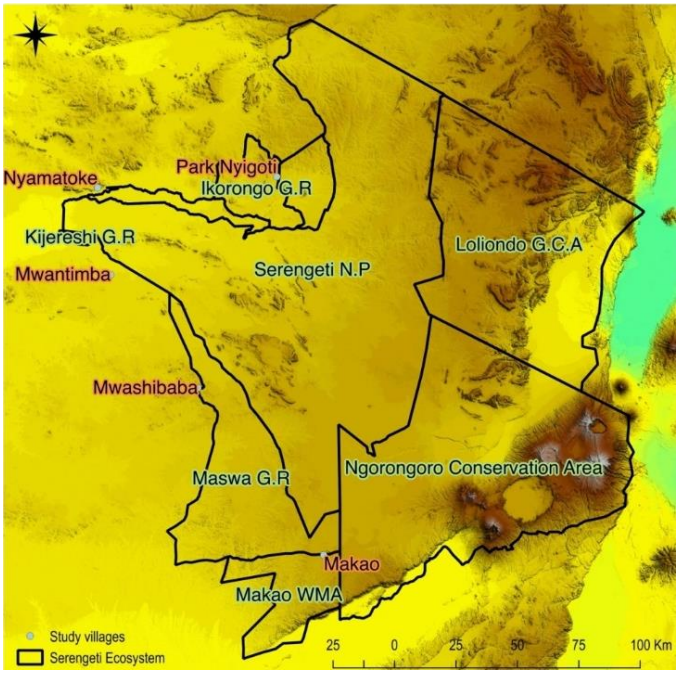

Figure 1. Map of Serengeti ecosystem showing the study sites in western Serengeti.

Study design

The study was designed to sample vegetation and assess soil texture along 4 km transects that traversed across different land use types including: domestic livestock grazing; mixed grazing by domestic livestock and wildlife; and wildlife grazing. This method was chosen because it can be easily applied in rapid vegetation surveys when funds and time are limited. Two transects separated by 5 km were established for each of the 5 villages. Each transect started in village land traversing 0 to 1.5 km in grazing land dominated by livestock grazing followed by 1.5 to 2.5 km crossing the border between village land and protected area which was dominated by mixed grazing, and the rest 2.5 to 4.0 km was in protected area dominated by wildlife grazing. The starting and end points of each transect were established by recording GPS readings. In addition to the two 4 km transects, for each village a separate 1 km transect was established in grazed fallow land, with up to 4–5 year-old vegetation. This sampling transect separation was necessary because crop/fallow land is usually not in proximity to grazing lands.

Vegetation sampling

Vegetation sampling for determination of plant species diversity was done at the peak blooming period of herbaceous plants during April and May 2016 and 2017. At the same time, soil cover by plants was determined by visual estimation. Herbaceous plant species were recorded within 0.25 m^2 quadrats at every 0.1 km along each transect. Plants were identified by following plant nomenclature according to Agnew and Agnew (1994). Each species encountered was categorized in terms of functional attributes, e.g. life form (grass, forb and small shrub), life span (annual and perennial), feeding merit (edible and inedible) and desirability for grazing animals (undesirable, slightly desirable, moderately desirable and highly desirable). The desirability of the identified species was based on experience of research workers, subjective opinion of the rangers and livestock keepers as well as support from literature.

Expected number of herbaceous plant species in land types

Expected number of species encountered in each land use type was estimated by using species accumulation curves according to Bunge and Fitzpatrick (1993) so as to ascertain the possibility of encountering all herbaceous plant species that exist in the study area. To establish the species accumulation curves the 'Vegan' R package (Oksanen et al. 2013) was used and the curves were fitted using the Michaelis-Menten function as follows:

$$S = (b_0 \times A_b)/(b_1 + A)$$

where:

S is the number of species (the dependent variable); A is the sampling unit (the independent variable); and b_0 and b_1 are the 2 (estimated) parameters. The best function for each land use type was chosen based on the lowest corrected Akaike Information Criterion (AICc) of the fitted model (Grueber et al. 2011).

Soil sampling

Soil samples, 0–30 cm horizon, were taken at the central point of every fourth quadrat after clipping of plants (10 samples per transect) for determination of soil texture according to the standard procedure described by Brady (1974).

Statistical analyses

Analysis of data was done using R software version 3.5.0. Shapiro test was used for testing normality of data collected. Log-transformation was applied to the data that did not conform to normal distribution so as to enable application of normally distributed analysis of data. The herbaceous plant species composition in land use types was ordinated by PCA according to Legendre and Legendre (2012). An ordination diagram was developed in order to assess species composition in relation to land use type. Pearson correlation coefficients were established among the soil texture and herbaceous species variables. One variable was chosen from highly correlated variables for inclusion in a model. Then, stepwise elimination of variables in a model was used to find out their contribution to variance observed in species ground cover across land use types.

Herbaceous plant species diversity

The plant species diversity among different land use types was determined in terms of Shannon-Wiener diversity index according to the following formula:

$$\text{Diversity Index (H)} = -\sum p_i \ln p_i$$

where:

$p_i = n_{i/N}$ is the proportion of the total number of all species in a quadrat and ln = natural logarithm to base e.

Herbaceous plant species ground cover modelling

Collinearity analysis was conducted by construction of Spearman's correlation matrix for each dataset, and if 2 variables had correlations >0.60, one variable was deleted from the model selection stage in accordance with the procedure of Zuur et al. (2009). A global mixed effects model using lmer package of R statistical software (Kuznetsova et al. 2017) was used where herbaceous species ground cover was considered as the response variable. Ground cover is an important parameter in determination of rangeland degradation due to soil erosion. The predictor variables included number of species (species richness), inedible species, edible species, undesirable species, slightly desirable species, moderately desirable species, highly desirable species, perennial species, annual species, grass species and forbs, while land use type (livestock, mixed and wildlife) was defined as a random effect. The input variables were standardized using Gelman's approach (Gelman 2008) and the dredge function in package MuMIn (Barton 2009) was used to perform automated model selection with subsets for each of the standardized global models. The best fitting model procedure was used to select the most accurate model. Model averaging was used to calculate model averaged parameters and used the second-order Akaike information criterion (AICc) (Burnham and Anderson 2002) to obtain the top model based on variables with highest relative importance.

Results

Herbaceous plant species composition across land use types

A total number of 123 herbaceous plant species from 20 families were recorded in the vegetation survey (Appendix 1). Species accumulation curves (Figure 2) indicated the highest species richness occurred in protected areas and the lowest in fallow. Results from Figure 2 further indicated that species richness reached an asymptote within sample size from different land use types. Maximum herbaceous plant species richness in different land use types fell in the ranges: 80–100, 50–60, 40–50, 15–20 and 10–15 for protected areas (Wildlife), livestock and wildlife (Mixed), continuous livestock grazing (Livestock) and Fallow respectively.

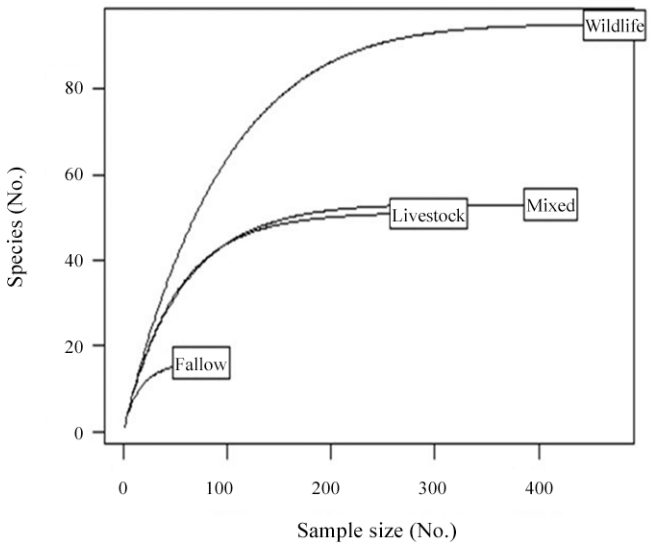

Figure 2. Herbaceous plant species accumulation curves in different land use types in western Serengeti, Tanzania.

Ordination (Figure 3) indicated shift of herbaceous species composition towards *Themeda triandra* in Wildlife grazing areas, *Cynodon dactylon* in Livestock grazing areas and *Chloris pycnothrix* in the Mixed grazing sites. Fallowing of cultivated lands developed herbaceous species composition rich in *Sphaeranthus suaveolens*.

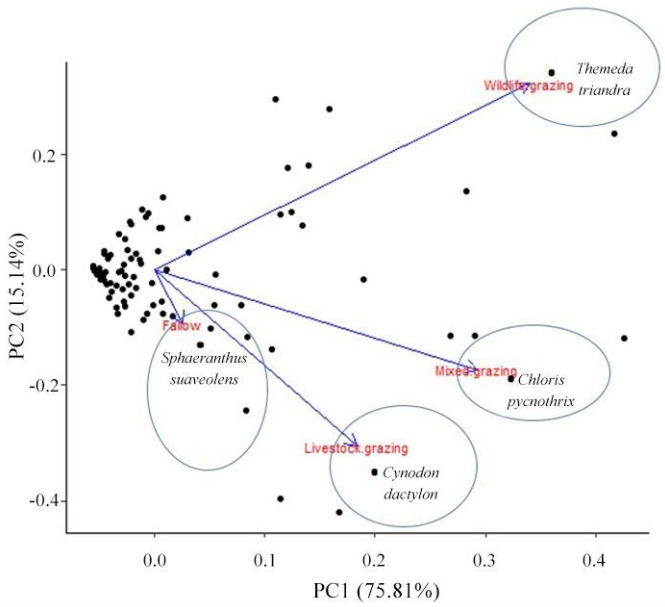

Figure 3. Ordination of plant species composition based on land use type.

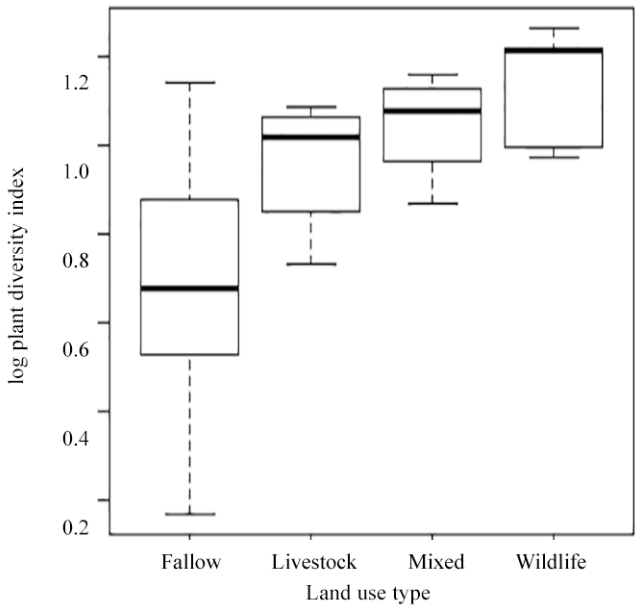

Figure 5. Comparison of herbaceous plant diversity among different land use types.

The dendrogram (Figure 4) grouped species composition into 3 clusters. Communal Livestock grazing and Mixed grazing were closely related, while plant species composition under Fallow land and Wildlife grazing were not closely related, i.e. they were rather separated from the Livestock and Mixed clusters.

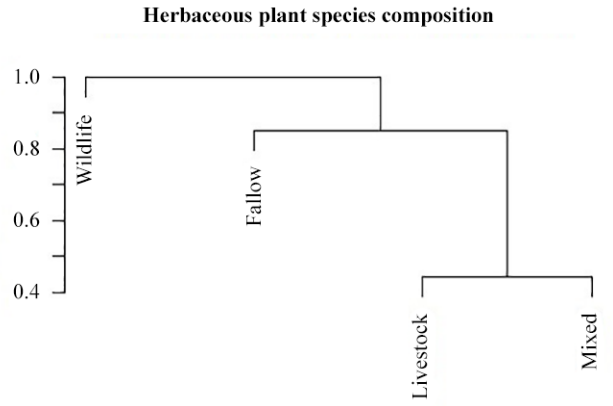

Figure 4. Cluster analysis of herbaceous plant species composition for different land uses.

Herbaceous plant species diversity

Results (Figure 5) indicated highest plant diversity in protected areas (Wildlife) and lowest in fallow lands (Fallow).

Influence of agro-pastoral activities on availability of herbaceous plant species

Figure 6a indicates that the number of species increased along transects from communal grazing lands towards protected areas as did the number of grass species (Figure 6b).

While the number of perennial species increased from communal lands (Livestock grazing) into the protected area (Wildlife grazing), the reverse was true for annual species (Figures 7a and 7b).

This indicated that annual species contributed significantly as a feed resource for livestock grazing in communal grazing lands. Plants highly desired for grazing animals were less available in communal lands than in protected areas, presumably because they were reduced by heavy grazing (Figure 8).

Availability of herbaceous plants in different land use types contributed to different patterns of ground cover. Results indicated an increase in ground cover along transects from communal grazing lands to protected areas (Figure 9). Vegetation gradients observed along transects from communal lands into protected areas indicated variation in coverage of ground by different forms of herbaceous plants.

Numbers of undesirable herbaceous plant species were higher in communal grazing lands and declined towards the protected area (Figure 10).

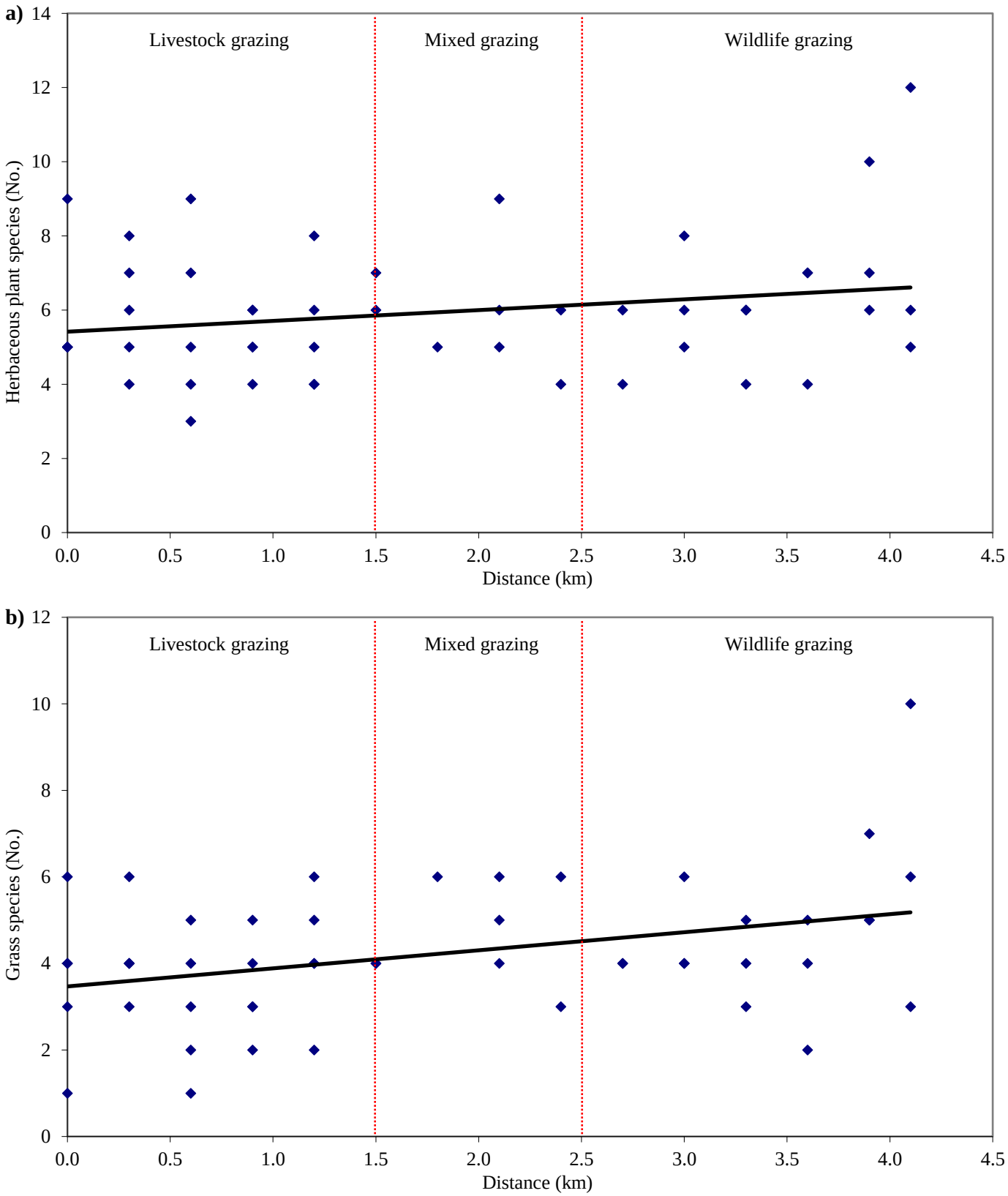

Figure 6. Availability of herbaceous plant species in western Serengeti as a function of location (distance from village to protected areas) and grazing strategy: a) all species; b) grasses only.

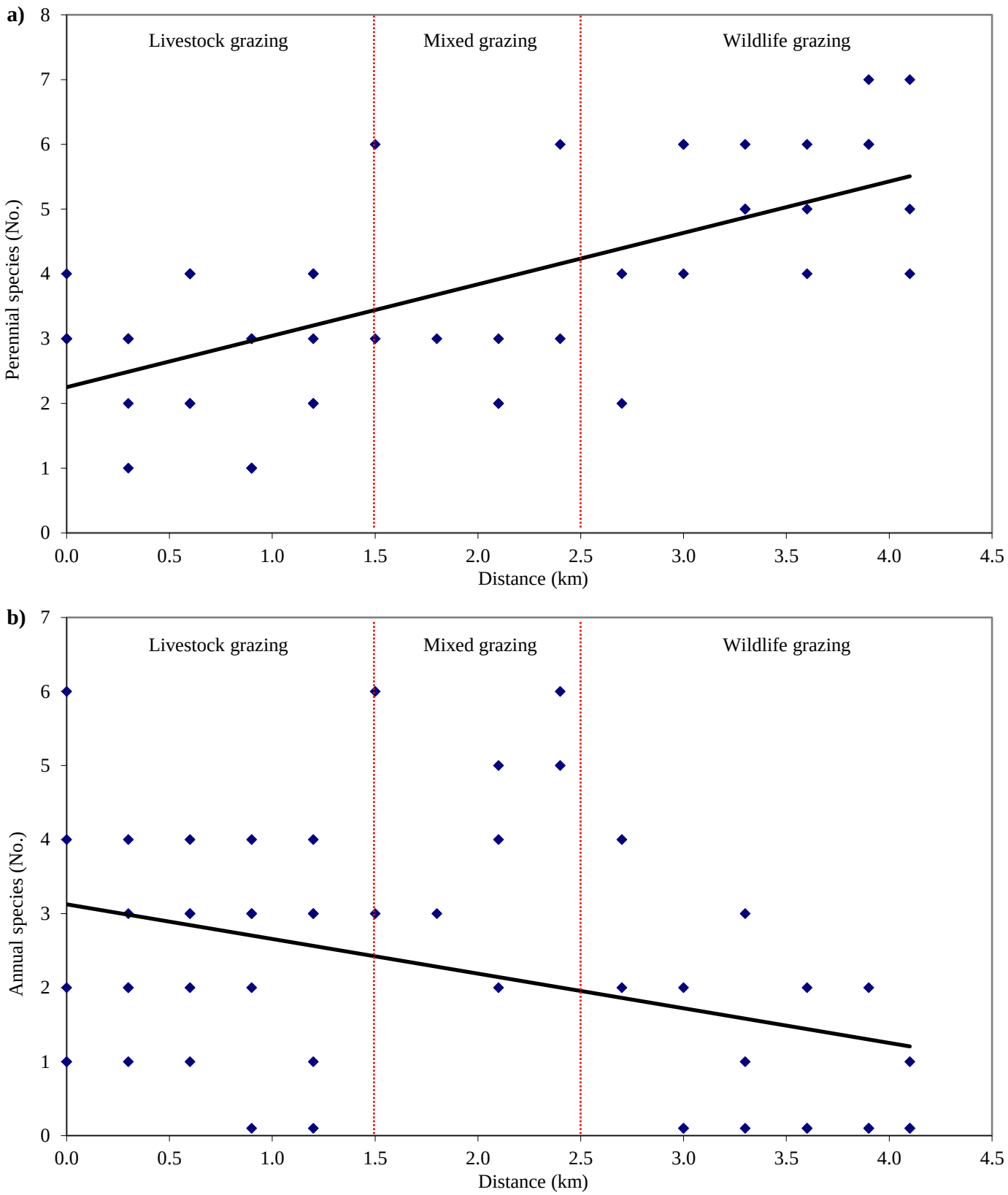

Figure 7. Availability of perennial (a) and annual (b) species as a function of location (distance from village to protected areas) and grazing strategy.

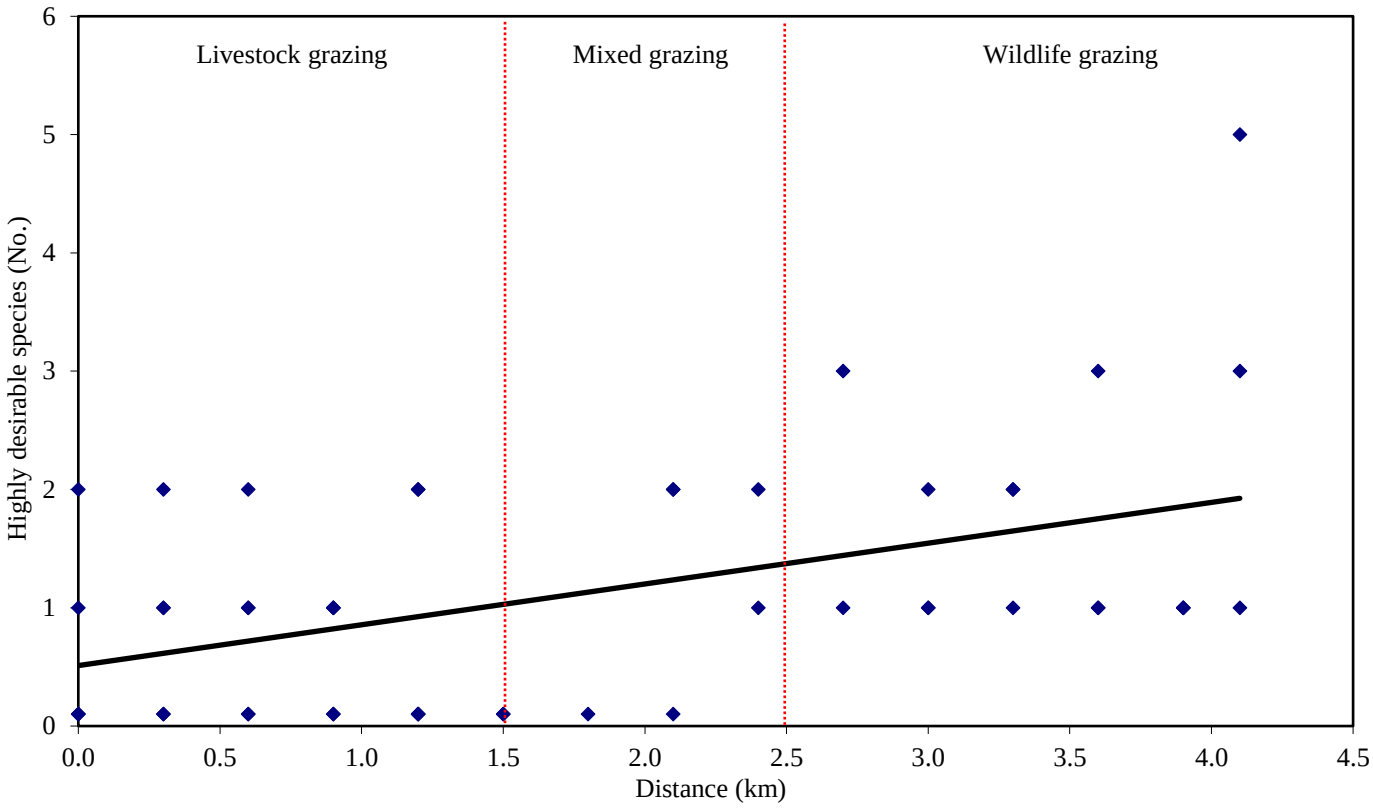

Figure 8. Availability of highly desirable herbaceous plants as a function of location (distance from village to protected areas) and grazing strategy.

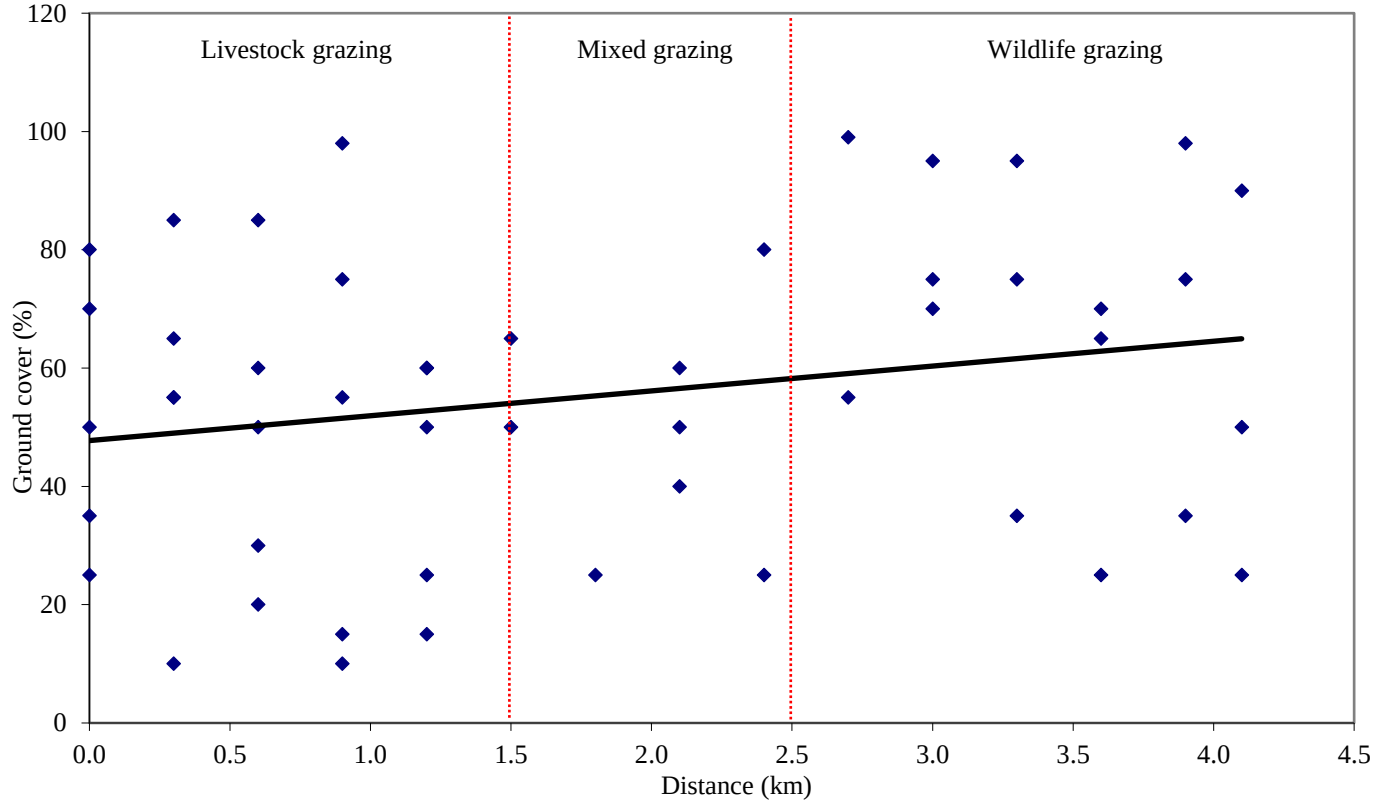

Figure 9. Ground cover of herbaceous plants as a function of location (distance from village to protected areas) and grazing strategy.

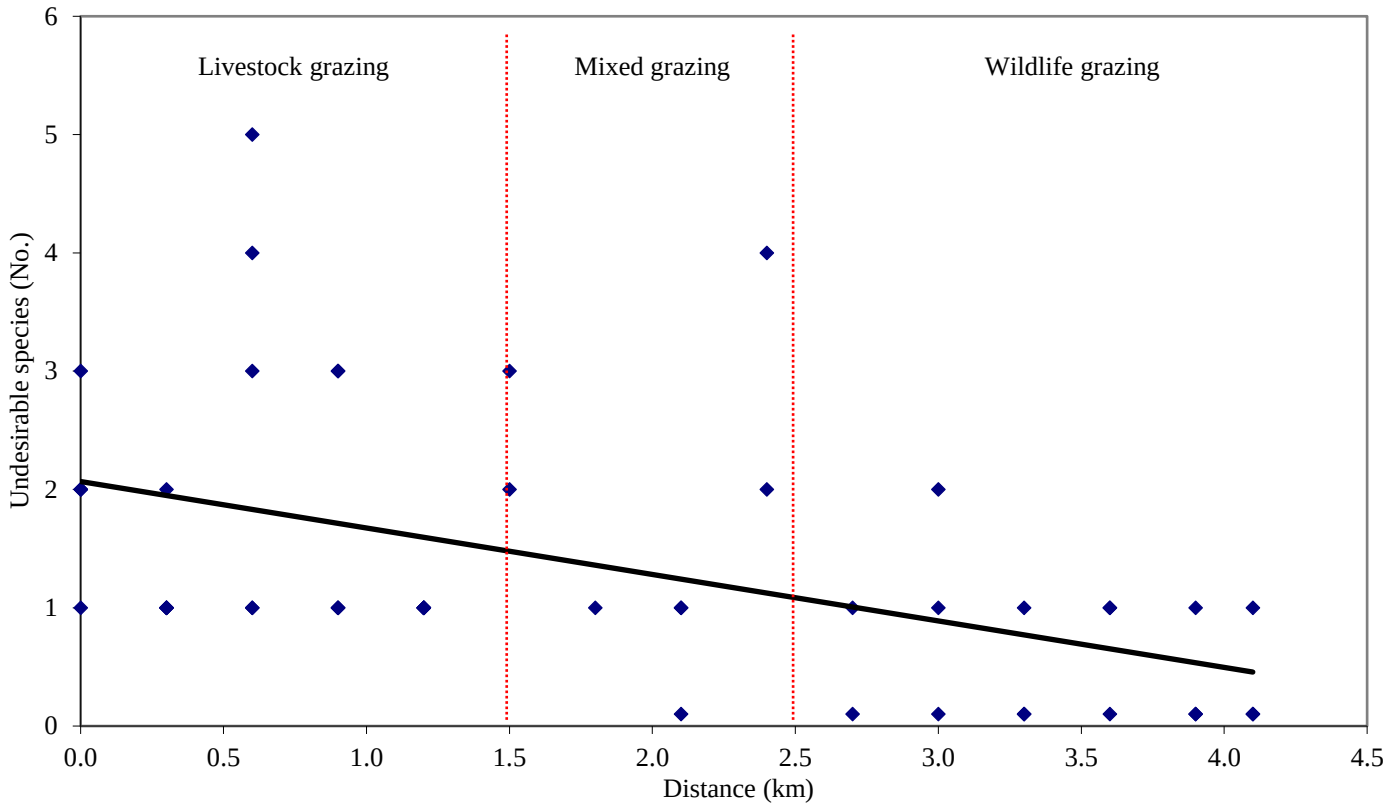

Figure 10. Undesirable herbaceous plants as a function of location (distance from village to protected areas) and grazing strategy.

Herbaceous plant ground cover model

Top model variables are shown in Table 1 along with their rankings that were used for selection of variables for the final model.

Contributions by variables, such as soil texture (clay, sand and silt), life span (perennial and annual species), desirability for grazing animals (undesirable, slightly desirable, moderately desirable and highly desirable), species richness and land use type, to the variance observed in herbaceous plant species ground cover were evaluated by coefficient of determination (R^2) of the model formed by exclusion of either a group or a single variable (Table 2).

Land use type was included in Model 1 as a random effect that encompassed: high grazing pressure in communal lands due to continuous livestock grazing; intermediate grazing pressure on borders between communal and protected lands due to mixed grazing of livestock and wildlife; and low grazing pressure due to wildlife grazing on large protected areas. Coefficient of determination in Model 1 indicated that almost 68% of the variance in herbaceous plant species ground cover was attributed to other factors not considered in the model. The difference in terms of coefficients of determination between Model 1 and Model 2 indicated that land use type contributed <1% of the variance observed in herbaceous plant ground cover. Differences in coefficients of determination among Models 2, 3, 4 and 5 indicated the contributions of desirability of plant species, species richness, plant life span and soil texture to variance in herbaceous plant ground cover were 6.8, 5.7, 6.6 and 12.6%, respectively. This shows little contribution of soil texture to establishment of herbaceous species under grazing pressure in the western Serengeti. The model shows also little influence of soil texture (clay, silt and sand), plant life span (annual or perennial), plant desirability for grazing animals (undesirable, slightly desirable, moderately desirable and highly desirable) and plant species richness on ground cover under grazing in western Serengeti.

Table 1. Products of automated model selection of different soil texture and vegetation variables.

Parameter	Coefficient	s.e.	z Value	Pr (>z)	Significance	Relative importance of variable
Intercept	-2104.9	722.6	2.717	0.00409	**	
Clay	21.450	7.251	2.916	0.00355	**	1.00
Slightly desirable species	-4.167	2.136	1.922	0.05465	NS	0.68
Perennial species	2.302	1.229	1.845	0.06499	NS	0.66
Sand	21.514	7.222	7.327	0.00332	**	1.00
Silt	20.568	7.316	2.771	0.00558	**	1.00
Species richness	3.238	1.260	2.533	0.01131	*	1.00
Undesirable species	4.284	2.349	1.687	0.09155	NS	0.54
Forbs	2.111	1.765	1.178	0.23881	NS	0.09

Table 2. Herbaceous plant ground cover variation attributed to different variables.

Model	R^2 (%)
Model 1: GrC = Clay+Sand+Silt+ASp+PSp+SR+MDS+SDS+MDS+HDS+UDS+LTYP	31.9
Model 2: GrC = Clay+Sand+Silt+ASp+SDS+PSp+SR+MDS+HDS+UDS	31.6
Model 3: GrC = Clay+Sand+Silt+ASp+PSp+ SR	24. 9
Model 4: GrC = Clay+Sand+Silt+ASp+PSp	19.2
Model 5: GrC = Clay+Sand+Silt	12.6

GrC = Ground cover; ASp = Annual species; PSp = Perennial species; SR = Species richness; SDS = Slightly desirable species; MDS = Moderately desirable species; HDS = Highly desirable species; UDS = Undesirable species; and LTYP = Land use type.

Discussion

This study has highlighted the relationships among agro-pastoral activities, herbaceous plant attributes, wildlife conservation and soil texture in western Serengeti, contributing to our knowledge of how these factors impact on the prevalence and sustainability of herbaceous plants in the ecosystem.

Plant species diversity is commonly used as one of the important indices of determining ecosystem status, i.e. the health of the system (Sharafatmandrad et al. 2014), and species diversity, richness and composition present in an ecosystem determine organismal traits that influence ecosystem processes (Chapin III et al. 2000). Diversity of plant species plays an important role in water purification, climate mitigation, air quality improvement and prevention of soil erosion (Pyne 1997). The lower numbers of herbaceous species (10–50 species) in areas highly involved in agro-pastoral activities (Figure 2), i.e. Fallow, Livestock and Mixed land use types, than in protected Wildlife areas (80–100 species) is not surprising. It is in agreement with findings by Luna-Jorquera et al. (2011), who described level of human impact as the main variable that explained variation in species composition of vegetation in British Columbia's southern Gulf Islands. Figure 4 in our study shows that the Fallow cluster (rested cultivated areas) was separate from the Livestock and Mixed clusters implying that the effect of cultivation on herbaceous plant species composition is different from the effect caused by grazing animals.

Results from this study agree with research conducted by Buba (2016) in Nigeria that showed a decrease in species composition following cultivation. After repeated cultivation, land that is fallowed to allow it to recover could not be expected to display a wide array of species as seed supplies of many plant species would be depleted over time. A similar situation, but possibly to a lesser degree, could be expected on areas grazed continuously by livestock. Poor management practices such as keeping of large herds of livestock within a small grazing area or grazing continuously on the same range area for the whole year exert pressure on edible herbaceous species, especially highly palatable ones, limiting recovery of grazed plants. Unlimited expansion of cultivated land involving land clearing and weeding reduces the array of herbaceous species on cropped areas and fallow lands. Studies conducted in different ecosystems by Johnstone et al. (2016) showed that disturbance altered the state of ecosystems, making them prone to degradation: large areas of protected pastures and restriction of human activities resulted in low pressure on herbaceous plants and consequently more diverse species composition.

The increase in herbaceous species richness from village land towards protected areas (Figure 7a) indicates that agro-pastoral activities conducted in the village caused a decline in number of perennial herbaceous species and an increase in annual species. Bare areas

within village lands started at about 600 m from the village-protected area boundary, occurring in overgrazed areas, crop farms and settlements. Analogous to this study, Coppolillo (2000) reported from the Sukuma agro-pastoral system in Rukwa Valley, Tanzania, that more settlements (and more cattle) depleted grazing resources and forced herds to travel farther away from the settlements to find suitable and palatable forage.

As well as providing the feed resource base for ruminants, the herbaceous plants particularly grasses serve other important roles including water retention, biodiversity reserves, cultural and recreational needs and potentially a carbon sink to reduce greenhouse gas emissions (Boval and Dixon 2012). The number of grass species, especially perennials, increased along transects from communal grazing lands towards protected areas as also reported by Sabo et al. (2009) and Pour et al. (2012). Perennial grasses are very important in rangeland health as they are usually more productive than annuals, allow extended grazing periods and improve soil quality as their extended root zones enable recapture of leached nutrients and water (Manahan 2007). Unavailability of perennial grasses in communal grazing lands reduces forage availability within village lands, increasing intrusion of livestock into protected areas and resulting in border disputes. Land clearing for crop farming involves uprooting of perennial grasses, which are considered as notorious weeds in crops, and continuous heavy grazing limits the ability of perennial grasses to set seed for perpetuation of the species. While fallowing of crop farms could possibly increase availability of perennial grasses in communal lands, cultivation in village lands usually opens up new niches and encourages the proliferation of annual forbs (Davis et al. 2000).

Vegetation is usually considered a good indicator of rangeland condition with poor condition described as low grass cover, preponderance of grasses of low palatability, change in species composition where annuals replace perennials as the dominant herbaceous species, and increase in bush encroachment (Bayene 2003). Results from this study (Figure 8b) support this hypothesis, suggesting the current agro-pastoral practices in villages of western Serengeti contribute significantly to rangeland deterioration. Highly desirable herbaceous species, such as the grasses *Brachiaria semiundulata*, *Digitaria milanjiana*, *Cenchrus ciliaris* and *Panicum coloratum*, were more plentiful in protected areas than in communal grazing lands (Figure 9). In contrast, undesirable herbaceous species were more plentiful in communal grazing land than in protected areas (Figure 10).

Changes in species composition are central to grazing land management for sustainable production and conservation of plant species diversity. According to Crawley (1997) grazing-sensitive or highly desirable species decline in abundance, while undesirable plant species become more abundant under high grazing pressure. The decline in highly desirable and increase in undesirable herbaceous species in communal grazing lands as observed in this study indicate existence of high grazing pressure.

According to Naylor et al. (2002) the major effects of vegetation on soil are bio-protection and bio-construction. Plant cover protects soil against erosion by reducing water runoff (Rey 2003; Puigdefábregas 2005; Durán Zuazo et al. 2006, 2008) and by increasing water infiltration into the soil matrix (Ziegler and Giambelluca 1998; Wainwright et al. 2002). Herbaceous plant ground cover increased from communal grazing lands to protected areas in our study. Communal grazing lands with limited plant cover, especially of perennial species, are vulnerable to soil erosion, leading to poor soil condition and consequently low plant productivity, if the situation is not reversed.

While there were suggestions that soil type affects the range of species present in different locations (Cottle 2004), the overall absence of significant relationships between soil texture and species composition observed in this study indicated that other factors like grazing pressure had the major influence on pasture species growing at different locations. The model developed in the present study indicated that plants and soil texture had small influence on ground cover of herbaceous plants in western Serengeti. This supports other studies that showed rainfall as a major factor influencing ground cover in Sub-Saharan Africa (Ellis and Swift 1988; Oba et al. 2000). It implies that linkage of climatic variables, plants and grazing could provide better understanding of dynamics of herbaceous plant ground cover in western Serengeti. Oba et al. (2000) emphasized that climate is the principal driver of ground cover and biomass dynamics, while grazing influences biomass, species diversity and the efficiency with which plants use rainwater.

Our study indicated perennial herbaceous species were present in all areas though at a lower frequency in communal areas than in protected areas. This indicates the possibility of rejuvenation of perennial herbaceous plants in presence of rainfall by resting of grazing land as shown by Hughes (2002), where frequency of perennial grasses increased in Arizona after resting from livestock grazing. A study conducted by Oduor et al. (2018) in a semi-arid rangeland in Kenya showed a higher percentage of perennial grasses in enclosures than in open grazing areas supporting the hypothesis that grazing lands can be rejuvenated by restricting livestock grazing. Reece et al.

(2007) showed deferring grazing, when air temperature and soil water were simultaneously favorable, helped to maintain and improve vigor of grasses in grazing lands because rapid growth of grasses could occur under these positive conditions for plant growth. Therefore understanding of how plants grow and how environmental factors affect their growth is critical for planning restoration of herbaceous plants in grazing lands.

Conclusions

- Current agro-pastoral activities carried out in western Serengeti affected herbaceous plant diversity and availability of highly desirable plant species.
- Cultivation, continuous livestock grazing and settlements reduced the diversity of herbaceous species in village lands.
- The array of pasture species still present in communal grazing areas suggests that rejuvenation of these areas could be still possible if different management strategies were adopted.

Recommendations

- Rehabilitation of denuded lands in village areas is imperative if the current trend of declining perennial and highly desirable herbaceous species is to be reversed to ensure future availability of feed resources for grazing animals in village lands.
- New strategies that involve resting of grazing lands should be developed with the aim of making livestock grazing sustainable and productive in communal lands. The better condition of pastures in wildlife areas with greater species diversity indicates that managing village areas in a similar way could improve the condition of pastures in communal areas

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Appendix 1. Plant species encountered during vegetation survey (taxonomy according to the Plant List (theplantlist.org).

	Species	Life form	Life span	Merit	Utilization	Desirability
1	*Abutilon mauritianum* (Jacq.) Medik. (Malvaceae)	Shrub	Annual	Edible	Eaten by livestock and wildlife	Desirable
2	*Achyranthes aspera* L. (Amaranthaceae)	Forb	Annual	Edible	Wildlife (seeds eaten by birds)	Less desirable
3	*Aeschynomene indica* L. (Leguminosae)	Forb	Annual	Edible	Eaten by livestock and wildlife	Less desirable
4	*Albuca kirkii* (Baker) Brenan (Asparagaceae)	Bulb	Perennial	Inedible	None (rodents)	Undesirable
5	*Alternanthera pungens* Kunth (Amaranthaceae)	Forb	Perennial	Inedible	None	Undesirable
6	*Andropogon greenwayi* Napper (Poaceae)	Grass	Perennial	Edible	Wildlife (wildebeest, buffalo, gazelle)	Desirable
7	*Aristida adoensis* Hochst. ex A. Rich. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Less desirable
8	*Aristida kenyensis* Henrard (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
9	*Asparagus africanus* Lam. (Asparagaceae)	Forb	Perennial	Edible	Eaten by livestock (especially goats)	Less desirable
10	*Aspilia mossambicensis* (Oliv.) Wild (Compositae)	Shrub	Perennial	Inedible	None (medicinal for chimpanzees)	Undesirable
11	*Bidens schimperi* Sch.Bip. ex Walp. (Compositae)	Forb	Annual	Edible	Eaten by livestock	Less desirable
12	*Blepharis linariifolia* Pers. (Acanthaceae)	Forb	Perennial	Inedible	None (medicinal)	Undesirable
13	*Blepharis maderaspatensis* (L.) B. Heyne ex Roth (Acanthaceae)	Forb	Perennial	Edible	Eaten by livestock (particularly flowers)	Less desirable
14	*Bothriochloa insculpta* (A. Rich.) A. Camus (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Desirable
15	*Brachiaria brizantha* (A. Rich.) Stapf (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
16	*Brachiaria jubata* (Fig. & De Not.) Stapf (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
17	*Brachiaria semiundulata* (Hochst.) Stapf (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Highly desirable
18	*Brachiaria serrata* (Thunb.) Stapf (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
19	*Cenchrus ciliaris* L. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
20	*Centrapalus pauciflorus* (Willd.) H. Rob. (Compositae)	Forb	Annual	Edible	Eaten by livestock and wildlife	Less desirable
21	*Chamaecrista mimosoides* (L.) Greene (Leguminosae)	Forb	Annual	Inedible	None	Undesirable
22	*Chloris gayana* Kunth (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
23	*Chloris pycnothrix* Trin. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
24	*Chloris virgata* Sw. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
25	*Chrysochloa orientalis* (C.E. Hubb.) Swallen (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Less desirable
26	*Cleome monophylla* L. (Cleomaceae)	Forb	Annual	Inedible	None	Undesirable
27	*Clitoria ternatea* L. (Leguminosae)	Forb	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
28	*Commelina africana* L. (Commelinaceae)	Forb	Perennial	Edible	Eaten by livestock and wildlife	Desirable
29	*Commelina aspera* G. Don ex Benth. (Commelinaceae)	Forb	Annual	Edible	Eaten by livestock and wildlife	Desirable
30	*Commelina benghalensis* L. (Commelinaceae)	Forb	Perennial	Edible	Eaten by livestock and wildlife	Desirable
31	*Corchorus aestuans* L. (Malvaceae)	Forb	Annual	Edible	Preferably eaten by rabbits	Desirable
32	*Corchorus trilocularis* L. (Malvaceae)	Forb	Annual	Inedible	None	Undesirable
33	*Craterostigma plantagineum* Hochst. (Linderniaceae)	Forb	Perennial	Inedible	None	Undesirable
34	*Crotalaria spinosa* Benth. (Leguminosae)	Forb	Annual	Inedible	None	Undesirable
35	*Cycnium tubulosum* (L. f.) Engl. (Orobanchaceae)	Forb	Perennial	Inedible	None	Undesirable
36	*Cymbopogon caesius* (Hook. & Arn.) Stapf (Poaceae)	Grass	Perennial	Inedible	None	Undesirable
37	*Cynodon dactylon* (L.) Pers. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Desirable
38	*Cynodon plectostachyus* (K. Schum.) Pilg. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Desirable
39	*Cyperus dubius* Rottb. (Cyperaceae)	Sedge	Perennial	Edible	Eaten by livestock and wildlife	Less desirable
40	*Cyperus pulchellus* R. Br. (Cyperaceae)	Sedge	Perennial	Edible	Eaten by livestock and wildlife	Desirable
41	*Cyphostemma serpens* (Hochst. ex A. Rich.) Desc. (Vitaceae)	Forb	Perennial	Inedible	None	Undesirable
42	*Dactyloctenium aegyptium* (L.) Willd. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Less desirable
43	*Desmodium tortuosum* (Sw.) DC. (Leguminosae)	Forb	Annual	Edible	Eaten by livestock and wildlife	Desirable
44	*Digitaria abyssinica* (A. Rich.) Stapf (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Desirable
45	*Digitaria bicornis* (Lam.) Roem. & Schult. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Highly desirable
46	*Digitaria eriantha* Steud. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable

47	*Digitaria longiflora* (Retz.) Pers. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Highly desirable
48	*Digitaria* macroblephara (Hack.) Paoli (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
49	*Digitaria milanjiana* (Rendle) Stapf (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
50	*Digitaria ternata* (A. Rich.) Stapf (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Less desirable
51	*Dyschoriste radicans* (Hochst. ex A. Rich.) Nees (Acanthaceae)	Forb	Perennial	Inedible	None	Undesirable
52	*Echinochloa pyramidalis* (Lam.) Hitchc. & Chase (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
53	*Eleusine indica* (L.) Gaertn. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Less desirable
54	*Eragrostis aspera* (Jacq.) Nees (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
55	*Eragrostis cilianensis* (All.) Janch. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife (but unpleasant odor when fresh)	Less desirable
56	*Eragrostis patula* (Kunth) Steud. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
57	*Eragrostis racemosa* (Thunb.) Steud. (Poaceae)	Grass	Perennial	Edible	Wildlife (buffalo, elephant)	Desirable
58	*Euphorbia inaequilatera* Sond. (Euphorbiaceae)	Forb	Annual	Inedible	None	Undesirable
59	*Eustachys paspaloides* (Vahl) Lanza & Mattei (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
60	*Gomphrena globosa* L. (Amaranthaceae)	Forb	Annual	Edible	Eaten by livestock	Less desirable
61	*Gutenbergia cordifolia* Benth. ex Oliv. (Compositae)	Forb	Annual	Inedible	Pollinators	Undesirable
62	*Gutenbergia petersii* Steetz (Compositae)	Forb	Annual	Inedible	None	Undesirable
63	*Harpachne schimperi* A. Rich. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Desirable
64	*Heliotropium steudneri* Vatke (Boraginaceae)	Forb	Annual	Edible	Wildlife (tortoise)	Less desirable
65	*Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife (when young)	Desirable
66	*Hygrophila auriculata* (Schumach.) Heine (Acanthaceae)	Forb	Annual	Inedible	None	Undesirable
67	*Hyparrhenia hirta* (L.) Stapf (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Less desirable
68	*Hyperthelia dissoluta* (Nees ex Steud.) Clayton (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Less desirable
69	*Hypoxis hirsuta* (L.) Coville (Hypoxidaceae)	Forb	Perennial	Inedible	None	Undesirable
70	*Indigofera basiflora* J.B. Gillett (Leguminosae)	Shrub	Perennial	Inedible	None	Undesirable
71	*Indigofera hochstetteri* Baker (Leguminosae)	Forb	Perennial	Inedible	None	Undesirable
72	*Indigofera spicata* Forssk. (Leguminosae)	Forb	Perennial	Inedible	None	Undesirable
73	*Indigofera volkensii* Taub. (Leguminosae)	Forb	Perennial	Edible	Eaten by livestock (especially sheep)	Less desirable
74	*Ipomoea mombassana* Vatke (Convolvulaceae)	Forb	Perennial	Inedible	None	Undesirable
75	*Justicia betonica* L. (Acanthaceae)	Forb	Perennial	Edible	Eaten by livestock and wildlife	Desirable
76	*Justicia exigua* S. Moore (Acanthaceae)	Forb	Annual	Edible	Eaten by livestock and wildlife	Highly desirable
77	*Justicia glabra* K.D. Koenig ex Roxb. (Acanthaceae)	Forb	Perennial	Inedible	None	Undesirable
78	*Justicia matammensis* (Schweinf.) Oliv. (Acanthaceae)	Forb	Perennial	Inedible	None	Undesirable
79	*Kyllinga nervosa* Steud. (Cyperaceae)	Sedge	Perennial	Edible	Eaten by livestock and wildlife	Desirable
80	*Kyllinga odorata* Vahl (Cyperaceae)	Sedge	Perennial	Edible	Eaten by livestock and wildlife	Desirable
81	*Lactuca inermis* Forssk. (Compositae)	Forb	Perennial	Inedible	None	Undesirable
82	*Lactuca virosa* Habl. (Compositae)	Forb	Annual	Inedible	None	Undesirable
83	*Lepidagathis scabra* C.B. Clarke (Acanthaceae)	Forb	Perennial	Inedible	None	Undesirable
84	*Leucas aspera* (Willd.) Link (Lamiaceae)	Forb	Annual	Inedible	None (medicinal)	Undesirable
85	*Leucas deflexa* Hook. f. (Lamiaceae)	Forb	Perennial	Inedible	None	Undesirable
86	*Leucas martinicensis* (Jacq.) R. Br. (Lamiaceae)	Forb	Perennial	Inedible	None	Undesirable
87	*Macroptilium atropurpureum* (DC.) Urb. (Leguminosae)	Forb	Perennial	Edible	Eaten by livestock and wildlife	Desirable
88	*Melhania ovata* Spreng. (Malvaceae)	Forb	Perennial	Inedible	None	Undesirable
89	*Microchloa kunthii* Desv. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Less desirable
90	*Mollugo nudicaulis* Lam. (Molluginaceae)	Forb	Annual	Inedible	None	Undesirable
91	*Ocimum basilicum* L. (Lamiaceae)	Shrub	Perennial	Inedible	None	Undesirable
92	*Ocimum gratissimum* L. (Lamiaceae)	Shrub	Perennial	Inedible	None	Undesirable
93	*Ormocarpum kirkii* S. Moore (Leguminosae)	Shrub	Perennial	Inedible	None	Undesirable
94	*Ormocarpum* trichocarpum (Taub.) Engl. (Leguminosae)	Shrub	Perennial	Inedible	None	Undesirable
95	*Oxygonum sinuatum* (Hochst. ex Steud. & Meisn.) Dammer (Polygonaceae)	Forb	Annual	Inedible	None	Undesirable
96	*Panicum coloratum* L. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
97	*Panicum maximum* Jacq. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
98	*Pennisetum mezianum* Leeke (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Less desirable
99	*Portulaca oleracea* L. (Portulacaceae)	Forb	Annual	Edible	Eaten by livestock	Less desirable

100	*Portulaca quadrifida* L. (Portulacaceae)	Forb	Annual	Edible	Eaten by livestock (rich in vitamins E, A and C)	Desirable
101	*Rhynchosia minima* (L.) DC. (Leguminosae)	Forb	Perennial	Edible	Eaten by livestock and wildlife	Desirable
102	*Senna occidentalis* (L.) Link (Leguminosae)	Shrub	Annual	Inedible	None (bitter taste)	Undesirable
103	*Sesbania sesban* (L.) Merr. (Leguminosae)	Shrub	Perennial	Edible	Eaten by livestock and wildlife	Desirable
104	*Setaria pumila* (Poir.) Roem. & Schult. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
105	*Setaria sphacelata* (Schumach.) Stapf & C.E. Hubb. ex Moss (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Highly desirable
106	*Setaria verticillata* (L.) P. Beauv. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
107	*Sida acuta* Burm. f. (Malvaceae)	Forb	Perennial	Edible	Eaten by livestock and wildlife	Desirable
108	*Solanum incanum* L. (Solanaceae)	Shrub	Perennial	Edible	Wildlife (rhino, butterflies)	Less desirable
109	*Sphaeranthus suaveolens* (Forssk.) DC. (Compositae)	Forb	Perennial	Inedible	None	Undesirable
110	*Sporobolus africanus* (Poir.) Robyns & Tournay (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Less desirable
111	*Sporobolus cordofanus* (Hochst. ex Steud.) Hérincq ex Coss. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
112	*Sporobolus festivus* Hochst. ex A. Rich. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Desirable
113	*Sporobolus ioclados* (Trin.) Nees (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Desirable
114	*Sporobolus pyramidalis* P. Beauv. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Less desirable
115	*Tagetes minuta* L. (Compositae)	Forb	Annual	Inedible	None	Undesirable
116	*Talinum portulacifolium* (Forssk.) Asch. ex Schweinf. (Talinaceae)	Forb	Perennial	Edible	Eaten by livestock	Desirable
117	*Tephrosia pumila* (Lam.) Pers. (Leguminosae)	Forb	Perennial	Inedible	None	Undesirable
118	*Themeda triandra* Forssk. (Poaceae)	Grass	Perennial	Edible	Eaten by livestock and wildlife	Desirable
119	*Tragus berteronianus* Schult. (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
120	*Tribulus terrestris* L. (Zygophyllaceae)	Forb	Annual	Inedible	None	Undesirable
121	*Triumfetta rhomboidea* Jacq. (Malvaceae)	Forb	Annual	Inedible	None	Undesirable
122	*Urochloa brachyura* (Hack.) Stapf (Poaceae)	Grass	Annual	Edible	Eaten by livestock and wildlife	Desirable
123	*Xanthium strumarium* L. (Compositae)	Shrub	Annual	Inedible	None	Undesirable

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Research Paper

Evaluation of fodder biomass yield of hydroponically-grown barley and oats and the effects on intake, digestibility and weight gain of Washera sheep when fed as a supplement to a basal diet of natural pasture hay in Ethiopia

Rendimiento de biomasa, consumo y digestibilidad de cebada y avena cultivadas en medio hidropónico y su efecto en la ganancia de peso vivo de ovejas Washera en Etiopía

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Abstract

The feasibility of using hydroponically-grown (HGF) barley and oats forage as a supplement to natural pasture hay (PH) for sheep-feeding was investigated. Twenty-five intact Washera male lambs were used in both a 90 day feeding trial and a 10 day digestibility study. The treatments compared were: 100% PH (control, = T1); PH + a concentrate mix (CM) (= T2); PH + HGF barley (= T3); PH + HGF oats (= T4); and PH + 50% CM and 50% HGF mixture of barley and oats (= T5). Chemical composition of diets and refusals, feed intake and digestibility of DM and nutrients were recorded. The average HGF fresh biomass yields from 1 kg grain were 5.21 and 6.32 kg for barley and oats, respectively. The CP, NDF, ADF and ADL concentrations in HGF were 13.2, 45.6, 34.8 and 6.7% for barley and 13.7, 46.8, 36.6 and 7.6% for oats. All supplemented treatments had higher total DM intakes (12–21%) than the control ($P<0.05$) and all supplements produced marked substitution effects for PH (35–51%). Animals on the PH diet lost weight (17 g/d), while all supplemented groups gained weight (58–65 g/d). Partial budget analysis showed that the highest net return was for T5 followed by T2, T4 and T3. Hydroponically-grown oats forage could have potential to replace a commercial concentrate for supplementing sheep on native pastures, but both HGF and concentrates are probably unaffordable for the majority of smallholder farmers engaged in sheep production. Establishment of farmer cooperative hydroponic facilities could spread the overhead costs of the capital infrastructure and this approach should be investigated.

Keywords: Biomass yield, chemical composition, economics, substitution effects.

Resumen

En Bahir Dar, Etiopía, se investigó la viabilidad del uso de forraje de cebada y avena cultivadas hidropónicamente (HGF) como suplemento de heno de pasto nativo (PH) para la alimentación de ovejas. Se usaron 25 corderos Washera enteros en un ensayo de alimentación de 90 días y un estudio de digestibilidad de 10 días. Los tratamientos fueron: 100% PH (control, = T1); PH + una mezcla de concentrados (CM) (= T2); PH + HGF de cebada (= T3); PH + HGF de cebada (= T4); y PH + 50% CM y 50% mezcla de HGF de cebada y avena (= T5). Se evaluaron la composición química de las dietas y del forraje rechazado, el consumo del forraje y la digestibilidad de la MS y de los nutrientes. Los rendimientos promedio de biomasa fresca de HGF de 1 kg de grano fueron 5.21 y 6.32 kg para cebada y avena, respectivamente. Las concentraciones de CP, NDF, ADF y ADL en HGF fueron 13.2, 45.6, 34.8 y 6.7% para cebada y 13.7, 46.8, 36.6 y 7.6% para avena. El consumo total de MS fue para todos los tratamientos suplementados (T2–T5) más alto (12–21%) que para el control ($P<0.05$) y todos los suplementos mostraron marcados efectos de sustitución para PH (35–51%). Los animales

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con la dieta PH perdieron peso (17 g/día), mientras que los de los grupos suplementados aumentaron de peso (58–65 g/día). El análisis del presupuesto parcial mostró que el mayor rendimiento neto fue para T5 seguido de T2, T4 y T3. HGF de avena podría tener el potencial de reemplazar un concentrado comercial como suplemento para ovejas en pasturas nativas, pero tanto el HGF como los concentrados probablemente no sean asequibles para la mayoría de los pequeños agricultores dedicados a la producción de ovejas en Etiopía. El establecimiento de instalaciones hidropónicas en cooperativas de agricultores podría considerarse como mecanismo para dispersar los costos de capital necesario y este enfoque debería investigarse.

Palabras clave: Análisis económico, composición química, economía, efecto substitutivo, producción de forraje.

Introduction

Ethiopia has the largest livestock population in Africa, with an estimated 59.9 million cattle, 30.7 million sheep, 30.2 million goats, 56.5 million poultry and 1.23 million camels (CSA 2018). The livestock sector contributes significantly to the economy of the country, producing about 47% of agricultural gross domestic product (Behnke 2010). Livestock products and by-products in the form of meat, milk, honey, eggs, cheese and butter provide animal protein for the local human population. In addition, other items, such as live animals, meat, hides and skins, are exported to earn foreign exchange for the country and play a significant role in the social and cultural values of society (Tegegne et al. 2010).

However, livestock productivity in Ethiopia is still far below its potential, as animals are mostly left to graze on degraded pasture land and crop residues, which do not supply the nutrient requirements for maintenance of animals (Gebremedhin et al. 2009). As a consequence, livestock productivity in Ethiopia is one of the lowest in the world, with average carcass weights of 108, 10, 8.5 and 0.8 kg/head for cattle, sheep, goats and chickens, respectively (Negassa et al. 2011). In addition, Hagos and Melaku (2009) reported that the steady increase in the human population has resulted in grazing and browsing areas being converted to arable farming areas for food crop production, which further aggravates the scarcity of feed resources. Even the existing natural grazing land is characterized as overgrazed, poor in botanical composition, low in biomass yield (1.5–2 t DM/ha/yr) and of poor nutritive value. These grazing lands produce biomass only during the rainy season (July–October) and there is no green fodder in the remaining 8 months. Irrigation development for forage production is not widely exploited. Utilization of concentrate feeds in developing countries like Ethiopia is largely impractical as they are very expensive and not easily accessible by smallholder farmers.

Alternative fodder sources are needed if production levels are to increase. Green fodder production through hydroponic technology is one possible solution. Hydroponic fodder production is simple and easy (Uddin and Dhar 2018) and could be economically feasible in Ethiopia as the seed, construction of the plantation structure and management costs could be relatively inexpensive compared with costs of concentrates. The required materials for the construction and raw materials for growing fodder are available at the smallholder farmer's level. As climatic conditions in the country are suitable for fodder production through hydroponic technology, this production system should not incur significant costs (Bakshi et al. 2017). The materials used for hydroponic technology could also be varied based on the producer's investment capability.

Therefore, the majority of smallholder farmers could possibly establish this system using local materials, while urban and peri-urban dwellers could focus on a 'high tech' setup. Hydroponic fodder is targeted at supplementation of mainly highly-productive animals, not as the basal diet. However, information on possible production levels and utilization as a supplement for animals is quite limited.

This study was designed to assess the production of hydroponically-grown barley (*Hordeum vulgare*) and oats (*Avena sativa*) forage and the benefits from feeding this material as a supplement to sheep fed a basal diet of natural pasture hay.

Materials and Methods

Description of the experimental area

The study was conducted at the Zenzelema Campus of the College of Agriculture and Environmental Sciences of Bahir Dar University (11°37' N, 37°27' E; 1,900 masl), near Lake Tana, Ethiopia. Zenzelema is located at about 573 km northwest of Addis Ababa and 8 km north of Bahir Dar town. The average daily minimum and maximum temperatures are 7 °C and 29 °C, respectively, and mean annual rainfall is 1,445 mm.

Seed preparation before planting

Barley and oats grains were purchased from the local market and screened to remove debris and other foreign materials. The grains were washed using tap water and lemon juice soap at least 3 times and soaked in tap water for 4–6 hours. Seeds were then sterilized by soaking for 30 minutes in a 20% sodium hypochlorite solution with tap water to control the formation of mold growth. Before planting, soaked grains were stored in a gunny bag in a dark room for 24 hours until a root mat emerged.

Seed planting and watering

Sprouted seeds were spread on hydroponic trays which had a volume of 3,500 cm^3 (35×25×4 cm) with holes at the bottom to allow drainage of excess water from irrigation. The seeding rates used in this experiment were about 330–350 g barley and oats grain per tray (about 1–2 cm depth) layer. Tap water containing the nutrient solution was used to irrigate plants twice a day (early morning and late afternoon) throughout the growing period at a fixed rate of 500 mL/tray/day.

Hydroponically-grown green fodder biomass yield estimation

Seven days from planting, total green fodder yields were recorded and production per kg of grain sown was calculated. Representative fresh fodder samples (about 100–200 g) were selected at random and oven-dried at 70 °C for 24 hours to determine dry matter yields and for laboratory analysis.

Purchasing and management of experimental animals

Twenty-five yearling intact Washera sheep with average body weight of 15.2±1.18 kg (mean±SD) were purchased from Sekela local market and quarantined for 3 weeks to allow them to adapt to their new environment and to confirm that they were healthy. The sheep were de-wormed, treated for external parasite control and placed in individual well-ventilated pens. After the adaptation period they were weighed after overnight fasting and allocated by stratified randomization on the basis of initial body weight into 5 groups of 5 animals. Sheep were fed individually throughout the feeding trial.

Experimental design and treatments

The experimental design was a randomized complete block design (RCBD) with 5 treatments and 5 replications. The 5 groups of sheep were randomly assigned to the following treatments: T1 (control group) = 100% natural pasture hay (PH) ad libitum; T2 = PH ad libitum + 300 g concentrate mix (wheat bran and noug seed cake, 1:1 w/w); T3 = PH + 1 kg hydroponic barley (50% DM); T4 = PH + 948 g hydroponic oats; and T5 = PH + 150 g concentrate mix + 250 g hydroponic barley + 237 g hydroponic oats forage. These quantities of hydroponic oats and barley provided similar amounts of nitrogen.

Feeds and feeding management

Natural pasture hay was purchased and chopped to a length of approximately 4–5 cm to minimize preferential selection and wastage, weighed and offered to individual sheep ad libitum as a basal diet. Wheat bran and noug seed (*Guizotia abyssinica*) cake were purchased and mixed in the proportion of 1:1 (w/w) and offered to individual lambs. Barley and oats forage was harvested after 7 days growth and air-dried to 50% DM before feeding the following day. The daily rations of natural pasture hay, hydroponically-grown barley and oats and concentrate mixture were offered in separate troughs. The basal hay ration was fed ad libitum with 10% refusal adjustment every week in the morning and supplements were fed in 2 equal portions in the morning and afternoon. The lambs had free access to clean and fresh water and common salt at all times.

Feed and nutrient intakes

Daily mean basal feed intakes were measured as differences between feed offered and refusals. Natural pasture hay and hydroponic forage refusal samples were taken daily for each animal during both feeding and digestion studies, pooled on a treatment basis, and sub-sampled at the end of the experiment for chemical analysis. Dry matter intake (DMI) was estimated from voluntary feed intake (VFI) × percentage of DM. Intake values for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were calculated by multiplying feed intake by the corresponding percentages for each proximate component.

Bodyweight change and feed conversion efficiency

Animals were weighed at the beginning of the trial and then every 10 days during the 90 days of the feeding trial in the morning after overnight fasting, using a suspended weighing scale with a sensitivity of 100 g. Average daily bodyweight gains were calculated as the difference between final and initial weights divided by the number of feeding days. The feed conversion efficiency (FCE) of experimental animals was calculated as the daily feed

consumption divided by average daily bodyweight gain, i.e. g feed to produce a g of bodyweight gain.

Digestibility trial

For the digestibility trial prior to commencement of the feeding study, each sheep was fitted (harnessed) with a fecal collection bag for 3 days as an acclimatization period prior to total collection of feces for 10 consecutive days. Feeding and animal management during the digestibility trial were similar to those in the feeding trial. Feces voided were weighed and recorded every morning, thoroughly mixed and representative samples (20% of feces) were taken, frozen at -20 ºC and pooled over the collection period for each animal. At the end of the collection period, daily samples for individual animals were mixed and dried to calculate DM intakes and digestibility and were used for chemical analysis. The apparent digestibility values for DM, CP, NDF and ADF were determined using the following equations:

$$\text{Apparent DM digestibility coefficient} = \frac{\text{DMI} - \text{fecal DM output}}{\text{DMI}}$$

$$\text{Apparent nutrient digestibility coefficient} = \frac{\text{nutrient intake} - \text{fecal nutrient output}}{\text{nutrient intake}}$$

where:
DMI = dry matter intake.

Chemical analyses of experimental feeds

Experimental feeds were sub-sampled to determine DM concentration and ground to pass a 1 mm mesh sieve for chemical analyses. NDF, ADF and ADL were determined according to Van Soest and Robertson (1985), while CP concentration was estimated by the micro-Kjeldahl method (AOAC 1990).

Economic analysis

All input costs were recorded and the economic feasibility of the various treatments was determined through a partial budget analysis using the procedure of Upton (1979). The partial budget analysis involved the calculation of variable costs and benefits. The same purchase price was applied to sheep in all treatments initially regardless of differences in mean weights of groups. At the end of the study all sheep were sold within various treatments and values recorded. Differences in the values of sheep in each treatment before and after feeding were considered as gross returns (GR) in the analysis. For the calculation of variable costs, expenditures incurred on various feedstuffs were taken into consideration. Feed costs were computed by multiplying the actual feed intake for the whole feeding period by the prevailing market price, including the transportation costs incurred in moving the feeds to the experimental site. Total variable costs (TVC) included the costs of all inputs for the various treatments and net return (NR) was calculated as GR – TVC. The change in net return (ΔNR) was calculated by the difference between the change in gross return (ΔGR) and the change in total variable cost (ΔTVC), which is to be used as a reference criterion for decision on the adoption of a new technology, i.e. $\Delta NR = \Delta GR - \Delta TVC$. The marginal rate of return (MRR) measures the increase in net income (ΔNR) associated with each additional unit of expenditure (ΔTVC). This is expressed in percentage as: $MRR = (\Delta NR/\Delta TVC)$.

Statistical analysis

Performance data from the feeding trial including DMI, nutrient intake, digestibility, bodyweight change and feed conversion efficiency were analyzed using GLM procedure of SAS 9.1.3 based on the critical P value of 0.05. Differences among treatment means were tested using Duncan's multiple range test. The statistical model used in the study was:

$$Yij = \mu + Ti + \beta j + eij$$

where:
Yij = the general observation;
μ = the general mean;
Ti = the effect of the *i*th treatment;
Bj = the *j*th block; and
eij = the standard error.

Results

Biomass yields of hydroponically-grown barley and oats forage

The average green fodder biomass yields (50% DM) at 7 days after sprouting were 5.21 (2.61 kg DM) and 6.32 (3.16 kg DM) kg barley and oats per 1 kg barley and oats grain, respectively.

Chemical composition of treatment feeds

The chemical composition of the experimental diets is presented in Table 1. Hydroponic fodders had higher CP concentrations of 13.2 and 13.7% than the grains used to produce them (11.8 and 8.1% for barley and oats, respectively). Natural pasture hay contained 6.4% CP.

Table 1. Chemical composition of experimental feeds and refusals.

Item	Chemical composition (% DM)				
	Ash	CP	NDF	ADF	ADL
Feeds					
Barley grain	2.15	11.8	23.2	18.6	3.32
Barley forage	3.26	13.2	45.6	34.8	6.68
Oats grain	3.22	8.1	47.8	38.7	7.57
Oats forage	2.15	13.7	46.8	36.6	7.57
Wheat bran	3.19	14.4	15.6	10.6	2.24
Noug seed cake	7.44	30.1	41.2	29.8	5.58
PH	7.60	6.4	58.7	47.8	13.0
PH refusals					
T1	8.60	5.2	76.7	73.3	18.9
T2	7.60	4.8	71.4	58.7	15.9
T3	7.44	5.4	66.7	55.3	14.5
T4	9.67	5.0	88.8	76.6	20.0
T5	9.67	6.0	71.4	60.2	17.6

ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein; DM = dry matter; NDF = neutral detergent fiber; PH = natural pasture hay.
T1 = natural pasture hay only; T2 = PH + concentrate mix; T3 = PH + hydroponic barley forage; T4 = PH + hydroponic oats forage; T5 = PH + concentrate mix + hydroponic forage (barley and oats).

Daily intakes of dry matter and nutrients

The daily intakes of DM and nutrients in the various treatments are presented in Table 2. All supplements resulted in an increase (P<0.05) in total DM intake but there was no significant difference (P>0.05) in total DM intake between the 4 supplemented treatments (T2, T3, T4 and T5). Significant differences (P<0.001) were observed in intakes of almost all nutrients.

Table 2. Daily intakes (g/hd/d) of dry matter and nutrients by Washera sheep fed a basal diet of natural pasture hay ± supplements of concentrate mix and hydroponically-grown forage.

Component	T1	T2	T3	T4	T5	s.e.m.
Basal DM	453a	293a	221c	245c	225c	9.07
Suppl. DM	-	282c	310b	308b	341a	1.20
Total DM	453b	575a	531a	553a	566a	10.6
CP	33.5d	78.0a	61.6b	58.4c	77.8a	3.25
NDF	204d	214dc	331a	279b	239c	10.7
ADF	128d	164c	253a	216b	180c	9.59
ADL	37.5b	40.7b	52.9a	44.0b	39.0b	1.60

Means followed by different letters within rows are significantly different (P<0.05). ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein; DMI = dry matter intake; NDF = neutral detergent fiber.
T1 = natural pasture hay; T2 = hay + concentrate mix; T3 = hay + hydroponic barley forage; T4 = hay + hydroponic oats forage; T5 = hay + concentrate mix +hydroponic forage (barley + oats).

Apparent digestibility of dry matter and nutrients

Apparent digestibility coefficients for dry matter and nutrients are presented in Table 3. Apparent digestibilities of CP, NDF and ADF for supplemented treatments were generally higher (P<0.05) than those for the control treatment. However, only the treatment fed both concentrate and hydroponically-grown forage produced higher (P<0.05) DM digestibility than the control.

Table 3. Apparent digestibility of dry matter and nutrients (%) by Washera sheep fed a basal diet of natural pasture hay ± supplements of concentrate mix, hydroponically-grown forage (barley and oats) and their mixtures.

Nutrient	T1	T2	T3	T4	T5	s.e.m.
DM	49.7b	61.0ab	59.6ab	57.3b	71.1a	2.00
CP	47.8c	72.3ab	61.1b	55.5c	76.9a	2.66
NDF	43.0c	50.6b	51.7b	49.3b	57.1a	1.45
ADF	41.6b	50.0a	50.4a	47.2a	54.1a	1.10

Means within rows followed by different letters are significantly different (P<0.05). ADF = acid detergent fiber; CP = crude protein; NDF = neutral detergent fiber.
T1 = natural pasture hay; T2 = hay + concentrate mix; T3 = hay + hydroponic barley forage; T4 = hay + hydroponic oats forage; T5 = hay + concentrate mix +hydroponic forage (barley + oats).

Bodyweight change and feed conversion efficiency

Data on body weights and bodyweight changes are presented in Table 4. All supplemented treatments produced better (P<0.05) bodyweight gains and feed conversion efficiencies than the control.

Table 4. Bodyweight parameters and feed conversion efficiencies of Washera sheep fed a basal diet of natural pasture hay ± supplements of concentrate mix and hydroponically-grown forage (barley and oats).

Parameter	T1	T2	T3	T4	T5	s.e.m.
IBW (kg)	15.60a	14.60a	15.60a	14.50a	15.70a	0.35
FBW (kg)	14.10b	19.80a	20.78a	19.60a	21.56a	0.7
ADG (g/d)	-16.7b	57.8a	57.6a	56.7a	65.1a	6.02
FCE	27.1b	9.95a	9.23a	9.76a	8.69a	0.01

Means within rows followed by different letters are significantly different (P<0.05). ADG = average daily gain; FBW = final body weight; FCE = feed conversion efficiency (g feed/g bodyweight gain); IBW = initial body weight.
T1 = natural pasture hay; T2 = hay + concentrate mix; T3 = hay + hydroponic barley forage; T4 = hay + hydroponic oats forage; T5 = hay + concentrates + hydroponic forage (barley + oats).

Partial budget analysis

Results of the partial budget analysis of performance of the various groups of sheep is presented in Table 5. Animals in the control treatment lost weight, resulting in a substantial negative outcome financially. All supplemented rations resulted in positive financial outcomes with the highest net returns for the hay + concentrate + HGF treatment and the worst for the barley forage supplemented group.

Table 1. Partial budget analysis of Washera sheep fed a basal diet of natural pasture hay ± supplements of concentrate and hydroponically-grown forage (barley and oats).

Parameter	T1	T2	T3	T4	T5
Purchase price of sheep (ETB/hd)	914	914	914	914	914
Basal diet intake (kg/hd)	40.8	26.4	19.9	22.1	20.3
Concentrate intake (kg/hd)	-	27.0	-	-	13.5
Barley forage intake (as fed, kg/hd)	-	-	55.8	-	14.0
Oats forage intake (as fed, kg/hd)	-	-	-	55.4	13.9
Cost of basal diet (ETB/hd)	20.4	13.2	9.95	11	10.13
Cost of concentrate (ETB/hd)	-	148.5	-	-	74.25
Cost of barley forage (ETB/hd)	-	-	220.2	-	55
Cost of oats forage (ETB/hd)	-	-	-	137.6	34.4
Labor cost (ETB/hd)	150	180	184.2	184.2	182.1
Total variable cost (ETB/hd)	170.4	341.7	414.35	332.8	355.88
Selling price of sheep (ETB/hd)	850	1,469	1,450	1,435	1,510
Gross return (ETB/hd)	-64	555	536	521	596
Net return (ETB/hd)	-234.4	213.3	121.65	188.2	240.12
ΔNR		447.7	356.05	422.6	474.52
ΔTVC		171.3	243.95	162.4	185.48
MRR (ratio)		2.61	1.46	2.6	2.56

ETB = Ethiopian Birr; MRR = marginal rate of return; ΔNR = change in net return; ΔTVC = change in total variable cost.
T1 = natural pasture hay; T2 = hay + concentrate mix; T3 = hay + hydroponically-grown barley forage; T4 = hay + hydroponically-grown oats forage; T5 = hay + concentrate mix + hydroponically-grown forage (barley + oats).

Discussion

The forage production per kg of seed in the present study is lower than the 8 kg of hydroponic forage from 1 kg barley seed reported by Badran et al. (2017). Yields are also lower than earlier reports (Al-Karaki and Al-Hashimi 2012; Naik and Singh 2013; Kantale et al. 2017). The ranges of seedling heights of the shoots were 11–17 cm and 10–15 cm for barley and oats forage, respectively. These figures are lower than 18–20 cm for hydroponic barley forage (Al-Hashmi 2008), which was harvested at 8 and 9 days after sprouting. The differences in the biomass yield and length may be attributed to size of the seed, varieties of the grains and the environment where hydroponically grown.

The CP concentration in the natural pasture hay in the current study was lower than the 9.9% CP reported by Arefaine and Melaku (2017), and was below maintenance requirements for ruminants (Van Soest 1994) as evidenced by the weight loss recorded in sheep fed only hay.

In contrast, CP concentrations in the barley grain and barley forage in this study were 11.8 and 13.2%, respectively, values lower than the 19.7 and 19.8% reported by Fazaeli et al. (2012). The NDF and ADF concentrations in the barley forage (45.6 and 34.8%, respectively) were higher than the NDF and ADF concentrations of 31.6 and 14.6% reported by Helal (2015). While the oats grain contained only 8.1% CP, which was considerably lower than that of barley grain, the oats forage contained 13.7% CP, which was similar to that of the barley forage.

The increase in total DMI when supplements were fed was not surprising as the basal diet of native pasture hay contained only 6.4% CP, while diets where supplements were fed contained either concentrates or forage with CP concentrations of 13–22% and higher digestibility than the basal roughage. The increased N available to the rumen microorganisms would have increased rate of digestion and rate of passage of the basal diet resulting in increased appetite (Van Soest 1994). While all supplements increased total DMI, all produced a substantial substitution effect on hay intake with supplements containing forage resulting in a higher substitution effect (46–52%) than the concentrate (35%).

With the increased DMI recorded in supplemented groups, coupled with higher digestibility of the ration consumed, the improved bodyweight changes in these treatments were to be expected. Feeding supplements converted a weight loss of 17 g/d to gains of 57–65 g/d. However, the feed conversion efficiency values obtained were disappointing with intake of 10 kg DM needed to obtain a kg of bodyweight gain. The highest average daily gain (65.1 g/d) in T5 for the current study was similar to

the 64.6 g/d average daily weight gain for Arsi-bale sheep fed diets of different varieties of faba bean (*Vicia faba*) straw mixed with concentrate (Wegi et al. 2018) and 64.4 g/d for Washera sheep fed natural pasture hay plus 350 g/d concentrate mixture (hulls, wheat bran and noug seed cake) (Mesganaw 2014). Results from this study support the concept of Naik et al. (2014) that hydroponic sprouts can be a rich source of bioactive enzymes and may contain ingredients that improve the performance of livestock. While this confirms the biological feasibility of the process, assessment of the costs and returns is needed to confirm whether or not the procedure is financially viable.

The partial budget analysis of data from this study indicates that feeding any of the supplements produced a positive financial outcome and there were relatively small differences between feeding a concentrate supplement, oats forage and concentrate mixed with barley-oats forage. However, returns from barley forage as a supplement produced a much worse economic outcome than the other supplemented diets. The 21% higher biomass production of oats relative to barley per unit weight of grain under hydroponic conditions combined with a lower unit price of oats grain meant that the returns for the oats forage were more favorable than for barley. This suggests that hydroponic options could be adopted by farmers as hydroponic fodder production and utilization could be a possible option for boosting small-ruminant production by smallholder farmers. We consider it is possible to use low-cost techniques which are easy to operate and maintain and require simple infrastructure and low operational costs. For this intensive forage production strategy to be effectively used in the country, an option could be the production of hydroponic green forage by larger livestock producers as well as farmer-led commercial cooperatives. More studies are needed to confirm that the relative production levels of forage from barley and oats seeds are as found in this exercise.

Conclusions

The results of this study suggest that hydroponic green forage could be grown and fed as a replacement for conventional commercial concentrate mixtures for sheep during the dry season in Ethiopia. However, based on the costs and returns from this study plus the ease of feeding, the net benefits would still favor the concentrate supplements. In any case, unless simple low-cost methods for growing the forage can be developed, for this strategy to be effectively introduced in Ethiopia, hydroponic green forage may need to be produced in farmer-led commercial cooperatives to spread the capital cost.

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Short Communication

Seasonal influence on mineral concentration of forages on flooded pastures in South Sumatra, Indonesia

Concentración de minerales en forrajes nativos en pastizales estacionalmente inundados en South Sumatra, Indonesia

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Abstract

This study was conducted to evaluate macro- and micro-mineral concentrations in forages growing on seasonally flooded native pastures in non-tidal swamps of South Sumatra, Indonesia and grazed by buffalo. The upper part of native forage plants from 5 species of Poaceae, 4 species of Leguminosae, 3 species of Cyperaceae and 1 species of Onagraceae were sampled by hand-plucking during flooded and dry seasons. The results showed that mineral concentrations of forages varied greatly between seasons. In general concentrations of most minerals were adequate to supply the dietary needs of grazing ruminants in both wet and dry seasons. Phosphorus (P) concentrations were low in all species in both wet and dry seasons but growing animals should select a diet adequate in P, while lactating females could benefit from P supplementation. In cut-and-carry situations, animals would probably respond to additional P in the diet. These hypotheses need testing in the field.

Keywords: Buffalo, mineral deficiency, mineral toxicity.

Resumen

En South Sumatra, Indonesia se realizó un estudio para evaluar las concentraciones de macro- y micro-minerales en plantas forrajeras nativas comunes en pastizales estacionalmente inundados y utilizadas con búfalos. Para el estudio see muestrearon por el método ´hand-plucking´ plantas de 5 especies de Poaceae, 4 especies de Leguminosae, 3 especies de Cyperaceae y 1 especie de Onagraceae durante las estaciones inundada y seca. Los resultados mostraron que las concentraciones minerales variaron considerablemente entre estaciones. No obstante, las concentraciones de la mayoría de los minerales fueron adecuadas para satisfacer los requerimientos nutricionales de rumiantes en pastoreo en ambas estaciones. Las concentraciones de fósforo (P) fueron bajas en todas las especies en ambas estaciones, pero se considera que bajo condiciones de pastoreo los animales en crecimiento seleccionan una dieta adecuada en P, mientras que hembras lactantes podrían beneficiarse de P suplementado. En situaciones de corte y acarreo, los animales probablemente responderían a P adicional en la dieta. Estas hipótesis necesitan ser probadas en el campo.

Palabras clave: Búfalos, deficiencia mineral, toxicidad mineral.

Introduction

The concentrations of individual minerals in forages vary greatly depending on the interactions of a range of factors including soil, plant species, stage of maturity, yield, management factors and climate. Forages represent an important source of minerals for grazing ruminants and play essential roles in preventing diseases and inhibiting or stimulating ruminal microbial activity (Spears 1994). Natural pasture species in non-tidal swamps have been traditionally the main source of feed for swamp buffalo in e.g. Brazil (Camarão et al. 2004) and South Sumatra (Ali et al. 2014). Forage nutritive values fluctuate with seasons and the dry season is the most limiting in terms of nutrient

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supply to grazing buffalo. As most grazing livestock in tropical countries including Indonesia are usually unsupplemented, they must fulfill their mineral requirements from forage. Camarão et al. (2004) failed to find evidence of mineral deficiencies in grazing buffalo. Data on mineral concentrations in forage species and their variability between seasons are essential for correct and cost-effective ration formulation and swamp buffalo nutrition.

However, mineral profiles of tropical forages in flooded pasture have received little attention and limited studies showed that forages can be deficient in one or more elements. In this study we aimed to estimate and compare the concentrations of mineral elements including calcium (Ca), phosphorus (P), magnesium (Mg), sulphur (S), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) in native flooded pasture species in non-tidal swamps of South Sumatra, Indonesia during the flooded and dry seasons.

Materials and Methods

Site description

Research was conducted during February–September 2014 on communal pastures in non-tidal swamps in Rambutan subdistrict, Banyuasin district and Pampangan subdistrict, OKI (Ogan Komering Ilir) district of South Sumatra Province (between 3°05' and 3°11' S, and 104°55' and 104°58' E). The study area was selected as representative of non-tidal swamps used for grazing buffalo where the highest populations of swamp buffalo were found. The study area (± 567 ha) is part of the Batang hari river watershed and is surrounded by settlements and plantations of oil palm and rubber. The soils are poorly drained, acid fluvisols with low availability of Ca, P and Mg and high solubility of Fe and Zn (Ali et al. 2014).

The dry season extends from April to September while the rainy season normally occurs from October to March with annual rainfall of 2,100–3,264 mm. The study area is regularly inundated for 3–8 months during rainy seasons. Minimum temperatures range from 22 to 25 °C (at 05.00–08.00 h) and maximum temperatures range from 30 to 34 °C (at 11.00–14.00 h). Farmers also use the communal pastures for paddy fields and vegetable production (± 304 ha) when the water level is low.

Forage sampling

In the representative areas forage plants were never fertilized nor had they any management intervention. Observations of forage consumed by grazing buffalo during 08.00–11.00 h and 14.00–17.00 h (Setianah et al. 2004; Hirata et al. 2008) and in our previous study (Ali et al. 2014) revealed that native vegetation species consumed by buffalo included: Poaceae [*Urochloa mutica* (syn. *Brachiaria mutica*), *Leersia hexandra, Hymenachne amplexicaulis, Ischaemum rugosum, Oryza rufipogon*]; Leguminosae (*Mimosa pigra, Sesbania exasperata, Neptunia oleracea, Aeschynomene sensitiva*); Cyperaceae (*Actinoscirpus grossus, Scleria gaertneri, Eleocharis dulcis*); and Onagraceae (*Ludwigia peploides*).

The study area on both left and right sides of the river was divided into 2 subareas based on land use. Samples of the forages eaten by buffalo were randomly collected by hand-plucking during flooding in the wet season (March 2014) and in the dry season (August and September 2014) from at least 2 different sites within each subarea. A mix of leaf and stem (± 250 g) was hand-plucked from the upper parts of herbaceous plants in the pre-flowering stage and from younger twigs of the shrubs, *M. pigra* and *S. exasperata*. Samples for each species were washed with distilled water, chopped and dried at 50 °C. The oven-dried samples were pooled within the subarea and then coarsely milled to pass 1 mm screen for mineral analyses.

Mineral analyses

Representative samples (in duplicate) were digested stepwise with nitric acid (HNO_3). Concentrations of minerals in the forage samples were analyzed by using inductively coupled plasma emission spectrophotometer (SPS7700, Seiko Instruments Inc., Chiba, Japan) in the Laboratory of Feed Technology and Dairy Nutrition, Faculty of Animal Husbandry, Bogor Agricultural University.

Statistical analyses

Data were analyzed using StatView SAS (1999). The differences in means between seasons were determined using an unpaired t-test and were considered significant if $P<0.05$.

Results

While season had a significant effect ($P<0.05$) on concentrations of Ca, P, Mg and S, the effects were inconsistent across species (Table 1). Calcium concentrations in most forages were higher in the flooded season than in the dry season except for *U. mutica*, *N. oleracea*, *S. gaertneri*, *E. dulcis* and *L. peploides*. With the excep-

Table 1. Concentrations of macro- and micro-minerals in forages in swamps in South Sumatra during flooded and dry seasons.

Species	Season	Macro-mineral (% DM)				Micro-mineral (ppm DM)			
		Ca	P	Mg	S	Cu	Fe	Zn	Mn
Poaceae									
Urochloa mutica	dry	0.62b[1]	0.10b	1.59b	0.50b	11.6b	105a	41.8a	366b
	flooded	0.11a	0.01a	0.86a	0.04a	9.6a	107a	60.2a	128a
Leersia hexandra	dry	0.08a	0.16b	1.01b	0.13a	15.3a	193a	128.0b	274a
	flooded	0.12b	0.09a	0.84a	0.14a	12.3a	806b	97.8a	283a
Hymenachne amplexicaulis	dry	0.07a	0.11b	1.02a	0.17a	15.5a	139a	40.9a	225a
	flooded	0.19b	0.08a	1.09b	0.09a	17.5a	208b	56.8b	252a
Ischaemum rugosum	dry	0.08a	0.04a	1.03a	0.06a	13.2a	184b	57.0a	125a
	flooded	0.14b	0.07b	1.11b	0.38b	12.2a	121a	78.1a	297b
Oryza rufipogon	dry	0.12a	0.05a	1.19b	0.03a	16.8a	499b	45.8b	906a
	flooded	0.16b	0.08a	0.95a	0.03a	13.6a	188a	26.1a	1,044b
Leguminosae									
Mimosa pigra	dry	0.32a	0.14b	1.68b	0.30b	22.1b	206a	76.9b	252a
	flooded	0.39b	0.10a	1.54a	0.14a	8.6a	317a	33.7a	342a
Sesbania exasperata	dry	0.42a	0.17b	1.41b	0.30b	23.4a	100a	76.4a	153a
	flooded	0.64b	0.15a	1.17a	0.23a	21.3a	181b	64.3a	128a
Neptunia oleracea	dry	0.66b	0.07a	1.80a	0.05a	43.3b	202a	176.0b	1,076b
	flooded	0.43a	0.14b	2.01b	0.18b	21.7a	358b	43.2a	402a
Aeschynomene sensitiva	dry	0.46a	0.12a	1.98a	0.43a	29.2a	129a	86.7a	407b
	flooded	0.48b	0.13a	2.35b	0.40a	42.1b	145a	94.4a	286a
Cyperaceae									
Actinoscirpus grossus	dry	0.08a	0.21b	0.65a	0.31a	14.3b	89a	49.6b	291a
	flooded	0.20b	0.11a	1.32b	0.33a	9.9a	318b	43.3a	568b
Scleria gaertneri	dry	0.25b	0.06a	0.89b	0.05a	14.7b	69a	48.1a	487b
	flooded	0.10a	0.08a	0.74a	0.08b	9.6a	151b	62.3a	210a
Eleocharis dulcis	dry	0.08b	0.14a	1.01b	0.29a	20.1a	936a	55.1b	231b
	flooded	0.03a	0.20a	0.17a	0.49b	19.1a	1,629b	28.0a	34a
Onagraceae									
Ludwigia peploides	dry	0.56b	0.16a	2.62b	0.16a	31.2a	728a	198.0a	969b
	flooded	0.33a	0.21b	2.06a	0.15a	244.0b	926b	207.0a	691a
Critical level[2]		0.30	0.25	0.20	0.26	11	50	33	40
Toxic level[3]						25	500	750	1,000

[1]Means followed by different letters within a species between seasons are significantly different (P<0.05).
[2]Levels for growth and production of ruminant animals (McDowell 1997).
[3]Levels suggested to produce toxic symptoms in ruminant animals (McDowell 1997).

tion of *U. mutica*, Ca concentrations in Poaceae and Cyperaceae were lower than those in Leguminosae. Concentrations of P in most forages also varied (P<0.05) between seasons with the exception of *O. rufipogon*, *A. sensitiva*, *S. gaertneri* and *E. dulcis*. Some species had higher P concentrations in the dry while others had higher concentrations during flooding. As opposed to Ca concentrations, Mg concentrations in most forages were higher during the dry than in the flooded season.

Concentrations of micro-minerals also varied (P<0.05) between seasons and among species (Table 1). In most Poaceae species, Cu concentrations did not differ significantly between seasons, while Fe concentrations were usually higher during flooding periods and Zn and Mn concentrations were usually higher in the dry season.

Discussion

Macro-minerals

The variation between species in occurrence of peak concentrations of nutrients could be due to a combination of main growth periods, time of maturity and genetic variation (Minson 1990; Underwood and Suttle 1999).

The higher Ca concentrations in Leguminosae and most Poaceae during the flooded season than in the dry season differ from results of earlier studies in tropical regions (Espinoza et al. 1991; Pastrana et al. 1991; Lundu et al. 2012). The higher concentrations in legumes than in grasses agree with the findings of Nasrullah et al. (2004) in dry land of South Sulawesi of 0.3 (*Cenchrus ciliaris*)

to 4.0% (*Desmanthus virgatus*). Results of the current study showed that the range of Ca concentrations was similar to the range in the study of Pastrana et al. (1991) but lower than in other studies (Espinoza et al. 1991; Nasrullah et al. 2004; Lundu et al. 2012). Calcium concentration in the Leguminosae and Onagraceae species exceeded the 0.30% which meets the requirements of ruminants (McDowell 1997).

Phosphorus concentrations in all forages in this study were lower than the critical level for ruminants (0.25%; McDowell 1997) and fluctuated in both seasons. At the early heading stage, Nasrullah et al. (2004) reported that P concentration ranged from 0.20 [*Zea mays* subsp. *mexicana* (syn. *Euchlaena mexicana*)] to 1.80% (*Centrosema plumieri*) and was not significantly affected by season. A higher P concentration in rainy season than in dry season was found in highland forages on acid soils in Colombia (Pastrana et al. 1991) while Lundu et al. (2012) found in Zambia that P concentrations were higher in the hot dry season than in the wet season, ranging from 0.02 to 1.57%.

The low concentrations of Ca and P in forage in our study would be a function of acid soil conditions at this location with low availability of Ca and P for plants (Ali et al. 2014). Jumba et al. (1995) showed a significant positive correlation between soil P and herbage P, while Kanno et al. (2006) showed that soil pH related to not only grass growth and P uptake but also mycorrhizal dependency. Underwood and Suttle (1999) recommended that diets of livestock should have a Ca:P ratio in the range 1:1 to 2:1, and claimed this was more important than the absolute concentration for utilization by ruminants. In our study the Ca:P ratio for individual species ranged from 0.16:1 (*E. dulcis*) to 10:1 (*U. mutica*); thus grazing livestock would have a wide range from which to select an acceptable diet. Depending on the ability to select from available pasture, grazing buffalo, especially lactating females, could suffer from a P deficiency in the diet and would benefit from P supplementation. Studies by Jones (1990) and Miller et al. (1990) described some low-cost strategies on dryland pastures for overcoming P deficiency in grazing livestock. Direct P supplementation through the use of non-protein supplementation in urea-mineral blocks may serve as a more valuable alternative.

With the exception of *E. dulcis* in the flooded season, all forages had sufficient Mg concentration for requirements of ruminants (McDowell 1997); thus buffalo consuming forages in the area are unlikely to suffer Mg deficiency. The adequacy of Mg concentration in most forages has been reported in previous studies (Nasrullah et al. 2004; Lundu et al. 2012).

Sulphur is essential for microbial protein synthesis in the rumen and its concentration in ruminant diets is important. There was wide variation between seasons and species in S concentrations in the forages examined and some species contained sufficient S to satisfy ruminant requirements for effective rumen functioning. Availability of the various species and the opportunity to select by animals would determine the need for supplementation with sulphur. Similarly, S sufficiency was found in two legume trees consumed by goats in low land of Philippines (Uemura et al. 2014).

Micro-minerals

Based on mineral requirements (McDowell 1997) concentrations of Cu, Fe, Zn and Mn in most forages in flooded pasture exceeded the critical levels for grazing ruminants in tropical regions. Very high levels of Cu were found in *N. oleracea* in the dry season and in *A. sensitiva* and *L. peploides* throughout the year, the extremely high concentration in the flooded season in the latter species being particularly remarkable. If these species formed a significant portion of the diet, Cu toxicity could possibly occur in grazing animals. The range in Cu concentration in Poaceae and Leguminosae was higher than that reported by Fariani (2008) of 5.6–10.1 ppm and 3.8–16.6 ppm in 3 species of grasses and 4 species of legumes, respectively, in dry land of South Sumatra. Cu concentration ranges reported for forages in Pakistan were 10.9–25.7 ppm (Khan et al. 2006) and in West Sumatra, Indonesia 3.8–16.6 ppm (Evitayani and Warly 2010).

The generally higher Fe concentration in most Leguminosae, Cyperaceae and Onagraceae species during the flooded season than in the dry season was probably a function of a natural dilution process and translocation of minerals to the roots as plants mature. Khan et al. (2006) also reported that all forages had higher Fe concentrations in winter than in summer with a range from 93 to 202 ppm. As in our study, Khan et al. (2006) found that Zn and Mn concentrations in all forages were above critical levels and overall concentrations were higher in winter than in summer. Similar to Cu and Fe concentrations, ranges of Zn and Mn concentrations in our study were higher than studies in dry land (Khan et al. 2006; Fariani 2008).

Our findings could probably be explained by mineral level in soils (Minson 1990; Underwood and Suttle 1999). Soil condition in the study location was characterized by low pH, low availability of Ca and P, and excessive micro-mineral solubility for plants (Waluyo et al. 2012; Ali et al. 2014). High micro-

mineral concentrations in aquatic plants could be associated with the ability to accumulate these minerals in their tissues (Asikin and Thamrin 2012; Veschasit et al. 2012) and with excessive micro-mineral concentrations in soils and water, possibly caused by intensive agricultural practices. Research by Jan et al. (2011) showed that there were higher concentrations of Cu, Zn, Cr, Ni, Pb and Mn in meat and milk in contaminated areas than in control areas and concluded that consumption of the foods had significantly increased the mineral concentrations in human blood. Similar results have been reported by Skalická et al. (2005), Blanco-Penedo et al. (2006) and Miranda et al. (2009). Therefore, high concentrations of Cu, Fe and Mn in *N. oleracea*, *A. sensitiva*, *E. dulcis* and *O. rufipogon* might not only raise toxicity risks for grazing buffalo, when consumed in large amounts, but also contaminate meat and milk of buffalo when milked or slaughtered for human consumption. Hence, there is a need to further investigate the status of trace and toxic minerals in blood, liver, kidney, meat and milk of buffalo in the region.

Conclusion

Mineral concentrations of forages varied greatly between seasons on flooded pastures in South Sumatra. Concentrations of most minerals appeared adequate for the dietary needs of grazing buffalo given that the samples we collected were not whole plant samples but attempted to be similar to forage that buffalo would select. However, all forages had low P concentrations and grazing animals, especially lactating females, might suffer P deficiency in the diet, while stalled animals fed on a cut-and-carry basis would certainly suffer a deficiency. Feeding studies would be warranted to test benefits from supplementing animals with P.

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Nota Técnica

Estimación de la concentración de clorofila y su relación con la concentración de proteína cruda en tres especies del pasto *Urochloa* en el Piedemonte Llanero, Colombia

Estimating chlorophyll concentration and its relationship with crude protein concentration in three species of Urochloa *in the Piedemonte Llanero, Colombia*

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Resumen

Para conocer la relación entre la intensidad del color verde de las hojas y la concentración de proteína cruda (PC) foliar en los pastos *Urochloa brizantha*, *U. decumbens* y *U. humidicola* en el Piedemonte Llanero de Colombia, se midió la concentración de la clorofila en las hojas (en unidades SPAD), se relacionó con la intensidad del color verde según la Tabla Munsell y se comparó con la concentración de PC determinada por el método Kjeldahl. El análisis de regresión entre las concentraciones de clorofila y PC mostró coeficientes de determinación (r^2) entre 0.76 y 0.88. Aunque los datos obtenidos no permiten generar para las tres especies estudiadas tablas colorimétricas concluyentes basadas en la Tabla Munsell, las relaciones obtenidas son de utilidad para asistir a los productores en sus decisiones sobre la utilización y la fertilización de pasturas de la región.

Palabras clave: Clorofilómetro, índice de verdor, nitrógeno, SPAD, Tabla Munsell.

Summary

To establish the relationship between the intensity of the green color of leaves and their crude protein (CP) concentration in *Urochloa brizantha*, *U. decumbens* and *U. humidicola* pastures in the Piedemonte Llanero, Colombia, the leaf chlorophyll concentration was measured (in SPAD units), classified based on the Munsell color chart and compared with the leaf CP concentration determined by the Kjeldahl method. The regression analysis between chlorophyll and CP concentrations showed coefficients of determination (r^2) between 0.76 and 0.88. While collection of additional data will allow colorimetric charts based on the Munsell color chart to be developed which can be used to predict CP levels for each of these species, the relationships generated can be used to develop recommendations to assist farmers in the region in their decisions on use and fertilizing of these pastures.

Keywords: Chlorophyll meter, leaf greenness, Munsell color chart, nitrogen, SPAD.

Introducción

En gramíneas tropicales el conocimiento de la concentración de nitrógeno (N) es fundamental para la toma de decisiones en relación con el uso del forraje y la necesidad de fertilización (Pardo y Pérez 2010). Como una alternativa a la determinación de la concentración de N por métodos analíticos convencionales, como el

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método Kjeldahl, se ha propuesto la medición de la concentración de clorofila en las hojas y la correlación de la intensidad del color verde con la concentración de proteína cruda (PC) foliar. Esta metodología ha sido exitosamente probada no solo en cultivos como algodón (Neves et al. 2005) y maíz (Argenta et al. 2001; Zotarelli et al. 2003) sino también en algunos pastos, p.ej. *Cynodon dactylon* cv. Tifton 85 (Silva et al. 2009) y *Urochloa brizantha* cv. Marandu (Guimarães et al. 2011).

Esta metodología se basa en que el N es esencial para la formación de la clorofila y hace parte del anillo tetrapirrol que conforma esta molécula (Salisbury y Ross 1994). La concentración de la clorofila se determina con instrumentos como el clorofilómetro y se expresa en grados SPAD (Soil Plant Analysis Development) (Spectrum Technologies 2009). Además, para su determinación se pueden usar equipos con sensores remotos proximales y de contacto que se apoyan en las propiedades reflectivas de las plantas en diferentes regiones del espectro electromagnético (Morais et al. 2011; Zuffo et al. 2012). La intensidad del color verde es cuantificada por comparación utilizando la Tabla Munsell. Esta tabla de colores para tejido de plantas se divide en cinco clases principales: verde, rojo, amarillo, azul y púrpura y presenta subdivisiones como verde-amarillo y amarillo-rojo indicando por un lado, el grado de tono claro u oscuro y por otro el grado de saturación o matiz del color (Munsell Color 1977).

Debido a la alta acidez y baja fertilidad de los Oxisoles que predominan en los Llanos Orientales de Colombia, la producción de biomasa de los pastos cultivados es baja y la calidad nutritiva es afectada principalmente en términos de la concentración de PC (Rincón 2012). Una metodología que permita una rápida evaluación de la concentración de PC foliar en los pastos sería una herramienta útil para la toma de decisiones por parte de los ganaderos respecto al momento apropiado para el ingreso de los animales a las pasturas y para la aplicación de fertilizantes. En este contexto se realizó este estudio, con el objetivo de relacionar las concentraciones de clorofila y PC, y el color de las hojas, en especies de *Urochloa* ampliamente cultivadas en la región de los Llanos Orientales de Colombia.

Materiales y Métodos

El estudio se desarrolló durante la época de lluvias (abril–noviembre) en la Orinoquia colombiana, en la subregión del Piedemonte Llanero que hace parte de la cordillera oriental de los Andes en Colombia, en un Oxisol de la terraza media del Centro de Investigación La Libertad de la Corporación Colombiana de Investigación Agropecuaria (Agrosavia), ubicado en el municipio de Villavicencio, Meta, a 9°06' N y 73°4' O, a 330 m.s.n.m. Los pastos estudiados fueron *Urochloa decumbens* (sin. *Brachiaria decumbens*) cv. Decumbens, *U. humidicola* (sin. *B. humidicola;* antes: *B. dictyoneura*) cv. Llanero y *U. brizantha* (sin. *B. brizantha*) cv. Toledo.

Las muestras para los análisis fueron tomadas en pasturas manejadas con pastoreo rotacional y una edad de rebrote entre 25 y 35 días. El muestreo fue dirigido: de cada pasto se eligieron entre 350 y 400 hojas con diferentes tonalidades de color verde. En el tercio medio de cada hoja se determinó la concentración de clorofila (en grados SPAD) con un clorofilómetro portátil (Minolta Chlorophyll Meter SPAD-502®) para formar grupos de rangos de concentración de clorofila. La intensidad del color verde de 50 hojas en cada grupo fue comparada con los valores de referencia en la Tabla de colores Munsell. Las hojas en cada grupo fueron secadas a 60 °C durante 24 horas en estufa para determinar posteriormente la concentración de proteína cruda (%N × 6.25) por el método Kjeldahl en el Laboratorio de Nutrición Animal de Agrosavia. Para calcular las ecuaciones de regresión entre las concentraciones de clorofila (valores SPAD) y las de PC se usó el programa estadístico SAS 9.3.

Resultados

Los datos obtenidos para cada especie se agruparon en 7 rangos de grados SPAD, con variaciones de 5 unidades para cada rango (Cuadro 1).

Cuadro 1. Rangos de concentración de clorofila en especies de *Urochloa* agrupados por grados SPAD.

Grupo	Concentración de clorofila (grados SPAD)
1	≤20
2	21 – 25
3	26 – 30
4	31 – 35
5	36 – 40
6	41 – 45
7	46 – 50

En los Cuadros 2, 3 y 4 se presentan para cada una de las 3 gramíneas estudiadas las concentraciones de clorofila (en grados SPAD) y de PC, y el color de las respectivas hojas. Se observó un incremento de la concentración de PC y de la intensidad del color verde a medida que incrementó la concentración de clorofila.

Cuadro 2. Concentración de proteína cruda y color de las hojas en 7 grupos de concentración de clorofila en hojas de *Urochloa decumbens* cv. Decumbens. Llanos Orientales de Colombia.

Rango de concentración de clorofila (grados SPAD)	Proteína cruda (%)	Rango de códigos en Tabla Munsell	Rango de colores según Tabla Munsell
≤20	7.2	5 GY 6/8 – 7/10	
20 – 25	8.1	5GY 5/8 – 5/10	
26 – 30	9.5	5 GY 4/8 – 5/8	
31 – 35	10.7	5 GY 4/8 – 5/6	
36 – 40	12.6	5 GY 4/6 – 4/8	
41 – 45	15.9	5 GY 4/4 – 4/6	
46 – 50	17.7	5 GY 4/4 – 4/6	

Cuadro 3. Concentración de proteína cruda y color de las hojas en 7 grupos de concentración de clorofila en hojas de *Urochloa brizantha* cv. Toledo. Llanos Orientales de Colombia.

Rango de concentración de clorofila (grados SPAD)	Proteína cruda (%)	Rango de códigos en Tabla Munsell	Rango de colores según tabla Munsell
≤20	4.5	5 GY 6/8 – 7/8	
20 – 25	5.3	5GY 5/8 – 6/8	
26 – 30	7.4	5 GY 5/8 – 5/6	
31 – 35	7.7	5 GY 5/6 – 4/8	
36 – 40	8.8	5 GY 4/6 – 4/8	
41 – 45	9.6	5 GY 4/4 – 4/6	
46 – 50	12.0	5 GY 4/6 – 4/4	

Cuadro 4: Concentración de proteína cruda y color de las hojas en 7 grupos de concentración de clorofila en hojas de *Urochloa humidicola* cv. Llanero. Llanos Orientales de Colombia.

Rango de concentración de clorofila (grados SPAD)	Proteína cruda (%)	Rango de códigos en Tabla Munsell	Rango de colores según tabla Munsell
≤20	4.5	5 GY 6/8 – 7/8	
20 – 25	4.7	5GY 5/8 – 6/8	
26 – 30	5.7	5 GY 5/6 – 5/8	
31 – 35	6.6	5 GY 5/6 – 4/8	
36 – 40	7.5	5 GY 4/6 – 4/8	
41 – 45	9.2	5 GY 4/6 – 4/4	
46 – 50	12.5	5 GY 4/4 – 4/6	

En el Cuadro 5 se presenta la relación entre la concentración de clorofila y la de PC en las hojas de las gramíneas estudiadas, con coeficientes de determinación (r^2) que variaron entre 0.76 y 0.88.

Cuadro 5. Ecuaciones de regresión entre las concentraciones de clorofila (grados SPAD) y proteína cruda para los pastos *Urochloa decumbens* cv. Decumbens, *U. brizantha* cv. Toledo y *U. humidicola* cv. Llanero. Llanos Orientales de Colombia.

Pasto	Ecuación	r^2
cv. Decumbens	Y = 0.12 + 0.3646x	0.88
cv. Toledo	Y = 0.73 + 0.2289x	0.76
cv. Llanero	Y = 0.504 + 0.202x	0.84

Discusión

Los resultados mostraron una relación alta (r^2 = >0.76) entre la concentración de clorofila y la de PC foliar de las especies estudiadas. Los coeficientes de determinación son similares a los encontrados por Silva et al. (2009) para la relación entre N foliar (g/m^2) y grados SPAD en el pasto Tifton 85 (r^2 = 0.74) y por Guimarães et al. (2011) para la relación entre PC y grados SPAD en el pasto *U. brizantha* cv. Marandu (r^2 = 0.96).

Los resultados mostraron, además, que en un mismo rango de grados SPAD se presentaron diferencias entre los cultivares con respecto a la concentración de PC, mientras que los códigos de colores de la Tabla Munsell fueron similares. Por ejemplo, el valor más alto de PC correspondió al código 5GY 4/4–4/6 de la Tabla Munsell con 17% de PC para el cv. Decumbens, mientras que este mismo valor Munsell correspondió a 10.5 y 12% de PC para los cvs. Toledo y Llanero, respectivamente. Es posible que estas diferencias sean debidas a diferencias genéticas ya que el cv. Decumbens es un pasto adaptado al bajo suministro de N (Rao et al. 1998) y en condiciones de baja fertilidad presenta mayor concentración de PC (Pardo 1998).

Cuando se relacionó la concentración de clorofila con la de PC y los colores en la Tabla Munsell en un mismo cultivar, se presentó repetición en los códigos, encontrándose cercanía y traslape entre los tonos de verde de los grupos establecidos. Esto podría ser debido a la sensibilidad del ojo humano y la consiguiente subjetividad influenciada por la iluminación del ambiente. De todas maneras, no fue posible establecer para un mismo cultivar una relación entre la concentración de PC y los códigos de la Tabla de colores Munsell. Para una acertada asignación del valor de color Munsell o de algún otro patrón de color, habría que introducir en la metodología la conformación de páneles de análisis sensoriales de color.

Conclusión

Fue posible establecer relación entre la concentración de N (proteína cruda) y la de clorofila (medida en grados SPAD) para los cultivares estudiados. Por tanto el uso de un clorofilómetro portátil tiene el potencial de asisitir a los ganaderos en la toma de decisiones sobre prácticas de pastoreo y fertilización en el Piedemonte de los Llanos Orientales de Colombia.

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Short Communication

Growth characteristics, biomass yield and mineral concentrations in seven varieties of Napier grass (*Cenchrus purpureus*) at establishment in Kelantan, Malaysia

Características de crecimiento, producción de forraje y concentración mineral en la fase de establecimiento de siete variedades del pasto Napier (Cenchrus purpureus) *en Kelantan, Malasia*

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Abstract

Growth characteristics, biomass yield and mineral concentrations were evaluated in a completely randomized design study of 7 Napier grass varieties. Data on tiller number per plant, plant height, leaf length, leaf width, stem diameter, leaf:stem ratio and dry matter (DM) yield, as well as concentrations of nitrogen, calcium, magnesium, phosphorus, sodium, potassium, zinc, copper, manganese and iron, were obtained at 2 months growth. The growth characteristics, DM yields and mineral concentrations (except phosphorus) varied significantly ($P<0.01$) among varieties. The variety Indian was tallest (221 cm) and produced the highest DM yield (6.3 t/ha), whereas Dwarf had the highest tiller number and leaf:stem ratio. Purple had the longest and Taiwan and Indian the widest leaves. Kobe, Pakchong and Purple had the greatest stem diameter. Concentrations of Ca, K and Na were greatest in Zanzibar, while Dwarf had the highest concentrations of N, Zn, Mn and Fe. Studies beyond the establishment phase over a range of seasons and in a range of environments at different ages of harvest are needed to confirm the merits of different Napier grass varieties in the study zone.

Keywords: Dry matter yield, macro- and micro-minerals, plant height, tropical grasses.

Resumen

En Kelantan, Malasia se evaluaron las características de crecimiento, la producción de materia seca (MS) y las concentraciones minerales de 7 variedades del pasto Napier durante la fase de establecimiento. Dos meses después de loa siembra fueron determinados el número de brotes por planta, la altura de la planta, longitud de la hoja, ancho de la hoja, diámetro del tallo, rendimiento de MS y relación hoja:tallo, y se analizaron las concentraciones de nitrógeno, calcio, magnesio, fósforo, sodio, potasio, zinc, cobre, manganeso y hierro. Los parámetros de crecimiento, la producción de MS y las concentraciones minerales (con excepción del P) variaron significativamente ($P<0.01$) entre las variedades. La variedad Indian fue la más alta (221 cm) y produjo el mayor rendimiento de MS (6.3 t/ha), mientras que Dwarf tuvo el mayor número de brotes y la relación hoja:tallo más alta. Purple presentó las hojas más largas y Taiwan e Indian las más anchas. Kobe, Pakchong y Purple mostraron el mayor diámetro de tallo. Las concentraciones de Ca, K y Na fueron mayores en Zanzibar, mientras que Dwarf tuvo las mayores concentraciones de N, Zn, Mn y Fe. Se necesitan estudios más allá de la fase de establecimiento, durante un tiempo prolongado, con varias edades de corte y en diferentes condiciones ambientales para confirmar los méritos de las diferentes variedades del pasto Napier en la zona del estudio.

Palabras clave: Altura de planta, forraje, gramíneas tropicales, minerales macro y micro, producción de materia seca.

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Introduction

Ruminant livestock obtain their mineral requirements from concentrates and/or roughage sources, depending on the feeding circumstances. Intake of minerals in the diet must reach the recommended levels if livestock health, reproductive efficiency and performance are to be maintained at a satisfactory level (Ward and Lardy 2005). Obtaining mineral requirements from roughage sources is preferable, since roughage is usually less expensive than concentrates.

Different species of grasses have different capacities to absorb minerals from the soil. Napier grass is one of the most widely used forages for ruminants in developing countries, including Malaysia, because of its high biomass yield and ease of propagation (Halim et al. 2013). A number of varieties of Napier grass are cultivated, including Zanzibar, Indian, Kobe and Taiwan, and these different types vary in morphology, dry matter (DM) yield and nutritive value (Halim et al. 2013). However, little information has been published regarding the mineral composition of forage from various Napier grass varieties. Therefore, the objective of this study was to investigate the growth characteristics and mineral composition of 7 varieties of Napier grass, as a guide for subsequent studies.

Materials and Methods

Study site

This study was conducted at the Agro Techno Park, Universiti Malaysia Kelantan (UMK), Jeli campus (5°44.46′ N, 101°52.31′ E), Kelantan, Malaysia, from July to September 2018. Climatic conditions during the study and the past 9 years (2009–2018) are shown in Figure 1.

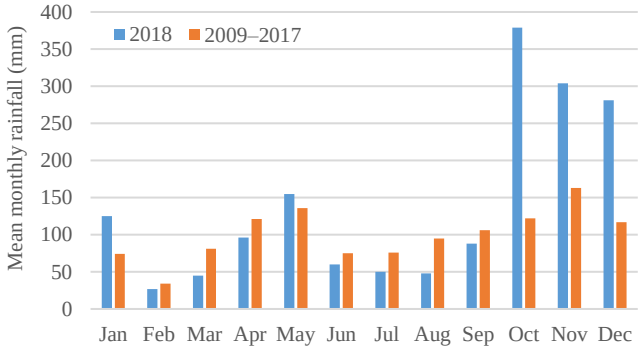

Figure 1. Monthly rainfall during 2018 and short-term mean rainfall (2009–2017) at the experimental station.

The soil was classified as a moderate reddish-brown lateritic soil with the following physical and chemical properties in the top 15 cm of soil as analyzed by conventional methods (Tan 2003): >20% clay; 25 mS/m electrical conductivity; 2.3% N; 4.6% organic matter; 2.7% organic carbon; and pH 5.3.

Experiment establishment

The area selected for planting was cleared and ploughed to provide a good tilth. Stem cuttings of 7 varieties of Napier grass (Taiwan, Zanzibar, Dwarf, Pakchong, Purple, Kobe and Indian) were purchased from a local supplier and planted in rows at spacings of 0.5 × 0.5 m by placing them horizontally in a furrow and covering with soil. Twenty-one plots were established in a completely randomized design to give 3 plots for each variety. The area of each plot was 2.5 × 2.5 m, and the plots were separated by 1.0 m paths.

Before planting, goat manure was applied to the land at a rate of 10 t/ha as a basal fertilizer. Each kilogram of goat manure contained 22.1 g nitrogen (N), 13.4 g phosphorus (P), 2.6 g potassium (K), 25.1 g calcium (Ca), 3.6 g magnesium (Mg), 1.1 g sodium (Na), 18.4 mg copper (Cu), 72.5 g zinc (Zn), 289 mg manganese (Mn) and 150 mg iron (Fe). Lime was also applied at a rate of 50 kg $Ca(OH)_2$/ha. An N:P:K fertilizer (15:15:15) was applied at the rate of 50 kg/ha during Napier grass establishment. Irrigations were applied as determined by weather conditions.

Growth parameters

After 60 days growth the following growth parameters were determined on 5 randomly selected plants per plot: plant height (PH; from the soil surface to the tip of the longest leaf); leaf length (LL); leaf width (LW); stem diameter (SD); and leaf:stem ratio (LSR). The number of plants and total tiller numbers (TN) of all plants were counted for each plot. Plants were harvested at about 10 cm above the ground. Leaf was considered to include leaf blade and leaf sheath. The harvested material was weighed to determine fresh weight, and a representative sample was taken for determination of total dry matter (DM) yield and LSR. For estimation of LSR, plant material was sorted into leaf and stem fractions. Whole plant, leaf and stem were chopped and dried in an oven for 48 hours at 70 °C for dry weight determination. Dried samples of chopped whole plant were ground and sieved through a 1 mm sieve for further chemical analyses.

Chemical analyses

Nitrogen concentration was determined by the Kjeldahl procedure (AOAC 2005) whereas mineral composition (Ca, P, Mg, K, Na, Cu, Zn, Mn and Fe) was determined as described by Cottenie (1980).

Statistical analysis

Data were analyzed by using one-way analysis of variance, and Duncan's Multiple Range Test was used to determine differences between treatment means at P<0.05.

Results and Discussion

Tiller numbers per plant and plant height

Tiller numbers per plant were higher (P<0.05) in the Dwarf variety (9.2 tillers) than in the other 6 (tall) varieties, with an overall mean of 3.6 tillers/plant (Table 1). In contrast with this study, Halim et al. (2013) observed 19.6 tillers per plant in the Dwarf variety compared with 14.8 tillers in tall varieties. This difference might reflect an effect of age and cutting frequency, because the results of the current study were obtained at the first cutting following planting. Similarly, PH differed (P<0.05) by variety, with Indian being the tallest (221 cm) and Dwarf being the shortest (77 cm). The average PH for the 6 taller varieties in the present study was 192 cm (range 158–221 cm), which contrasts with the average PH of 55.7 cm (range 41.6–69.8 cm) for 12 varieties in Kenya reported by Nyambati et al. (2010). These differences might reflect variations in soil fertility, management or environment in the experimental areas (Singh et al. 2013).

Leaf length, leaf width and stem diameter

The LL ranged from 51.5 to 93.0 cm and varied (P<0.05) depending on variety. The LL in this study was similar to that determined by Mdziniso (2012), who reported a range of LLs for Napier grass of 30–120 cm. Similarly, LW varied (P<0.01) among the varieties, with the lowest value measured for Kobe (1.8 cm) and the highest value for both Taiwan and Indian (2.5 cm) (Table 1). The measurements of LW in the present study also agree with the findings of Mdziniso (2012), who reported a range of LWs for Napier grass of 1.0–5.0 cm. Stem diameter also varied (P<0.05) among the varieties, with the lowest value (5.4 cm) for Dwarf and the highest value (6.8 cm) for Kobe, Pakchong and Purple. Nyambati et al. (2010) found that the SDs of 12 Napier grass varieties ranged from 4.0 to 6.8 cm, which was greater than the range in our study. These differences in LL, LW and SD could differ among the varieties due to genetics, as well as other factors like soil fertility and climatic conditions (Singh et al. 2013).

Biomass yield

Dry matter yield at 60 days growth differed (P<0.05) among the varieties, with the lowest yields for Kobe (1.6 t DM/ha) and Dwarf (2.0 t DM/ha) and the highest yield (6.3 t DM/ha) for Indian. These DM yields were much lower than those reported in a previous study (Turano et al. 2016) and could be attributed to the lower rainfall experienced early in the Napier grass establishment period. As expected, in the present study, the Dwarf variety showed a LSR of 3.0 (P<0.05), higher than those of the tall varieties, which ranged from 0.7 to 1.8 (Table 1). Halim et al. (2013) also found the highest LSR in the Dwarf variety.

Table 1. Growth characteristics and dry matter yields for Napier grass varieties.

Parameter[1]	Variety (Mean ± standard deviation)							Overall
	Taiwan	Zanzibar	Kobe	Pakchong	Purple	Indian	Dwarf	
TN/plant	2.5a[2]	3.1a	3.2a	2.2a	2.4a	2.9a	9.2b	3.6
	±0.4	±0.6	±0.2	±0.8	±0.4	±0.3	±1.0	±2.4
PH (cm)	202d	172bc	158b	195cd	203d	221d	77a	175
	±16.6	±9.4	±14.1	±12.3	±28.4	±17.3	±3.2	±47.5
LL (cm)	88.2cd	80.4b	79.3b	83.3bc	93.0d	86.8bcd	51.5a	80.4
	±2.6	±5.1	±3.3	±4.9	±6.9	±4.7	±1.0	±13.4
LW (cm)	2.5c	1.9a	1.8a	2.3bc	2.0ab	2.5c	2.0ab	2.1
	±0.2	±0.3	±0.2	±0.2	±0.2	±0.3	±0.1	±0.3
SD (cm)	6.5bc	6.2b	6.8c	6.8c	6.8c	6.7c	5.4a	6.5
	±0.3	±0.5	±0.1	±0.5	±0.4	±0.2	±0.1	±0.5
LSR	1.0a	1.8a	0.7a	0.7a	0.8a	0.7a	3.0b	1.2
	±0.2	±1.9	±0.1	±0.1	±0.1	±0.2	±0.4	±1.0
Mean DM yield (t/ha)	3.6ab	2.6ab	1.6a	4.7bc	4.2abc	6.3c	2.0ab	3.6
	±1.5	±1.0	±0.2	±1.9	±2.0	±1.8	±0.2	±2.0

[1]TN – tiller number; PH – plant height; LL – leaf length; LW – leaf width; SD – stem diameter; LSR – leaf:stem ratio.
[2]Means within rows followed by different letters are different at P<0.05.

Macro-mineral concentrations in plants

Concentrations of N, Ca, Mg, K and Na in plants varied (P<0.05) among varieties (Table 2), while P concentration did not differ (P>0.05), with an overall mean of 4.8 g P/kg DM. The variation in mineral composition among different varieties suggests that an opportunity exists for cultivation or selection of a suitable variety if the objective is to obtain high levels of dietary minerals from the plants.

Dwarf variety contained higher (P<0.05) N than other varieties. Even Taiwan and Indian varieties, which showed the lowest N concentrations in forage, would provide almost sufficient N for effective functioning of the rumen and for maintenance of growing ruminants if fed as cut-and-carry forage, while the Dwarf variety was well above the maintenance level. Supplementing with N in the form of non-protein-nitrogen, e.g. urea, could increase intake. Where animals were allowed to select from available forage, sufficient N to support weight gains would be obtained.

The Ca concentration in the present study ranged from 2.54 to 6.50 g/kg DM, whereas the recommended Ca requirement for ruminants is 3 g/kg DM (Table 2). Hence, all varieties, apart from Purple and Indian, in this study contained sufficient amounts of Ca to provide adequate dietary Ca for ruminants. The present data showed that P concentrations in Napier grass ranged from 4.1 to 5.6 g/kg DM, with no differences among the varieties, which would supply the normal requirements for ruminants (2.5 g/kg DM) (Table 2).

The differences in K concentration found between the 7 varieties (range 20.2 to 40.4 g/kg DM) were in line with those documented by Turano et al. (2016), who reported a range of K concentrations in forages from 21.5 to 39.1 g/kg DM. These values are higher than the recommended K concentration (6–8 g/kg DM) for ruminants (Table 2). The Mg concentration in forages for ruminants should be 2.0 g/kg DM, as recommended by McDowell and Arthington (2005), but this value is higher than the Mg concentration of the Napier grass varieties studied in the present work. Therefore, all the studied Napier grass varieties need to be fortified with Mg to eliminate Mg deficiency, if these varieties are to be used as the sole feed for ruminants. Most of the Napier grass varieties in this study contained adequate amounts of Na for ruminants (Table 2), but Pakchong, Purple and Indian were deficient in Na.

Micro-mineral concentrations in plants

Table 3 shows that the Dwarf variety contained higher (P<0.05) concentrations of Zn than Pakchong and Purple. Kobe contained higher (P<0.05) concentrations of Cu than all other varieties, except for Zanzibar. The data presented in this study suggest a deficiency in the levels of Cu and Zn for ruminants fed Napier grass. All varieties showed levels of Zn slightly lower than those required by ruminants. Similarly, most of the studied Napier grass varieties showed levels of Cu (except for Zanzibar, Kobe and Dwarf varieties) slightly lower than those required (Table 3).

Concentrations of Mn were higher (P<0.05) in Dwarf than in all other varieties. Similarly, the Dwarf variety contained higher (P<0.05) concentrations of Fe than all other varieties. By contrast, Fe and Mn concentrations in this study were at levels considered adequate to excessive in all 7 Napier grass varieties (Table 3).

Table 2. Macro-mineral concentrations (g/kg DM) in Napier grass varieties.

Mineral	Variety (Mean ± SD)							Overall	Critical level (g/kg DM)
	Taiwan	Zanzibar	Kobe	Pakchong	Purple	Indian	Dwarf		
N	10.9a ± 0.6	11.4a ± 1.4	12.1a ± 1.1	11.1a ± 2.1	11.7a ± 1.6	10.3a ± 1.2	19.7b ± 1.5	12.5 ±3.3	16.6[2]
Ca	3.6ab[1] ±1.6	6.5c ±1.3	4.5abc ±1.08	3.0a ±0.48	2.5a ±0.20	2.9a ±0.73	5.6abc ±1.60	4.1 ±1.7	3.0[3]
Mg	1.4abc ±0.2	1.4abc ±0.1	1.2ab ±0.21	1.3abc ±0.21	1.0a ±0.35	1.5bc ±0.22	1.6c ±0.19	1.3 ±0.3	2.0[3]
K	33.0bc ±5.7	40.4c ±5.9	36.4bc ±6.10	20.2a ±7.17	26.2ab ±7.40	28.8ab ±2.25	36.3bc ±1.81	31.6 ±8.1	6.0–8.0[3]
Na	0.6a ±0.8	2.2b ±0.4	1.0ab ±1.32	0.3a ±0.13	0.2a ±0.02	0.3a ±0.16	0.6a ±0.86	0.8 ±0.8	0.6[3]
P	5.6 ±0.7	5.1 ±2.1	5.4 ±0.5	4.1 ±1.4	4.4 ±1.0	4.1 ±1.2	4.7 ±0.4	4.8 ±1.2	2.5[3]

[1]Means within rows followed by different letters differ at P<0.05. [2]Critical level based on lactating cow needs (McDonald et al. 2011). [3]Critical level based on growing animal (ruminant) needs (McDowell and Arthington 2005).

Table 3. Micro-mineral concentrations (mg/kg DM) in Napier grass varieties.

Mineral	Variety (Mean ± SD)							Overall	Critical level (mg/kg DM)[2]
	Taiwan	Zanzibar	Kobe	Pakchong	Purple	Indian	Dwarf		
Zn	25.4ab[1]	23.1ab	23.6ab	21.5a	21.7a	26.4ab	28.8b	24.4	30
	±1.4	±2.0	±3.1	±3.1	±5.7	±1.9	±4.7	±3.8	
Cu	8.2a	11.3bc	12.2c	8.5a	9.1a	8.9a	10.0ab	9.7	10
	±0.5	±1.1	±0.3	±0.6	±1.0	±1.4	±1.4	±1.7	
Mn	76.7a	79.6a	93.1a	71.2a	81.8a	78.0a	157.4b	91.1	30–40
	±7.4	±13.0	±10.0	±6.0	±4.0	±29.8	±41.8	±33.3	
Fe	114a	116a	139a	87a	120a	134a	241b	136	30
	±12.1	±3.3	±27.5	±11.3	±7.9	±24.5	±87.3	±55.8	

[1]Means within rows followed by different letters differ at P<0.05. [2]Critical level based on growing animal (ruminant) needs (McDowell and Arthington 2005).

Conclusion

The results presented here indicate that Indian, Purple and Pakchong varieties may be better suited to the study area than the other tested varieties by virtue of better PH, LL, LW, SD and DM yield at establishment. Conversely, Dwarf variety showed better TN per plant and LSRs than the other varieties. Zanzibar variety could provide an adequate amount of macro-minerals, such as N, Ca, K and Na, while the Dwarf variety could provide an adequate amount of micro-minerals, such as Zn, Mn and Fe. However, the amount of Mg in all 7 Napier grass varieties may not be sufficient to meet the dietary requirements of ruminants.

Since the information provided here refers to an establishment phase of only 60 days, it should be considered preliminary. However, it is a useful basis for subsequent production phase studies at different stages of harvest and with different growth/cutting cycles with the final objective to allow farmers to choose the most suitable varieties of Napier grass based on their special needs to obtain higher quantity or higher quality of forage.

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