Research Paper

Effects of harvesting age and spacing on plant characteristics, chemical composition and yield of desho grass (*Pennisetum pedicellatum* Trin.) in the highlands of Ethiopia

Efectos de la edad a la cosecha y del espaciamiento en las características de planta, composición química y rendimiento del pasto desho (Pennisetum pedicellatum Trin.) en las tierras altas de Etiopía

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Abstract

The study was conducted to evaluate effects of harvesting age and plant spacing on plant characteristics, composition and forage yield of desho grass (*Pennisetum pedicellatum* Trin.). A factorial experiment with 3 harvesting ages (75, 105 and 135 days after planting) and 3 plant spacings (10×50 , 30×50 and 50×50 cm) with 3 replications was used. The data collected were morphological characteristics such as leaf length, plant height, number of tillers per plant and number of leaves per plant. Chemical analysis was conducted for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), and dry matter yield (DMY) was quantified. Results indicated that the only morphological characteristic significantly (P<0.05) affected by plant spacing was leaf length. However, harvesting age significantly (P<0.01) affected morphological characteristics and DMY as well as CP and NDF (P<0.05). Dry matter yield increased dramatically as harvesting dates were delayed but plant spacing had no significant effect on DMY. Crude protein concentration in forage declined as harvesting dates were delayed (10.9% at 75 d vs. 9.3% at 135 d). Factors such as weed control and amount of planting material required should be the criteria used by farmers to decide inter-row spacing as, within the conditions of our study, row spacing had minimal effect on yield. As only a single harvest at each age was conducted, the yields quoted in this study are not representative of the yields provided by multiple harvests at these intervals. Further studies are needed to quantify these differences.

Keywords: Biomass, harvesting day, morphological characteristics, nutritive value, plant spacing.

Resumen

En el distrito de Farta, Etiopía, se evaluaron los efectos de la edad a cosecha y la distancia de siembra de la hierba desho (*Pennisetum pedicellatum* Trin.) sobre las características morfológicas de la planta, la composición química y el rendimiento de forraje. Se utilizó un experimento factorial con 3 edades a la cosecha (75, 105 y 135 días después de la siembra), 3 espaciamientos de plantas (10×50 , 30×50 y 50×50 cm) y 3 repeticiones. Se evaluaron las características morfológicas: longitud de hoja, altura de planta, número de tallos por planta y número de hojas por planta; se determinaron las concentraciones de proteína cruda (PC), fibra detergente neutra (FDN), fibra detergente ácida (FDA) y lignina detergente ácida; y se cuantificó el rendimiento de materia seca (MS). Los resultados mostraron que la longitud de hoja fue la única característica morfológica que fue afectada (P<0.05) por el espaciamiento de siembra de las plantas,

Correspondence: B. Asmare, Department of Animal Production and Technology, College of Agriculture and Environmental Sciences, Bahir Dar University, P.O. Box 5501, Bahir Dar, Ethiopia. E-mail: limasm2009@gmail.com mientras que la edad a la cosecha afectó tanto las características morfológicas y el rendimiento de MS (P<0.01) como la PC y la FDN (P<0.05). El rendimiento de MS aumentó marcadamente a medida que la edad a cosecha fue mayor, mientras que el espaciamiento de las plantas no tuvo un efecto significativo sobre los rendimientos obtenidos. La concentración de PC en el forraje disminuyó a medida que el intervalo de cosecha fue mayor (10.9% a 75 días vs. 9.3% a 135 días). Los resultados de este estudio sugieren que buenas prácticas de manejo como el control de malezas y la cantidad adecuada de material de siembra deben ser los criterios que deben utilizar los agricultores para seleccionar el espaciamiento entre hileras de siembra, ya que en las condiciones del estudio, la distancia no tuvo efecto sobre el rendimiento. Como se realizó una sola cosecha a cada edad, los rendimientos obtenidos en este estudio no son necesariamente representativos de los rendimientos a obtenerse por múltiples cosechas en estos intervalos. Se necesitan más estudios para cuantificar estas diferencias.

Palabras clave: Características morfológicas, día de cosecha, espaciamiento de plantas, producción de biomasa, valor nutritivo.

Introduction

Livestock production is an integral part of the subsistence crop-livestock systems in the Ethiopian highlands, as livestock provide draft power for land preparation and threshing, plus a source of cash income and assets and nutrition for the rural communities. In addition, livestock are considered as a mobile bank that can be hired, shared, inherited and contracted by rural households (Amede et al. 2005). However, the contribution of this subsector to date has been suboptimal (CSA 2015). One of the important constraints causing low productivity of livestock is low quality and insufficient supply of forage (FAO 2010). Overgrazing is common, resulting in land degradation and low carrying capacity. As a result, the decline in desirable plant species and nutritional value of the available feed resources, particularly protein, means most animals are unable to obtain their maintenance requirements from grazing (Mengistu 1987).

To combat this situation, the use of indigenous forage plants as a feed source, e.g. desho grass (Pennisetum pedicellatum Trin.), is recommended (Leta et al. 2013; Asmare 2016). Desho grass is a perennial grass from Chencha district in southern Ethiopia (Welle et al. 2006) and is currently utilized for soil conservation practices in the highlands of Ethiopia (Heuzé and Hassoun 2015). It is a highly popular, drought-tolerant species, and is used as one of the major feeds for ruminants (Bogdan 1977; FAO 2010; Asmare 2016) with high production potential under a multi-cut harvesting regime (MRDP 1990). However, the optimum plant spacing and intervals between harvests are not well known. The objective of this study was to assess the effects of harvesting age and plant spacing on morphological characteristics, dry matter vield and nutritive value of desho grass in the highlands of Ethiopia.

Materials and Methods

Description of the study area

The agronomic study was conducted in Farta district of northwestern Ethiopia located at 660 km northwest of Addis Ababa (11°32'–12°03' N, 37°31'–38°43' E; 2,720 masl). The topography of the district is 45% gentle slopes, 29% flat land and 26% steep slopes. In terms of land use, an estimated 65% of the area is cultivated and planted with annual and perennial crops, while the areas under grazing and browsing, forests and shrubs, settlements and wastelands account for about 10, 0.6, 8 and 17%, respectively. The total area of the district is estimated to be 1,118 km². The average minimum, maximum and mean temperatures are 9.3, 22.3 and 15.8 °C, respectively. The rainfall pattern is uni-modal (May–September) and mean annual rainfall is 1,445 mm (FDOA 2015).

Treatments and experimental design

A factorial arrangement of treatments was employed using a randomized complete block design with 2 factors (plant spacing and harvesting age) with 3 replications. Three plant spacings within rows (10, 30 and 50 cm) were compared at 3 harvesting dates (75, 105 and 135 days). In all treatments inter-row spacing was 50 cm. The total experimental area was 10×19 m (190 m²) with individual plot size of 3 m² and spacing between plots and replications of 50 and 100 cm, respectively. The land was prepared thoroughly by plowing at the start of the rainy season. Planting material of desho grass was collected from a nursery site at Farta District Office of Agriculture and planted on 15 July 2015. Urea fertilizer was applied at the rate of 100 kg/ha at planting and ammonium phosphate (DAP) was added at 25 kg/ha 21 days after establishment according to the local recommendations (Leta et al. 2013).

Methods of data collection

Data were recorded throughout the experimental period (June 2015-October 2015) on leaf length (LL), plant height (PH), leaf number per plant (LN) and number of tillers per plant (NT). Six plants in each plot were randomly selected for recording data at each harvesting date. The total herbage on each plot at the fixed dates was harvested leaving out border rows. From each plot, an area of 2.2 m² was used to calculate dry matter (DM) yield. Harvesting was done by hand using a sickle, leaving a stubble height of 10 cm, and the harvested herbage was weighed fresh in the field using a field balance. Random samples of fresh forage were taken and oven-dried at 60 °C for 72 h to determine DM concentration, before calculating dry matter yield (DMY). The dried desho grass samples were ground to pass through a 1 mm sieve (Wiley mill) and stored in airtight plastic bags until required for laboratory chemical analysis. Total ash concentration was determined according to AOAC (1990). Nitrogen was determined by the Kjeldahl method (AOAC 1990) and crude protein (CP) concentration was calculated as N% \times 6.25. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations were determined according to Van Soest et al. (1991).

Methods of data analysis

All data were analyzed using the General Linear Model (GLM) procedure of SAS (2007) for least squares analysis of variance. Mean comparisons were done using Duncan's Multiple Range Test (DMRT) for variables whose F-values indicated significant difference. Differences were considered statistically significant at P<0.01 and P<0.05. The statistical model for the analysis of data was: $Y_{ijk} = \mu + H_i + S_j + H_i^*S_j + e_{ijk}$ where:

yijk = all dependent variables (morphological data and chemical composition) collected

 $\mu = overall mean$

 $\begin{array}{l} H_i = the \; effect \; of \; i^{th} \; harvesting \; date \; (75, 105 \; and \; 135 \; days) \\ S_j = the \; effect \; of \; j^{th} \; spacing \; between \; plants \; (10, \; 30 \; and \; 50 \; cm) \end{array}$

 $H_i * S_j$ = the interaction of harvesting date and spacing e_{ijk} = random error.

For correlation analyses of parameters such as morphological characteristics, chemical composition and yield, simple bivariate Pearson correlation was employed.

Results

Morphological characteristics and dry matter yield of desho grass as affected by harvesting age and plant spacing

Overall, there were no significant interactions between the effects of the main treatment variables (plant spacing and harvesting age) so main effects only are presented. The effects of harvesting age and plant spacing on morphological characteristics and dry matter yield of desho grass are shown in Table 1. Mean leaf length at harvesting ages of 75 and 105 days was significantly (P<0.05) greater than at 135 days (18.1, 18.8 vs. 17.4 cm, respectively).

Similarly, mean length of leaves was significantly (P<0.05) greater at the narrow spacing (10 cm) than at the intermediate (30 cm) and wide spacings (50 cm) (19.0 vs. 18.2 and 17.7 cm, respectively). Harvesting age had a significant effect on plant height (P<0.01) with height increasing progressively from 46.2 cm at 75 days harvesting age to 69.8 cm at 105 days and 83.1 cm at 135 days (Table 1). Plant spacing had no significant (P>0.05) effect on plant height with a mean height overall of 66.4 cm.

Table 1. Morphological characteristics and dry matter yield of desho grass as affected by harvesting age and plant spacing.

Parameter		Harvesting	g age (days)	Plant spacing (cm)					
	75	105	135	Mean	10×50	30×50	50×50	Mean	
Leaf length (cm)	19.0a	18.2b	17.7b	18.3	18.1a	18.8a	17.4b	18.1	
Plant height (cm)	46.2c	69.8b	83.1a	63.4	67.3	66.2	65.7	66.4	
Number of tillers/plant	36.4c	93.1b	106.4a	78.6	75.3b	76.7ab	83.9a	78.6	
Number of leaves/plant	249c	554b	710a	508	497	498	517	504	
Dry matter yield (t/ha)	7.1c	15.7b	25.5a	16.1	16.5	16.0	15.7	16.1	

Mean values within rows followed by a different letter are significantly different at P<0.05.

Both harvesting age and plant spacing had significant effects on tiller numbers (Table 1). Mean tiller number per plant increased from 36.4 at 75 days growth to 106.4 at 135 days (P<0.01), while corresponding numbers for different plant spacings were 75.3 tillers/plant at 10 cm and 83.9 tillers/plant at 50 cm (P<0.05). Leaf number per plant, which, in part, determines the photosynthetic capacity of the plants, was significantly (P<0.01) affected by harvesting age, while plant spacing had no effect on this parameter (P>0.05) (Table 1). Number of leaves per plant increased from 249 leaves at 75 days to 410 leaves at 135 days.

The DM yield of desho grass was significantly (P<0.01) affected by harvesting age but not by plant spacing (P>0.05) (Table 1). Total DM harvested increased progressively from 7.1 t/ha at 75 days of age to 25.5 t/ha at 135 days of age. Mean DM yield overall for the different plant spacings was 16.1 t/ha.

Chemical composition of desho grass as affected by harvesting age and plant spacing

The chemical composition of desho grass as affected by harvesting age and plant spacing is shown in Table 2. The DM concentration showed minimum variation and ranged from 88.2 to 89.1%. Crude protein (CP) concentration was significantly affected (P<0.05) by harvesting age, declining from 10.9% at 75 days to 9.3% at 135 days. Crude protein yields (CPY) increased progressively and significantly (P<0.01) as growth period increased (0.76 t/ha at 75 days to 2.36 t/ha at 135 days; Table 2). By contrast, plant spacing had no significant effect (P>0.05)

on CPY with an overall mean of 1.57 t/ha. Ash concentration declined significantly (P<0.01) as harvesting age increased with values at 75 days exceeding those at 105 and 135 days (Table 2). Progressive increases in plant spacing resulted in significant increases in ash concentration (Table 2).

Organic matter (OM) concentration increased progressively (P<0.01) as harvesting age increased and decreased progressively (P>0.05) as plant spacing increased (Table 2). While NDF concentration increased significantly as harvesting age (P<0.05) and plant spacing (P<0.05) increased, ADF concentration increased significantly (P<0.01) with increase in harvesting age but was unaffected (P>0.05) by plant spacing (Table 2). The highest ADF concentration (48.1%) was recorded in desho grass harvested at 135 days and grown at 50 cm plant spacing.

Correlation among morphological characteristics and chemical composition of desho grass

The relationships among morphological parameters, nutritional parameters and yield of desho grass are shown in Table 3. The analysis showed that DM % and DMY were positively correlated (P<0.01). These parameters were also positively correlated with most chemical parameters, e.g. CPY plus NDF, ADF, ADL and OM concentrations. This indicated that, as the DM % increased, cell wall constituents also contributed to the increase in DMY. However, DM % and DMY were negatively correlated (P<0.01) with CP % and total ash %.

Table 2. Chemical composition of desho grass as affected by harvesting age and plant spacing.

Parameter		Harvestin	g age (days)	Plant spacing (cm)					
	75	105	135	Mean	10×50	30×50	50×50	Mean	
Dry matter (%)	88.2b	88.4b	89.0ab	88.5	89.1a	88.3b	88.2b	88.5	
Ash (%)	9.16a	7.89b	7.0b	8.0	6.15c	8.15b	9.74a	8.1	
Organic matter (%)	79.1c	80.6b	82.0a	80.6	83.0	80.2	78.5	80.9	
Crude protein (%)	10.9a	10.2ab	9.3b	10.2	9.6	10.2	10.7	10.2	
Crude protein yield (t/ha)	0.8c	1.6b	2.4a	1.57	1.5	1.6	1.6	1.6	
Neutral detergent fiber (%)	45.2b	46.2b	51.7a	47.7	45.2c	47.8ab	50.1a	47.7	
Acid detergent fiber (%)	33.1c	37.6b	42.6a	37.8	37.6	38.1	41.5	38.1	
Acid detergent lignin (%)	17.3b	18.3b	20.7a	18.8	16.9c	18.8b	20.5a	18.7	

Within parameters and treatments, means with different letters within rows are significantly different (P<0.05).

Table 3. Correlation coefficients among morphological parameters, nutritional parameters and yields of desho grass.

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	DM	DMY	СР	CPY	Ash	OM	NDF	ADF	ADL	LL	NT	PH	NL
DM	1	0.55**	-0.43*	0.44*	-0.69**	0.81**	0.41*	-0.064	0.063	-0.02	0.31	0.44*	0.40*
DMY		1	-0.62**	0.95**	-0.45*	0.50**	0.49**	0.59**	0.59**	-0.42*	0.87**	0.94**	0.92**
СР			1	-0.29	0.26	-0.32	-0.26	-0.17	-0.08	0.18	-0.40*	-0.44*	-0.44*
CPY				1	-0.41*	0.44*	0.44*	0.63**	0.62**	-0.43*	0.87**	0.93**	0.91**
Ash					1	-0.98**	-0.41*	0.029	0.28	-0.23	-0.35	0.47*	0.44*
OM						1	0.44*	-0.40	-0.24	-0.18	0.36	0.49**	0.46*
NDF							1	0.12	-0.009	-0.27	0.03	0.47*	0.42*
ADF								1	0.6**	-0.46*	0.65**	0.58**	0.63**
ADL									1	0.62**	0.55**	0.53**	0.54**
LL										1	-0.29	-0.30	-0.30
NT											1	0.95**	0.97**
PH												1	0.97**
NL													1

Level of significance: ** = P < 0.01; * = P < 0.05; DM = dry matter %; DMY = dry matter yield; CP = crude protein %; CPY = crude protein yield; Ash = ash %; OM = organic matter %; NDF = neutral detergent fiber %; ADF = acid detergent fiber %; ADL = acid detergent lignin %; LL = leaf length; NT = number of tillers per plant; PH = plant height; and NL = number of leaves per plant.

Discussion

Plant characteristics and their relation with DM yield

The absence of any significant effect of plant spacing on dry matter yield (DMY) (P>0.05) was at variance with the findings of Melkie (2005), who demonstrated the highest DMY at narrow spacing, which he attributed to the greater number of plants per unit area. In our study the higher plant population at narrow plant spacing was counteracted to some extent by the greater number of tillers per plant produced at wider plant spacing, although individual leaves were longer at narrow plant spacing. The finding that narrow plant spacing (10 and 30 cm) produced longer leaves than wider spacing (50 cm) supports the results of Yasin et al. (2003), who reported that narrow spacing in Napier grass increased interplant competition, causing individual plants to grow taller with longer internodes, plus slender, thin and weak stalks due to poor light exposure and hence poor photosynthetic output. However, Melkie (2005) and Alemu et al. (2007) reported the opposite effect for Bana grass (Pennisetum purpureum × Pennisetum americanum hybrid), where leaf length at relatively narrow plant spacing was shorter than at medium and wider plant spacings.

The higher dry matter yields at later stages of harvesting were to be expected as plants were taller, had more tillers per plant and more leaves per plant. All these characteristics would contribute to increased photosynthetic activity and hence higher DM production. Ansah et al. (2010) showed that total herbage yield in Napier grass increased with increase in harvesting age (60<90<120 days), which these authors attributed to the increase in tiller number, leaf formation, leaf elongation and stem development. Similarly, Melkie (2005) reported that yield of Bana grass increased as harvesting stage increased.

The observed high number of leaves per plant at later stages of harvesting reinforces the findings of Asmare (2016) with the same grass species, Butt et al. (1993) and Melkie (2005) with Bana grass and Zewdu et al. (2002) with Napier grass. Generally, the longer the vegetative phase and the taller the plant, the greater the number of leaves produced (Hunter 1980), a situation reflected in our study as the number of leaves from new tillers generally increased with increase in age at harvesting. The increase in plant height with harvesting age was not unexpected. In the same grass species, Asmare (2016) showed that plant height increased as plant age at harvest advanced to 120 days. Increments in plant height at later harvest stages could be due to massive root development and efficient nutrient uptake, allowing the plant to continue to increase in height as mentioned by Melkie (2005).

The current finding that the number of tillers per plant increased as plant spacing increased agrees with Melkie (2005), who reported similar results for Bana grass. At wider spacing, light can easily penetrate to the base of the plant and this may have stimulated tiller development. Moreover, under wider spacing competition for nutrients is less, so individual plants can support more tillers. For Napier grass, Yasin et al. (2003) reported that, when sufficient space is available to the individual plant, there is capacity to increase the number of tillers per plant with the variation among the different spacings being ascribed to variable nutritional areas and access to light. At narrow spacing, plants reach maturity before the achievement of optimal leaf area, which is important for estimating pasture productivity. Thus, the lower tiller counts at narrow plant spacing may be due to high plant competition for resources, namely light, space and nutrients. The increased competition for light causes reduced growth and tillering capacity. Interplant competition in grass causes rapid and exhaustive height increments, so that overcrowding results in neighboring plants producing weak tillers (Boonman 1993). Therefore, the competitor plants are forced to grow upright to dominate other tillers produced on the same plant rather than expanding laterally by bearing more tillers.

Desho grass harvested at young age in this study had excellent nutritional value, particularly high CP concentration, a limiting nutrient in tropical forages. Even forage cut at 135 days of age had CP concentrations well above 7.0%, which is the level below which voluntary intake of ruminants might be depressed. All of the forage produced would provide sufficient energy and protein to support some level of production above a maintenance level. However, harvesting at the early stage resulted in low DM yields at that harvest. Allowing the plants to grow until 135 days of age resulted in much higher yields without a great reduction in quality despite some reduction in CP concentration and increase in NDF. In any pasture situation, compromises between quality and yield must be made when deciding at what stage to harvest or graze a crop or pasture.

With regard to plant spacing and forage production, both narrow and wide plant spacings have implications for different aspects of forage production (Rao 1986) as the number of plants per unit area is the primary source of competition. Generally, narrow plant spacing suppresses the emergence of various weeds, but additional planting material is required. When density is maintained above optimum, there will be greater total demand for resources that results in stress in the plants (Trenbath 1986). Wider plant spacing requires less planting material and enables greater tillering capacity in forage grasses but the probability of weed invasion increases and may lead to extra cost of weeding. Again compromises must be made. Individual farmers may find that the optimum plant density and total population differ from those of others based on the resources at their disposal. Yasin et al. (2003) indicated that the correct use of relatively inexpensive and simple management practices such as correct plant spacing, regular weeding, appropriate cutting systems and application of fertilizers can help increase the level of fodder production.

Chemical composition and its relation to yield

Chemical analyses of forage in this study revealed that results conformed with other studies in terms of the effects of age at harvest on quality parameters. As would be expected, the highest CP concentration was obtained at the earliest stage of harvesting, with values declining as harvesting was delayed. This result agrees with the findings of Asmare (2016) for the same species. Similarly, Bayble et al. (2007) and Ansah et al. (2010) reported for Napier grass a decreasing trend of CP with increase in harvesting age (60>90>120 days). This phenomenon is referred to as a growth dilution effect with increase in structural carbohydrate content of forage materials harvested at late maturity reducing the percentage of protein in the forage.

Despite the reduction in CP percentage with time, crude protein yield (CPY) increased significantly as harvesting was delayed. Similar findings have been reported by Asmare (2016) for the same grass species and by Melkie (2005), who recorded mean CPYs at 60, 90 and 120 days of age of 0.47, 0.91 and 0.85 t/ha, respectively, in Bana grass. Obviously, decisions on the optimal time to harvest desho grass will depend on a compromise between yield and quality of forage.

However, plant spacing had no marked effect on CPY. Since CPY is the product of total DM yield and CP concentration in the plant and there were no significant effects of plant spacing on either of these parameters, one would not expect to record a significant outcome. Our results are at variance with those of Melkie (2005), who found lower CPY at a spacing of 75×75 cm than at 100×50 cm.

As would be expected, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations all increased significantly (P<0.05) as harvesting time was delayed. Increase in plant spacing also resulted in higher NDF and ADL levels, although ADF was unaffected. While increase in stem percentage and increased lignification with maturity would account for the age effects, the increases with wider plant spacing would possibly reflect larger tiller development in the wider-spaced plants. Zewdu et al. (2002) and Bayble et al. (2007) reported that the predominant features of increasing plant density or narrow spacing were a marked reduction in leaf:stem ratio, which in turn resulted in an increase in cell wall and lignin concentrations in Napier grass. The increasing trend of NDF concentration with increase in harvesting age agrees with Asmare (2016) for the same grass species, where NDF concentration increased from 72.8% at 90 days to 77.7% at 150 days of

age. Bayble et al. (2007) recorded a similar trend when Napier grass was harvested at 60, 90 and 120 days.

Conclusions

This study has documented the increases in yield of desho grass as days to harvest are increased and has highlighted the reduction in quality, especially reduced CP concentrations and increased NDF, ADF and ADL concentrations, with advancing maturity. Farmers could use this information to assist in making decisions based on the relative importance of forage yield and quality in their operations. While delayed harvesting results in increased DMY, this is at the expense of a reduction in quality. However, these data do not present a complete picture as the pasture harvested early would regrow and the reduction in yield we observed would be much greater than actually would occur, where repeated cuttings would be made for the earlier ages of harvest.

The absence of any differences in yield with variation in plant spacing indicates that farmers can make their decisions on what spacing to use based on other factors, e.g. forage quality issues, weed control etc. Our findings suggest that plant spacing within rows can be varied quite markedly without any variation in forage yield and within the bounds of the spacings we used, farmers can choose a spacing to suit their conditions. An important limitation of this study was that the measurements of total forage yield at different harvest frequencies were not carried out. This information would be needed before a farmer could use these data effectively in decision making. Moreover, leaf:stem ratio was not measured in this study, a good indicator of forage quality. Both limitations of this study could be addressed in future studies.

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(Received for publication 26 December 2016; accepted 26 March 2017; published 31 May 2017)

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