

Chemical composition and fatty acid profile of a seed-propagated elephant grass genotype, ensiled at different regrowth ages

Composição química e perfil de ácidos graxos de um genótipo de capim-elefante propagado por sementes, ensilado em diferentes idades de rebrota

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Highlights

Forage harvested at 75-120 days of regrowth presented low nutritional value.

Silage produced at 75-120 days of regrowth presented low nutritional value.

Forage and silage presented moderate to high linoleic acid content.

Forage and silage presented moderate to low α -linolenic acid content.

Abstract

This study aimed to evaluate the chemical composition and fatty acid (FA) profile of forage and silage of a seed-propagated elephant grass genotype called "PCEA" harvested at 75, 90, 105 and 120 days of regrowth. A randomized block design with five replications was used. The results were analyzed by mixed models ($P < 0.05$) that included treatment (regrowth age) as a fixed effect and block as a random effect. Linear and quadratic effects of the treatments were analyzed using orthogonal contrasts. There was linear increase on total dry matter (DM) forage production and linear decrease on leaf:stem ratio as a function of the increase in regrowth age ($P < 0.05$). In response to the advance of regrowth age, "PCEA" forage and silage showed linear decreases on crude protein (CP) content (g kg^{-1} DM) and *in vitro* DM digestibility (g kg^{-1}); and linear

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increases on DM (g kg^{-1}), lignin (g kg^{-1} DM), and acid detergent insoluble protein (%CP) contents ($P < 0.05$). All "PCEA" silages had DM content $< 200 \text{ g kg}^{-1}$, $\text{pH} > 4.0$, and ammonia N content $> 10\%$ total N. In response to the advance of regrowth age, linear decreases ($P < 0.05$) were observed on forage and silage linoleic, α -linolenic, and total FA contents (g kg^{-1} DM). From 75 to 120 days of regrowth, the seed-propagated elephant grass genotype "PCEA" presents forage and silage with low nutritional quality for feeding dairy cattle, moderate to high linoleic acid contents, and moderate to low α -linolenic acid contents. The "PCEA" forage and silage obtained from 75 to 120 days of regrowth presents low potential for production of milk naturally enriched with bioactive FAs beneficial to human health.

Key words: *Cenchrus purpureus*. Linoleic acid. Linolenic acid. *Pennisetum purpureum*. Silage.

Resumo

Objetivou-se por meio deste estudo avaliar a composição química e o perfil de ácidos graxos (AG) da forragem e da silagem de um genótipo de capim-elefante propagado por sementes denominado "PCEA", colhido aos 75, 90, 105 e 120 dias de rebrota. O delineamento experimental foi em blocos casualizados com cinco repetições, sendo os resultados analisados por modelos mistos ($P < 0,05$), que incluíram tratamento (idade de rebrota) como efeito fixo, e bloco como efeito aleatório. Os efeitos lineares e quadráticos dos tratamentos foram analisados por contrastes ortogonais. Houve aumento linear na produção total de matéria seca (MS) de forragem e decréscimo linear na relação folha:colmo, em função do aumento na idade de rebrota do capim ($P < 0,05$). Em resposta ao avanço na idade de rebrota, houve redução linear no teor de proteína bruta (PB) (g kg^{-1} MS) e na digestibilidade *in vitro* da MS (g kg^{-1}); e aumento linear nos teores de MS (g kg^{-1}), lignina (g kg^{-1} MS) e proteína insolúvel em detergente ácido (% da PB) da forragem e da silagem de "PCEA" ($P < 0,05$). Todas as silagens produzidas apresentaram teor de MS $< 200 \text{ g kg}^{-1}$, $\text{pH} > 4,0$ e teor de N amoniacal $> 10\%$ do N total. Em resposta ao avanço da idade de rebrota, decréscimos lineares ($P < 0,05$) foram observados nos teores (g kg^{-1} MS) dos ácidos linoleico, α -linolênico e AG totais. De 75 a 120 dias de rebrota, o genótipo de capim-elefante propagado por sementes "PCEA" apresenta forragem e silagem com baixa qualidade nutricional para alimentação do gado leiteiro, com moderado a alto teor de ácido linoleico, e moderado a baixo teor de ácido α -linolênico. A forragem e a silagem do "PCEA" obtidas de 75 a 120 dias de rebrota apresentam baixo potencial para produção de leite naturalmente enriquecido com AG bioativos benéficos à saúde humana.

Palavras-chave: Ácido linoleico. Ácido linolênico. *Cenchrus purpureus*. *Pennisetum purpureum*. Silagem.

Introduction

Elephant grass [*Cenchrus purpureus* (Schumach.) Morrone (syn. *Pennisetum purpureum* Schumach.)] is a tropical grass widely used for feeding cattle in milk production systems in Brazil. In addition to the high production of forage of good nutritional quality and acceptability by cattle, it has great versatility in the form of use, and

it is recommended under rotational grazing as well as in a cut-and-carry system to supply chopped forage into the trough or for its ensilage (Pereira et al., 2016b; Oliveira, 2018).

Regardless of its intended usage, elephant grass is vegetatively propagated through stems. Considering only the processes related to obtaining stems (cutting, selection, preparation, and transport), this

corresponds to ~7.3-12% of the total cost of formation of 1 ha of elephant grass (Empresa de Assistência Técnica e Extensão Rural do Distrito Federal [EMATER-DF], 2019; Pereira et al., 2021). Additionally, the method of vegetative propagation is associated with several operations in the field, such as those presented by Figueiredo et al. (2019) and Pereira et al. (2021): opening of planting furrows, distribution of stems in planting furrows and hand chipping, and cover of stems with soil etc. These operations make it difficult and costly to implement the crop. Other disadvantages of the vegetative propagation method include: the need for a public or private system responsible for the production/distribution of propagules with guaranteed sanitary quality and varietal purity; impossibility of storing propagules for long periods; and a more restricted planting season. Moreover, the continuous multiplication of vegetative parts can lead to the accumulation of diseases, contributing to less persistence of the plant, less productivity, and lower quality of the forage. On the contrary, propagation through seeds has the advantages of easier harvesting, storage, and transport of seeds as well as faster and easier planting with lower labor costs, resulting in lower cost of implanting the crop (Pereira et al., 2003).

In 1991, Embrapa Dairy Cattle (Juiz de Fora, MG, Brazil) initiated a breeding program of elephant grass addressing the development of improved cultivars for feeding ruminants, and one of the objectives was to obtain seed-propagated cultivars thus reducing the costs of implanting the crop and accelerating the diffusion of the cultivars (Pereira et al., 2003). As a result of this program, a seed-propagated elephant grass intraspecific genotype called

"PCEA" was developed from crosses carried out within a population of *C. purpureus*.

Currently, there is a growing interest in the consumption of milk and derivatives naturally enriched with bioactive components potentially beneficial to human health, such as rumenic acid (*cis*-9, *trans*-11 CLA) (Alves et al., 2017). This fatty acid (FA) is the major isomer of conjugated linoleic acid (CLA) present in ruminant milk fat (Bernard et al., 2018), and anticarcinogenic, antidiabetogenic (type 2 diabetes), antiatherogenic, and immunomodulatory properties have been attributed to rumenic acid (B. Yang et al., 2015). α -linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3) is the main FA in forages and the most useful for enhancing the milk fat quality (Glasser et al., 2013). Cows with diets high in α -linolenic, linoleic acid (*cis*-9, *cis*-12 C18:2), and oleic acid (*cis*-9 C18:1) have the potential to produce milk with an FA profile that is more nutritionally desirable for human health. From this perspective, elephant grass has been under investigated regarding factors known to influence the FA composition, such as regrowth age and forage conservation method.

This study was designed to investigate the agronomic characteristics of the plants and the chemical composition and FA profile of forage and silage of PCEA during a range of maturity at which the elephant grass cultivars are usually harvested and fed to dairy cattle.

Materials and Methods

The study was conducted from December 2015 to February 2017 at Embrapa Dairy Cattle, located in the municipality of Coronel Pacheco, Minas Gerais State,

Brazil, whose geographical coordinates are 21°33'22" S latitude, 43°6'15" W longitude with an average altitude of 410 m. According to the Köppen classification, the climate of the region is type Cwa (mesothermal), with a well-defined hot/rainy season during spring-summer from October to March, and a cold/

dry season during autumn-winter from April to September. The climatic data during the period of plant growth until harvest were obtained from a meteorological station located approximately 1 km away from the experimental area (Figure 1).

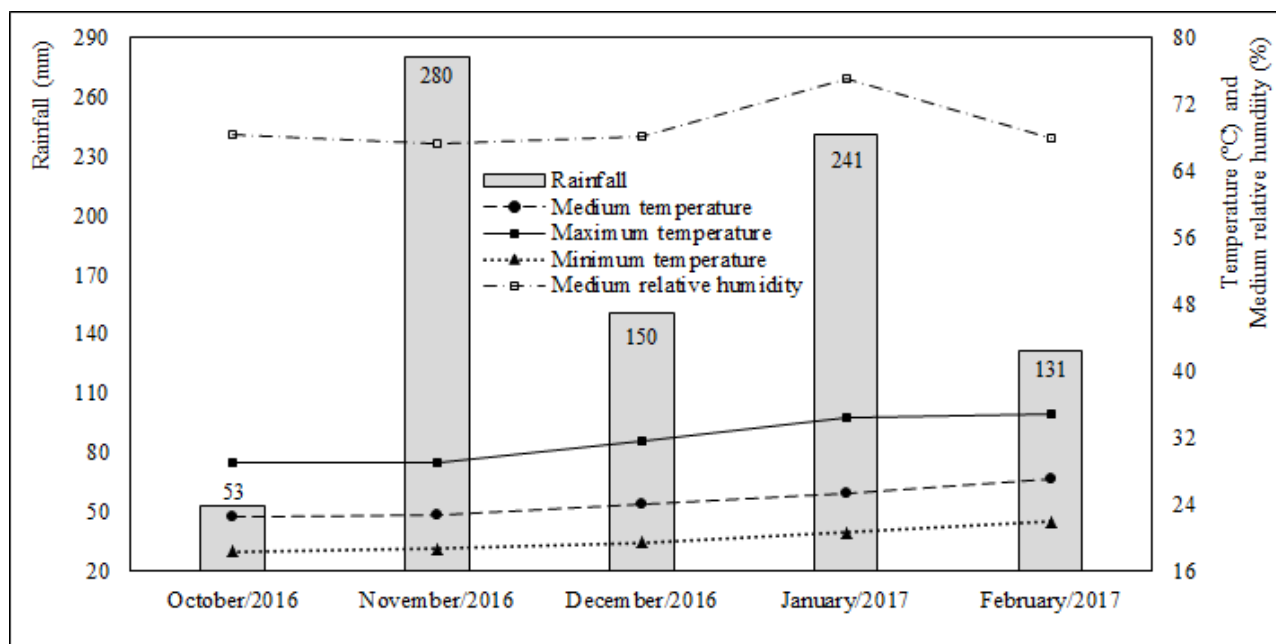


Figure 1. Climatic data during the period of growth of the plants until the moments of forage cutting.

Source: Instituto Nacional de Meteorologia - INMET (<https://bdmep.inmet.gov.br/>)

To evaluate the agronomic characteristics of PCEA grass, the forage and silage chemical composition and fatty acid (FA) profiles, and the silage fermentation parameters, four regrowth ages were defined: 75, 90, 105 and 120 days. These regrowth ages were determined according to Pereira et al. (2016a), considering that the main objective of the study was to evaluate the forage for silage production. Pereira et al.

(2016a) recommended, to produce BRS Capiáu elephant grass silage, that the harvest should be performed when the plants reach ages between 90 and 110 days of regrowth. Considering the regrowth age of 90 days and to space the cut-off ages by 15 days, an earlier cut-off age of 75 days and two later cut-off ages of 105 and 120 days were included in the study.

A randomized block design with five replications was used, totaling 20 experimental plots. The blocks were established according to the slope of the terrain in the experimental area. Each parcel was composed of four lines at 5 m in length and spaced 1 m. The useful area of the plot was formed by a 2 m center of two internal lines, totaling 4 m². The PCEA grass was established in a flat area (600 m²) on red-yellow latosolic soil with a clay texture containing the following chemical characteristics: pH = 5.9; organic matter = 25.3 g kg⁻¹; H + Al = 2.8 cmolc dm⁻³; P = 6.4 mg dm⁻³; K = 53 mg dm⁻³; Ca²⁺ = 1.6 cmolc dm⁻³; Mg²⁺ = 0.9 cmolc dm⁻³; and Al³⁺ = 0.0 cmolc dm⁻³. Sowing was performed on December 11, 2015, with a sowing density of 135 viable seeds per m²; 100 kg ha⁻¹ of P₂O₅ was used during sowing. In January 2016 the experimental area was fertilized with 200 kg ha⁻¹ of NPK 10-10-10, and on February 12, 2016, another fertilization was conducted with 350 kg ha⁻¹ of NPK 20-05-20. On October 10, 2016, a standardization cut in the plants was executed at 10 cm from the ground level, and the plots were fertilized on November 7, 2016, with 400 kg ha⁻¹ of NPK 20-05-20, when the plants reached approximately 0.30 m in height.

The four cuts were made at 15-day intervals, the first being on December 24, 2016, and the last on February 7, 2017. The cuts were made in the morning in the useful areas of the plots at a height of 10 cm in relation to the ground level using a 35 cm steel blade machete. Immediately after cutting, the plants belonging to each useful area of each plot were disintegrated in a stationary electric forage chopper (model EN-9F3B; Nogueira Máquinas Agrícolas, São João da Boa Vista, SP, Brazil) and regulated to obtain forage

particles with average size of 2 cm. The forage of each plot was manually homogenized, and two 400 g subsamples were collected and frozen at -20°C until chemical composition and FA profile analyses. The first subsample was thawed, pre-dried at 55°C, milled to 1 mm, and analyzed at the Food Analysis Laboratory of Embrapa Dairy Cattle (Juiz de Fora, MG) for DM at 105°C (INCT-CA G-003/1 method), mineral matter – MM (INCT-CA M-001/1 method), crude protein – CP (INCT-CAN-001/1 method), ether extract – EE (INCT-CA G-0051/1 method), acid detergent fiber – ADF (INCT-CA F-003/1 method), neutral detergent fiber corrected for ash and protein – NDF_{ap} (INCT-CA F-001/1 method), lignin (INCT-CA F-005/1 method), and acid detergent insoluble protein – ADIP (INCT-CA N-005/1 method) according to Detmann et al. (2012). In this subsample, *in vitro* DM digestibility (IVDMD) was determined according to D. J. Silva and Queiroz (2002), and carbohydrate fractionation analysis was performed according to Sniffen et al. (1992). Total carbohydrates (TC) were calculated using the formula TC = 100 - (CP + EE + ash), and non-fibrous carbohydrates (NFC), which constitute fractions A and B1, were obtained using the formula NFC = TC – NDF_{ap}. Fraction C was obtained by multiplying the lignin value by 2.4, and fraction B2 = NDF_{ap} - fraction C. The second subsample was lyophilized (model L120, Liotop, Liobras, São Carlos, SP, Brazil), ground (1 mm), and analyzed for FA composition at the Laboratory of Chromatography of Embrapa Dairy Cattle (Juiz de Fora, MG) according to the procedures described by Lopes et al. (2021).

Before ensiling, the forage of each plot was manually homogenized and directly ensiled without wilting using no additives or bacterial inoculants. The ensiling was carried

out in experimental polyvinyl chloride (PVC) silos at 10 cm in diameter and 30 cm height. The compaction of forage in the silo was performed by hand with the aid of a wooden socket, obtaining an average final density of forage mass of $\sim 800 \text{ kg m}^{-3}$. The silos were closed with vulcanized rubber caps attached with a clamp adapted with *Bunsen* valves to allow the escape of gas due to fermentation. After 90 days of storage at room temperature in a location protected from sunlight, the silos were weighed, and the silages were removed. After discarding 5 cm layers of silage from the bottom and top (which are often moldy), the contents of each silo were homogenized, and two subsamples of 400 g were collected and frozen at -20°C until later analysis of chemical and FA composition as described for forage. A third sample of silage of 100 g was pressed in a hydraulic press to obtain a juice, which was strained and measured for pH (model T-1000; Tekna Indústria Comércio e Serviços de Manutenção de Instrumentos Analíticos Ltda., São Bernardo do Campo, SP, Brazil). One 10 mL aliquot of silage juice was added to flask containing 0.4 mL sulfuric acid (50% v/v) and frozen until ammonia N content analysis according to the INCT-CA N-007/1 method (Detmann et al., 2012).

The losses through fermentation gases (% initial DM) in the ensiling process and the DM recovery index were calculated as described by Furtado et al. (2019).

The height of the plants was measured with a graduated ruler with 10 cm intervals at four points per plot before each cut was made. The production of green forage biomass was determined by weighing all forage harvested in the plot using a dynamometer graduated in grams. At the time of harvesting, three plants

from each plot were taken to the laboratory for separation into leaf lamina, stem (stems + leaf sheaths), and dead material. After separation, these fractions were chopped, weighed, and pre-dried in a forced ventilation oven at 55°C until constant weight, and the results were used to obtain the dry mass of the different components of the vegetation as well as the leaf:stem ratio.

The results were analyzed according to a randomized block design replicated five times using the procedure for mixed models of SAS version 9.0. The model included the fixed effect of treatment (regrowth age), and block and experimental error were considered random effect. Linear and quadratic effects of the treatments were analyzed using orthogonal contrasts. The results are reported as least square means, and effects were considered significant when $P < 0.05$. Regression analyses were performed using the REG procedure of SAS, and Pearson's correlation studies were performed using the CORR procedure of SAS.

Results and Discussion

This is the first known study to assess the chemical compositions and FA profiles of forage and silage of a seed-propagated elephant grass developed from crosses conducted within a population of *C. purpureus* (intraspecific).

There were linear increases ($P < 0.05$) in DM forage production and plant height, linear reductions ($P < 0.05$) on leaf:stem ratio and proportion of leaves (Figure 2), and quadratic effect ($P = 0.0026$) for the proportion of dead material on PCEA forage in response to the increase in regrowth age (Table 1).

Corroborating Carvalho et al. (2018), the plant height was positively correlated with forage DM production. In response to the linear increase in plant height (x , m), the forage DM production (\hat{y} , t ha⁻¹) also increased linearly from 75 to 120 days of regrowth, as shown by the equation $\hat{y} = 15.84003x - 32.57223$ ($r^2_{\text{adj}} = 0.88$; $P < 0.0001$). During this period, daily increases of 1.61 cm in the height of the plants ($P = 0.0001$) and 210 kg ha⁻¹ in the forage DM production ($P < 0.0001$) were observed (Figure 2). These values are lower than 1.99 cm and 230 kg ha⁻¹ previously obtained by Ferreira et al. (2019) and 3.50 cm and 382 kg ha⁻¹ obtained by Monção et al. (2019), who evaluated the elephant grass cultivars BRS Canará and BRS Capiçu at the regrowth ages from 42 to 105 days, and from 30 to 150 days, respectively, during the rainy season of Brazil. These two elephant grass cultivars are characterized by being tall plants with high forage production (Pereira et al., 2016b). The forage DM productions for BRS Capiçu at 90 and 120 days of regrowth were 23.78 and 33.29 t ha⁻¹, respectively (Monção et al., 2019) while BRS Canará presented forage DM productions of 18.55 and 22.26 t ha⁻¹ at 91 and 105 days of regrowth, respectively (Ferreira et al., 2019); those are all higher than DM productions obtained for PCEA at respective regrowth ages. In another study also performed during the rainy season in Brazil, Bhering et al. (2008) reported daily increments of 112 kg ha⁻¹ in forage DM production of elephant grass cultivar Napier Roxo at the regrowth ages from 30 to 105 days. The forage DM productions at 75, 90, and 105 days of regrowth were 7.55, 9.33, and 8.44 t ha⁻¹, respectively, which are all lower than those obtained for PCEA at these

regrowth ages (Table 1). In a study carried out in Costa Rica with elephant grass cultivars Taiwan, King, Gigante and Cameroon, Mora and Figueroa (2005) reported forage DM productions at 70 and 126 days of regrowth that ranged from 4.94 to 7.35 and 9.77 to 17.23 t ha⁻¹, respectively, which are also all lower than those obtained for PCEA at 70 and 120 days of regrowth (Table 1). Thus, compared to the results of these four studies, the PCEA generally presented medium forage DM production.

However, in response to the increase in plant height with the advance of regrowth age, there was a rapid and sharp decline in the proportion of leaves (Figure 2) and, consequently, in the leaf:stem ratio (Table 1). Both metrics are of great importance for the nutritional value to dairy cattle and for the agronomic management of the forage plants. As elephant grass develops and advances towards physiological maturity, there is an increasing competition for light inside the canopy. Therefore, a strategy to increase the photosynthetic capacity of the leaves without compromising the structure of the plant is to elevate the apical meristem through the internode elongation and thickening of the stems, with the objective of positioning the leaves on the top of the canopy, maximizing light interception (Ferreira et al., 2019). However, this morphological alteration mechanism of the canopy architecture promotes a substantial increase in the proportion of stems at the expense of leaves (Queiroz et al., 2000; Bhering et al., 2008; Ferreira et al., 2019).

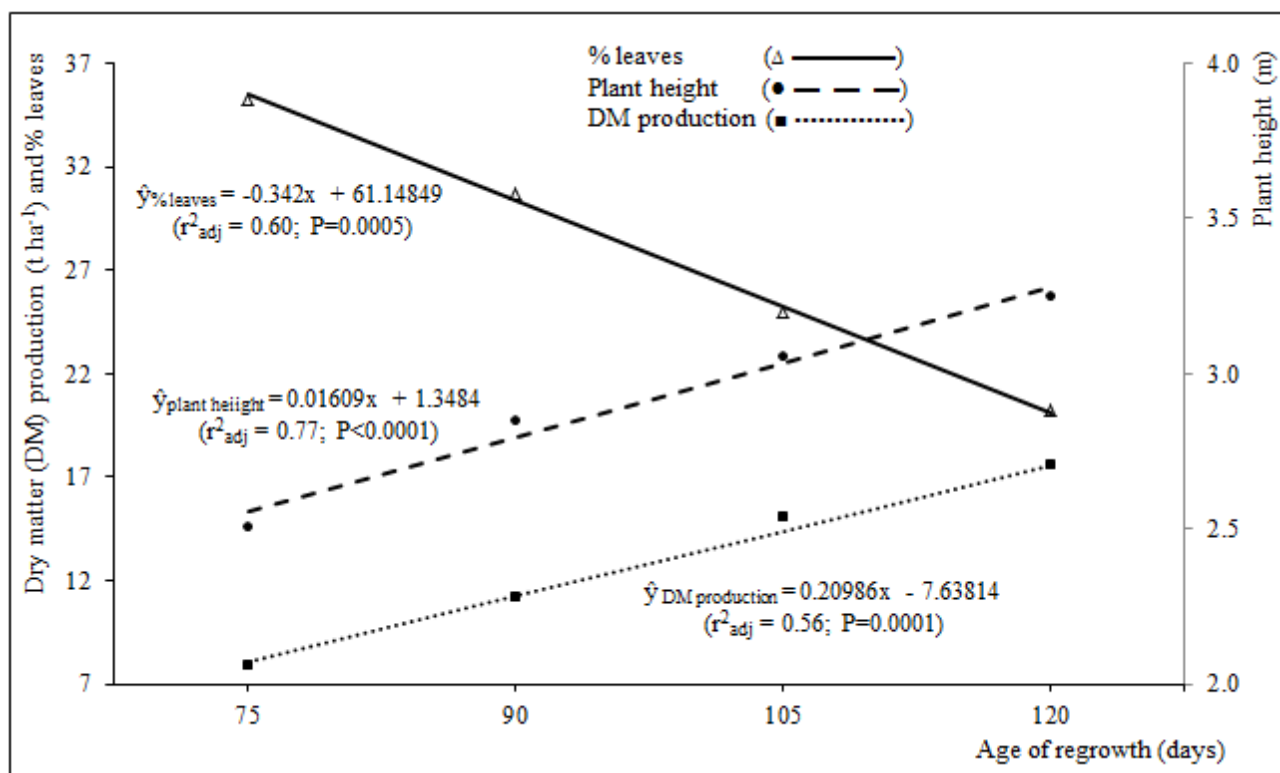


Figure 2. Agronomic characteristics of a seed-propagated elephant grass genotype at various regrowth ages.

Table 1

Agronomic characteristics of a seed-propagated elephant grass genotype at different regrowth ages

Item	Regrowth ages (days)				SEM	P-value	
	75	90	105	120		Linear	Quadratic
Plant height, m	2.51	2.85	3.06	3.25	0.07	<0.0001	0.1599
Leaf: stem ratio	0.67	0.51	0.38	0.28	0.04	<0.0001	0.3932
% leaves	35.3	30.7	25.0	20.2	2.46	0.0005	0.9577
% dead material	3.2	8.3	8.9	9.2	0.75	<0.0001	0.0026
Forage DM production, t ha ⁻¹	7.9	11.2	15.1	17.6	1.60	0.0001	0.7667

In response to this greater vertical development of elephant grass, there is death of tillers and shading of the lower leaves inside the canopy (Sanches et al., 2019),

promoting an increase in the proportion of dead material (Queiroz et al., 2000; Ferreira et al., 2019) which, in PCEA, reached an estimated maximum value of 9.7% at 109

days of regrowth. Proportionally, the greatest increase (+159%) in the proportion of dead material occurred from 75 to 90 days of regrowth, coinciding with the period in which the greatest proportional increase in plant height (+14%) was also observed (Table 1). At 75 days of regrowth, the proportion of dead material was similar to that of 3.43% reported by Ferreira et al. (2019) in rainy season (Table 1). However, the values observed at 90 and 105 days of regrowth were 126% and 120% higher than those obtained by Ferreira et al. (2019) at 91 and 105 days of regrowth, respectively. The daily increase in the proportion of dead material reported by Ferreira et al. (2019) was 0.07 compared to 0.12 percentage points in PCEA. This marked increase in the proportion of dead material with the advance of regrowth age is indicative of the speed with which the process of senescence in PCEA occurred, and, associated with the marked reduction in proportion of leaves, this increase contributed greatly to the nutritional quality losses of the forage.

The leaves are the photosynthetic organs of the plants, responsible for sunlight interception and photoassimilate production, and are, therefore, rich in cellular contents, which have high nutritional value for the growth and development of dairy cattle. Alternatively, despite the important contribution to the total production of DM forage, the stems present high levels of cell wall components, such as structural carbohydrates and lignins, which have low nutritional quality, because they are supporting structures of the plant (Ledea-Rodríguez et al., 2018). Corroborating this, it was demonstrated in studies conducted

in Costa Rica, Ghana, Cuba, and Brazil, that the leaves compared to the stems of several elephant grass varieties presented higher levels of CP (Mora & Figueroa, 2005; Ansah et al., 2010; Sanches et al., 2019) and lower contents of NDF, ADF, and lignin (Ansah et al., 2010; Sanches et al., 2019).

The proportion of leaves in the PCEA grass showed negative correlations ($P < 0.05$) with components associated with the reduction in nutritional quality of forage, namely: ADF, NDF_{ap} , lignin, ADIP, and fraction C of carbohydrates contents ($r = -0.70, -0.77, -0.57, -0.51$ and -0.55 , respectively). On the other hand, positive correlations ($P < 0.05$) were observed between the proportion of leaves and those components that are associated with increases in the nutritional quality of forage, specifically IVDMD, NFC, CP, and total FA contents ($r = 0.65, 0.74, 0.68$ and 0.59 , respectively). Figures 3a, 3b, and 3c illustrate the negative response that the linear reduction ($P = 0.0005$) in the proportion of leaves with the advance of regrowth age promoted on the nutritional value of PCEA forage. Linear decreases ($P < 0.0001$) in the CP and NFC contents were observed, and linear increases ($P < 0.01$) in the lignin levels, ADF, ADIP, and fraction C of carbohydrates were seen. Consequently, a linear decrease ($P < 0.0001$) in the forage IVDMD was observed. Additionally, there were linear increases ($P < 0.0001$) on forage DM (Figure 4a) and NDF_{ap} contents; linear decrease ($P = 0.0085$) on MM content were seen, and there was no effect ($P > 0.05$) of regrowth age on the forage EE content, which ranged from 11.5 to 14.2 g kg^{-1} DM (Table 2).

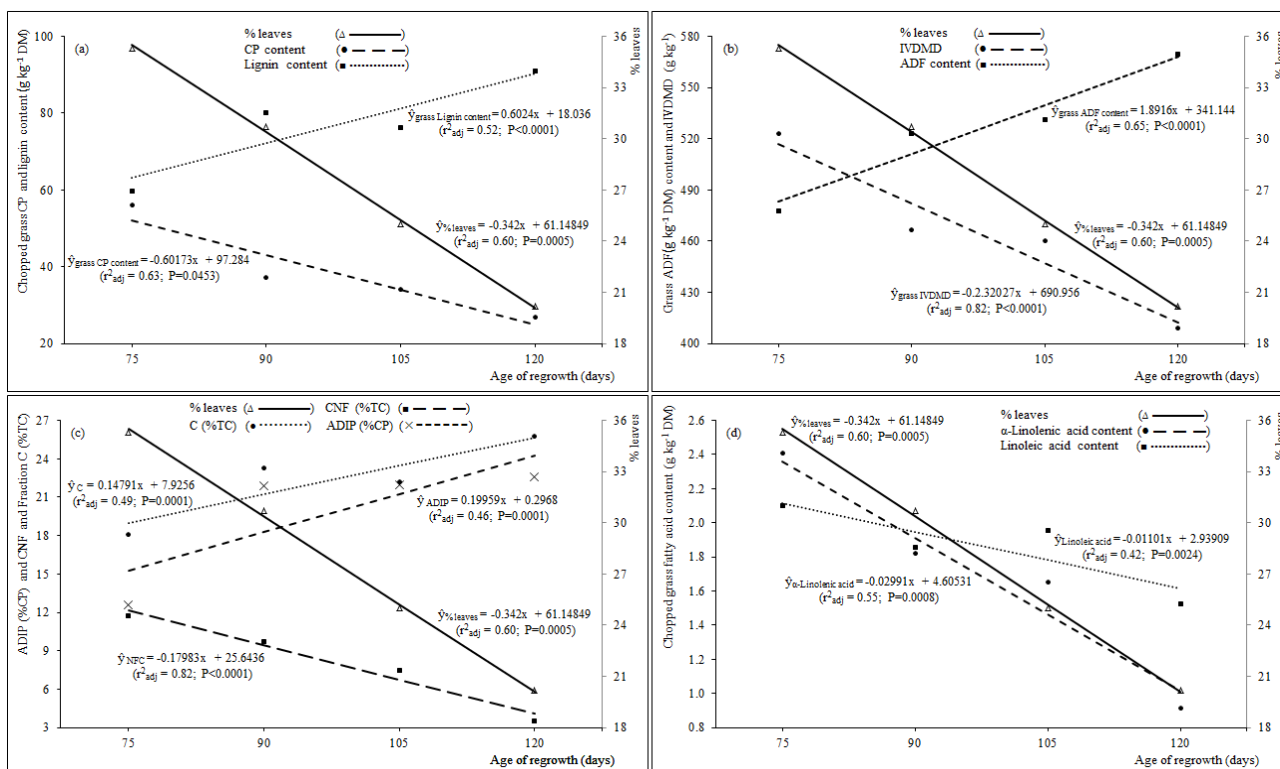


Figure 3. Proportion of leaves (%) and (a) crude protein (CP) and lignin contents (g kg⁻¹ dry matter - DM); (b) acid detergent fiber (ADF) content (g kg⁻¹ DM) and *in vitro* DM digestibility (IVDMD, g kg⁻¹); (c) acid detergent insoluble protein – ADIP (%CP), non-fibrous carbohydrate – NFC (%total carbohydrate – %TC) and Fraction C of carbohydrate (%TC); and (d) linoleic and α-linolenic acid contents (g kg⁻¹ DM) in forage of a seed-propagated elephant grass genotype harvested at different regrowth ages.

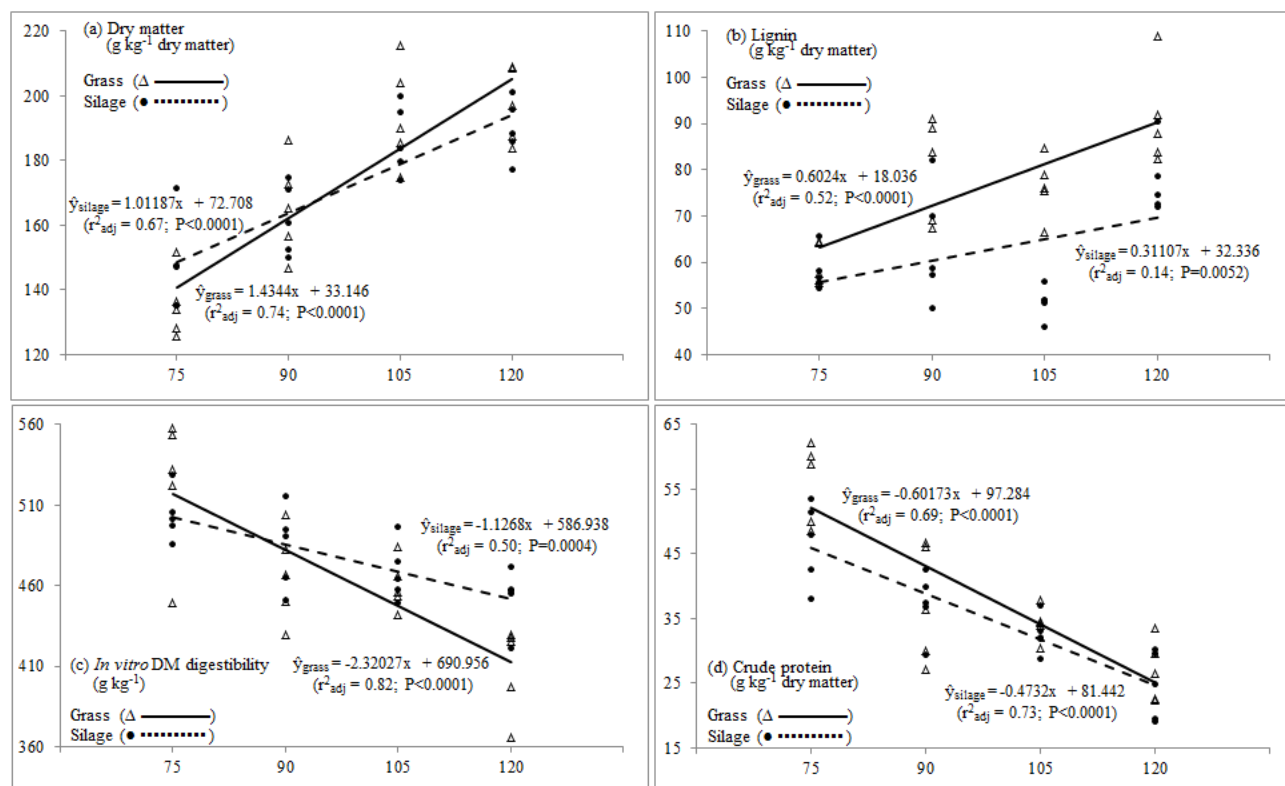


Figure 4. Chemical composition (g kg⁻¹ dry mater) of chopped forage and silage of a seed-propagated elephant grass genotype harvested at different regrowth ages (days).

Table 2

Chemical composition (g kg⁻¹ dry mater – DM) of chopped forage and silage of a seed-propagated elephant grass genotype harvested at different regrowth ages

Item	Regrowth ages (days)				SEM	P-value	
	75	90	105	120		Linear	Quadratic
Chopped forage							
DM (g kg ⁻¹ as fed)	135.1	165.5	194.1	197.3	6.01	<0.0001	0.0230
Mineral matter	138.4	124.7	126.5	116.7	5.91	0.0085	0.6717
IVDMD (g kg ⁻¹) ^a	523.0	466.6	460.3	409.1	13.70	<0.0001	0.8252
Ether extract	14.1	14.2	13.6	11.5	1.38	0.1810	0.4099
Acid detergent fiber	478.0	523.0	531.5	569.8	10.46	<0.0001	0.7358
Neutral detergent fiber ^b	719.5	759.2	777.9	828.0	7.94	<0.0001	0.4550
Lignin	59.6	80.1	76.3	91.0	3.88	<0.0001	0.4234
Crude protein (CP)	56.0	37.3	34.2	26.9	2.72	<0.0001	0.0453
ADIP (%CP) ^c	12.6	21.9	22.0	22.6	1.19	0.0001	0.0031
Fraction B2 (%TC) ^d	70.2	67.0	70.3	70.7	0.86	0.1973	0.0406
NFC (%TC) ^e	11.7	9.7	7.5	3.5	0.63	<0.0001	0.1011
Fraction C (%TC)	18.1	23.3	22.2	25.8	1.00	0.0001	0.4044
Silage							
DM (g kg ⁻¹ as fed)	147.4	161.8	186.5	189.8	5.20	<0.0001	0.2499
Mineral matter	142.6	128.7	135.5	129.6	4.98	0.1216	0.3823
IVDMD (g kg ⁻¹) ^a	503.9	483.5	468.4	452.5	8.92	0.0004	0.7789
Ether extract	26.5	24.1	22.5	19.5	1.63	0.0042	0.8269
Acid detergent fiber	497.9	483.4	477.3	503.6	14.18	0.8501	0.1293
Neutral detergent fiber ^b	731.9	733.7	732.3	758.9	11.67	0.1005	0.2388
Lignin	58.0	63.6	51.3	77.7	3.54	0.0052	0.0054
Crude protein (CP)	46.8	37.2	32.6	24.7	2.28	<0.0001	0.7281
ADIP (%CP) ^c	7.2	7.2	10.3	13.5	1.24	0.0011	0.1720
Fraction B2 (%TC) ^d	74.5	70.9	74.3	67.9	1.09	0.0057	0.2186
NFC (%TC) ^e	7.7	10.3	10.3	9.6	1.16	0.3128	0.1889
Fraction C (%TC)	17.8	18.8	15.4	22.5	0.96	0.0129	0.0035

^a*In vitro* DM digestibility; ^bFree from ash and protein; ^cAcid detergent insoluble protein; ^dTC = Total carbohydrates; ^eNFC = non-fibrous carbohydrate.

In a study conducted with crossbred steers fed exclusively elephant grass cultivar Cameroon with decreasing levels of CP and NFC and increasing levels of ADIP, NDF_{ap} , ADF, and lignin due to the increase from 33 to 93 days in the regrowth age, Machado et al. (2008) observed a linear reduction in DM intake, as well as in the apparent digestibility of DM, CP, and NDF_{ap} . According to the study, the low CP intakes observed in response to the advance in regrowth age of the grass were insufficient in meeting the requirements of ruminal microorganisms which compromised fiber digestion and reduced DM intake. Machado et al. (2008) also highlighted the low CP contents in the forage at regrowth ages of 78 and 93 days (53.2 and 51.8 g kg⁻¹ DM, respectively), which were above the minimum dietary content of 70 g kg⁻¹ DM required to sustain microbial growth and support efficient fibrous carbohydrate digestion (Lazzarini et al., 2009). It should be noted that the PCEA forage at 75 days of regrowth showed CP content above the recommended minimum dietary level (56.0 g kg⁻¹ DM, Table 2). Additionally, the low DM and NFC contents with the high NDF_{ap} and ADF contents (Table 2) indicate that dairy cattle fed exclusively with PCEA forage at 75 days of regrowth will present a compromise in productive performance. At 90 days of regrowth, despite the important 23% increase in DM content, the nutritional quality of PCEA forage continued to fall dramatically, mainly due to the 11%, 33%, and 17% reductions of the IVDMD, CP, and NFC content, respectively; 74%, 34%, and 29% increases of the ADIP, lignin, and fraction C of carbohydrates content, respectively, were observed (Table 2). At 120 days there were still increases in the levels of NDF_{ap} , ADF, lignin, and fraction

C of carbohydrates and decreases in IVDMD and NFC content, except for the CP content which, compared to the forage at 105 days of regrowth, stabilized at an extremely low level (26.9 g kg⁻¹ DM) (Table 2). Thus, from 75 to 120 days of regrowth, the PCEA forage presented low nutritional quality for feeding dairy cattle.

Interestingly, the proportions of leaves and the leaf:stem ratios at 75, 90, and 105 days of regrowth of PCEA grass (Table 1) are all higher than those reported by Ferreira et al. (2019) at 75, 91, and 105 days of regrowth of elephant grass cultivar BRS Canará harvested during the rainy season. However, the nutritional quality of the forages obtained at these regrowth ages were generally always superior to those of PCEA grass (Table 2), ranging from 511.0 – 555.1, 60.3 – 81.0, 646.1 – 733.8, 428.7 – 485.2, and 12.48 – 13.74 for total digestible nutrients and the contents of CP, NDF_{ap} , ADF, and ADIP, respectively (Ferreira et al., 2019). These results show characteristics that are intrinsic to the PCEA grass regarding changes in the plant's morphological composition (proportions of leaves, stems, and dead material) and in the metabolism of cellular content and cell wall components in response to physiological maturity; there is an accelerated process of senescence of the plants and rapid loss of the forage nutritional quality. Marked differences between elephant grass cultivars in growth rate, degree of lignification, and accumulation of structural carbohydrates as the harvesting age increased were reported by Zailan et al. (2016) and demonstrate different mechanisms in the genotypic expression of the cultivars in response to the external factors influence, such as edaphoclimatic conditions and plant management (Ledea-Rodríguez et al., 2018).

Using the equations generated (Figures 2, 3a, 3b, and 3c), the chemical composition of PCEA forage at the regrowth ages of 45 and 60 days were estimated at: 97.7 and 119.2, 586.5 and 551.7, 139.1 and 118.3, 70.2 and 61.2, 426.3 and 454.6, and 45.1 and 54.2 for DM, IVDMD, NFC, CP, ADF, and lignin contents, respectively. It should be noted that only at the regrowth age of 45 days was the CP content higher than the recommended minimum dietary level of 70 g kg⁻¹ DM, but the forage presented low DM content, while the levels of ADF, lignin, and NFC were within acceptable limits for dairy cattle nutrition. However, the estimated forage DM production was extremely low (1.8 t ha⁻¹), which could compromise the profitability of PCEA for feeding livestock.

In general, the losses in nutritional quality in PCEA forage due to the increase in the regrowth age were reflected in PCEA silages. In response to the advance in regrowth age, a linear increase ($P < 0.0001$) was observed in the silage DM content, which always remained above of 200 g kg⁻¹ (Table 2) and can be considered challenging for silage production as it requires a rapid acidification of the silage to pH ≤ 4.0 to allow an adequate fermentation and efficient conservation of ensiled material (Tomich et al., 2003). There were also significant linear increases in the silage contents of ADIP (Table 2), lignin (Table 2, Figure 4b), and fraction C of carbohydrates (Table 2); and a significant linear decrease ($P = 0.0042$) in the EE content was observed (Table 2, Figure 5), which indicates marked losses in the silage nutritional quality.

Reflecting these losses, the silage IVDMD was also significantly, linearly reduced ($P = 0.0004$) with the advance in the regrowth age, and IVDMD presented consistently low values, which ranged from 452.5 – 503.9 g kg⁻¹ (Table 2; Figure 4c). There was no effect ($P > 0.05$) of the regrowth age on the silage contents of MM, ADF, NDF, and NFC (Table 2).

The forage CP content did not achieve the recommended minimum level of 70 g kg⁻¹ DM at any regrowth age (Table 2). This worsened in the silages, where the CP content significantly decreased linearly ($P < 0.0001$) and varied from 24.7 – 46.8 g kg⁻¹ DM, lower than those in the forages originally ensiled (Table 2; Figure 4d). The positive correlation between the forage CP content and the silage N ammonia content ($r = 0.60$; $P = 0.0079$) explains part of the reduction in the silage CP content. The greatest proportional decrease in CP content occurred at 75 days of regrowth (-16%; 56.0 *versus* 46.8 g kg⁻¹ DM), likely because the lowest DM content in the forage harvested at this regrowth age (Table 2) allowed a greater proliferation of unwanted microorganisms. This, in the presence of high moisture content when ensiling wet crops (Muck, 2010; Yuan et al., 2020) and under silage pH above 4.0 (Khota et al., 2016), as all those in the present study (Table 3), promoted: undesirable secondary fermentations; protein, carbohydrate, and lactic acid degradation; acetic and butyric acids, carbon dioxide, hydrogen, amines, and ammonia N production; and DM losses (Muck, 2010; Khota et al., 2016).

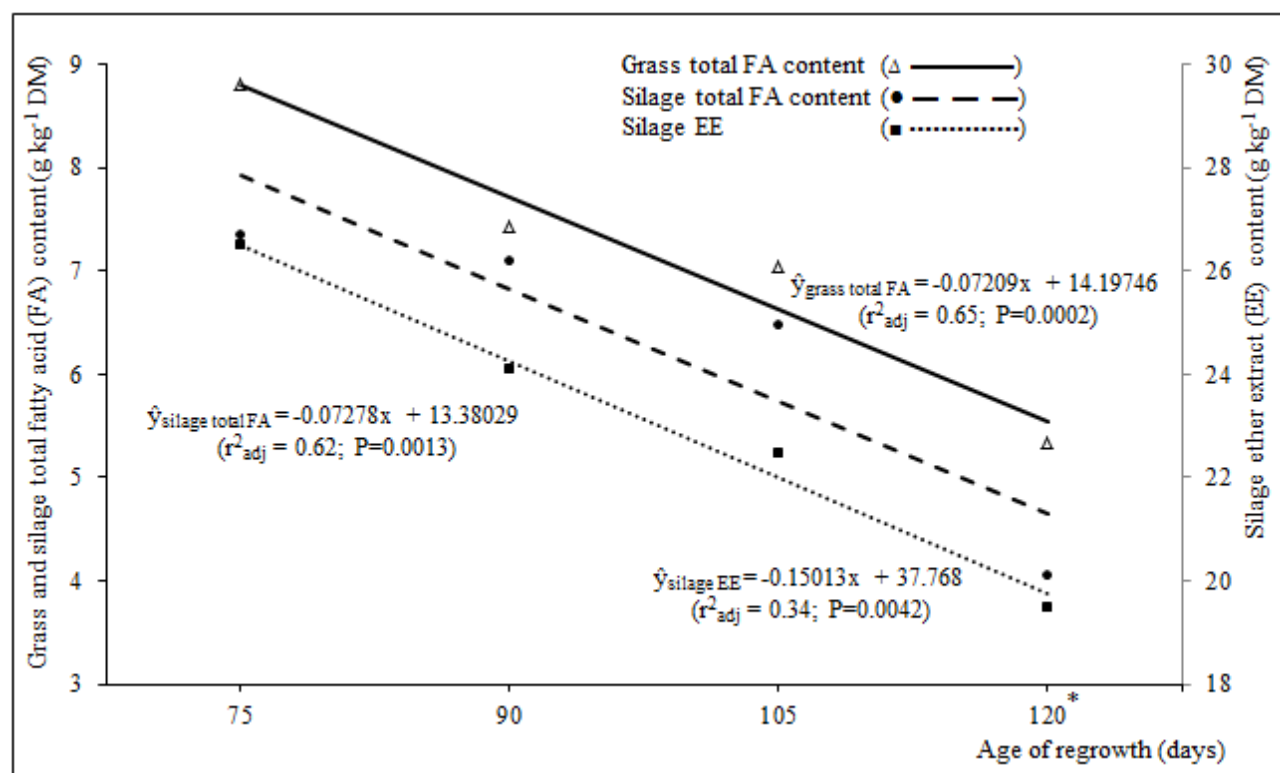


Figure 5. Total fatty acid contents (g kg^{-1} dry matter - DM) in chopped grass and silage and ether extract content in silage (g kg^{-1} DM) of a seed-propagated elephant grass genotype at different regrowth ages.

Table 3

Fermentation parameters of silage of a seed-propagated elephant grass genotype, harvested at different regrowth ages

Item	Regrowth ages (days)				SEM	P-value	
	75	90	105	120		Linear	Quadratic
pH	4.65	4.23	4.38	4.86	0.14	0.2057	0.0047
Ammonia N content (% total N)	27.0	11.9	15.3	15.7	1.86	0.0083	0.0025
Dry matter (DM) recovery index	92.0	95.2	90.3	94.9	1.62	0.5927	0.6322
LG (% initial DM) ^a	0.91	0.22	0.06	0.05	0.05	<0.0001	<0.0001

^aLosses through fermentation gases.

The silage with the lowest DM content (Table 2), highest pH, and highest N ammonia content was obtained at 75 days of regrowth, culminating in greater losses through fermentation gases (Table 3). This is a result of undesirable fermentations caused by secondary gas-producing microorganisms, such as enterobacteria and bacteria of the genus *Clostridium* (Muck, 2010). According to Tomich et al. (2003), silages with DM contents above 200 g kg⁻¹, such as those in the present study (Table 2), should have pH ≤ 4.0 to achieve good qualification regarding the quality of fermentation in the silo. According to these authors, only silages with ammonia N content ≤ 10% total N present efficient fermentation for conservation of ensiled material. The minimum estimated values for pH and silage ammonia N content were 4.23 and 12.0 mg dL⁻¹, respectively. None of the PCEA silages had pH ≤ 4.0 and ammonia N content < 10% total N, which indicates the occurrence of losses of DM, which were similar at all regrowth ages (Table 3).

In conclusion, the PCEA silages demonstrate a compromised nutritional quality, with marked reductions in CP content and with potentially negative effects on the consumption and acceptability by cattle. However, considering the competitive advantages of the seed-propagated elephant grass to livestock feeding, especially those related to the cost of planting the crop, as previously discussed in the introduction, it is extremely important to continue the process of improving this characteristic in elephant grass simultaneously with the increase in its forage production capacity with higher nutritional quality. Additionally, there are several technological practices that can be implemented in the elephant grass ensilage

process to increase the DM content of the forage, aiming at its proper fermentation and conservation in the silo as well as simultaneously increasing the nutritional value of the silage. For example, Moran (2005) and Ribas et al. (2021) demonstrated that wilting the forage prior to ensilage is a strategy that can adjust the moisture content of elephant grass forage to 25-30%, recommended by Kung et al. (2018), aiming at proper fermentation of grasses in the silo. However, it should be noted that wilt can promote significant losses in the levels of important FAs such as α-linolenic and linoleic acids (Van Ranst et al., 2009a; Wyss, 2012) and increase harvesting losses in the field (Daniel et al., 2019). Another technique that improves the quality of fermentation in the silo consists of adding dry feedstuffs, such as citrus pulp, soybean hulls, or maize grain, immediately prior to ensiling (Daniel et al., 2019). Because they are fermentable and absorbent substrates, ensiling tropical grasses in mixture with these by-products can produce silages with minimal leachate all while improving their nutritional value. However, the adoption of this technique will always depend on the price and local availability of the additives(s). Other strategies, such as the use of enzymatic-bacterial inoculant (Ribas et al., 2021) and banana peel hay (A. F. Silva et al., 2021), were successfully used at ensilage to increase the DM content and the fermentative profile of elephant grass silage. All these strategies can be useful and could be utilized to improve fermentation quality and the nutritional value of PCEA silage, although such research has not yet been investigated.

Compared to forage, at all regrowth ages, expressive increases ranging from 65 – 88% were observed in the silage EE contents

(Table 2). These can be explained by the loss of water-soluble nutrients in the silo effluents or the fermentative products resulting in the concentration of EE in the silage DM (Baumont et al., 2011; Boichicchio et al., 2015) and corroborates the results of Boichicchio et al. (2015) and Lopes et al. (2021).

The higher concentration FAs in the PCEA were palmitic (C16:0), α -linolenic, and

linoleic acids, accounting for approximately 88 – 92% and 82 – 89% of the total FAs in forage and silage, respectively (Tables 4 and 5). Compared to ranges compiled by Lopes et al. (2015) for tropical grasses, PCEA forage and silage presented moderate to high linoleic acid content and moderate to low α -linolenic acid content (Table 5).

Table 4

Fatty acid (FA) composition (g kg⁻¹ dry mater) of chopped forage and silage of a seed-propagated elephant grass genotype harvested at different regrowth ages

Fatty acid (g kg ⁻¹ dry mater)	Regrowth ages (days)				SEM	P-value		
	75	90	105	120		Linear	Quadratic	
Chopped forage								
C12:0	0.028	0.029	0.033	0.033	0.013	0.1094	0.8832	
C14:0	0.034	0.034	0.033	0.032	0.002	0.5224	0.7502	
C16:0	3.607	2.842	2.696	2.280	0.154	<0.0001	0.2398	
C18:0	0.145	0.123	0.117	0.101	0.006	<0.0001	0.5274	
<i>cis</i> -9 C18:1	0.564	0.423	0.399	0.318	0.027	<0.0001	0.2440	
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.102	1.857	1.956	1.527	0.095	0.0024	0.3338	
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	2.410	1.821	1.651	0.915	0.256	0.0008	0.7259	
Total FA ^a	8.800	7.440	7.036	5.347	0.448	0.0002	0.7066	
Silage								
C12:0	0.049	0.062	0.072	0.059	0.004	0.0230	0.0028	
C14:0	0.050	0.070	0.074	0.060	0.004	0.0786	0.0015	
C16:0	2.691	2.848	2.753	2.130	0.178	0.0375	0.0369	
C18:0	0.116	0.110	0.112	0.098	0.006	0.0624	0.4142	
<i>cis</i> -9 C18:1	0.350	0.386	0.372	0.263	0.034	0.0524	0.0235	
<i>cis</i> -9, <i>cis</i> -12 C18:2	1.815	1.616	1.451	0.792	0.107	0.0001	0.0420	
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	2.279	1.632	1.308	0.590	0.171	<0.0001	0.8193	
Total FA ^a	7.356	7.095	6.484	4.062	0.490	0.0013	0.0441	

^aΣ C12:0 + C14:0 + C16:0 + C18:0 + *cis*-9 C18:1 + *cis*-11 C18:1 + *cis*-9, *cis*-12 C18:2 + *cis*-9, *cis*-12, *cis*-15 C18:3 + C20:0 + C22:0 + C24:0.

Table 5

Fatty acid (FA) composition (g 100 g⁻¹ FA) of chopped forage and silage of a seed-propagated elephant grass genotype harvested at different regrowth ages

Fatty acid (g 100 g ⁻¹ FA)	Regrowth ages (days)				SEM	P-value		
	75	90	105	120		Linear	Quadratic	
Chopped forage								
C12:0	0.305	0.423	0.484	0.648	0.064	0.0012	0.6820	
C14:0	0.379	0.497	0.477	0.618	0.047	0.0035	0.7835	
C16:0	40.972	41.551	38.564	43.038	1.653	0.6417	0.2103	
C18:0	1.644	1.807	1.669	1.903	0.092	0.1156	0.6690	
<i>cis</i> -9 C18:1	6.439	6.208	5.670	6.008	0.367	0.2614	0.4175	
<i>cis</i> -9, <i>cis</i> -12 C18:2	24.013	24.996	27.815	28.698	0.706	0.0001	0.9388	
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	26.546	24.070	23.155	16.463	2.336	0.0051	0.2822	
Silage								
C12:0	0.656	0.953	1.090	1.370	0.115	0.0006	0.9354	
C14:0	0.687	1.073	1.134	1.414	0.146	0.0028	0.4524	
C16:0	35.489	43.024	41.803	47.971	2.094	0.0018	0.7300	
C18:0	1.512	1.681	1.703	2.234	0.111	0.0007	0.1439	
<i>cis</i> -9 C18:1	4.572	5.835	5.628	6.010	0.512	0.0840	0.3713	
<i>cis</i> -9, <i>cis</i> -12 C18:2	21.408	22.741	22.445	19.410	1.316	0.2520	0.1038	
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	30.831	23.030	19.871	14.581	1.856	0.0001	0.4645	

There were no effects ($P>0.05$) of regrowth age on the forage lauric (C12:0) and myristic (C14:0) acid contents (Table 4). By contrast, the linear decreases on the forage contents of palmitic (Figure 6a), stearic (C18:0) (Figure 6b), oleic (Figure 6c), linoleic (Figure 6d), α -linolenic (Figure 6e), and total FAs (Figure 5) in response to the advance in the regrowth age of PCEA grass (Table 4) corroborate results presented by Lopes et al. (2021) for elephant grass BRS Capiapu harvested during the rainy season at the regrowth ages from 50 to 110 days. However, the daily rates of decrease in the levels of α -linolenic acid (0.030 g kg⁻¹ DM) and total FAs (0.072 g kg⁻¹ DM) were, on average, 28 – 30% higher in PCEA forage

than those reported by Lopes et al. (2021) of 0.021 and 0.052 g kg⁻¹ DM, respectively. These results indicate, once again, that the PCEA grass presents an accelerated process of senescence in the plants with rapid loss of nutritional quality attributed to, at this time, the negative impacts on the forage FA content and profile. This reduces its potential for milk production with an FA profile that is more nutritionally desirable for human health, since the α -linolenic acid is the most useful FA for enhancing milk fat quality (Glasser et al., 2013). When comparing the forage α -linolenic acid contents at the regrowth ages of 75 and 120 days, there was a dramatic loss of 62% (Table 4).

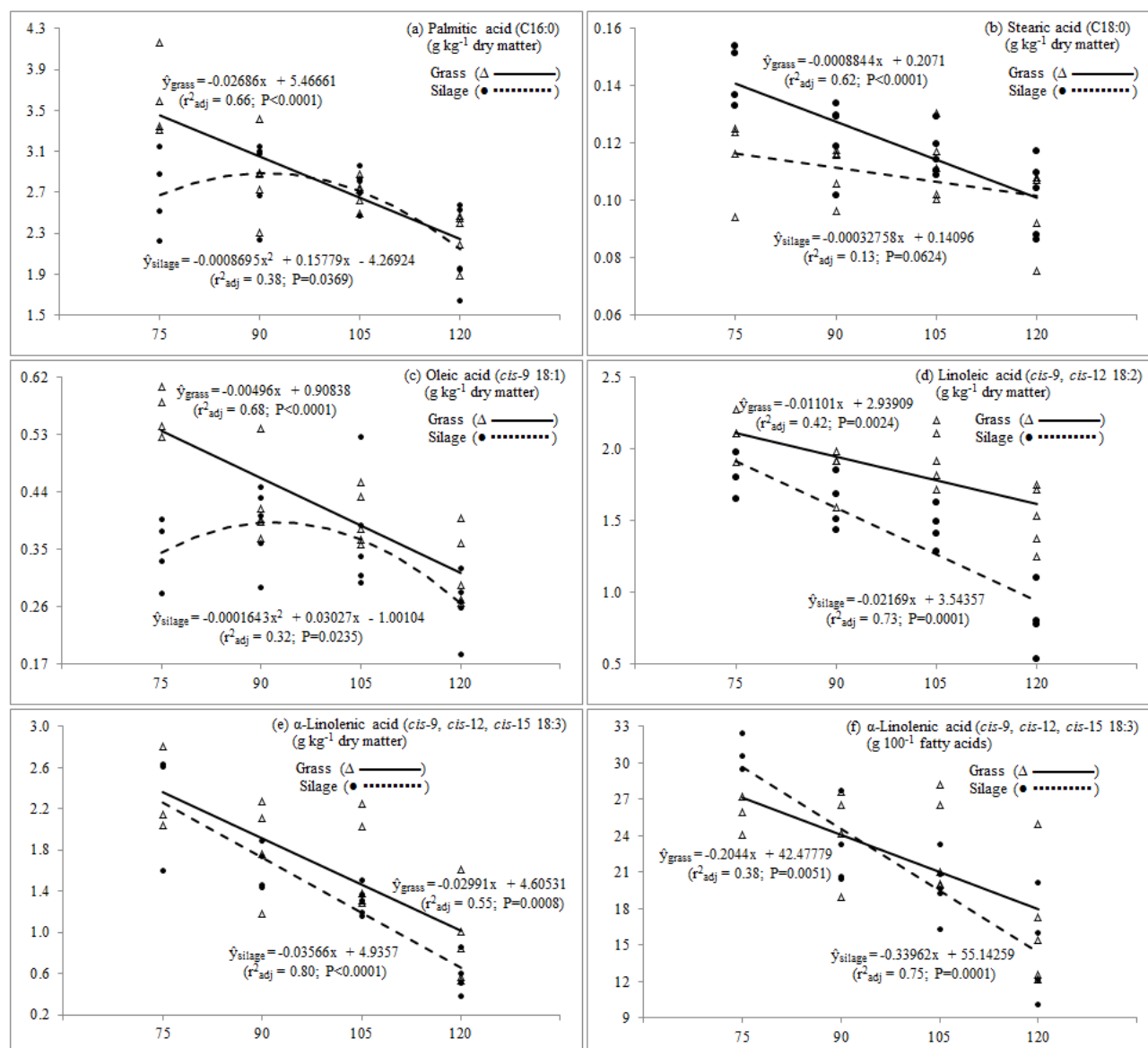


Figure 6. Fatty acids (FA) contents (g kg⁻¹ dry matter or g 100 g⁻¹ total FA) in chopped grass and silage of a seed-propagated elephant grass genotype harvested at different regrowth ages (days).

Decreases in the forage contents (g kg⁻¹ DM) of palmitic, linoleic, α -linolenic, and total FAs in response to the advance in the plant maturity were also reported by Khan et al. (2015) in elephant grass cultivar Mott (early to late maturity stage) and by Mojica-Rodríguez et al. (2017) in elephant grass cultivar Elefante (3 to 9 weeks of regrowth).

Linoleic and α -linolenic acids are the main dietary FAs that are precursors for beneficial FAs in products of ruminants; their contents (g kg⁻¹ DM, Table 4) in PCEA forage were much higher than those reported by Mojica-Rodríguez et al. (2017) and much lower than those presented by Khan et al. (2015). Except for the extremely low content

of α -linolenic acid at 120 days of regrowth ($0.915 \text{ g kg}^{-1} \text{ DM}$), they were generally similar to those reported by Lopes et al. (2021) in BRS Capiapu forage. The use of the elephant grass cultivar Mott in the study by Khan et al. (2015), which has a high leaf:stem ratio (Mora & Figueroa, 2005), may be a possible explanation for the extremely high contents of linoleic and α -linolenic acids obtained by these authors ($2.36 - 2.90$ and $7.29 - 13.81 \text{ g kg}^{-1} \text{ DM}$, respectively) since those FAs are present predominantly in thylakoid membranes of chloroplasts (Khan et al., 2015) and leaves have chloroplasts in greater number and size than stems (Li et al., 2001). The positive correlations ($P < 0.05$) between the proportion of leaves and the PCEA forage contents ($\text{g kg}^{-1} \text{ DM}$) of palmitic, stearic, oleic, linoleic, α -linolenic, and total FAs ($r = 0.62, 0.69, 0.57, 0.48, 0.50$ and 0.59 , respectively) explain the decreases in the levels of all these FAs in forage in response to the advance in the regrowth age of PCEA grass (Table 4). Furthermore, during leaf senescence, there is a gradual reduction in the number of chloroplasts per mesophyll cell (Mae et al., 1984; Ono et al., 1995). In this sense, Z. Yang and Ohlrogge (2009) reported FA losses of 80% in leaves of the tropical grass *Panicum virgatum* between 60 and 121 days after sowing. In the present study, from 75 to 120 days of regrowth, there was a 39% reduction in the PCEA forage total FA content (Table 4). Therefore, it can be concluded that the drastic linear decrease in the leaf proportion (Table 1, Figure 2), as well as the maturation of the leaves due to the rapid PCEA grass senescence process were the main culprits for the linear decreases in the levels of the major FAs, highlighting linoleic and α -linolenic acids (Figure 3d). The absence of effect of regrowth age on the forage contents of lauric

and myristic acids (Table 4) can be partly explained by the non-significant ($P > 0.05$) correlations between them and the proportion of leaves.

According to current literature, only in a recently published study conducted with BRS Capiapu (Lopes et al., 2021) are there results of FA composition in elephant grass silage which generally presented similar levels of the major FAs to those in the PCEA silages (Table 4).

There was no effect ($P > 0.05$) of regrowth age on the silage stearic acid content (Figure 6b), but linear decreases ($P < 0.05$) on silage contents of linoleic (Figure 6d), α -linolenic (Figure 6e), and total FAs (Figure 5) were observed. Quadratic effects ($P < 0.05$) on silage contents of lauric, myristic, palmitic (Figure 6a), and oleic acids (Figure 6c) were observed (Table 4). The adverse effect of the advance in the regrowth age on the forage PCEA contents of the major FAs, especially linoleic and α -linolenic acids, was intensified in the PCEA silages (Table 4, Figures 6d and 6e). While in the PCEA forage, the daily rates of reduction in the contents of these FAs were 0.011 and $0.030 \text{ g kg}^{-1} \text{ DM}$, respectively, in PCEA silage they reached 0.022 and $0.036 \text{ g kg}^{-1} \text{ DM}$, respectively (Figures 6d and 6e). Despite this, the daily rates of reduction in the total FAs contents in forage and silage were similar (0.072 versus $0.073 \text{ g kg}^{-1} \text{ DM}$, Figure 5). Compared to forage, decreases ranging from 13 – 48%, 5 – 36%, and 5 – 24% were observed in the silage contents of linoleic, α -linolenic, and total FAs at all regrowth ages (Table 4, Figures 6d and 6e), showing the low potential of the PCEA silages as exclusive roughage for milk production with a FA profile more nutritionally desirable for human health.

The contents expressed on g 100 g⁻¹ FA of the five major FAs (palmitic, α -linolenic, linoleic, oleic, and stearic acids) in the forage (Table 5) are close to the ranges compiled by Lopes et al. (2015) for chopped elephant grass. Compared to BRS Capi  u (Lopes et al., 2021), PCEA showed, both in forage and silage, higher levels of palmitic, oleic, and linoleic acids with lower stearic acid (g 100 g⁻¹ FA; Table 5) and total FA (g kg⁻¹ DM, Table 4) contents; and they showed similar contents of α -linolenic acid (Table 5) and EE (Table 2).

There were no effects ($P>0.05$) of the regrowth age on the forage palmitic, stearic, and oleic acid contents, but linear ($P<0.05$) increases on the forage contents of lauric and myristic acids were observed (Table 5). However, the main changes in the forage FA profile expressed in g 100 g⁻¹ FA in response to the advance in the regrowth age of PCEA were the linear reduction ($P=0.0051$) in the α -linolenic acid content (Figure 6f) and the linear increase ($P=0.0001$) in the content of linoleic acid (Table 5). However, while the daily rate of reduction in the content of α -linolenic acid was extremely high (-0.204 g 100 g⁻¹ FA; Figure 6f), this rate was moderate for the linoleic acid content ($+0.112$ g 100 g⁻¹; data not shown). Apparently, the α -linolenic acid was more sensitive than linoleic acid to the drastic reduction in the proportion of leaves that was observed from 75 to 120 days of regrowth of PCEA grass (Figure 3d). In a study conducted with elephant grass cultivar Pioneiro managed under grazing, Dias et al. (2017) reported higher proportions of α -linolenic acid in the leaves (61%) compared to the stems (18%) and, by contrast, higher proportions of linoleic acid in the stems (30%) compared to the leaves (14%). Based on results by Dias et al. (2017), it could be hypothesized

that, in response to the higher proportion of stems with a higher linoleic acid content than in the leaves, the linoleic acid content in the forage was less influenced by the decrease in the proportion of leaves, promoting a slight increase in linoleic acid content in the forage in response to the advance in regrowth age (Table 5). Corroborating this, the PCEA leaf:stem ratio showed a negative correlation with the PCEA forage linoleic acid content ($r = -0.74$; $P=0.0006$), and a positive correlation with the PCEA forage α -linolenic acid content ($r = 0.47$; $P=0.0657$).

Regrowth age did not influence ($P>0.05$) silage oleic and linoleic acid contents expressed in 100 g⁻¹ FA in response to the advance in the regrowth age of PCEA grass (Table 5), but three main changes in the silage FA profile deserve to be highlighted: i) the linear increases ($P<0.05$) on the contents of all saturated FAs (lauric, myristic, palmitic, and stearic acids); ii) the linear reduction ($P=0.0001$) in the α -linolenic acid content (g 100 g⁻¹ FA), where the daily rate of decrease was extremely high (0.340 g 100 g⁻¹ FA; Figure 6f); and iii) the general decreases ($P<0.05$) in the contents (g 100 g⁻¹ FA) of the unsaturated FAs in the silages compared to those in the original forages in each regrowth age (Table 5). The decrease in the levels of the three unsaturated FAs in the silages compared to those in the original forages in each regrowth age (Table 5) may be associated with the action of plant lipases, which are activated after tissue injury of forage during harvesting operations (Baumont et al., 2011). However, the lipases remain functional during the first days of ensiling, declining with a reduction in silage pH, as well as when the residual oxygen is consumed with the progress of ensiling (Han & Zhou, 2013). As all the PCEA silages

presented DM contents $<200 \text{ g kg}^{-1}$ and none had $\text{pH} \leq 4.0$ with an ammonia N content $\leq 10\%$ total N (Table 3), it is possible that a prolonged and inefficient fermentation process in the silo occurred (Tomich et al., 2003). This potentially promoted lipolysis on the silage lipids (Van Ranst et al., 2009a), accumulation of free FAs in the silo (Van Ranst et al., 2009b), and further oxidation and decomposition by the action of plant lipoxygenases, mainly for α -linolenic and linoleic acids (Han & Zhou, 2013), as well as their biohydrogenation into stearic acid by microbes in the silo (Liu et al., 2019). Because it is not oxidized by lipoxygenases, the palmitic acid released from lipids by lipases tends to increase in silage (Liu et al., 2019), and it is feasible that this also occurs with the other saturated FAs (lauric, myristic, and stearic acids). All these processes help to illuminate the different patterns of decrease or increase in the contents ($\text{g } 100 \text{ g}^{-1}$ FA) of unsaturated and saturated FAs in the PCEA silage compared to the forage as well as in response to the advance in the regrowth age of PCEA grass (Table 5).

Conclusions

From 75 to 120 days of regrowth, the seed-propagated elephant grass genotype presents forage and silage with low nutritional quality for feeding dairy cattle, moderate to high linoleic acid contents, and moderate to low α -linolenic acid contents.

The forage and silage obtained from 75 to 120 days of regrowth presents low potential for production of milk naturally enriched with bioactive fatty acids potentially beneficial to human health.

Considering the importance of elephant grass in Brazilian milk production systems

and all the benefits inherent to the use of a seed-propagated elephant grass genotype, it is extremely important to continue the process of improvement of this characteristic in elephant grass simultaneously with the increase in its forage production capacity with higher nutritional quality. Likewise, research on strategies that could be employed to improve fermentation quality and the nutritional value of silage of seed-propagated elephant grass genotype should also be conducted in future investigations.

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