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Chemical composition and fatty acid profile of BRS Capiaçu ensiled at different regrowth ages

Composição química e perfil de ácidos graxos do BRS Capiaçu ensilado em diferentes idades de rebrota

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Highlights

BRS Capiaçu harvested at up to 70 days of regrowth presented good nutritional value.
Capiaçu forage at 50-70 days of regrowth showed the highest linoleic acid levels.
Capiaçu forage at 50-70 days of regrowth showed the highest α-linolenic acid levels.
At 90-110 days of regrowth, the silage showed the highest levels of linoleic acid.
At 90-110 days of regrowth, the silage showed the highest levels of α-linolenic acid.

Abstract

This study aimed to evaluate the chemical composition and fatty acid (FA) profile of chopped forage and silage of BRS Capiaçu elephant grass at four regrowth ages: 50, 70, 90 and 110 days. A randomized block design with five replications was used. The ensiling was carried out manually in experimental silos without wilting using no additives or bacterial inoculants. The results were analyzed using mixed models ($P < 0.05$). The model 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 were linear increases in the dry matter (DM, g kg^{-1}) and lignin (g kg^{-1} DM) contents and linear reductions in the *in vitro* DM digestibility (g kg^{-1}) of chopped grass and silage as a function of regrowth age ($P < 0.001$). Quadratic effects ($P \leq 0.01$) were observed for the chopped grass contents (g kg^{-1} DM) of crude protein (CP), ether extract (EE) and neutral detergent fiber (NDF) as a function of regrowth age. There were linear decreases ($P < 0.0001$) in the CP content (g kg^{-1} DM) and pH and linear increases ($P < 0.001$) in the EE and NDF contents (g kg^{-1} DM) in the silage as a function of regrowth age. There were linear decreases ($P < 0.01$) in the chopped grass contents and linear increases ($P < 0.05$) in the silage contents of total FAs, linoleic and α-linolenic acids (g kg^{-1} DM) as

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a function of regrowth age. BRS Capiaçu elephant grass must be harvested at up to 70 days of regrowth to obtain forage with good nutritional value and the highest levels of linoleic and α-linolenic acids (g kg^{-1} DM). To produce silages with adequate pH values and the highest levels of linoleic and α-linolenic acids (g kg^{-1} DM), BRS Capiaçu must be harvested between 90 and 110 days of regrowth.

Key words: Linoleic acid. Linolenic acid. *Pennisetum purpureum.* Silage. Tropical grass.

Resumo

O estudo objetivou avaliar a composição química e o perfil de ácidos graxos (AG) da forragem e da silagem do capim-elefante BRS Capiaçu em quatro idades de rebrota (50, 70, 90 e 110 dias), sob delineamento em blocos casualizados, com cinco repetições. A ensilagem foi realizada manualmente em silos experimentais, sem pré-murchamento, aditivos ou inoculantes bacterianos. Os resultados foram analisados usando modelos mistos ($P < 0,05$), que incluíram o efeito fixo de tratamento (idade de rebrota) e efeito aleatório de bloco. Os efeitos lineares e quadráticos foram analisados por meio de contrastes ortogonais. Houve aumento linear nos teores de matéria seca – MS (g kg^{-1}) e lignina (g kg^{-1} de MS), e reduções lineares na digestibilidade *in vitro* da MS (g kg^{-1}) da forragem e da silagem em função da idade de rebrota ($P < 0,001$). Efeitos quadráticos ($P \leq 0,01$) foram observados na forragem para os teores (g kg^{-1} de MS) de proteína bruta (PB), extrato etéreo (EE) e fibra em detergente neutro (FDN) em função da idade de rebrota. Houve reduções lineares ($P < 0,0001$) no teor de PB (g kg^{-1} de MS) e no pH, e aumentos lineares ($P < 0,001$) nos teores de EE e FDN (g kg^{-1} de MS) da silagem em função de idade de rebrota. Para os teores dos AG totais e dos ácidos linoleico e α-linolênico (g kg^{-1} de MS), houve reduções lineares ($P < 0,01$) na forragem e aumentos lineares ($P < 0,05$) na silagem em função da idade de rebrota. O BRS Capiaçu deve ser colhido até 70 dias de rebrota para obter forragem com bom valor nutricional e maiores teores dos ácidos linoleico e α-linolênico (g kg^{-1} de MS). Para silagem com adequado pH e maiores teores dos ácidos linoleico e α-linolênico (g kg^{-1} de MS), o BRS Capiaçu deve ser colhido entre 90 e 110 dias de rebrota.

Palavras-chave: Ácido linoleico. Ácido linolênico. Gramínea tropical. *Pennisetum purpureum.* Silagem.

Introduction

Elephant grass [*Cenchrus purpureus* (Schumach.) Morrone (syn. *Pennisetum purpureum* Schumach.)] is one of the most traditional tropical grasses used for feeding dairy cattle in Brazil. Although it is recommended for rotational grazing, the most common form of use is chopped grass supplied in the trough by a cutting and transport system (Pereira, Morenz, Ledo, & Ferreira, 2016b).

In 1991, Embrapa Dairy Cattle (Juiz de Fora, MG, Brazil) initiated an elephant grass breeding program, addressing the development of improved cultivars for use in feeding ruminants. In 2015, as part of this program, the cultivar BRS Capiaçu was launched; BRS Capiaçu presented high forage productivity with good nutritional value in addition to other desirable agronomic characteristics (Pereira et al., 2016a; Pereira, Lédo, & Machado, 2017). BRS Capiaçu presented 35 to 67% higher productivity and 27 to 31% higher crude protein (CP) content than other elephant grass cultivars (Mineiro and Cameroon) (Pereira et al., 2017). BRS Capiaçu has excellent adaptation to mechanical harvest, and it has a lower production cost per hectare of dry matter (DM), CP and total digestible nutrients than other forages traditionally used for silage production (corn, sorghum and sugarcane) (Pereira et al., 2016a).

Despite their low FA content, forages are important sources of polyunsaturated fatty acids (PUFAs), notably α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3), and due to the high proportion of forages in dairy cattle diets, they strongly influence PUFA intake in dairy cows (Dewhurst, Shingfield, Lee, & Scollan, 2006; Khan et al., 2015). Cows consuming forages containing high levels of α-linolenic and linoleic acids (*cis*-9, *cis*-12 C18:2) have the potential to produce milk with an FA profile that is more nutritionally desirable for human health. These PUFAs and, to a lesser extent, oleic acid (*cis*-9 C18:1) are substrates for vaccenic acid (*trans*-11 C18:1) production by ruminal microorganisms. Vaccenic acid is the precursor for the synthesis of 64-97% rumenic acid (*cis*-9, *trans*-11 CLA) in the mammary gland, being the major isomer of CLA (conjugated linoleic acid) in bovine milk (Shingfield, Bernard, Leroux, & Chilliard, 2010). Anticarcinogenic, antidiabetogenic (type 2 diabetes), antiatherogenic and immunomodulatory properties have been attributed to this FA (B. Yang et al., 2015).

In a cut-and-carry system, when well-managed, elephant grass forage contains high levels of linoleic and α-linolenic acids, with 13.3-28.0 and 13.8-55.0 g 100 g^{-1} of total FA, respectively (Lopes, Silva, Almeida, & Gama, 2015). However, elephant grass has been scarcely investigated with respect to factors known to influence the forage FA profile. Aspects related to species/cultivar, age of regrowth, season of the year, nitrogen fertilization, temperature, light intensity and pasture management modulate the FA profiles of forages (Boufaïed et al., 2003; Dewhurst et al., 2006; Khan, Cone, Fievez, & Hendriks, 2012; Glasser, Doreau, Maxin, & Baumont, 2013; Elgersma, 2015; Khan et al., 2015). Knowledge of these factors could help to define management strategies to increase precursors for beneficial FAs in products of ruminants (Elgersma, 2015), especially α-linolenic acid, the main FA in forages and the most useful for enhancing milk fat quality (Glasser et al., 2013).

For BRS Capiaçu grown in the rainy season of the Mata Atlântica Biome of Brazil, considering the biomass production and the nutritional quality of the forage, it is recommended that it be harvested at 50 to 70 days of regrowth to be supplied in the fresh chopped form. On the other hand, for silage production, mainly as a function of the DM content of the forage and to reach desirable fermentation in the silo, harvesting must be carried out when the plants reach ages between 90 and 110 days of regrowth (Pereira et al., 2016a). The forage FA content declines with plant maturity due to a decrease in the leaf/stem ratio and initiation of flowering and leaf senescence (Khan et al., 2015). Only two studies have presented the FA composition in elephant grass forage as a function of plant maturity: Khan et al. (2015) and Mojica-Rodríguez, Castro-Rincón, Carulla-Fornaguera and Lascano-Aguilar (2017) observed a reduction ($P<0.05$) in the forage content of α-linolenic acid as a function of plant maturity. In turn, the conservation method can also influence the FA composition, and the reported effects of ensiling on the total FA and α-linolenic acid contents are contradictory. Many studies have reported maintenance or reduction (Arvidsson, Gustavsson, & Martinsson, 2009; Alves, Cabrita, Jerónimo, Bessa, & Fonseca, 2011; Ding, Long, & Guo, 2013; Liu, Dong, & Shao, 2018; Liu, Wu, & Shao, 2019), while in others, increases in the total FA and/or in the α-linolenic acid contents have been observed (Boufaïed et al., 2003; Bochicchio, Comellini, Marchetto Faeti, & Della Casa, 2015), with this latter case also including unwilted (Glasser et al., 2013) or additive-treated silages (Dewhurst et al., 2006; Alves et al., 2011). However, no study was found on the FA profile of elephant grass silage.

This study was, therefore, designed to investigate the changes in forage and silage chemical composition, with particular emphasis on fatty acid composition, over a range of maturity levels at which BRS Capiaçu elephant grass is usually harvested and fed to dairy cattle.

Materials and Methods

The study was carried out at Embrapa Dairy Cattle (Coronel Pacheco, MG, Brazil) from February to September 2015. The geographical coordinates are 21°33′22″ S latitude and 43°6′15″ W longitude, and the average altitude is 410 m. The climate of the region, according to the Köppen classification, is of the Cwa type (mesothermal), with a well-defined hot/rainy season (spring-summer) from October to March and a cold/dry season (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 500 m away from the experimental area (Figure 1).

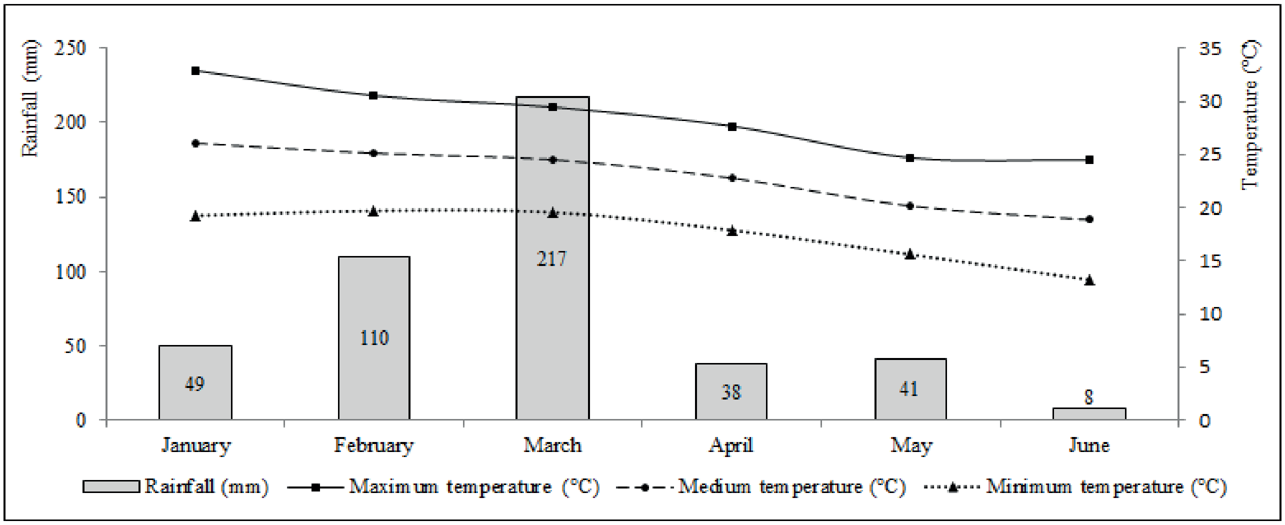

Figure 1. Climatic data during the period of plant growth until forage harvesting.
Source: Instituto Nacional de Metereologia [INMET] (2020).

To evaluate the chemical composition and fatty acid (FA) profile of chopped forage and silage of BRS Capiaçu, four regrowth ages were defined: 50, 70, 90 and 110 days. These regrowth ages were chosen because this is the range of maturity at which BRS Capiaçu is usually harvested and fed to dairy cattle (Pereira et al., 2016a). A randomized block design with five replications was used, totaling 20 experimental plots. Each parcel was composed of 4 lines that were 5 m in length with a spacing of 1 m. The useful area of the plot was formed by a 3 m center of 2 internal lines, totaling 6 m^2. On February 23, 2015, in a flat area established with BRS Capiaçu, a standardization cutting of the plants was carried out at 10 cm from the ground level, with the plots fertilized with 370 kg ha^{-1} of NPK 20-05-20. The plots in the useful area were harvested manually on the mornings of April 14, May 4, May 24 and June 13, 2015, corresponding to the regrowth ages of 50, 70, 90 and 110 days, respectively, at a height of 10 cm from ground level using a 35 cm steel blade machete. Immediately after cutting, the plants belonging to each useful area of each plot were disintegrated (average forage particle size of 1-2 cm) in a stationary electric forage chopper. The forage of each plot was manually homogenized, and two 400 g subsamples were collected and frozen (-20°C) to analyze the chemical composition and FA profile. The first subsample was thawed, predried (55°C, 72 h), milled (1 mm) and analyzed at the Food Analysis Laboratory of Embrapa Dairy Cattle (Juiz de Fora, MG) for DM (at 105°C), mineral matter (MM), crude protein (CP), ether extract (EE), acid detergent fiber (FDA), neutral detergent fiber (NDF), lignin and *in vitro* DM digestibility (IVDMD), according to Detmann et al. (2012). The second subsample was lyophilized (model L120, Liotop, Liobras, São Carlos, SP, Brazil), ground (1 mm) and analyzed for FA composition, according to Sukhija and Palmquist (1988), with adaptations (Palmquist & Jenkins, 2003), at the Laboratory of Chromatography of Embrapa Dairy Cattle (Juiz de Fora, MG). Briefly, samples containing between 10 and 50 mg of total FA were subjected to lipid extraction and transesterification using a one-step method based on acid catalysis (10% methanolic HCl solution, v/v) under heating (70°C water bath for 2 h). Subsequently, the FA methyl esters (FAMEs) were separated and quantified in a gas chromatograph (Agilent 6890, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a flame ionization detector at 250°C, N_2 makeup (30 mL min^{-1}) and a 10:1 ratio. By means of an automatic sampler, FAMEs were injected (volume of 1.0 μL, split 1:50, temperature of 250°C) into a capillary column of high polarity (HP-FFAP, 25 m x 0.2 mm x 0.33 μm), with a stationary phase of modified polyethylene glycol nitroterafitalic acid, using H_2 as the carrier gas at 1.0 mL min^{-1}. The initial temperature of the oven was adjusted to 100°C, with a heating ramp from 15°C min^{-1} to 230°C, maintained until the complete elution of the FAs of interest. The total FA content in the samples was calculated in g kg^{-1} DM based on the extrapolation of the internal standard area (C19:0; nonadecanoic acid; Sigma Aldrich CAS 646-30-0), added to the samples before extraction. FAs were identified by comparison of their retention times with those of reference FAME standards (Sigma Aldrich Inc.), being expressed in g 100 g^{-1} of total FA. Unfortunately, the forage sample for FA profile analysis corresponding to the regrowth age of 90 days was lost.

Before ensiling, the forage of each plot was manually homogenized and directly ensiled without wilting using no additives or bacterial inoculants. Ensiling was carried out in experimental PVC (polyvinyl chloride) silos (10 cm diameter and 30 cm height). The compaction of forage in the silo was carried out by hand with the aid of a socket made of wood, obtaining an average final density of forage mass of ~700 kg m^{-3}. The silos were closed with vulcanized rubber caps attached to a clamp, adapted with *Bunsen* valves to allow the escape of gases from fermentation. After 90 days of storage at room temperature in a covered location protected from sunlight, the silages were removed from the silos. After discarding 5 cm layers of silage from the bottom and top (which are often moldy), the contents of the silos were homogenized, and two subsamples of 400 g were collected and frozen (-20°C) for chemical composition and FA profile analyses, as previously described for chopped forage. A third sample of silage (100 g) was pressed in a hydraulic press to obtain a juice, whose pH was determined with a digital potentiometer.

The results were analyzed according to a randomized block design replicated five times using the procedure for mixed models of SAS. The model included the fixed effect of treatment (regrowth age), and block was considered a 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 of the variables as a function of regrowth age were performed using the REG procedure of SAS. Pearson's correlation studies were performed using the CORR procedure of SAS. Specifically, for forage FA profile results, due to the loss of forage samples collected at 90 days of regrowth, the levels of the treatments (regrowth age) were not equally spaced. Therefore, the ORPOL function in the CONTRAST statement in the Interactive Matrix Language (IML) procedure of SAS was used to determine orthogonal polynomial trend contrast coefficients. To compare the chemical composition (50, 70, 90 and 110 days of regrowth) and FA profile (50, 70 and 110 days of regrowth) of silage in relation to chopped forage, paired t-tests using the TTEST procedure of SAS were conducted for each regrowth age.

Results and Discussion

There were linear increases in DM (g kg^{-1}) and lignin (g kg^{-1} DM) contents and a linear reduction in IVDMD (g kg^{-1}) of forage as a function of regrowth age ($P<0.0001$; Table 1; Figures 2a, 2f and 3). Quadratic effects were observed for the contents (g kg^{-1} DM) of CP, EE, MM, ADF and NDF ($P<0.05$; Table 1; Figures 2b, 2c, 2d and 2e). The DM (g kg^{-1}), EE, ADF, NDF and lignin contents (g kg^{-1} DM) were linearly increased ($P\leq0.0001$), the CP content (g kg^{-1} DM) and the IVDMD (g kg^{-1}) were linearly decreased ($P<0.0001$), and a quadratic effect was observed ($P=0.0002$) for MM content (Figure 2d) of silage as a function of regrowth age (Table 1; Figures 2c, 2e, 2f and 3). There was a linear decrease ($P<0.0001$) in the silage pH as a function of regrowth age (Table 1; Figure 3). Regardless of the regrowth age, the silage DM, EE and NDF contents were always higher ($P<0.05$) than those in chopped grass, which, in turn, always had higher IVDMD and MM contents than the silage (Table 1; Figures 2a, 2c, 2d, 2e and 3). For ADF and lignin, the silage had higher ($P<0.05$) contents only at 50 days, being similar ($P>0.05$) to the chopped grass at the other regrowth ages (Table 1; Figure 2f). Except at 90 days, when the CP contents were similar ($P=0.0785$), in the other regrowth ages, the chopped grass always had higher ($P<0.05$) CP contents than the silage (Table 1; Figure 2b).

Table 1
Chemical composition (g kg^{-1} dry mater – DM) of chopped forage and silage of BRS Capiaçu elephant grass harvested at different regrowth ages

Item	Regrowth ages (days)[a]				SEM	P-value	
	50	70	90	110		Linear	Quadratic
			Chopped forage				
DM (g kg^{-1} as fed)	85.3 Bd	126.2 Bc	149.4 Bb	179.3 Ba	3.5533	<0.0001	0.0300
Mineral matter	160.0 Aa	121.9 Ab	115.0 Ac	110.1 Ac	2.8121	<0.0001	<0.0001
IVDMD (g kg^{-1})[b]	743.0 Aa	665.4 Ab	651.2 Ab	586.5 Ac	10.5676	<0.0001	0.5215
Ether extract	12.6 Ba	12.1 Bab	10.6 Bb	13.5 Ba	0.5538	0.6423	0.0100
ADF[c]	433.4 Bc	471.5 Ab	502.8 Aa	511.9 Aa	6.4407	<0.0001	0.0159
NDF[d]	604.5 Bc	662.5 Bb	682.4 Ba	685.8 Ba	6.0857	<0.0001	0.0002
Lignin	37.7 Bd	58.1 Ac	69.9 Bb	76.8 Aa	1.5870	<0.0001	<0.0001
Crude protein	95.1 Aa	77.2 Ab	61.6 Ac	56.2 Ad	1.7650	<0.0001	0.0003
			Silage				
DM (g kg^{-1} as fed)	102.0 Ad	138.7 Ac	169.7 Ab	190.8 Aa	4.5587	<0.0001	0.0423
Mineral matter	144.8 Ba	111.3 Bb	100.5 Bc	97.4 Bc	2.5444	<0.0001	0.0002
IVDMD (g kg^{-1})[b]	621.8 Ba	584.8 Bab	547.7 Bbc	518.2 Bc	1.2160	<0.0001	0.7631
Ether extract	19.8 Ab	21.0 Ab	27.1 Aa	27.0 Aa	1.3099	0.0001	0.5732
ADF[c]	465.9 Ac	476.0 Abc	491.9 Bb	510.3 Aa	5.3477	<0.0001	0.4466
NDF[d]	673.6 Ac	706.2 Ab	722.4 Aab	737.9 Aa	5.8294	<0.0001	0.1686
Lignin	60.6 Ab	62.3 Ab	75.9 Aa	78.1 Aa	3.4613	0.0008	0.8871
Crude protein	70.3 Ba	63.9 Bb	53.3 Bc	51.2 Bc	2.0001	<0.0001	0.3057
pH	5.12 a	5.18 a	3.71 b	3.85 b	0.0834	<0.0001	0.6822

[a]For each variable, means followed by the same letters, which are uppercase in the columns in the comparison between chopped forage *versus* silage in each regrowth age (paired t-test) and lowercase in the rows for comparison among days of regrowth, do not differ at 5% probability; [b]*In vitro* DM digestibility; [c]Acid detergent fiber; [d]Neutral detergent fiber.

There were linear ($P<0.001$) increases in the contents (g kg^{-1} DM) of lauric (C12:0) and myristic (C14:0) acids in the chopped grass, while there were linear reductions ($P<0.01$) in the contents of palmitic (C16:0), stearic (C18:0), oleic, linoleic, α-linolenic and total FAs as a function of regrowth age (Table 2; Figures 4a to 4d). For each day of increase in regrowth age between 50 and 110 days, reductions in chopped grass contents of 0.019, 0.001, 0.004, 0.010, 0.021, and 0.052 g kg^{-1} DM were observed for palmitic, stearic, oleic, linoleic, α-linolenic and total FAs, respectively. There were linear reductions ($P<0.001$) in the silage contents (g kg^{-1} DM) of lauric, palmitic and oleic acids, while the levels of linoleic and α-linolenic acids and total FAs linearly increased ($P<0.05$) as a function of regrowth age (Table 2; Figures 4a to 4d). There was no effect ($P>0.05$) of the age of regrowth on the silage myristic acid content, while a quadratic effect ($P=0.0429$) was observed for stearic acid (Table 2). At 50 and 70 days of regrowth, the contents (g kg^{-1} DM) of oleic, linoleic, α-linolenic and total FAs were always higher ($P<0.05$) in chopped grass than in silage. However, at 110 days of regrowth, there was no difference (P>0.05) between chopped grass and silage in the contents of linoleic, α-linolenic and total FAs, with the higher oleic acid content (P=0.0143) remaining in the chopped grass (Table 2; Figures 4a to 4d). Figure 5 presents the percentage changes (%) in the silage contents (g kg^{-1} DM) of linoleic, α-linolenic and total FAs compared to those of chopped grass harvested at different regrowth ages. Regardless of the regrowth age, there was no difference (P>0.05) in stearic acid contents between silage and chopped grass. For palmitic acid, only at 70 days of regrowth was a difference observed (P=0.0272), with a higher content in chopped grass than silage (Table 2). Higher (P<0.05) lauric and myristic acid contents were observed at 50 days of regrowth in silage, and lower (P<0.001) contents were observed at 110 days. At 70 days of regrowth, there was no difference (P>0.05) between silage and chopped grass for the lauric acid content, while the chopped grass showed a higher (P=0.0170) content of myristic acid.

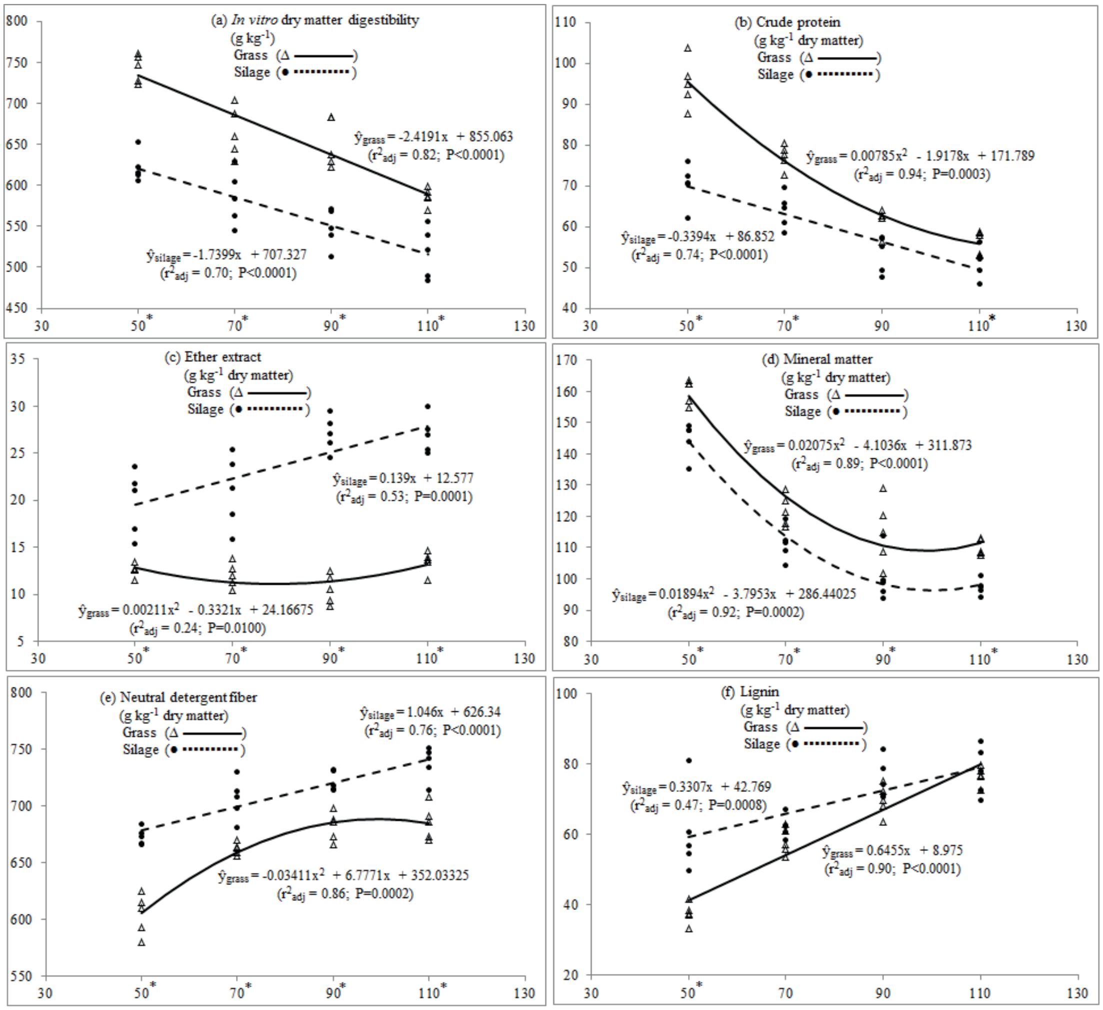

Figure 2. Changes in the chemical composition of chopped grass and silage of BRS Capiaçu harvested at different regrowth ages (days). *Asterisks beside the regrowth age indicate a difference (P<0.05) between chopped grass *versus* silage (paired t-test).

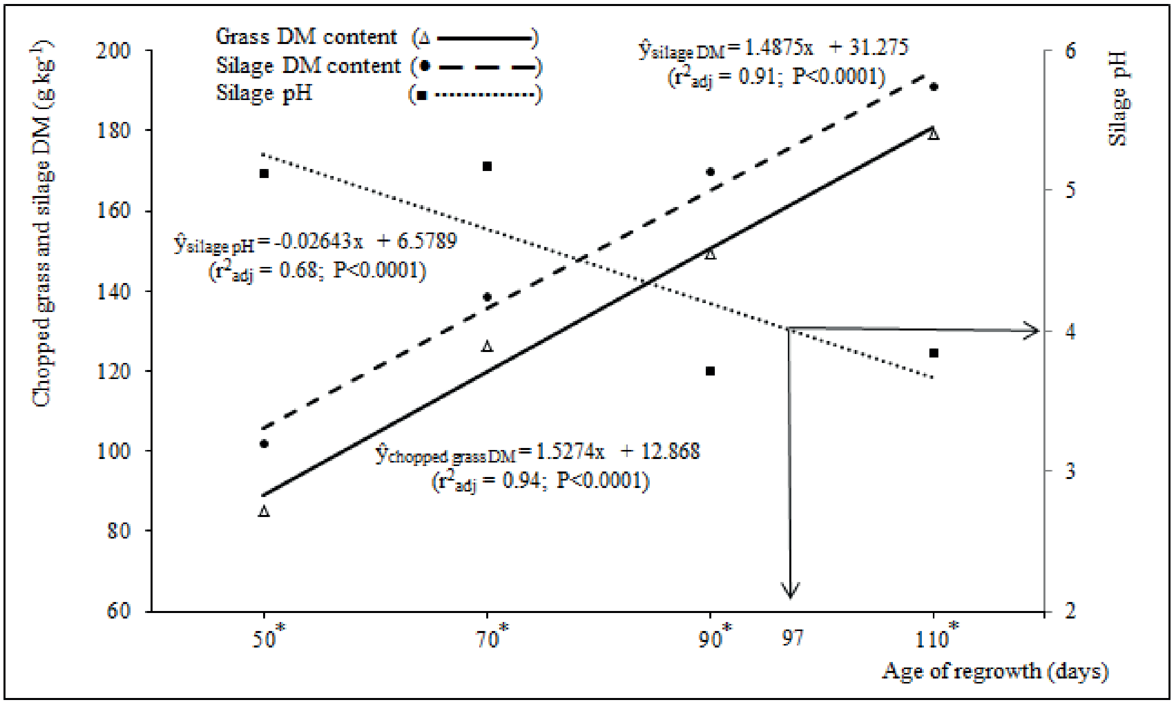

Figure 3. Changes in the silage pH and in the dry matter (DM) contents of chopped grass and silage of BRS Capiaçu harvested at different regrowth ages (days). *Asterisks beside the regrowth age indicate a difference (P<0.05) between chopped grass *versus* silage (paired t-test).

Table 2

Fatty acid (FA) composition (g kg^{-1} dry mater) of chopped forage and silage of BRS Capiaçu elephant grass harvested at different regrowth ages

Fatty acid	Regrowth ages (days)[a]				SEM	P-value	
	50	70	90	110		Linear	Quadratic
Chopped forage							
C12:0	0.071 Bb	0.083 Ab	*	0.134 Aa	0.0069	0.0001	0.3158
C14:0	0.047 Bc	0.056 Ab	*	0.077 Aa	0.0022	<0.0001	0.7693
C16:0	3.264 Aa	2.645 Ab	*	2.043 Ac	0.0708	<0.0001	0.0147
C18:0	0.209 Aa	0.171 Ab	*	0.165 Ab	0.0062	0.0006	0.0060
cis-9 C18:1	0.618 Aa	0.438 Ab	*	0.339 Ac	0.0160	<0.0001	0.0024
cis-9, *cis*-12 C18:2	2.136 Aa	2.054 Aa	*	1.541 Ab	0.0882	0.0003	0.2284
LnA[b]	2.716 Aa	2.525 Aa	*	1.498 Ab	0.1955	0.0014	0.3911
Total FA	10.350 Aa	9.188 Ab	*	7.180 Ac	0.3851	<0.0001	0.7961
Silage							
C12:0	0.173 Aa	0.108 Ab	0.082 c	0.074 Bc	0.0072	<0.0001	0.0023
C14:0	0.055 Aa	0.044 Bb	0.051 ac	0.048 Bbc	0.0027	0.1620	0.0895
C16:0	2.870 Aa	2.395 Bb	2.348 b	2.062 Ab	0.1248	0.0008	0.4622
C18:0	0.201 Aa	0.162 Ab	0.166 b	0.162 Ab	0.0077	0.0071	0.0429
cis-9 C18:1	0.331 Ba	0.277 Bb	0.255 bc	0.214 Bc	0.0142	<0.0001	0.6314
cis-9, *cis*-12 C18:2	0.895 Bb	1.144 Bb	1.542 a	1.532 Aa	0.1178	0.0009	0.2920
LnA[b]	1.039 Bb	1.176 Bb	1.874 a	1.891 Aa	0.1870	0.0021	0.7530
Total FA	6.618 Ba	6.687 Ba	7.307a	7.522 Aa	0.3158	0.0316	0.8161

*Not analyzed; [a]For each variable, means followed by the same letters, which are uppercase in the columns in the comparison between chopped forage *versus* silage in each regrowth age (paired t-test) and lowercase in the rows for comparison among days of regrowth, do not differ at 5% probability; [b]α-Linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3).

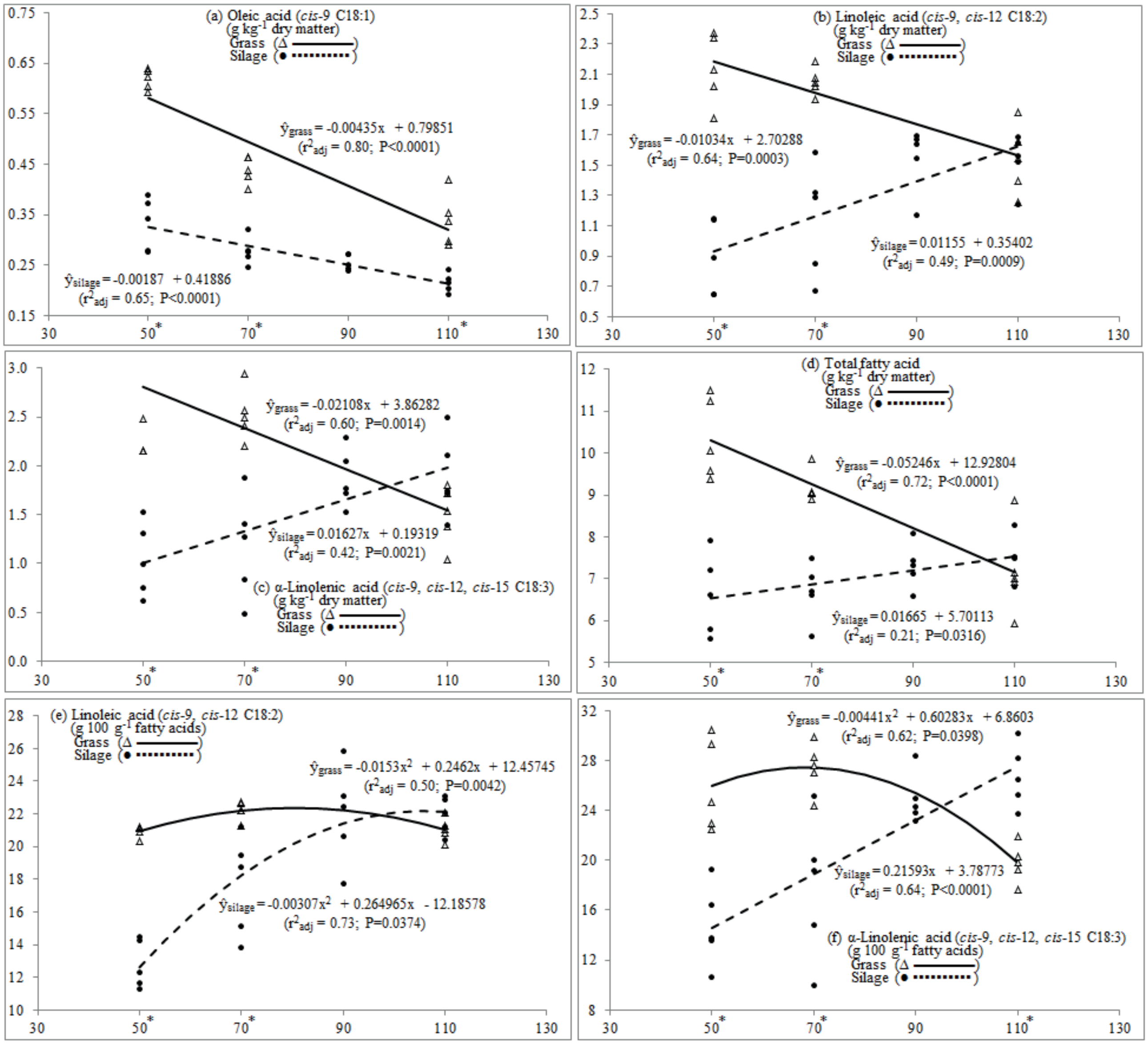

Figure 4. Changes in fatty acid contents (expressed in g kg^{-1} dry matter or g 100 g^{-1} fatty acids) in chopped grass and silage of BRS Capiaçu harvested at different regrowth ages (days). *Asterisks beside the regrowth age indicate a difference ($P<0.05$) between chopped grass *versus* silage (paired t-test).

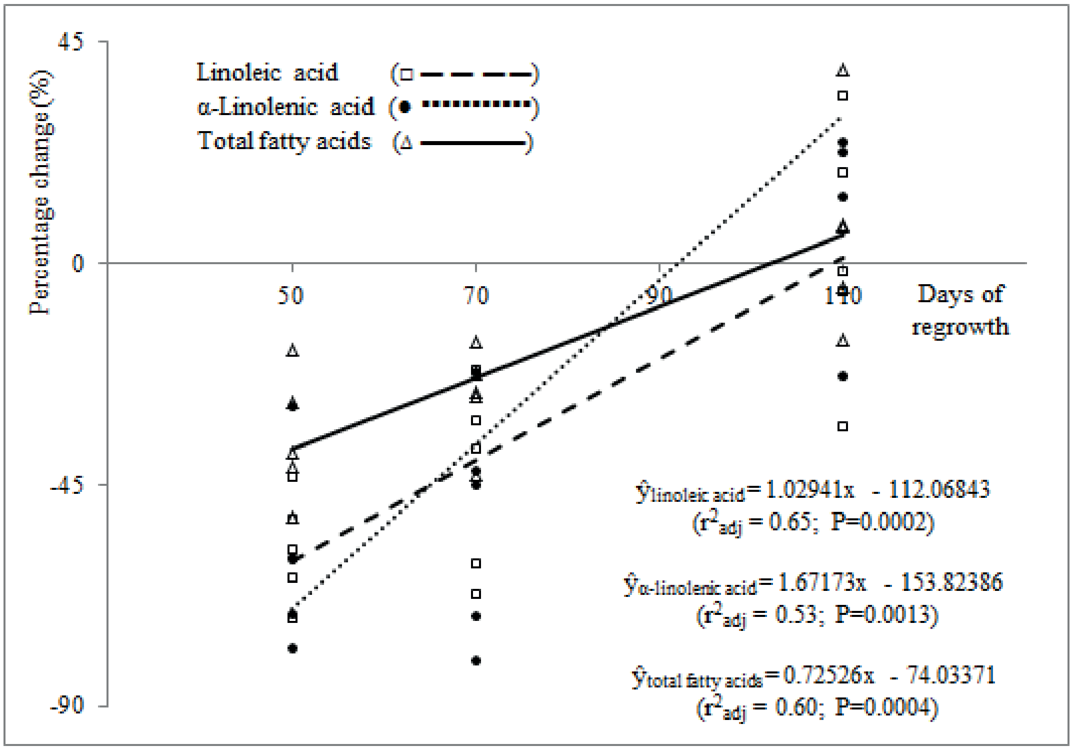

Figure 5. Percentage changes (%) in the silage contents (g kg^{-1} dry matter) of linoleic, α-linolenic and total fatty acids in relation to those of chopped BRS Capiaçu elephant grass harvested at different regrowth ages.

There were linear (P<0.0001) increases in the contents (g 100 g^{-1} FA) of lauric and myristic acids in the chopped grass, while quadratic effects (P<0.05) were observed in the palmitic, stearic, oleic, linoleic and α-linolenic acid contents as a function of regrowth age (Table 3; Figures 4e, 4f). The maximum contents of linoleic acid (22.367 g 100 g^{-1} FA) and α-linolenic acid (27.461 g 100 g^{-1} FA) were estimated to occur at 80 and 68 days of regrowth, respectively. There were linear reductions (P<0.01) in the silage lauric, palmitic, stearic and oleic acid contents (g 100 g^{-1} FA) as a function of regrowth age (Table 3). There was no linear or quadratic effect (P>0.05) on the silage myristic acid content, while a quadratic effect (P=0.0374) was observed on the silage linoleic acid content as a function of regrowth age (Table 3; Figure 4e); the maximum content (22.183 g 100 g^{-1} FA) was estimated to occur at 106 days of regrowth. The silage α-linolenic acid content increased linearly (P<0.0001) as a function of regrowth age (Table 3; Figure 4f). Compared to those of chopped grass, the silage lauric and palmitic acid contents (g 100 g^{-1} FA) were higher (P<0.05) at 50 and 70 days and lower (P<0.05) at 110 days of regrowth (Table 3). For myristic acid (g 100 g^{-1} FA), the silage had a higher (P=0.0046) content at 50 days, a lower content at 110 days (P=0.0002) and a similar (P>0.05) content to that of the chopped grass at 70 days (Table 3). Higher (P<0.05) stearic acid levels (g 100 g^{-1} FA) were observed in the silage at 50 and 70 days, which was similar (P>0.05) to that of chopped grass at 110 days (Table 3). The contents of oleic acid (g 100 g^{-1} FA) were higher ($P<0.05$) in chopped grass at 50 and 110 days and similar ($P>0.05$) to that of silage at 70 days of regrowth (Table 3). Higher ($P<0.05$) linoleic acid contents (g 100 g^{-1} FA) were observed in chopped grass at 50 and 70 days, which was similar ($P>0.05$) to that of the silage at 110 days of regrowth (Table 3; Figure 4e). For α-linolenic acid (g 100 g^{-1} FA), the chopped grass showed higher ($P<0.01$) levels at 50 and 70 days and a lower ($P<0.0001$) content at 110 days of regrowth (Table 3; Figure 4f).

Table 3

Fatty acid (FA) composition (g 100 g^{-1} FA) of chopped forage and silage of BRS Capiaçu elephant grass harvested at different regrowth ages

Fatty acid	Regrowth ages (days)^a				SEM	P-value	
	50	70	90	110		Linear	Quadratic
			Chopped forage				
C12:0	0.692 Bc	0.902 Bb	*	1.870 Aa	0.0587	<0.0001	0.1498
C14:0	0.462 Bc	0.612 Ab	*	1.196 Aa	0.0490	<0.0001	0.2946
C16:0	30.908 Bab	29.307 Bb	*	31.100 Aa	0.4973	0.4409	0.0278
C18:0	1.951 Bb	1.855 Bb	*	2.311 Aa	0.0567	0.0009	0.0156
cis-9 C18:1	6.021 Aa	4.745 Ab	*	4.724 Ab	0.2050	0.0044	0.0108
cis-9, *cis*-12 C18:2	20.954 Ab	22.215 Aa	*	21.073 Aa	0.2477	0.6894	0.0042
LnA^b	25.973 Aa	27.442 Aa	*	19.792 Bb	1.1541	0.0025	0.0398
			Silage				
C12:0	2.434 Aa	1.781 Aa	1.104 b	1.055 Bb	0.2578	0.0006	0.3148
C14:0	0.826 Aa	0.718 Aa	0.685a	0.689 Ba	0.0638	0.1578	0.3989
C16:0	41.734 Aa	38.856 Aa	32.542 b	29.498 Bb	1.8938	0.0003	0.9657
C18:0	2.943 Aa	2.628 Aab	2.234 b	2.318 Ab	0.1541	0.0064	0.2187
cis-9 C18:1	4.818 Ba	4.457 Aa	3.423 b	3.359 Bb	0.2226	0.0002	0.5183
cis-9, *cis*-12 C18:2	12.800 Bc	17.699 Bb	21.948 a	21.934 Aa	1.0499	<0.0001	0.0374
LnA^b	14.719 Bb	17.846 Bb	24.929 a	26.754 Aa	1.6393	<0.0001	0.6981

*Not analyzed; ^{a}For each variable, means followed by the same letters, which are uppercase in the columns in the comparison between chopped forage *versus* silage in each regrowth age (paired t-test) and lowercase in the rows for comparison among days of regrowth, do not differ at 5% probability; ^{b}α-Linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3).

In the Mata Atlântica Biome of Brazil, depending on the form of use (chopped grass or silage), BRS Capiaçu is recommended for harvesting and supplying to dairy cattle at 50 to 110 days of regrowth (Pereira et al., 2016a). In response to the advance in the regrowth age, there are intense physiological, morphological and structural changes in the plant, which promotes inevitable losses in the nutritional quality of the forage and, consequently, in the silage. Decreases in the leaf/stem ratio, leaf maturation and senescence, cell wall thickening and lignification are among the main changes in the morphology and structure of elephant grass resulting from the increase in regrowth age (Ferreira, Abreu, Martinez, Braz, & Ferreira, 2018; Maranhão et al., 2018). These aspects contribute to losses in the forage nutritional quality, which, in the present study, were mainly related to the decrease in the CP content, increases in the NDF, lignin and ADF levels and, consequently, reduction in IVDMD with the advance of regrowth age (Table 1; Figures 2a, 2b, 2e and 2f). For each increment of 1 g kg^{-1} DM of CP, NDF, ADF and lignin, changes ($P<0.0001$) of +3.96, -1.57, -1.67 and -3.74 in g kg^{-1}, respectively, were observed in chopped grass IVDMD, showing that CP and lignin were the factors most influential on IVDMD.

The main change in the chemical composition of the chopped grass occurred between the regrowth ages of 50 and 70 days, when reductions of 19% in CP content and 10% in IVDMD and increases of 9%, 10% and 54% in the contents of ADF, NDF and lignin, respectively, were observed ($P<0.05$; Table 1). However, there was an important increase ($P<0.05$) of 48% in the DM content in the comparison between the forages harvested at 50 and 70 days of regrowth. In a study carried out with lactating cows fed chopped elephant grass-based diets, Soares et al. (2009) reported lower ($P<0.05$) DM forage intake with 30 days of regrowth in relation to those cut at 45 and 60 days, which may be associated with its lower DM content. It should be noted that at 70 days of regrowth, the CP content is still 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). Considering all changes in the chemical composition, the forage obtained at 70 days still presented good nutritional value compared to that harvested at 50 days of regrowth. Between the regrowth ages of 70 and 90 days, the forage nutritional quality continued to fall, mainly due to the reduction of 20% in the CP content, which was below the recommended dietary minimum (Figure 2b), and the increase of 20% in the lignin content (Figure 2f), which was reflected in increases of 7% and 3% in the ADF and NDF levels (Figure 2e), respectively ($P<0.05$; Table 1). Between the regrowth ages of 90 and 110 days, there was a decrease ($P<0.05$) of 9% in the CP content and 10% in the IVDMD, and although the ADF and NDF levels stabilized ($P>0.05$), there was also a 9% increase ($P<0.05$) in lignin content (Table 1).

Zailan, Yaakub and Jusoh (2018) studied the nutritive quality of fresh and ensiled elephant grass cultivars and concluded that the process of fermentation clearly decreased the nutritive value of the original forage. In the present study, this was also observed. Except for the EE content, which was always higher in the silages than in chopped grass regardless of the regrowth age, there were reductions of 12% to 16% in the IVDMD and increases of 6% to 11% in the NDF content of the silages produced at different ages of regrowth (Table 1). Except at 90 days, at the other regrowth ages, there were reductions of 9% to 26% in the silage CP contents compared to those of the chopped grass, and the CP contents in these silages were always below the minimum of 70 g kg^{-1} DM recommended in the diet. The increase in the EE content in the silage compared to that of the original chopped grass corroborates the results of Arvidsson et al. (2009) and Bochicchio et al. (2015) and can be explained by the loss of water-soluble nutrients in the silo effluents or the fermentative products, thus concentrating the EE in the DM silage (Baumont, Arrigo, & Niderkorn, 2011; Bochicchio et al., 2015).

The effluent losses can reach 28.5% of the original wet mass of a chopped grass with 13% DM ensiled manually in experimental silos made of PVC (Savoie, Amyot, & Thériault, 2002). According to these authors, the average effluent chemical composition was 125, 290, 3.1, and 126 g kg^{-1} DM of MM, CP, ammonia N, and soluble sugars, respectively. These results also partially explain the lower MM and CP contents in silages than in chopped grass (Table 1). The effluent DM losses and those due to fermentative activity (catabolization of nonlipid organic compounds, mostly water-soluble carbohydrates and proteins) could also contribute to NDF enrichment in silages (Alves et al., 2011; Liu et al., 2019). Indeed, regardless of the regrowth age, the silages had more NDF than chopped grass (Table 1).

There is a general perception that tropical grasses are difficult to ensile, partly because the maximum nutritional quality of their forage is reached when the DM content is still too low to allow desirable fermentation in the silo. Wilting the forage to 30% DM prior to ensiling is a technique that can be used by small holders (Moran, 2005), but according to Daniel, Bernardes, Jobim, Schmidt and Nussio (2019), elephant grass is tall and has thick stems, which are difficult to wilt; moreover, wilting tropical grasses may increase field losses. In addition, wilting prior to ensiling reduces the total FA contents with significant losses in the contents of individual FAs, such as α-linolenic and/or linoleic acids (Boufaïed et al., 2003; Dewhurst et al., 2006; Van Ranst, Fievez, De Riek, & Van Bockstaele, 2009a). These losses are associated with the lipoxygenase system, a plant defense mechanism initiated in damaged tissues (Dewhurst et al., 2006). In the present study, wilting was not used to improve the fermentability of the ensiled forage mass. Thus, the best regrowth age for silage production is that in which there was a balance between the maximum nutritional quality of the forage to be ensiled and a DM content that allows rapid acidification of the silage and therefore efficient conservation of the ensiled forage. In the present study, for each increment of 1 g kg^{-1} DM in the chopped grass, there was a reduction of 0.01 in the pH value of the silage ($P<0.0001$; $r^2 = 0.70$; Figure 3). Tomich, Pereira, Gonçalves, Tomich and Borges (2003) proposed a system for the qualification of the silage fermentation process, associating the pH value with the silage DM content. In this respect, silages with DM contents less than 200 g kg^{-1}, such as all those obtained in the present study (Table 1), should have pH ≤ 4.0 to achieve good fermentation quality, while those with pH > 4.8 would present low fermentation quality. Considering these criteria, only silages produced at the regrowth ages of 90 and 110 days would have good fermentation quality (Table 1) in addition to being equivalent in nutritional quality, since, except for the ADF content that was 4% higher ($P<0.05$), the silage produced at 110 days of regrowth presented similar ($P>0.05$) MM, EE, ADF, lignin and CP contents and IVDMD (Table 1). Using the linear equation, a silage pH of 4.0 was estimated to be obtained at 97 days of regrowth (Figure 3).

The increase in the silage DM content as a function of regrowth age reflects the increases in the chopped grass DM content. For each increment of 1 g kg^{-1} in chopped grass DM content, an increase of 0.95 ($P<0.0001$; $r^2 = 0.97$) was observed in the silage DM content. This increase was proportionally greater at the age of regrowth of 50 days (+26%) than at 70, 90 and 110 days (+10%, +14% and +6%, respectively). This indicates, at least in part, that the effluent DM losses and/or those due to anaerobic fermentative activity were possibly greater at 50 days of regrowth, being gradually reduced as the DM content of the forage increased with the regrowth age. These supposed losses contributed to DM content enrichment of the silages, and since EE and NDF are less sensitive to anaerobic degradation in the silo (Alves et al., 2011), these nutrients were concentrated in the silage. For each increment of 1 g kg^{-1} in silage DM content, there were increases of 0.08 g kg^{-1} DM (P=0.0070; r^2 = 0.44) and 0.70 g kg^{-1} DM (P<0.0001; r^2 = 0.82), respectively, in the silage EE and NDF contents. On the other hand, as a function of the age of regrowth, it is expected that the nutrients most likely to leach, *i.e.*, MM, CP, ammonia N, and soluble sugars (Savoie et al., 2002) and most sensitive to anaerobic fermentative activity, *e.g.*, water-soluble carbohydrates and proteins (Alves et al., 2011) had their levels reduced in the silages. Compared to the ensiled forages, the silage CP contents were reduced by 26%, 17%, 14% and 9% at regrowth ages of 50, 70, 90 and 110 days, respectively. For each increment of 1 g kg^{-1} in silage DM content, there were reductions (P<0.001) of 0.20 g kg^{-1} DM (r^2 = 0.70) and 0.51 g kg^{-1} DM (r^2 = 0.88) in silage CP and MM contents, respectively.

On average, the FAs showing higher concentrations in the chopped grass were palmitic, α-linolenic and linoleic acids, accounting for approximately 76% of the total FAs. The average contents (g 100 g^{-1} FA) of the five major FAs (palmitic, α-linolenic, linoleic, oleic, and stearic acids) were in the ranges compiled by Lopes et al. (2015) for chopped elephant grass. Linear reductions were observed in the chopped grass contents (g kg^{-1} DM) of all of these FAs as well as in the content of the total FAs as a function of regrowth age (Table 2; Figures 4a to 4d). α-Linolenic acid had the largest rate of decrease with maturity, corroborating the results of Khan et al. (2015), followed by palmitic acid and linoleic acid at -0.021, -0.019 and -0.010 g kg^{-1} DM day^{-1}, respectively. The daily reduction in the chopped grass total FA content was -0.052 g kg^{-1} DM.

The chopped grass contents of oleic and linoleic acids (g kg^{-1} DM) were the most reduced, by approximately 45%, in the comparison between the regrowth ages of 50 and 110 days. In this period, the reductions in the contents of the total FAs and linoleic and palmitic acids were 31%, 28% and 37%, respectively. The magnitude of the individual FA reductions at each regrowth age in relation to reductions in the total FA contents was reflected in the results of chopped grass FA contents expressed in g 100 g^{-1} FA (Table 3), where the maximum contents of linoleic acid (22.367 g 100 g^{-1} FA) and α-linolenic acid (27.461 g 100 g^{-1} FA) were estimated to occur at 80 and 68 days of regrowth, respectively. That is, at approximately 70 days of regrowth, the BRS Capiaçu forage showed high levels of the two main precursors for the formation of vaccenic acid in the rumen and, subsequently, for the synthesis of rumenic acid in the mammary gland.

Only in two previous studies were the elephant grass FA compositions presented as a function of plant maturity. Similar to the present study, Mojica-Rodríguez et al. (2017) also observed a reduction (P<0.05) in the elephant grass forage contents of stearic and α-linolenic acids due to the increase in the regrowth age; the α-linolenic acid levels at 21, 42 and 63 days of regrowth were 0.46, 0.35 and 0.06 g kg^{-1} DM, respectively, showing much lower levels than those obtained in the present study. Additionally, corroborating the results of the present study, Khan et al. (2015) also observed reductions ($P<0.05$) in the contents of palmitic, linoleic, α-linolenic and total FAs in elephant grass forage with advancing plant maturity. The linoleic, α-linolenic and total FA contents of 2.36-2.90, 7.29-13.81, and 14.25-22.93 g kg^{-1} DM, respectively, were much higher than those obtained in the present study. The use of the elephant grass Mott cultivar, which has a high leaf:stem ratio, may be a possible explanation for this superiority, since according to Cabiddu et al. (2017) and Goossen, Kraft and Bosworth (2018), leaves have higher contents of total FAs than stems.

FAs in forage are present in membrane lipids, predominantly in thylakoid membranes of chloroplasts (Khan et al., 2015), and chloroplasts are the major site for *de novo* FA synthesis in the plant cell (Hölzl & Dörmann, 2019). Leaves have chloroplasts in greater number and size than stems and other nonleaf organs (Li, Bai, Hu, Kuang, & Lin, 2001), but during leaf senescence, there is a gradual reduction in the number of chloroplasts per mesophyll cell (Mae, Kai, Makino, & Ohira, 1984; Ono, Hashimoto, & Katoh, 1995). Chloroplast lipids are subject to constant turnover and are degraded during senescence (Hölzl & Dörmann, 2019). In a study with the tropical grass *Panicum virgatum* (switch grass), Z. Yang and Ohlrogge (2009) demonstrated that at 60 days after sowing, the total FA content of the leaves began to drop, and at the late senescence stage of 121 days, the leaves had lost 80% of FAs. According to these authors, the daily average degradation rate of FAs was approximately 0.69 μg mg^{-1} DM. In elephant grass, advancement at the age of regrowth increases the proportion of stems and senescent leaves, reducing the leaf proportion in the plant and subsequently reducing the leaf/stem ratio (Ferreira et al, 2018; Maranhão et al., 2018). Therefore, corroborating Khan et al. (2015), it can be assumed that the decrease in leaf/stem ratio and leaf senescence with advancing maturity promoted a decline in chloroplast lipids, decreasing the forage FA content (Table 2).

Positive relationships ($P<0.01$) were observed between chopped grass CP content (g kg^{-1} DM) and IVDMD (g kg^{-1}) *versus* the contents of the total FAs and oleic, linoleic and α-linolenic acids (g kg^{-1} DM). On the other hand, negative relationships ($P<0.05$) were observed between the contents of the total FAs and oleic, linoleic and α-linolenic acids (g kg^{-1} DM) *versus* the DM, NDF, ADF and lignin contents (g kg^{-1} DM) of chopped grass (data not shown). All of these responses are closely associated with the proportion and age of the leaves and stems of BRS Capiaçu. For each increment of 1 g kg^{-1} DM of CP, ADF and lignin in chopped grass, changes of +0.077, -0.031 and -0.075; +0.014, -0.006 and -0.013; and +0.029, -0.012 and -0.028, respectively, in the total FA, linoleic acid and α-linolenic acid contents (g kg^{-1} DM) were observed in chopped grass ($P<0.05$). Stems have higher contents of NDF, ADF and lignin, while leaves are richer in CP, total FAs, linoleic and α-linolenic acids (Cabiddu et al., 2017). Therefore, the close relationship between CP and the total FA content can be explained, at least in part, by these nutrients being primarily located in the leaves (photosynthetic organs) rather than in stems (Glasser et al., 2013). As already discussed, the leaves have chloroplasts in greater number and size than the stems (Li et al., 2001), and chloroplasts are rich in proteins and lipids (Gedi et al., 2017). These latter authors showed that chloroplast-rich fractions extracted from green leaf tissues of the tropical grass *Paspalum notatum* presented 302 and 304 g kg^{-1} DM of protein and lipids. Therefore, in several studies, positive relationships between CP or N *versus* total FA contents were also observed (Boufaïed et al., 2003; Glasser et al., 2013; Cabiddu et al., 2017; Goossen et al., 2018).

Several studies have evaluated the effect of regrowth age on the forage FA profile, but few have studied this effect in silage. At first, it could be expected that the reduction in the forage FA levels due to the age of regrowth (as shown in the present study; Tables 2 and 3) will also occur in silage, since, according to Khan et al. (2015), most of the variation in FA content in grass silages was caused by differences in maturity at harvest. In fact, except for myristic acid, the silage contents of all other FAs were influenced by the age of regrowth (Tables 2 and 3). However, harvesting and ensiling agricultural practices and their influence on the biochemical changes related to the fermentation process in the silo can substantially alter the chemical composition of the forage that has been ensiled (Baumont et al., 2011) as well as its FA profile (Boufaïed et al., 2003; Van Ranst et al., 2009a; Glasser et al., 2013). Considering the dietary FAs that are precursors for beneficial FAs in products of ruminants, the linear increases in the silage linoleic and α-linolenic acid contents, as well as in the total FAs, as a function of regrowth age, stand out (Table 2; Figures 4b to 4d). Thus, while this effect of regrowth age is not clear, promoting an increase in the silage contents of these FAs, changes that occur inside the silo can help in understanding these results.

In the present study, the percentage changes in the total FA, linoleic acid and α-linolenic acid contents (g kg^{-1} DM) of the silages compared to those of the chopped grass were -58%, -62% and -36% at 50 days; -46%, -53% and -27% at 70 days; -13%, -5% and -11% at 90 days (values for FA contents estimated by the linear equations); and -0.6%, + 26% and + 5% at 110 days of regrowth. At 50 and 70 days of regrowth, the total FA, linoleic acid and α-linolenic acid contents (g kg^{-1} DM and g 100 g^{-1} FA) were always higher ($P<0.05$) in chopped grass than in silage (Tables 2 and 3). However, at 110 days of regrowth, there was no difference ($P>0.05$) in the contents (g kg^{-1} DM) of these FAs between chopped grass and silage (Table 2; Figures 4b to 4d). The greatest losses in the total FA, linoleic acid and α-linolenic acid contents of the silages compared to those of the chopped grass were observed at 50 and 70 days of regrowth (Table 2).

The main changes in the FA profile during ensiling are related to the action of plant lipases, which are activated after injury of forage tissue during harvesting operations, especially the leaves, which are less resistant than the stems because they are less lignified and therefore more sensitive to the action of tools (Baumont et al., 2011). Lipase and lipoxygenase activities are elevated soon after harvesting or in poorly preserved silages (Bueno, Lazzari, Jobim, & Daniel, 2020), and the microbial lipases in silos do not contribute to a large extent to the lipolysis of forage membrane lipids (Ding et al., 2013; Gadeyne et al., 2016).

According to Tomich et al. (2003), silages with DM contents less than 200 g kg^{-1}, such as all those obtained in the present study (Table 1), should have pH≤4.0 to achieve good fermentation quality. This is related to the quality of fermentation in the silo. Plant lipases remain functional, and their activity is higher during the first two days of ensiling, declining with a reduction in silage pH, and residual oxygen is consumed with the progress of ensiling (Han & Zhou, 2013). Thus, considering that the rapid acidification of the silage might preclude FA cleavage (Bueno et al., 2020), the low DM contents of forages with 50 and 70 days of regrowth probably promoted a slow, prolonged and inefficient fermentation process in the silo (as indicated by the pH>5.0; Table 1), leading to higher lipolysis in silage lipids (Van Ranst et al., 2009a), resulting in free FA accumulation in the silo (Van Ranst, Fievez, Vandewalle, De Riek, & Van Bockstaele, 2009b). Considering that the most common substrates for plant lipoxygenases are α-linolenic and linoleic acids, these FAs can be further oxidized by the actions of these enzymes to form hydroperoxides and are finally decomposed into aldehydes and ketones (Han & Zhou, 2013) as well as be biohydrogenated into stearic acid by microbes in the silo (Liu et al., 2019).

According to Liu et al. (2019), lipase could also degrade lipids to release palmitic acid, which, because it is not oxidized by lipoxygenases, tends to increase in silage. Proportional increases (P<0.05) in the contents (g 100 g^{-1} FA) of palmitic and stearic acids in the silages in relation to the chopped grass at the different regrowth ages were +35% and +51% at 50 days; +33% and +42% at 70 days; and +11% and +13% at 90 days (values for FA contents estimated by the quadratic equations). At 110 days of regrowth, the silage contents (g 100 g^{-1} FA) of palmitic acid were 5% lower (P<0.05) than those of chopped grass, and there was no difference (P>0.05) in the silage contents of stearic acid compared to that of chopped grass (Table 3). The greatest increases in the contents (g 100 g^{-1} FA) of palmitic and stearic acids in the silages in relation to those in chopped grass observed at 50 and 70 days of regrowth (Table 3) can be considered indicative that, at these regrowth ages, there was an intense activity of lipases, releasing palmitic, α-linolenic and linoleic acids; the last two of these were further oxidized by lipoxygenases and biohydrogenated to stearic acid by microbes in the silo (Liu et al., 2019), as demonstrated by the significant reductions in their silage contents (Table 3). On the other hand, it is possible that the higher DM contents in forages with 90 and 110 days of regrowth promote a more rapid acidification of silage, precluding the oxidation of FAs (Bueno et al., 2020). This can be shown, at least in part, by the similar total FA, linoleic acid and α-linolenic acid contents (g kg^{-1} DM) in the silages compared to the chopped grass at 110 days of regrowth (Table 2). The silage contents of the total FAs and linoleic and α-linolenic acids showed correlations (P<0.05) that were positive with the silage DM content (r = 0.52 to 0.76) and negative with the silage pH (r = -0.39 to -0.61), which is indicative of the importance of these factors in the preservation of FAs in silages, primarily by a shorter fermentation and, therefore, fewer lipolysis, oxidation and biohydrogenation reactions, especially in linoleic and α-linolenic acids.

At 110 days of regrowth, there was similarity (P>0.05) in the total FA, linoleic acid and α-linolenic acid contents (g kg^{-1} DM) in silages compared to those in chopped grass (Table 2). Considering the α-linolenic acid content expressed in g 100 g^{-1} FA, there was even an increase (P<0.05) in the contents in silage compared to those in chopped grass (Table 3). In studies in which there was maintenance or even an increase of the total FA and/or linoleic and α-linolenic acid contents in the silages compared to chopped grass (Boufaïed et al., 2003; Alves et al., 2011; Glasser et al., 2013; Bochicchio et al., 2015), the main explanation for this result is the degradation of silage DM during anaerobic fermentation (catabolization of nonlipid organic compounds, mostly water-soluble carbohydrates and proteins) and/or a loss of soluble components in silage effluent, which is rich in MM and CP (Savoie et al., 2002), with a resultant increase in the levels of the other components, such as EE, total FAs, linoleic and α-linolenic acids (Boufaïed et al., 2003; Van Ranst et al., 2009b; Bochicchio et al., 2015). In fact, negative correlations ($P<0.05$) were observed between the silage contents of CP and MM *versus* the silage contents of EE ($r = -0.64$ and -0.54), total FAs ($r = -0.46$ and -0.44), linoleic acid ($r = -0.74$ and -0.66) and α-linolenic acid ($r = -0.62$ and -0.58).

Conclusions

BRS Capiaçu elephant grass must be harvested at up to 70 days of regrowth to obtain forage with good nutritional value and the highest levels of linoleic and α-linolenic acids (g kg^{-1} DM).

To produce silages with adequate pH values and the highest levels of linoleic and α-linolenic acids (g kg^{-1} DM), BRS Capiaçu must be harvested between 90 and 110 days of regrowth.

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