p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Strengthening for Research and Development No: 30/E/KPT/2018

Tropical Animal Science Journal, March 2022, 45(1):56-63
DOI: https://doi.org/10.5398/tasj.2022.45.1.56
Available online at http://journal.ipb.ac.id/index.php/tasj

The Influence of Plant Age and Microbes-Enzymatic Additives on Fermentation of Total Mixed Ration Silages of Capiaçu Grass *(Pennisetum purpureum, Schum)*

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(Received 11-05-2021; Revised 20-07-2021; Accepted 04-08-2021)

ABSTRACT

The conservation of elephant grass cultivar Capiaçu in the form of total mixed ration (TMR) silage can help to improve the quality of the fermentative process and optimize feed management. However, the best cutting age of grass and the necessity of using microbes-enzymatic additives to aid in the process have not been determined thus far. Therefore, the objective of this study was to evaluate cutting age and different microbes-enzymatic additives on the fermentative and nutritional quality of total mixed ration (TMR) silages based on BRS Capiaçu. A completely randomized design was used in a 3×3 factorial scheme, with three cutting ages of grass (60, 90, and 120 days of regrowth), associated with three types of additives [CON (control), HOM (homofermentative inoculant + fibrolytic enzyme), and COMBO (homofermentative inoculant + heterofermentative inoculant + fibrolytic enzyme)]. The fermentative losses, the production of organic acids, chemical composition, and the aerobic stability of the tested silages were measured. The TMR silages containing 60-day-old grass showed the lowest dry matter contents, highest effluent production, and lower aerobic stability. The COMBO inoculant application provided higher acetic acid contents and greater aerobic stability of the 90- and 120-day-old grass silages. The highest lactic acid concentrations were observed in silages produced with the 60- and 90-day-old grass silages. It is concluded that the TMR produced with BRS Capiaçu at 90 days of age and in association with COMBO is the best option to balance the nutritional and fermentative quality of this type of silage.

Keywords: ***β-glucanase; fermentation quality; Lactobacillus buchneri; Pediococcus pentosaceus; xylanase***

INTRODUCTION

BRS Capiaçu is an elephant grass cultivar (*Pennisetum purpureum*, Schum.) for which high dry mass production has been reported; additionally, the cultivar exhibits resistance to tumbling, drought stress, and is suitable for mechanical harvesting (Santos *et al*., 2013; Pereira *et al*., 2017). These qualities help identify BRS Capiaçu as a good alternative for the formation of grass areas when the objective is the production of silage (Monção *et al*., 2019; Monção *et al*., 2020).

Along with high productivity, BRS Capiaçu produces a substantial proportion of succulent stems, which result in the production of forage with low levels of dry matter (DM) (Pereira *et al*., 2017). Low levels of DM can cause excessive effluent production during silage fermentation and lead to undesirable fermentations, which can directly impact the nutritional quality of the silage (Gebrehanna *et al*., 2014; Borreani *et al*., 2018). Therefore, to increase the DM contents of elephant grass silage, several researchers recommend adding small proportions of dry concentrates with grass or suggest ensiling total mixed rations (TMR) (Weinberg *et al*., 2011; Bueno *et al*., 2020).

The TMR silages comprise the ensilage of a complete diet (fresh forage, protein concentrate, energy concentrate, vitamins, minerals, and additives), formulated to provide the nutritional requirements of a specific animal category (Bueno *et al*., 2020). Thus, TMR facilitates the feed management of farms because it eliminates the need for the daily preparation of diets and allows a more rational use of labor.

Gusmão *et al.* (2018) obtained positive results for improving the fermentative and nutritional quality of TMR silages obtained from elephant grass cv. Cameroon was cut at the height of 1.8 m (early cutting). In practice, elephant grass management can be performed at different heights or cutting ages (60 to 120 days). These factors interfere with biomass production and forage nutritional quality (Zailan *et al*., 2016; Adesogan *et al*., 2019), especially after 70 days of regrowth, when the reduction in the yield of crude protein digestible of elephant grass is higher (Muia *et al*., 1999). Consequently, these quality and chemical composition variations may lead to

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changes in the fermentative and nutritional parameters of the TMR silages produced. However, no studies have demonstrated the influence of the age of BRS Capiaçu on TMR production.

This cultivar was presented on the Brazilian market in 2016; however, information regarding its fermentative and nutritional quality when ensiled in TMR form needs to be better studied. Thus, the objective of the current study was to evaluate the fermentative and nutritional quality of TMR silages, based on BRS Capiaçu characteristics at different cutting ages, in association with varying types of microbes-enzymatic additives. Therefore, the current study has been conducted based on the following hypotheses: i) the use of dry concentrates for the composition of TMR reduces the production of silage effluent, regardless of the age of the plant; ii) the microbes-enzymatic additives provide better fermentative quality of TMR silages, and iii) the advancing age of BRS Capiaçu elephant grass negatively interferes with the nutritional quality, but positively affects the fermentative quality of TMR silages.

MATERIALS AND METHODS

The current experiment was conducted at Embrapa Agropecuária Oeste (CPAO) between the years 2019 and 2020. The analyses of the chemical composition and *in vitro* dry matter digestibility (IVDMD) of the silages were performed at the Laboratory of Utilization of Agricultural Waste of the Federal University of Grande Dourados (UFGD). Both research institutions are located in the municipality of Dourados, MS (22°11′ S, 54°56′ W; 452 m asl).

A completely randomized design was used in a 3x3 factorial scheme, considering three cutting ages of BRS Capiaçu (60, 90, and 120 days of regrowth), associated with the use of three types of additives [CON (control - distilled water), HOM (homofermentative inoculant + fibrolytic enzyme), and COMBO (homofermentative inoculant + heterofermentative inoculant + fibrolytic enzyme)], with five replicates performed per treatment (experimental silos).

The HOM inoculant used consisted of the following: *Pediococcus pentosaceus* NCIMB 12455 (1.8×10^5 CFU/g of forage), *Lactobacillus plantarum* CNCM I- 3736 (2.0×10^4 CFU/g of forage), β-glucanase (8,000 IU/g of product), and Xylanase (9,000 IU/g of product). The COMBO inoculant used consisted of the following: *P. pentosaceus* NCIMB 12455 (1×10^5 CFU/g of forage), *Lactobacillus buchneri* NCIMB 40788 (7.5×10^4 CFU/g of forage), *Lactobacillus hilgardii* CNCM I-4785 (7.5×10^4 CFU/g of forage), β-glucanase (5,750 IU/g of product), and Xylanase (30,000 IU/g of product). Both additives were produced by Lallemand Animal Nutrition, Brazil, GO.

The elephant grass cultivar used to prepare the silage was BRS Capiaçu; the cultivar was obtained from a 540-m^2 area, and it had been planted two years earlier. Before the commencement of the experiment, the area was divided into three parts. A standardization cut was performed (15 cm from the soil surface) at different times in each part, to obtain grass with different regrowth ages. In the first part of the area, a standardization cut was performed on 12/12/2019, while in the second part, the standardization cut was performed on 01/12/2020 and, in the third part, the standardization cut was performed on 02/12/2020. Fertilization with 200 kg of N/ha and 50 kg of K_2O/ha, using urea and potassium chloride as the nitrogen and potassium source, respectively, was performed after the conduction of each standardization cut. With the staggering standardization cuts, it was possible to obtain grass at cutting ages of 60, 90, and 120 days on 04/12/2020 (harvest day).

On the day of harvest, the grass was cut at 15 cm from the soil surface and ground in a chopper (Trapp model TR500) to obtain an average particle size of 2 cm. A grass sample of each age was used to determine the dry matter (DM) content of the grass, which was used to calculate the proportions of roughage and concentrate in the TMR.

The microbes-enzymatic additives were diluted in distilled water, and application of the additives was performed using a manual sprayer on the TMR of each plot, following the manufacturer's instructions (10 g of the additive diluted in 90 mL of water per ton of fresh forage). Subsequently, the material from each plot was homogenized once again and used for filling the experimental silos. During the filling of the experimental silos, two samples of TMR were collected from each treatment; the first sample, approximately 300 g, was used to determine the chemical composition and *in vitro* dry matter digestibility (IVDMD), and the second sample, of approximately 70 g, was frozen for further processing and determination of pH and buffering capacity values.

The mass of TMR of each treatment was stored in experimental silos constructed using polyvinyl chloride tubes (PVC, 10 cm of diameter and 50 cm of height), with a volume of 3.8 L. The material was compacted manually with wooden sticks to obtain the average density of 820 kg/m^3. At the bottom of each silo, a layer of approximately 4.5 cm of sand (300 g) was placed to facilitate the drainage of effluents. A fine mesh of cotton fabric was used to avoid contact between the forage and the sand. After the filling performance, the silos were sealed with double-sided plastic canvas (black and white) and adhesive tape and stored in the laboratory at room temperature for 90 days.

To calculate the fermentative losses, all components of the silos (PVC tube, sand, and fabric), as well as the mass of ensiled TMR, were weighed before and after the ensiling process. Then, the DM recovery (g/kg of ensiled DM), gas loss (g/kg of ensiled DM), and effluent production (g/kg of ensiled forage) were calculated according to the equations reported by Li *et al*. (2017).

After the silos were opened, the material present in each silo was removed and subjected to homogenization for sampling. Two samples were collected from each treatment; one sample was used to evaluate the chemical composition and IVDMD. The other sample was frozen for later evaluation of ammonia nitrogen ($N\text{-}NH_3$) generation and the organic acid profile of silages.

Approximately 2 kg of silage from each treatment was used and added to their respective silos to evaluate aerobic stability. The material was covered with a moistened cotton fabric to allow air entry and avoid dehydration of the silage. The air and silage temperatures were monitored for seven consecutive days, every 30 minutes, using a data logger. The disruption of aerobic stability was considered when the silage temperature exceeded the air temperature by 2 °C (Kung Jr *et al*., 2018).

Before and after subjection to ensiling, the TMR samples were pre-dried in an oven with forced circulation at 55 °C for 72 h. Subsequently, these samples were ground in a Willey mill using a 1.0-mm mesh sieve to determine the chemical composition and IVDMD of the silages. The contents of DM, organic matter (MO; method 942.05), and crude protein (CP; method 976.06) were determined according to AOAC (2005). The contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin were analyzed according to Mertens (2002), with the use of thermostable amylase for NDF. The value of IVDMD was determined according to the methodology described by Tilley & Terry (1963). The ruminal fluid (4.0 L) was collected in the morning from two Holstein x Zebu cattle (450 ± 6.86 kg) (CEUA/UFG-ethical commission on the use of animals in experimentation protocol number 023/2015), provided with a ruminal cannula, grazing *Urochloa brizantha* (syn Brachiaria), and receiving only mineral supplementation. The collected ruminal fluid was processed in a blender, filtered through 4 layers of cheesecloth into a warm (39 °C) insulated flask, and purged with CO_2. The buffering capacity of the TMR in the pre-silage was determined according to the method described by Playne & McDonald (1966). The chemical composition and the IVDMD of BRS Capiaçu and the TMR used in the experiment are presented in Table 1.

The frozen samples (initial and final) were thawed to obtain an aqueous extract, and this extract was used to evaluate the pH (initial and final), buffering capacity (only in the initial material), ammoniacal nitrogen, and organic acid contents (only in the final material). To obtain the aqueous extract, 25 g of forage was diluted in 225 mL of distilled water and was subjected to homogenization in an industrial blender for approximately 5 minutes. The pH of the extract was determined using a digital potentiometer (mPA210 MS Tecnopon), and the buffering capacity was determined according to the method described by Playne and McDonald (1966). A portion of this extract was filtered through filter paper, centrifuged for 15 min at 10,000 rpm, and the supernatant was frozen at -20 °C for conducting further analysis of volatile organic acids. Organic acid profiles were determined by performing gas chromatography with a mass detector (GCMS QP 2010 Plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, USA, 60 m, 0.25 mm Ø, 0.25 µm polyethylene cross bond carbowax glycol). The concentration of lactic acid was determined by performing the colorimetric method according to Vendramini *et al*. (2016). The level of $N\text{-}NH_3$ was determined according to the methodology described by Carlson (1978).

The data were analyzed using the statistical program RStudio (R, 2019). The mean values were compared by using the Scott Knott test, with a significance level of 5%, according to the following model:

$$Yijk = \mu + Ti + Pj + TPij + \mathcal{E}ijk$$

where Yij represents the response, μ represents the general average; Ti represents the effect of the additive, Pj represents the effect of the cutting ages of BRS Capiaçu, TPij represents the interaction between the additives and the cutting ages, and Ɛij represents the error associated with each observation. Each experimental unit was represented by an experimental silo, with five observations per treatment (k), a total of 45 experimental units. The significant interaction between the additives and the cutting ages was shown in the figure, and the main components' effect was disregarded in this case.

RESULTS

The cutting age of the grass did not influence the values of DM recovery of the silage (Table 2). However, silages subjected to treatment with the additive COMBO and CON showed the highest values for DM recovery. No differences were observed for gas losses among the treatments conducted.

Effluent production was affected by the age of the grass, with the highest value observed for TMR silages

Table 1. Chemical composition of ingredients and total mixed rations (TMR) used in the experiment

Items	pH	BC	DM	OM	CP	NDF	ADF	IVDMD
			g/kg of DM					
Elephant grass (60 days)	-	-	134	878	53	714	260	602
Elephant grass (90 days)	-	-	165	882	38	722	266	658
Elephant grass (120 days)	-	-	208	916	28	738	280	520
Dry ground corn	-	-	890	809	81	81	63	890
Soybean meal	-	-	891	810	458	69	45	870
Calcitic limestone	-	-	952	0	0	0	0	0
TMR (60 days)	6.1	15	234	925	104	437	144	748
TMR (90 days)	6	16	269	927	96	451	168	732
TMR (120 days)	5.9	18	324	944	91	461	175	681

Note: pH= hydrogen potential; BC= buffering capacity (meq HCl/100g of forage); DM= dry matter, OM= organic matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; IVDMD= *in vitro* dry matter digestibility. For the production of TMR, a standard diet was used, with the following proportions of ingredients (g/kg of DM): 516 g/kg elephant grass, 388 g/kg dry ground corn, 91 g/kg soybean meal, and 5 g/kg calcitic limestone.

produced with grass cut at 60 days of age, followed by grass cut at 90 days of age, with the lowest production observed in grass cut at 120 days of age. The CON treatment also presented higher effluent production when compared to the silages inoculated with HOM and COMBO.

The pH of the silages was not influenced ($p>0.05$) by the age at which the grass was cut or by the additives used. The highest lactic acid concentrations were observed in TMR silages produced using grass at 60 and 90 days of regrowth. The lactic acid contents decreased with the use of the additives HOM and COMBO when compared to CON.

For acetic acid and butyric acid contents, the interaction was established between the grass's age and the type of additive used (Figure 1). The silages inoculated with COMBO presented higher concentrations of acetic acid regardless of the age of the grass. For grass cut at 90 days of age, the CON treatment presented the lowest concentration of acetic acid. For the grass cut at 120 days of age, the HOM treatment presented a lower concentration of acetic acid. The highest contents of butyric acid (average of 2.9 g/kg of DM) were observed for the TMR silage made with the grass cut at 60 and 90 days of age and without additive inclusion (CON). For the 60- and 90-day-old grass samples, the use of additives (HOM and COMBO) resulted in lower butyric acid contents in the silages. The TMR silage produced with BRS Capiaçu, cut at 120 days of regrowth and inoculated with COMBO, presented the lowest value of butyric acid among the treatments conducted.

The iso-valeric acid yields were higher in the treatments that involved additives (HOM or COMBO) compared to CON. For the contents of propionic acid and valeric acid, no differences were observed between treatments. TMR silages produced with grass cut at 90 days of age showed the lowest values of $N\text{-}NH_3$, and no influence of the additives was observed on this parameter.

An interaction was observed between the ages of the grass and the microbes-enzymatic additives in the values of aerobic stability (Figure 1). For TMR silages produced with grass cut at 60 days of age, no effect of the use of microbes-enzymatic additives was exerted on the aerobic stability of the silages; however, the disruption of stability occurred at approximately 38 hours for these treatments. Furthermore, for TMR silages produced with the grass cut at 90 and 120 days of age, the addition of the COMBO inoculant resulted in greater aerobic stability (128 and 132 hours, respectively) in comparison to 60 days of age grass silages.

Higher contents of DM were observed for silages with the addition of grass cut at 120 days of age. The highest CP levels were observed in the TMR silages produced using 60-day-old grass. Higher CP levels were also observed in the silages that were subjected to treatment with the COMBO additive. No differences were

Table 2. Fermentative variables and chemical composition of total mixed rations (TMR) silages containing BRS Capiaçu grass at different cutting ages (60, 90, and 120 days) and microbes-enzymatic additives

Variables	Cutting age (days)			Additive			EPM	p value		
	60	90	120	CON	HOM	COMBO		Ag	Add	Ag*Add
Recovery of DM, g/kg DM	973	965	971	973^{A}	959^{B}	977^{A}	2.1	ns	**	ns
Gas loss, g/kg DM	31	36	31	31	30	37	1.4	ns	ns	ns
EP, kg/ton DM	327^{a}	179^{b}	84^{c}	221^{A}	175^{B}	193^{B}	16.5	**	**	ns
Final pH	4.4	4.4	4.5	4.6	4.4	4.5	0.41	ns	ns	ns
Lactic acid, g/kg DM	61^{a}	58^{a}	55^{b}	64^{A}	56^{B}	54^{B}	1.2	*	**	ns
Acetic acid, g/kg DM	35^{b}	43^{a}	32^{b}	30^{C}	37^{B}	54^{A}	2.3	*	*	**
Butyric acid, g/kg DM	2.5^{a}	2.5^{a}	1.2^{b}	2.9^{A}	1.2^{C}	2.1^{B}	0.26	**	**	**
Propionic acid, g/kg DM	3.1	4.1	4.2	3.6	4.2	3.8	0.22	ns	ns	ns
Iso-valeric acid, g/kg DM	9.4	6.9	3.1	4.9^{B}	7.4^{A}	7.2^{A}	0.62	ns	*	ns
Valeric acid, g/kg DM	8	16	10	7	12	14	1.1	ns	ns	ns
$N\text{-}NH_3$, g/kg TN	42^{a}	28^{b}	46^{a}	42	36	38	2.1	**	ns	ns
Aerobic stability, hours	38^{c}	118^{a}	73^{b}	99^{A}	67^{B}	63^{B}	6.3	**	**	**
Dry matter, g/kg fresh forage	297^{b}	301^{b}	332^{a}	310	307	313	3.6	**	ns	ns
Organic matter, g/kg DM	934^{b}	928.51^{c}	947.55^{a}	931.41	940.53	938.82	1.745	**	ns	ns
Crude protein, g/kg DM	101.4^{a}	96.8^{b}	98.8^{b}	97.8^{B}	98.3^{B}	101.0^{A}	0.64	**	**	ns
NDF, g/kg DM	540	524	548	552	522	538	6.3	ns	ns	ns
ADF, g/kg DM	158^{c}	173^{b}	191^{a}	169	177	176	3.3	**	ns	ns
Cellulose, g/kg DM	138^{c}	152^{b}	167^{a}	148	155	154	2.9	**	ns	ns
Hemicellulose, g/kg DM	392^{a}	351^{b}	356^{b}	393^{A}	341^{B}	365^{B}	7.8	**	**	ns
Lignin, g/kg DM	19.9^{b}	20.5^{b}	25.5^{a}	20.9	22.4	22.6	0.67	**	ns	ns
IVDMD, g/kg DM	800^{a}	731^{b}	742^{b}	757	746	770	6.4	**	ns	**

Note: EP= effluent production; DM= dry matter; $N\text{-}NH_3$= ammonia nitrogen; TN= total nitrogen; NDF= neutral detergent fiber; ADF= acid detergent fiber; IVDMD= *in vitro* dry matter digestibility; CON= control; HOM= homolactic additives + fibrolytic enzymes; COMBO= homolactic additive + heterolactic additive + fibrolytic enzymes; SEM= standard error of the mean. Means followed by different letters differ from each other according to Scott Knott test at 5% probability. Capital letters represent differences between additives (Add) and lowercase letters represent differences between plant ages (Ag). ns= non-significant; * = significant at 5% probability; ** = significant at 1% probability. The significant interaction between the additives and the cutting ages were shown in the Figure 1

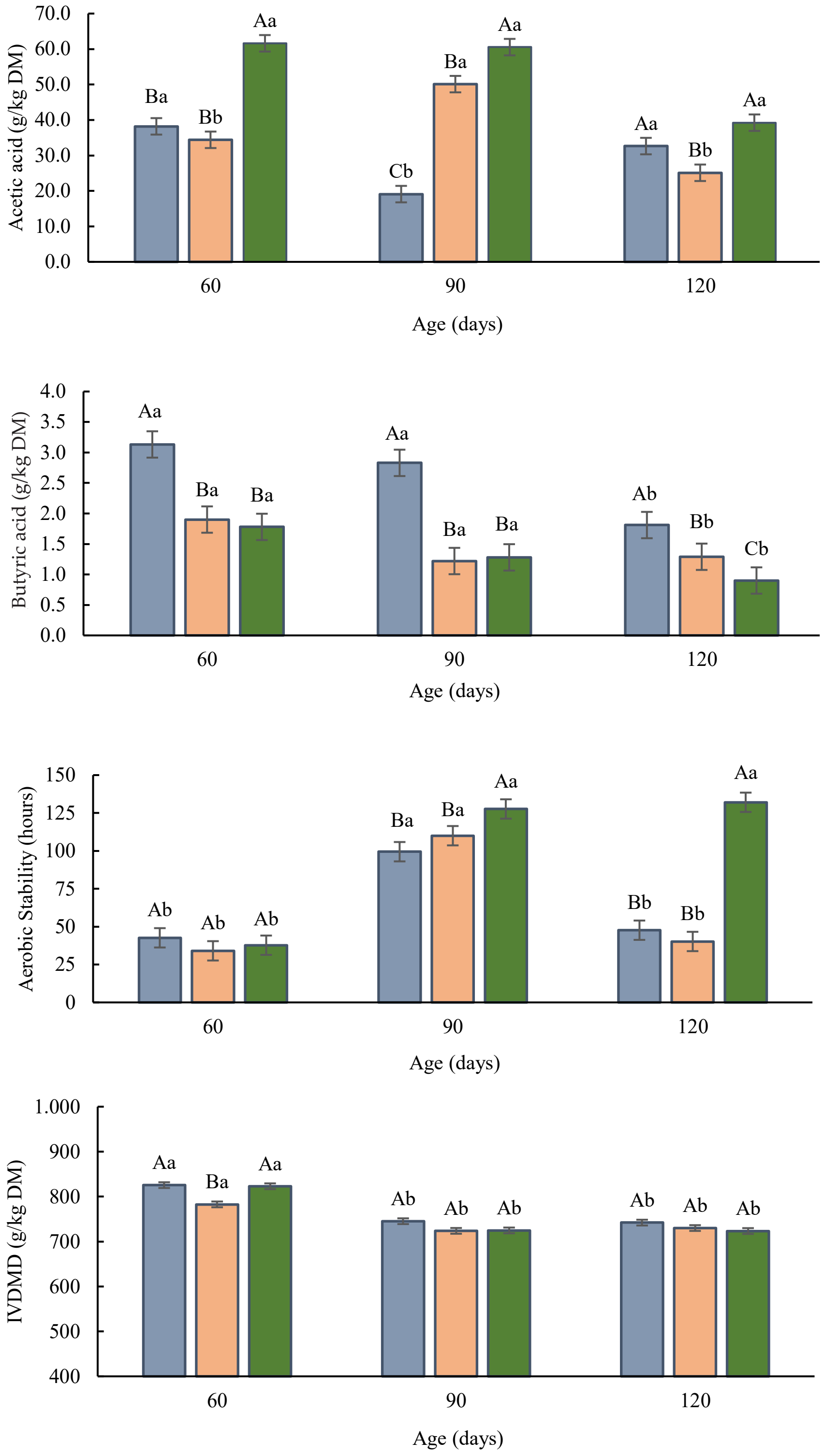

Figure 1. Levels of acetic acid and butyric acid, aerobic stability, and *in vitro* DM digestibility of total mixed rations (TMR) silages containing BRS Capiaçu grass at different cutting ages (60, 90, and 120 days) and microbes-enzymatic additives. Means followed by different letters differed according to Scott Knott test at 5% probability. Capital letters represent the differences between the additives used, and lowercase letters represent differences between plant ages. 🟦 = CON; 🟧 = HOM; 🟩 = COMBO.

observed in the NDF levels in the treatments evaluated. However, the ADF levels and cellulose were influenced by cutting ages, with the lowest averages observed in silages produced using 60-day-old grass.

In the case of hemicellulose, the lowest contents were observed in the TMR silages produced using 90 and 120-day-old grass. Lower hemicellulose contents were also observed in silages that were subjected to treatment with the COMBO and the HOM additives.

In lignin content, grass aged 60 and 90 days presented with the lowest averages, with no differences observed between the applied additives.

For the IVDMD, the establishment of interaction was observed between the period of grass regrowth and the use of additives (Figure 1). The TMR silages produced using grass cut at 60 days of age with additives COMBO, and CON showed the highest IVDMD. The lowest IVDMD values were observed in TMR silages produced using grass at 90 and 120 days of age, regardless of the type of inoculation.

DISCUSSION

The TMR silages, based on elephant grass, cultivar BRS Capiaçu, presented DM levels ranging from 290 g/kg to 330 g/kg, depending on the age of the grass. Regardless of the age of regrowth, BRS Capiaçu grass presented low DM values at the time of cutting (134.2 g/kg to 208.9 g/kg of DM, as shown in Table 1). The DM values of the TMR silages obtained in the present study were lower than those reported for TMR silages based on corn or sorghum, which present with DM contents ranging from 400 g/kg to 650 g/kg (Bueno *et al*., 2020).

The lower values of DM observed for the TMR silages prepared using grass at 60 days of age explain the higher production of effluent observed for this treatment. According to the findings reported by Borreani *et al*. (2018), silages with DM contents lower than 350 g/kg resulted in higher effluent productions.

The low content of DM can lead to a lower recovery of DM and loss in the nutritional quality of the silage attributed to the leaching of nutrients (Orrico Junior *et al*., 2015; Orrico Junior *et al*., 2020). The use of HOM and COMBO additives may have accelerated the initial process of fermentation of the ensiled mixture, thus reducing the period of action of aerobic microorganisms that use the carbohydrates present in the ensiled mass for the production of energy, CO_2, and metabolic water (Borreani *et al*., 2018). The lower metabolic water production might explain lower effluent production exhibited by silages inoculated with HOM and COMBO additives compared to those without subjection to inoculation.

The preferred use of strains with homofermentative metabolism (HOM) was not efficient in promoting greater recovery of DM and higher lactic acid production compared to the control treatment. Recovery of DM and gas production in silages is strongly correlated with fermentation that occurs inside the silos. Based on the findings reported by Borreani *et al.* (2018), lactic acid fermentation results in minimal losses of DM and energy, while the acetic acid, alcoholic, and butyric acid fermentation result in greater losses of DM and gas production (mainly CO_2).

Likely, the competition between the homofermentative bacterial strains present in the microbial additive and the epiphytic microbiota present in the plants may have led to the lower production of lactic acid (Michel *et al*., 2016). Another factor that can justify such findings is the use of calcitic limestone present in the TMR formulation. Application of calcitic limestone increases the osmotic pressure of the medium with buffering action, which can trigger changes in the populations of microorganisms due to the inhibition of certain organic acids produced during fermentation (Bueno *et al*., 2020).

There are reports on the literature on the lack of efficiency of homofermentative microbial additives in reducing fermentative losses in tropical grass silages. Cunha *et al*. (2020) and Vendramini *et al*. (2016) also observed no differences in the recovery of DM of silages inoculated with commercial microbial additives that contained *P. pentosaceus* and *L. plantarum*. Likewise, Orrico Junior *et al.* (2020) did not observe improvements in DM recovery values when silages were inoculated with *P. pentosaceus* and *L. buchneri.*

The pH of the silages remained slightly elevated in the current experiment, even with the high lactic acid levels. The main factor contributing to a lower decrease in pH values during the fermentation process was the presence of limestone in the TMR formulation. The presence of mineral mixtures and protein concentrates can change the buffering capacity of the ensiled material (Table 1), hindering the decrease in pH values.

Based on the findings reported by Bueno *et al.* (2020), increasing the buffering capacity of silages can result in high acid production (mainly lactic acid) because high acid production is necessary for the silage to achieve pH stability. In the literature, studies reporting high lactic acid production (above 50 g/kg of DM) in TMR silages are common (Gusmão *et al*., 2018), with pH values reportedly ranging between 3.9 and 4.8. This behavior was also observed in the current experiment, where high lactic acid levels did not correspond to low pH values.

The higher moisture content, associated with a pH of 4.39 of the silages produced with younger grass (60 days), may have contributed to the higher levels of butyric acid and $N\text{-}NH_3$. Based on the findings reported by Kung Jr *et al.* (2018), the higher production of butyric acid and $N\text{-}NH_3$ occurs as a result of the activity of bacteria of the genus *Clostridium sp* under conditions of high humidity and higher pH. The activity of these microorganisms compromises the efficiency of forage conservation because they can utilize soluble sugars, proteins, or lactic acid present in silages as a substrate for their growth (Orrico Junior *et al*., 2017; Kung Jr *et al*., 2018). However, it is important to mention that the values of butyric acid and $N\text{-}NH_3$ observed in the present study are within the recommended values for good-quality silages (butyric acid below 10 g/kg of DM and $N\text{-}NH_3$ below 100g/kg of NT) (Kung Jr *et al*., 2018).

The silages produced with the use of the COMBO additive were more efficient at producing acetic acid when compared to the other treatments. This was probably due to the action of *L. buchneri*, which synthesizes acetic acid via lactic acid metabolism (Comino *et al*., 2014; Gandra *et al*., 2016; Kung Jr *et al*., 2018). Since acetic acid has a higher dissociation constant compared to lactic acid, it is usually the most important organic acid involved in inhibiting the growth of yeast and molds (Wilkinson & Davies, 2013). This might explain the reason underlying the highest aerobic stability values for the treatments that exhibited the highest levels of acetic acid.

The lowest aerobic stability values were observed for the silages produced using the 60-day-old regrowth grass. The values might be associated with the high levels of lactic acid produced due to these treatments. In addition to showing less efficacy at inhibiting yeast and mold growth, lactic acid can be used as an energy source for yeast growth. Few experiments have shown that well-fermented silages containing high lactic acid contents (relative to the total organic acids produced) commonly exhibit lower aerobic stability values (Maxin *et al*., 2017).

The values of the buffering capacity of TMR may also have contributed to the TMR silages, prepared using grass cut at 90 and 120 days of age, had shown higher values of aerobic stability. This is because buffering capacity is not only responsible for hindering the decline in the pH value of the silage but is also responsible for hindering the elevation of the pH value after the opening of the silo, which subsequently reduces the rate of growth of aerobic microorganisms (longer time required by the silage to reach the instability phase).

The lower values of CP and IVDMD and the higher proportions of fiber observed in the TMR silages prepared using grass cut at 90 and 120 days of regrowth result from the effect of grass age on its nutritional quality. BRS Capiaçu is a plant that can reach 4.2 m in height at older (Pereira *et al*., 2017), requiring substantial proportions of fiber in the supporting tissues to maintain plant structure. Thus, with advancing age and increasing plant height, there is inevitably a marked decline in the nutritional quality of the forage (Adesogan *et al*., 2019).

The use of the microbial additives with the enzymes β-glucanase and xylanase may have contributed to the partial degradation of the plant cell wall, thus explaining the reduction in hemicellulose contents in these silages corroborating the findings of the study conducted by Li *et al.* (2018). Despite the reduction in hemicellulose content, the addition of the inoculants containing fibrolytic enzymes was not efficient in improving the digestibility of TMR silage.

Notably, we used a standard concentrate and the same proportion of roughage and concentrate (base of DM) for all TMRs tested. Only the age of grass regrowth and the inclusion of different types of additives were subject to variations. Thus, adjustments in the proportions of TMR ingredients can correct the decrease in the nutritional value of the grass as a function of age at regrowth. The use of microbes-enzymatic additives showed efficiency only at reducing losses attributed to affluent and the concentrations of butyric acid and hemicellulose; hence these additives should be evaluated with caution considering the costs of its application.

CONCLUSION

It is concluded that the TMR produced with BRS Capiaçu at 90 days of age and in association with COMBO is the best option to balance the nutritional and fermentative quality of the silages, besides promoting better aerobic stability of TMR. The formulation of TMR with elephant grass cv. BRS Capiaçu, cut at 60 days of regrowth, showed better nutritional value; however, it did not help maintain the effluent production within acceptable levels, apart from providing silages with higher levels of butyric acid and lower aerobic stability.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

Appreciation is extended to the Coordination for the Improvement of Higher Education Personnel (CAPES; Brasilia, DF, Brazil) and the National Council of Technological and Scientific Development (CNPq; Brasilia, DF, Brazil) for their investments in the authors.

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