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Protein and carbohydrate profiles of 'Massai' grass silage with pelleted citrus pulp and microbial inoculant

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Abstract – The objective of this work was to evaluate the effects of the inclusion of pelleted citrus pulp and microbial inoculant on the fermentation characteristics, carbohydrate and protein fractionation, and total digestible nutrient contents of 'Massai' grass (*Megathyrsus maximus* × *Megathyrsus infestus*) silage. The experimental design was completely randomized, in a 2×4 factorial arrangement (application or not of inoculant × 0, 10, 20, and 30% citrus pulp) with five replicates. There was an increase in dry matter recovery as affected by citrus pulp levels. The values obtained for pH and ammonia nitrogen indicated an adequate fermentation process. Under increasing citrus pulp levels, there was a reduction in neutral detergent fiber and an increase in nonfibrous carbohydrate contents and in protein linked to fiber. Total digestible nutrient content reaches a peak of 513.1 g kg^{-1} with the inclusion of 12.50% citrus pulp. The microbial inoculant, when isolated, does not influence carbohydrate and protein profiles. Inclusions between 10 and 20% citrus pulp are able to maintain adequate fermentative parameters and improve the digestible nutrient profile, with an adequate dry matter recovery.

Index terms: *Megathyrsus maximus* × *Megathyrsus infestus*, additive, nonfibrous carbohydrates.

Perfis de proteínas e carboidratos de silagem de capim 'Massai' com polpa cítrica peletizada e inoculante microbiano

Resumo – O objetivo deste trabalho foi avaliar os efeitos das inclusões de polpa cítrica peletizada e de inoculante microbiano sobre características fermentativas, fracionamento de carboidratos e proteínas, e teores de nutrientes digestíveis totais de silagem de capim 'Massai' (*Megathyrsus maximus* × *Megathyrsus infestus*). O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 2×4 (aplicação ou não de inoculante × 0, 10, 20 e 30% de polpa cítrica), com cinco repetições. Houve aumento da recuperação de matéria seca em função dos níveis de polpa cítrica. Os valores obtidos para pH e nitrogênio amoniacal indicaram adequado processo fermentativo. Sob níveis crescentes de polpa cítrica, houve redução dos teores de fibra insolúvel em detergente neutro e aumento do teor de carboidratos não fibrosos e proteína ligada à fibra. O teor de nutrientes digestíveis totais atinge ponto máximo de 513,1 g kg^{-1} com a inclusão de 12,50% de polpa cítrica. O inoculante microbiano, quando isolado, não influencia os perfis de carboidratos e proteínas. Inclusões entre 10 e 20% de polpa cítrica são capazes de manter parâmetros fermentativos adequados e melhorar o perfil de nutrientes digestíveis, com adequada recuperação de matéria seca.

Termos para indexação: *Megathyrsus maximus* × *Megathyrsus infestus*, aditivo, carboidratos não fibrosos.

Introduction

Many farmers have used grass-based silages for animal feed to maintain herd performance, mainly in the dry season (Daniel et al., 2019). When compared with traditional crops, such as corn (*Zea mays* L.) and sorghum (*Sorghum* spp.), tropical grasses can present greater yield, besides an easier cropping management because they are perennials and require a lower soil fertility (Bernardes et al., 2018).

Among grasses with the potential to be ensiled, cultivars from *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs (Syn. *Panicum maximum* Jacq.) have been used in production systems in Brazil (Carvalho et al., 2021). The Massai cultivar, which is a spontaneous hybrid from *M. maximus* and *Megathyrsus infestus* (Andersson) B.K.Simon & S.W.L.Jacobs (Syn. *Panicum infestum* Andersson), was registered and commercially released in 2001 (Rodrigues et al., 2014). This cultivar stands out because it presents a high forage accumulation, adaptability to reduced annual rainfall, a greater leaf:stem ratio, adequate crude protein contents in leaves and stems, and a good resistance to leafhopper infestation (Fernandes et al., 2017; Silva et al., 2018).

However, like for other tropical grasses, there are limitations for the ensilage of 'Massai' grass, such as its high moisture content and lower soluble carbohydrate contents at harvest time (Bernardes et al., 2018). Moreover, there are reports that 'Massai' grass managed in pastures has a low nutritional value, which leads to a decrease in animal performance if feed is not supplemented (Emerenciano Neto et al., 2018).

Considering these limitations, additives that reduce the moisture content, increase the nutritional value, and improve the fermentation process of silages have been encouraged in recent decades (Muck et al., 2018). Fermentation characteristics are improved by a favorable environment for lactic fermentation, with a fast pH reduction and the mitigation of dry matter losses (Kung Jr. et al., 2018). To better understand the fermentation process, during which the consumption of soluble carbohydrates and bacterial proteolysis occurs, it is important to study the fractionation of both carbohydrates and proteins (Bernardes et al., 2018).

In this context, pelleted citrus pulp has been recommended as an additive to improve the quality of grass-based silages and other industrial residues that are ensiled too (Macedo et al., 2015; Yasuoka et al., 2015; Grizotto et al., 2020). Biological additives, as microbial inoculants, also have this capacity because they modulate the fermentation process, increasing the population of lactic bacteria, mainly those of the *Lactobacillus* genus. For this reason, these microbial inoculants contribute to lower contents of acetic acid, butyric acid, and ammonia nitrogen in silages, as well as to a greater dry matter recovery (Muck et al., 2018).

The objective of this work was to evaluate the effects of the inclusion of pelleted citrus pulp and microbial inoculant on the fermentation characteristics, carbohydrate and protein fractionation, and total digestible nutrient contents of 'Massai' grass silage.

Materials and Methods

The experiment was carried out at the Animal Science Institute of Universidade Federal Rural do Rio de Janeiro, located in the municipality of Seropédica, in the state of Rio de Janeiro, Brazil (22º45'S, 43º41'W, at 33 m of altitude). The climate of the region is Aw’, rainy tropical, according to Köppen-Geiger’s classification. The dry season occurs from April to September, and the rainy season, from October to March. The average annual rainfall is 1,354 mm, and the average annual temperature is 28°C. The soil of the experimental area is classified as a Planossolo Háplico (Santos et al., 2018), i.e., an Albaqualf.

The experimental design was completely randomized, in a 2×4 factorial arrangement with five replicates (silos). The treatments consisted of: application or not of the microbial inoculant and the addition of 0, 10, 20, and 30% citrus pulp, which were both assessed as additives of 'Massai' grass silage.

For ensilage, 'Massai' grass was harvested in 2015 from an experimental area of 250 m^2. The forage was harvested after cut to a uniform height, at 50 days of regrowth, when canopy and stubble heights were 35 and 5.0 cm, respectively. Fertilization was carried out according to the following soil chemical properties: pH (H_2O) 6.0, 15.3 g dm^{-3} organic matter, 2.05 $cmol_c$ dm^{-3} Ca, 0.98 $cmol_c$ dm^{-3} Mg, 0.07 $cmol_c$ dm^{-3} K, 0.06 $cmol_c$ dm^{-3} Na, 1.98 $cmol_c$ dm^{-3} H+Al, 17 mg dm^{-3} P (Mehlich-1), cation exchange capacity of 5.14 $cmol_c$ dm^{-3}, and base saturation of 61%. Rates of 100 kg ha^{-1} N and 67 kg ha^{-1} K_2O were applied as urea and potassium chloride (Silva et al., 2018).

Forage was chopped into particles of 2.0 to 3.0 cm with the aid of a stationary forage machine. From this material, an aliquot of 1.0 kg was collected to determine the chemical composition of 'Massai' grass before ensilage (Table 1). Then, pelleted citrus pulp, donated by Instituto Federal do Rio de Janeiro, was added at 0, 10, 20, and 30% based on the fresh matter of 'Massai' grass. Table 1 shows the composition of the pelleted citrus pulp, with maximum levels of 1.0% impurities and 20 parts per billion of aflatoxins. During the filling of the silos, a commercial microbial inoculant – SiloMax Matsuda Centurium (SiloMax, Rolândia, PR, Brazil) composed of *Lactobacillus plantarum* ($2.6x10^{10}$ CFU per gram) and *Pediococcus pentosaceus* ($2.6x10^{10}$ CFU per gram) –, 85% starch, and 1.0 g L^{-1} silicate aluminum (25 mL per silo with a capacity of 15.7 L) were added to and homogenized with the forage. The inoculant was added through an aqueous solution (distilled water) with the aid of a manual sprayer for a uniform distribution on the forage mass.

The harvested material was compacted into cylindrical PVC tubes (50 cm height, 10 cm diameter, and 15.7 L volume), which were sealed by lids equipped with Bunsen-type valves for gas escape. A nonwoven fabric bag, containing 350 g washed and dried sand, was placed on the bottom of each silo to drain silage effluents. Silos were weighed before being filled and remained sealed for 90 days under an average density of 600 kg m^{-3} based on fresh matter.

Table 1. Chemical composition of 'Massai' grass (*Megathyrsus maximus* × *Megathyrsus infestus*) and used additive before ensilage.

Variable[(1)]	'Massai' grass	Pelleted citrus pulp
DM (g kg^{-1} FM)	345.4	848.1
Ashes (g kg^{-1} DM)	117.4	56.8
Ether extract (g kg^{-1} DM)	16.7	32.0
Crude protein (g kg^{-1} DM)	48.3	82.4
NDF (g kg^{-1} DM)	729.6	246.6
ADF (g kg^{-1} DM)	446.0	160.4
Lignin (g kg^{-1} DM)	50.9	33.1
Cellulose (g kg^{-1} DM)	336.6	142.5
Hemicellulose (g kg^{-1} DM)	295.7	86.1
NFC (g kg^{-1} DM)	62.2	602.7
NH_3 (g kg^{-1} total N)	39.9	39.7
NDIP (g kg^{-1} CP)	101.7	27.7
ADIP (g kg^{-1} CP)	35.8	60.7
TDN (g kg^{-1} DM)	460.3	772.1

[(1)]DM, dry matter; FM, fresh matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; NFC, nonfibrous carbohydrate; NH_3, ammonia nitrogen; NDIP, neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; and TDN, total digestible nutrients.

After this period, the silos were opened and weighed again to determine gas losses and effluent and dry matter recovery, using the equations described by Jobim et al. (2007). Gas losses (GL) were obtained by: GL (g kg^{-1} DM) = {[FWen (kg) - Wen (kg)] × DMen (%DM)} - {[FWo (kg) - Wen (kg)] × DMo (%DM)} × 100 ÷ {[FWen (kg) - Wen (kg)] × DMen (%DM)}, where DM is dry matter, FWen is full silo weight during ensilage, Wen is silo weight, DMen is forage DM during ensilage, FWo is full silo weight during opening, and DMo is forage DM content during silo opening. Effluent losses (EL) were calculated by: EL (kg Mg^{-1} FM) = {[Wo (kg) × Wen (kg)] / FMef (kg)} × 1,000, where Wo is the empty set weight (silo + cover + wet sand + bag) at silo opening, Wen is the empty set weight (silo + cover + dry sand + bag) during ensilage, and FMef is the fresh mass of the ensiled forage. DM recovery (DMR) was estimated with the equation: DMR (g kg^{-1} DM) = {[FMf (kg) × DMf (kg)] / [FMi (kg) × DMi (kg)]} × 100, where FMf is forage mass at silo opening, DMf is forage dry matter content at silo opening, FMi is forage mass at silo closure, and DMi is forage dry matter content at silo closure.

The assessed silages were divided into three portions, and the roughage from the superior part of silos (5.0 cm) was disregarded. The first portion was used right after silo opening for pH determination (Bernardes et al., 2019). The second was dried in a forced-air oven, at 55°C, for 72 hours and chopped to 1.0 mm in a Willey-type mill to determine DM, crude protein, ash, and ether extract contents using AOAC methods 934.01, 954.01, and 920.29 according to Horwitz (2005). In addition, contents of neutral (NDF) and acid (ADF) detergent fibers and of lignin were analyzed following the methods described by Van Soest et al. (1991).

Contents of total carbohydrates (TC) were obtained with the equation of Sniffen et al. (1992): TC = 10 - (CP + EE + ashes), where CP is crude protein and EE is ether extract. For carbohydrate fractionation, nonfibrous carbohydrates (fraction A + B_1) were obtained according to Hall (2003), using the equation: NFC = 100 - NDFcp - CP - EE - ashes, where NFC are nonfibrous carbohydrates and NDFcp is the content of NDF completely free of proteins and ashes.

Hemicellulose and cellulose contents (fraction B_2), respectively, were obtained by differences between NDF and ADF contents and between ADF and lignin contents. Fraction C was obtained by the correction of lignin, which was multiplied by 2.4.

Regarding protein fractionation, contents of nonprotein nitrogen (fraction A), soluble protein (fraction B_1), fraction B_2, and neutral (fraction B_3) and acid (fraction C) detergent insoluble proteins were determined according to Licitra et al. (1996). The last portions of silages were used to obtain ammonia nitrogen content according to Bolsen et al. (1992). Finally, the content of total digestible nutrients (TDN) was obtained by the theoretical model described by Weiss et al. (1992): TDN = (0.98 × NFC) + (0.93 × CP) + 2.25 × (EE-1) + 0.75 × (NDFp - lignin) × [1 - (lignin ÷ NDFp) × 0.667] - 7, where NFC is nonfibrous carbohydrates, and NDFp is the NDF completely free of protein.

Data were analyzed with the aid of Proc GLM from SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC, USA), being subjected to Shapiro-Wilk's residual normality test, at 5% probability, to the analysis of variance by the F-test ($p<0.05$) for inoculant effect, and to the regression analysis ($p<0.05$) for citrus pulp effect. The used mathematical model was: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$, where Y_{ijk} is the observed value, μ is the population average, α_i is the effect of the microbial inoculant (1 to 2), β_j is the effect of the pelleted citrus pulp (1 to 4), $(\alpha\beta)_{ij}$ is the interaction effect between the pelleted citrus pulp and the microbial inoculant, and ε_{ij} is the residual error.

Results and Discussion

The chemical composition of 'Massai' grass harvested for ensilage showed acceptable nutrient contents considering the species and cutting frequency (Table 1). The grass was harvested at 50 days of regrowth and had greater DM and crude protein contents than at 40 days of regrowth (Borja et al., 2012; Lima et al., 2013); the greater DM content, which likely improved fermentation parameters, can be explained by the lower harvesting frequency adopted in the present study. Lignin and cellulose contents were 50.9 and 336.6 g kg^{-1} DM (Table 1), respectively, lower than those of 104.3 and 381.9 g kg^{-1} DM reported by Borja et al. (2012) and Lima et al. (2013), respectively. The contents of nonfibrous carbohydrates were of 62.2 g kg^{-1}, lower than that of 159 g kg^{-1} for 'Massai' grass ensiled after 40 days of regrowth (Oliveira et al., 2018).

An interaction was observed between the inclusions of microbial inoculant and citrus pulp on silage pH (Table 2). In the silage with 10% citrus pulp, but without the inoculant, pH (3.99) was greater than when the microbial inoculant was added (3.89). The levels of citrus pulp had a quadratic effect on the pH of the inoculated silages, but all silages showed adequate values. According to Bernardes et al. (2018), pH values between 3.8 and 4.2 indicate a good fermentation kinetics during the time silos are sealed. Yasuoka et al. (2015) found values between 4.08 and 4.2, respectively, when 10 and 30% pelleted citrus pulp were added to silages of 'Xaraés' grass [*Urochloa brizantha* (A.Rich.) R.D.Webster]. Additives like citrus pulp often increase desirable fermentation, as the lactic one, which facilitates maintaining adequate pH levels (Muck et al., 2018; Grizotto et al., 2020).

Ammonia nitrogen levels linearly increased as affected by citrus pulp levels (Table 2). Oliveira et al. (2018) also verified a linear effect on this fermentation parameter due to the inclusion of different levels of licuri (*Syagrus coronata* Becc.) cake in 'Massai' grass silages. According to the authors, these results can be explained by the great crude protein content of this additive. Before ensilage in the present study, the crude protein content of citrus pulp was considerably greater (82.4 g kg^{-1} DM) than that of 'Massai' grass (48.3 g kg^{-1}), which possibly increased the proteolysis in silages with the pulp. However, all ammonia nitrogen contents were lower than 100 g kg^{-1} crude protein, which is an indicative that excessive proteolysis by *Clostridium* bacteria did not occur (Gusmão et al., 2018; Muck et al., 2018).

There was a quadratic effect of citrus pulp levels on the DM recovery of 'Massai' grass silage with the microbial inoculant (Table 2), besides a linear increase in DM contents (Table 3). Absorbent additives often increase DM content and reduce losses of grass-based silages, which have a lower moisture content than tropical grasses (Muck et al., 2018). Several authors have already reported significant improvements on silage DM recovery due to the addition of ingredients such as citrus pulp (Grizotto et al., 2017, 2020), sunflower meal (Borja et al., 2012), peanut meal (Lima et al., 2013), and *Parkia platycephala* Benth. pod meal (Costa et al., 2020). Increasing DM recovery after ensilage is important to avoid gas and effluent losses,

Table 2. Fermentation parameters and losses of silages of 'Massai' grass (*Megathyrsus maximus* × *Megathyrsus infestus*) with or without microbial inoculant plus different levels of pelleted citrus pulp[(1)].

Inoculant	Citrus pulp level (% fresh matter)				Regression equation	R^2	p-value	CV (%)
	0	10	20	30				
	pH							
Without inoculant	4.00	3.99	3.95	3.97	$\hat{Y} = 3.98$	0.06	0.4694	1.4
With inoculant	4.00	3.89	3.94	4.02	$\hat{Y} = 3.9966 - 0.0129x + 0.0005x^2$	0.55	0.0004**	
	NH_3 (g kg^{-1} total N)							
Without inoculant	38.8	43.3	47.9	52.4	$\hat{Y} = 38.772 + 0.445x$	0.56	0.0002*	6.6
With inoculant	38.9	43.6	48.3	52.9	$\hat{Y} = 38.962 + 0.467x$	0.71	<0.0001*	
	GL (g kg^{-1})							
Without inoculant	0.36	0.25	0.22	0.23	$\hat{Y} = 0.26$	0.16	0.1555	41.0
With inoculant	0.23	0.24	0.21	0.23	$\hat{Y} = 0.23$	0.01	0.7210	
	DMR (g kg^{-1})							
Without inoculant	767.3	857.8	934.9	998.5	$\hat{Y} = 767.3 + 9.7313x - 0.06725x^2$	0.88	<0.0001**	4.7
With inoculant	778.4	856.1	927.4	992.5	$\hat{Y} = 778.4 + 8.0800x - 0.03140x^2$	0.81	<0.0001**	

[(1)]pH, hydrogen potential; NH_3, ammonia nitrogen; GL, gas losses; DMR, dry matter recovery; x, level of pelleted citrus pulp; R^2, coefficient of determination; and CV, coefficient of variation. * and **Significant effect of linear regression and of quadratic regression, respectively, at 5% error probability.

Table 3. Chemical composition of silages of 'Massai' grass (*Megathyrsus maximus* × *Megathyrsus infestus*) with or without microbial inoculant plus different levels of pelleted citrus pulp[(1)].

Inoculant	Citrus pulp level (% fresh matter)				Regression equation	R^2	p-value	CV (%)
	0	10	20	30				
	DM (g kg^{-1})							
Without inoculant	349.1	391.3	357.5	361.7	$\hat{Y} = 349.1 + 4.2185x$	0.60	0.0002*	5.8
With inoculant	353.1	356.1	359.2	362.2	$\hat{Y} = 353.1 + 3.029x$	0.82	<0.0001*	
	Ashes (g kg^{-1} DM)							
Without inoculant	122.9	115.8	112.3	111.9	$\hat{Y} = 115.7$	0.15	0.1296	9.7
With inoculant	132.2	121.3	110.5	99.6	$\hat{Y} = 132.2 - 1.085x$	0.62	<0.0001*	
	NDF (g kg^{-1} DM)							
Without inoculant	737.6	683.4	629.2	575.0	$\hat{Y} = 737.6 - 5.4187x$	0.92	<0.0001*	2.6
With inoculant	745.9	676.5	607.1	537.6	$\hat{Y} = 745.9 - 6.9419x$	0.90	<0.0001*	
	ADF (g kg^{-1} DM)							
Without inoculant	434.6	407.0	379.3	351.8	$\hat{Y} = 434.6 - 2.7603x$	0.73	<0.0001*	3.2
With inoculant	441.8	415.2	388.7	362.1	$\hat{Y} = 441.8 - 2.6560x$	0.79	<0.0001*	
	Lignin (g kg^{-1} DM)							
Without inoculant	54.9	60.5	55.8	66.8	$\hat{Y} = 59.5$	0.14	0.1378	1.2
With inoculant	53.5	59.4	65.6	71.6	$\hat{Y} = 53.5 + 0.604x$	0.33	0.0112*	
	CP (g kg^{-1} DM)							
Without inoculant	47.1	54.4	61.6	68.9	$\hat{Y} = 47.1 + 0.7276x$	0.69	<0.0001*	5.5
With inoculant	49.4	53.0	56.6	60.3	$\hat{Y} = 49.4 + 0.3626x$	0.52	0.0005*	
	EE (g kg^{-1} DM)							
Without inoculant	26.6	23.8	21.0	18.2	$\hat{Y} = 26.6 - 0.2784x$	0.42	0.0034*	8.8
With inoculant	23.6	23.1	22.8	25.3	$\hat{Y} = 24.0$	0.19	0.2601	
	TDN (g kg^{-1} DM)							
Without inoculant	485.7	512.7	503.7	458.6	$\hat{Y} = 485.7 + 4.5048x - 0.1802x^2$	0.57	0.0009**	3.5
With inoculant	513.6	509.9	504.7	503.8	$\hat{Y} = 508.0$	0.05	0.6608	

[(1)]DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; EE, ether extract; TDN, total digestible nutrient; x, level of citrus pulp; R^2, coefficient of determination; and CV, coefficient of variation. * and **Significant effect of linear regression and of quadratic regression, respectively, at 5% error probability.

especially since proteins, soluble carbohydrates, and vitamins are part of these losses (Lemos et al., 2021).

The ash contents of 'Massai' grass silage were linearly reduced by the levels of citrus pulp in the presence of inoculants (Table 3). Oliveira et al. (2018) and Costa et al. (2020) also observed linear reductions in mineral matter content in silages made of tropical grasses plus levels of absorbent ingredients. According to Wascheck et al. (2008), silages with a low content of ashes are indicative of a good conservation of organic matter after the fermentation process, which is beneficial for the final quality of the product. Likewise, the contents of NDF and ADF were linearly reduced as affected by citrus pulp levels, whereas lignin content was increased by this additive only when the microbial inoculant was added (Table 3). Grizotto et al. (2020) also reported a reduction in NDF content in silages made of orange peels and citrus pulp, while Macedo et al. (2015) found the same effect on 'Marandu' grass (*U. brizantha*) silage. This type of additive also improves the nutritional value of silages because it presents greater contents of total digestible nutrients and nonfibrous carbohydrates, as well as a lower content of fibrous carbohydrates (Muck et al., 2018; Andrade et al., 2020). Conversely, the presence of this compound in citrus pulp slightly increased lignin content.

In addition, there was an increment in crude protein contents according to the levels of citrus pulp (Table 3), since, as mentioned before, this additive had a greater content of crude protein than 'Massai' grass before ensilage (Table 1). Ether extract content, however, decreased with citrus pulp levels in silages without the inoculant. Ding et al. (2013) concluded that the lipolysis during the fermentation process is associated with plant lipases and not with microbial ones, which justified the results obtained for ether extract contents, which, in the present study, were quite low before ensilage both for 'Massai' grass and the pelleted citrus pulp. Comparatively, Borja et al. (2012) observed a linear increase in the ether extract contents of 'Massai' grass silage due to the addition of sunflower meal, which they attributed to the high fat content present in this byproduct obtained from the sunflower oil industry.

There was a quadratic and positive effect of pelleted citrus pulp levels only on the total digestible nutrient contents of silages without the microbial inoculant (Table 3). A peak of 513.1 g kg^{-1} DM was reached with inclusion of 12.5% of the additive. Oliveira et al. (2018)

Table 4. Carbohydrate fractionation of silages of 'Massai' grass (*Megathyrsus maximus* × *Megathyrsus infestus*) with or without microbial inoculant plus different levels of pelleted citrus pulp[(1)].

Inoculant	Citrus pulp level (% fresh matter)				Regression equation	R^2	p-value	CV (%)
	0	10	20	30				
	Total carbohydrates (g kg^{-1} DM)							
Without inoculant	802.5	808.9	806.4	809.7	Ŷ = 806.9	0.03	0.4428	1.4
With inoculant	794.5	803.5	812.6	821.6	Ŷ = 794.5 + 0.9046x	0.44	0.0019*	
	NFC (g kg^{-1} DM)							
Without inoculant	120.4	185.1	249.8	314.5	Ŷ = 120.4 + 6.4725x	0.74	<0.0001*	14.8
With inoculant	126.2	176.1	226.0	275.9	Ŷ = 126.2 + 4.9926x	0.76	<0.0001*	
	Hemicellulose (g kg^{-1} DM)							
Without inoculant	303.0	276.4	249.8	223.3	Ŷ = 303.0 - 2.6584x	0.80	<0.0001*	6.2
With inoculant	304.1	261.2	218.4	175.5	Ŷ = 304.1 - 4.2852x	0.83	<0.0001*	
	Cellulose (g kg^{-1} DM)							
Without inoculant	378.3	351.7	325.2	298.6	Ŷ = 378.3 - 2.6548x	0.74	<0.0001*	4.0
With inoculant	388.2	355.6	323.0	290.4	Ŷ = 388.2 - 3.2613x	0.83	0.0024*	
	Fraction C (g kg^{-1} DM)							
Without inoculant	131.9	145.1	133.9	160.5	Ŷ = 142.8	0.14	0.1378	13.2
With inoculant	126.9	145.2	144.3	153.8	Ŷ = 142.6	0.33	0.1581	

[(1)]DM, dry matter; NFC, nonfibrous carbohydrates; Fraction C, lignin content × 2.4; x, levels of pelleted citrus pulp; R^2, coefficient of determination; and CV, coefficient of variation. *Significant effect of linear regression, at 5% error probability observed a linear increase from 402 to 484 g kg^{-1} in the contents of total digestible nutrients of 'Massai' grass silage when adding 0 to 24% licuri cake. These results were slightly lower than those obtained in the present study, which varied from 458.6 to 513.6 g kg^{-1}. The considerable increase in soluble nutrients as nonfibrous carbohydrates (Table 4) due to the inclusion of citrus pulp probably contributed to these results.

Citrus pulp levels linearly increased the contents of total carbohydrates only in the silage with the microbial inoculant, but increased the nonfibrous carbohydrate content (fraction A + B_1) while reducing hemicellulose and cellulose (fraction B_2) regardless of the addition of the inoculant (Table 4). However, there was no significant effect on fraction C content. Gusmão et al. (2018) also reported an increase in the nonfibrous carbohydrate content of elephant grass (*Pennisetum purpureum* Schumach.) forage when cornmeal and citrus pulp were added, ensiling this material as total mixed ration. The increase in nonfibrous carbohydrates in silages suggests a good conservation of the ensiled mass because there is a considerable consumption of soluble nutrients during the fermentation process (Bernardes et al., 2018). Moreover, feeds with high contents of starch, saccharose, and other fast-digestible sugars are great energy sources for the ruminal microbiota (Sniffen et al., 1992). However, the inclusion of protein sources, with fast or variable degradation, is important for an adequate synchronization between energy and nitrogen release (Carvalho et al., 2007).

Considering the obtained results, citrus pulp levels had a quadratic effect on soluble protein content (fraction B_1) – whose highest values were obtained with the inclusion of 9.11% of the additive – only in the inoculated silages. Also in the inoculated silages, fraction B_2 was reduced up to 19.67% citrus pulp inclusion, whereas, in silages without the microbial inoculant, this fraction was greater (Table 5). Conversely, a linear increase in ADIP content (fraction C) occurred in all silages. Generally, the results of the present study do not suggest improvements in the protein profile due to the used additives, mainly considering the increase in protein linked to lignin represented by ADIP (Lemos et al., 2021). Regarding the microbial inoculant, no improvement in the carbohydrate profile was observed.

Table 5. Protein fractionation of silages of 'Massai' grass (*Megathyrsus maximus* × *Megathyrsus infestus*) with or without microbial inoculant plus different levels of pelleted citrus pulp[(1)].

Inoculant	Citrus pulp level (% fresh matter)				Regression equation	R^2	p-value	CV (%)
	0	10	20	30				
	NPN (g kg^{-1} CP)							
Without inoculant	450.0	432.8	441.7	418.2	$\hat{Y}$ = 435.7	0.18	0.0782	4.1
With inoculant	432.1	442.9	437.3	451.4	$\hat{Y}$ = 440.1	0.11	0.1608	
	Soluble protein (g kg^{-1} CP)							
Without inoculant	106.3	77.0	85.2	68.4	$\hat{Y}$ = 84.2	0.46	0.0828	17.5
With inoculant	87.0	95.6	83.3	50.0	$\hat{Y} = 87.0 + 1.9161x - 0.1051x^2$	0.69	0.0048**	
	Fraction B_2 (g kg^{-1} CP)							
Without inoculant	187.0B	215.0A	179.5A	195.8A	$\hat{Y}$ = 194.4	0.01	0.0284**	15.7
With inoculant	221.2A	166.7B	149.2B	169.9B	$\hat{Y} = 221.2 - 7.3261x + 0.1862x^2$	0.70	0.1170	
	NDIP (g kg^{-1} CP)							
Without inoculant	142.2	143.0	146.7	177.7	$\hat{Y}$ = 152.4	0.28	0.5074	15.4
With inoculant	149.8	164.9	156.8	147.6	$\hat{Y}$ = 161.5	0.09	0.2024	
	ADIP (g kg^{-1} CP)							
Without inoculant	109.1	138.6	152.4	150.2	$\hat{Y} = 109.1 + 3.7477x - 0.0792x^2$	0.74	0.0171**	14.5
With inoculant	105.6	152.2	198.8	245.4	$\hat{Y} = 105.6 + 4.6620x$	0.77	0.0004*	

[(1)]NPN, nonprotein nitrogen; CP, crude protein; Fraction B_2 = NDIP - ADIP; NDIP, neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; x, levels of pelleted citrus pulp; R^2, coefficient of determination; and CV, coefficient of variation. * and **Significant effect of linear regression and of quadratic regression, respectively, at 5% error probability.

Conclusions

1. The addition of pelleted citrus pulp improves the fermentation characteristics of 'Massai' grass (*Megathyrsus maximus* × *Megathyrsus infestus*) silage, mainly dry matter recovery.
2. The microbial inoculant, from an isolated perspective, does not influence significantly both carbohydrate and protein profiles of 'Massai' grass silage, although it can increase soluble protein content when associated with pelleted citrus pulp.
3. Inclusions between 10 and 20% pelleted citrus pulp, based on fresh matter, jointly improve the fermentation characteristics and the carbohydrate and protein fractionation of 'Massai' grass silage, with an adequate dry matter recovery.
4. Total digestible nutrient content reaches its peak with a citrus pulp inclusion of 12.50%.

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