

Effect of microbial inoculants on the chemical composition and aerobic stability of Tanzania guinea grass silages

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Abstract

The present study evaluated the effects of microbial inoculants on chemical changes and aerobic stability efficiency in Tanzania guinea grass silage. The treatments consisted of C: silage without inoculant; I: silage inoculated with *Lactobacillus plantarum* (CCT 0580) 8.0×10^9 CFU g⁻¹, *Bacillus subtilis* (CCT 0089) 2.0×10^9 CFU g⁻¹, and *Pediococcus acidilactici* (CCT 2553) 1.0×10^{10} CFU g⁻¹, and L: silage inoculated with *Lactobacillus plantarum* (CCT 0580) 2.6×10^{10} CFU g⁻¹ and *Pediococcus pentosaceus* (CCT 7659) 2.6×10^{10} CFU g⁻¹. The experimental design was completely randomized, with five replications. There were no treatment effects on the nutritional composition of the silages, but both inoculants were effective in reducing the pH of the silage to 4.80 and 4.83 for I and L, respectively, compared with 5.04 for C. Silage with L had a lower ammonia nitrogen (N-NH₃) content than the other silages (9.83%). Despite the lower pH values, the use of inoculants did not reduce fungal and yeast counts or improve the aerobic stability of Tanzania guinea grass silages.

Keywords: aerobic deterioration, ammonia nitrogen, lactic bacteria, *Panicum maximum*, spoilage microorganisms

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Introduction

Cultivars of *Panicum maximum* Jacq. are characterized by accelerated growth and high potential for fresh matter production per unit area. Tanzania guinea grass is one of the most used *Panicum* cultivars in pasture formation and in the production of preserved feed. When harvested for silage production, its apex of nutritional quality coincides with the stage where forage has low dry matter (DM) and soluble carbohydrates, and high buffer capacity (Zanine *et al.*, 2018), which may impair fermentation and favour *Clostridium* (Santos *et al.*, 2008). These characteristics affect the nutritional quality of the silage, and also the aerobic stability when the silo is opened (Santos *et al.*, 2014).

The use of lactic acid bacteria as inoculants to minimize the deterioration of grass silage has been studied (Santos *et al.*, 2008; Tabacco *et al.*, 2009; Khota *et al.*, 2016; Zanine *et al.*, 2016). However, the results of these studies have been contradictory and inconclusive. It is believed that differences among the strains of bacteria that were used contributed to the disparate effects (Dong *et al.*, 2020). Ávila *et al.* (2009) described improvements in the fermentation profile and reduction of fungi concentration in Mombaça guinea grass silage that was inoculated with *Lactobacillus buchneri*. Santos *et al.* (2008) observed the nutritional superiority of inoculated Tanzania guinea grass silage compared with control silage. However, Paziani *et al.* (2006) found no positive effects from inoculation in Tanzania guinea grass silage. In most of these studies, the inoculants contained strains of *Lactobacillus buchneri* and *Lactobacillus plantarum*, with little insight into other strains with conservative potential.

Species of the genus *Bacillus* have known ability to produce antimicrobial substances, including antibiotics. However, few studies have evaluated them as possible agents for improvement of forage

fermentation. Although not classified as lactic bacteria, *Bacillus subtilis* has the potential to produce acetic acid. The use of *Bacillus* was observed to have beneficial effects on nutritional quality and aerobic stability in corn silage (Basso *et al.*, 2012). *Bacillus subtilis* can facilitate aerobic metabolism in the initial phase of fermentation when there is residual oxygen and yeasts develop exponentially. *Bacillus subtilis* may prevent the development of yeasts by producing anti-fungal substances or by competing for substrate. In a review, Muck *et al.* (2018) reported that inoculants with *Bacillus* improved aerobic stability of silages by controlling the growth of yeasts.

Effective production of lactic acid is important for rapid pH drop, reduced protein degradation, and reduced growth of undesirable microorganisms (Ávila *et al.*, 2009, Konca *et al.*, 2016). Homo-fermentative strains, such as *Pediococcus acidilactici* and *Pediococcus pentosaceus*, can be used for process efficiency (Konca *et al.*, 2016). Kleinschmit and Kung Jr. (2006). However, these results raise awareness of the importance of working with associations of microorganisms and further studies are needed to evaluate the most appropriate combinations. Thus, the goal of this study was to evaluate inoculants with mixtures of microbial strains for effects on the nutritional value and aerobic stability of Tanzania guinea grass silage.

Material and Methods

Tanzania guinea grass (*Panicum maximum* Jacq. Tanzania) was grown at the State University of Londrina, Londrina, State of Paraná, located at coordinates 23°23'S and 51°11'W at an altitude of 566 m. After 80 days of regrowth, the material was harvested, immediately ground in a stationary mill, the additives applied and ensiled. The additives were diluted in non-chlorinated water and homogeneously sprayed onto the ground material before being ensiled. The treatments evaluated were: (C) control silage with water applied in the same proportion as the other silages; (I) silage with an inoculum of *Lactobacillus plantarum* [CCT 0580] 8.0×10^9 CFU g⁻¹, *Bacillus subtilis* [CCT 0089] 2.0×10^9 CFU g⁻¹, and *Pediococcus acidilactici* [CCT 2553] 1.0×10^{10} CFU g⁻¹, and (L) silage with an inoculum of *Lactobacillus plantarum* [CCT 0580] 2.6×10^{10} CFU g⁻¹ and *Pediococcus pentosaceus* [CCT 7659] 2.6×10^{10} CFU g⁻¹.

Fifteen 18 liter polyethylene buckets closed with a plastic cap and sealed with adhesive tape were used as experimental silos. The silage had an average particle size of 31.98 mm and was packed to an average density of 497.7 kg fresh matter m⁻³ as described by Lammers *et al.* (1996) (Table 1).

Table 1 Particle retention on sieves with different mesh sizes, average particle size and density of tanzania guinea grass silages with different microbial inoculants

Silages	Particles retained on the sieve, %				Average particle size, mm	Density, kg FM m ⁻³
	19 mm	8 mm	4 mm	0 mm		
C	89.2	7.2	3.8	0.0	31.15	485.1
I	94.0	4.0	1.8	0.0	33.34	507.0
L	89.8	6.6	3.4	0.0	31.45	500.9

C: silage treated with water, I: silage treated with *Lactobacillus plantarum*, *Bacillus subtilis* and *Pediococcus acidilactici*, L: silage treated with *Lactobacillus plantarum* and *Pediococcus pentosaceus*

After 60 days, the silos were opened, and 10 cm portions from the top and bottom were discarded. Samples were collected immediately for fungi and yeast counts in which 25 g fresh silage was diluted in 225 mL deionized water to prepare the aqueous extract. Aliquots of 1 mL of each dilution were pipetted into sterile Petri dishes (100 × 20 mm), which were prepared in duplicate. Fifteen mL dextrose potato agar was added to each dish. It had previously been melted, cooled to 45 °C and acidified to pH 3.5 ± 0.1 with 10% tartaric acid. The mixture was solidified at room temperature and incubated inverted at 25 °C for 72 hours for the yeast count, and 120 hours for the fungal count (Downes & Ito, 2001). The number of microorganisms was enumerated as colony forming units (CFU) and log₁₀ transformed. This procedure was repeated after the silages had been exposed to air for seven days.

Silo temperature was measured daily at 08h00 and 16h00 with a digital immersion thermometer 10 cm below the surface, without stirring. Ambient temperature was also recorded at these times and pH was measured (Cherney & Cherney, 2003). On the first and last days, samples were taken to determine ammonia nitrogen (NH₃-N Total N⁻¹), dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), and ashes, according to AOAC (1995).

The experiment was a completely randomized design, with five replications. The data were tested with the Shapiro-Wilk and Bartlett tests to check the assumptions of normality and homogeneity of variance, respectively. Those response variables that were recorded one time on an experimental unit subjected to one-way analysis of variance using SAS (SAS Institute Inc., Cary, North Carolina, USA). Treatment effects were tested at the 5% level of probability and then with Tukey's test for comparison of means. For the variables that were recorded daily, a split plot in time model was used for the statistical analysis. For the study of temperature and pH over time, Box-plot charts were used to allow visualization of dispersion, asymmetry and discrepant data.

Results and Discussion

No differences were detected in the nutritional value of the silages (Table 2). Both inoculants were effective in reducing the pH relative to the control silage. Sufficient concentrations of soluble carbohydrates are required as the fuel for lactic acid bacteria (Tomaz *et al.*, 2018; Dong *et al.*, 2020). When this condition is met, the bacteria present in the inoculants produce lactic acid and acetic acid, resulting in lowered pH (Holzer *et al.*, 2003). As a consequence, microbial metabolic activity inside the silo is reduced and the forage can be preserved for longer periods (McDonald *et al.*, 1991). High pH conditions also favour development of *Clostridium* bacteria, which can take advantage of aerobic conditions after opening the silo to convert proteins into NH₃-N (Bumbieris Junior *et al.*, 2017; Zanine *et al.*, 2018). Controlling *Clostridium* bacteria in silages with inoculants can be difficult in humid conditions, and dry matter may be lost as a result (Tomaz *et al.*, 2018).

Proteolytic activity is consistently related to the NH₃-N contents of silage. However, in the present study only L reduced NH₃-N compared with C. Homolactic and heterolactic bacteria promote a faster rate of fermentation, providing less proteolysis (Muck, 2010). *Bacillus subtilis* present in I might have reduced the rate of fermentation, resulting in the similarity of this silage to C. Lower NH₃-N was seen in this study than in Tomaz *et al.* (2018), but the level was similar to Zanine *et al.* (2018). It should be noted that NH₃-N contents above 10% of total N are considered detrimental to the nutritional and sanitary qualities of the silages.

Table 2 Chemical composition of Tanzania guinea grass silages with various microbial inoculants

Variables	Silages			P-value
	C	I	L	
Dry matter, % FM	29.13 ± 0.59	29.68 ± 0.44	29.32 ± 0.51	0.6266
Crude protein, % DM	8.26 ± 0.34	8.34 ± 0.10	8.62 ± 0.12	0.1900
Neutral detergent fibre, % DM	67.64 ± 0.74	67.43 ± 0.80	67.25 ± 0.58	0.3019
Acid detergent fibre, % DM	42.46 ± 1.37	42.39 ± 1.62	43.76 ± 1.00	0.1232
Ash, % DM	12.56 ± 0.16	12.98 ± 0.20	12.78 ± 0.20	0.6609
pH	5.04 ^a ± 0.02	4.80 ^b ± 0.03	4.83 ^b ± 0.04	<0.0001
NH ₃ -N, % Total N	11.08 ^a ± 0.94	11.06 ^a ± 0.58	9.83 ^b ± 0.93	<0.0001

C: silage treated with water, I: silage treated with *Lactobacillus plantarum*, *Bacillus subtilis* and *Pediococcus Acidilactici*, L: silage treated with *Lactobacillus plantarum* and *Pediococcus pentosaceus*, FM: fresh matter, DM: dry matter, NH₃-N: ammonia nitrogen (N)

^{a,b} Within a row, means with a common superscript were not different with probability $P=0.05$

The yeast and fungal counts increased in time since opening the silos, but were similar among the treatments (Table 3). Tabacco *et al.* (2009) also detected no difference in fungal and yeast counts in corn and sorghum silages, irrespective of their having been inoculated with *Lactobacillus plantarum*. Basso *et al.* (2012) reported that inoculation with *Bacillus subtilis* at 5.0×10^5 CFU g⁻¹ controlled the growth of aerobic spoilage microorganisms and increased aerobic stability of in corn silage at a pH of 3.74. Growth of fungi and yeasts occurs mainly at pH higher than 4.5 (Muck, 2010), and in the present study, the high pH of Tanzania guinea grass silages may have favoured the accelerated development of these microorganisms, thus compromising silage preservation. In short, the present results corroborate much of the literature on the development of spoilage microorganisms under aerobic conditions in silages. In addition, the present study

is the first evaluation of *Bacillus subtilis* as an inoculant in Tanzania guinea grass silage, and additional studies are needed of its mechanism of action during the fermentation process.

Table 3 Fungal and yeast counts (log CFU g⁻¹) in Tanzania guinea grass silages with various microbial inoculants on the first and seventh day after opening the silos

Variables	Silages			Mean
	C	I	L	
Day 1, log CFU g ⁻¹	2.66	2.35	2.21	2.45 ^B
Day 7, log CFU g ⁻¹	5.20	5.89	5.75	5.70 ^A

CFU: colony-forming units; C: silage treated with water, I: silage treated with *Lactobacillus plantarum*, *Bacillus subtilis* and *Pediococcus acidilactici*, L: silage treated with *Lactobacillus plantarum* and *Pediococcus pentosaceus*

^{A,B} Within a column, means with a common superscript were not different with probability $P=0.05$

In C there was a quadratic effect ($P < 0.05$) on temperature with increasing days of exposure to air. However, the temperatures of I and L increased linearly over time after opening of silo. The increase in temperature over time is because of the enzymatic activity of the deleterious microorganisms that release energy (heat) during the process of nutrient degradation (Bumbieris Junior *et al.*, 2017). This is different from what occurs normally in corn silages (Tomaz *et al.*, 2018) and supports the concept that tropical grasses are more prone to deterioration by aerobic bacteria. This deterioration occurs because of the stability of the fermentation at pH above 4.5, high moisture content and absence of soluble carbohydrates. The difficulty in gaining thermal increase may also be related to the lower efficiency of aerobic bacteria in producing heat compared with yeasts and the greater energy requirement for heating in more moist environments.

Table 4 Regressions of Tanzania guinea grass silage temperature and pH on time after silo opening

Silages	Regression equation	R ²	P-value	CV, %
Temperature				
C	$24.80 + 0.0075x + 0.0374x^2$	0.7424	<0.0001	1.86
I	$24.25 + 0.2925x$	0.7140	<0.0001	1.68
L	$24.10 + 0.3298x$	0.8078	<0.0001	1.66
pH				
C	$5.06 - 0.0028x + 0.0087x^2$	0.9080	0.0006	4.05
I	$4.72 + 0.1721x - 0.0134x^2$	0.8170	<0.0001	1.58
L	$4.77 + 0.1465x - 0.0115x^2$	0.7845	<0.0001	1.60

C: silage treated with water, I: silage treated with *Lactobacillus plantarum*, *Bacillus subtilis* and *Pediococcus acidilactici*, L: silage treated with *Lactobacillus plantarum* and *Pediococcus pentosaceus*

The regression equation that represents the pH of C also differed from I and L. For C, the trend was increased at an increasing rate over time, whereas for I and L the trends in pH increased to maxima and decreased thereafter. Figures 1 and 2 help one to understand the temperature and pH in the hours after the silos were opened.

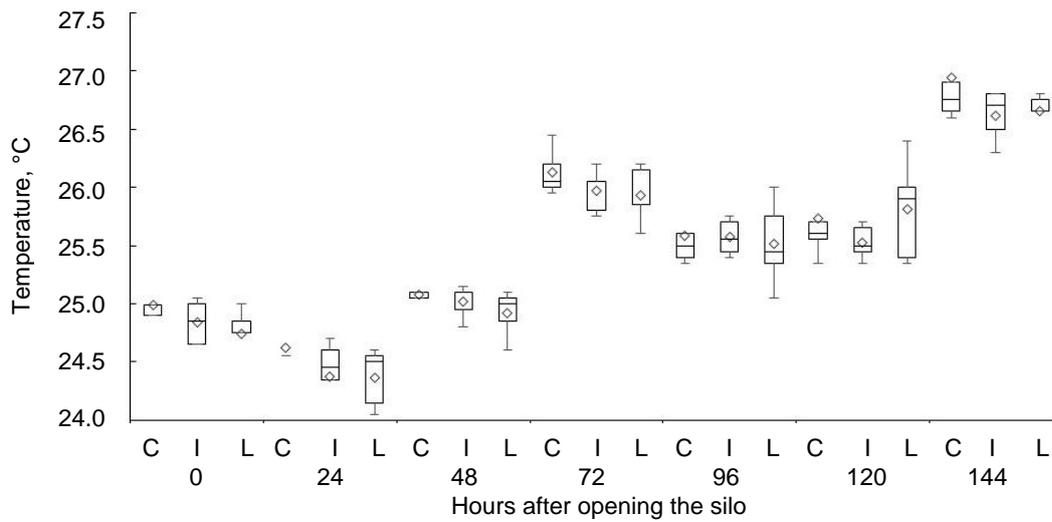


Figure 1 Mean temperature values of the silages over the hours of aerobic exposure
 C: silage treated with water, I: silage treated with *Lactobacillus plantarum*, *Bacillus subtilis* and *Pediococcus acidilactici*,
 L: silage treated with *Lactobacillus plantarum* and *Pediococcus pentosaceus*

The increase in temperature detected over the hours of aerobic exposure is generally observed in silages independent of the inoculant, forage species and evaluation methodologies. According to Adesogan (2009), higher environmental temperatures may cause growth of undesirable microorganisms in silages such as clostridium and enterobacteria. A similar effect can be inferred for pH, confirming the change in nutritional value can be attributed to deleterious microorganisms. Yeasts are in the forefront of the timing and severity of these effects. Higher concentrations of lactic bacteria provide for a faster decline in pH of the ensiled mass, and reducing the time for yeast to develop prior to the transformation to an anaerobic environment after silo sealing (McDonald *et al.*, 1991). This activity of yeasts does not resume when the silage is again exposed to air. It should be noted that both temperature and pH are related to the aerobic stability of the silage (Table 5).

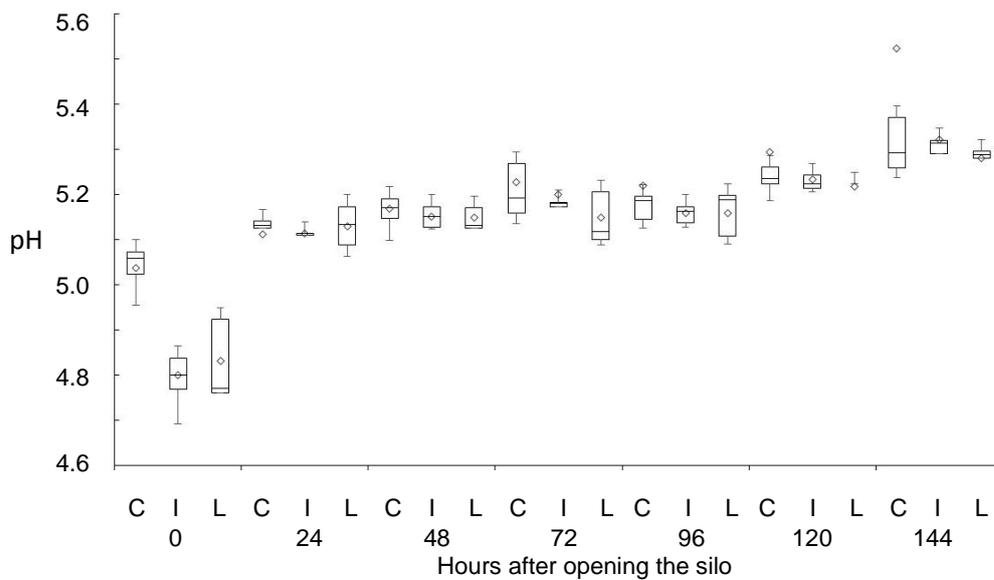


Figure 2 Mean pH values of the silages over the hours of aerobic exposure
 C: silage treated with water, I: silage treated with *Lactobacillus plantarum*, *Bacillus subtilis* and *Pediococcus Acidilactici*,
 L: silage treated with *Lactobacillus plantarum* and *Pediococcus pentosaceus*

The acetic acid production capacity of *Bacillus subtilis* led to the expectation that I would have higher aerobic stability. However, although the lower pH was seen initially, the effect dissipated within 24 hours of opening the silos and the difference between treatments in aerobic stability was not significant ($P > 0.05$) (Table 5).

Table 5 Values related to aerobic stability (\pm SD) of Tanzania guinea grass silages with different microbial inoculants

Variables	Silages			P-value
	C	I	L	
Aerobic stability, hours	115.2 \pm 9.60	120.0 \pm 0.00	110.4 \pm 11.15	0.1889
Time for maximum temperature, hours	144.0	144.0	144.0	-
pH after stability test, day 7	5.52 ^a \pm 0.04	5.32 ^b \pm 0.06	5.28 ^b \pm 0.05	0.0233

C: silage treated with water, I: silage treated with *Lactobacillus plantarum*, *Bacillus subtilis* and *Pediococcus acidilactici*, L: silage treated with *Lactobacillus plantarum* and *Pediococcus pentosaceus*

Although the regression models for temperature were different, the silages reached their maximum temperature at the same time. After seven days of evaluating aerobic stability, pH of I and L were significantly lower compared with C. Basso *et al.* (2012) reported mean pH values of 5.53 for corn silage inoculated with *Bacillus subtilis* and 6.73 for the control silage after 12 days of exposure to air. They suggested that this difference was due mainly to control of filamentous fungi, which otherwise became active from day 7 onwards. The present study ended on day 7 and thus this conclusion cannot be drawn. In agreement with Wilkinson and Davies (2012), success in controlling aerobic deterioration is not the result of a single intervention, and losses can be minimized at all stages of the ensiling process and during the time that the silage is being fed.

Conclusions

The inoculant containing *Lactobacillus plantarum* and *Pediococcus pentosaceus* was effective in controlling the level of $\text{NH}_3\text{-N}$ in Tanzania guinea grass silage. This inoculant and one containing *Lactobacillus plantarum*, *Bacillus subtilis* and *Pediococcus acidilactici* both reduced pH, but the aerobic stability of the silages was affected by neither inoculant.

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Authors' Contributions

FLMJ and DARM collected the data. VAPG and GJM developed the first draft of the article. EHH refined the manuscript. VHBJ designed the study, revised the manuscript, and analysed the data together with SG.

Conflict of Interest Declaration

There is no conflict of interest.

References

- Adesogan, A.T., 2009. Challenges of tropical silage production. In: G.A. Broderick (ed.) Proceedings of the 15th International Silage Conference, Madison, Wisconsin, 2009. Pp. 139-154.
- AOAC, 1995. Official methods of analytical chemists. 16th ed. Chapter 4: Animal feed. AOAC, Washington, DC.
- Ávila, C.L.A., Pinto, J.C., Figueiredo, H.C.P., Morais, A.R., Pereira, O.G. & Schwan, R.F., 2009. Estabilidade aeróbia de silagens de capim-mombaça tratadas com *Lactobacillus buchneri*. Rev. Bras. Zootecn. 38, 779-787.
- Basso, F.C., Lara, E.C., Assis, F.B.D., Rabelo, C.H.S., Morelli, M. & Reis, R.A., 2012. Características da fermentação e estabilidade aeróbia de silagens de milho inoculadas com *Bacillus subtilis*. Rev. Bras. Saúde Prod. Anim. 13, 1009-1019.
- Bumbieris Junior, V.H., Guimarães, V.A.D.P., Fortaleza, A.P.D.S., Massaro Junior, F.L., Moraes, G.J.D. & Meza, D.A.R., 2017. Aerobic stability in corn silage (*Zea mays* L.) ensiled with different microbial additives. Acta Sci Anim Sci. 39, 357-362.

- Cherney, J.H. & Cherney, D.J.R., 2003. Assessing silage quality. In: D.R. Buxton, R.E. Muck & J.H. Harisson. Silage science and technology. Agronomy Monographs. Madison, Wisconsin, USA <https://access.onlinelibrary.wiley.com/doi/abs/10.2134/agronmonogr42.c4>
- Dong, M., Li, Q., Xu, F., Wang, S., Chen, J., Li, W., 2020. Effects of microbial inoculants on the fermentation characteristics and microbial communities of sweet sorghum bagasse silage. *Sci. Rep.* 10, 1-9.
- Downes, F.P. & Ito, K., 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, DC. 107 pp.
- Holzer, M., Mayrhuber, E., Danner, H. & Braun, R., 2003. The role of *Lactobacillus buchneri* in forage preservation. *Trends Biotechnol.* 21, 282-287.
- Khota, W., Pholsen, S., Higgs, D. & Cai, Y., 2016. Natural lactic acid bacteria population of tropical grasses and their fermentation factor analysis of silage prepared with cellulase and inoculant. *J. Dairy Sci.* 99, 9768-9781.
- Kleinschmit, D.H. & Kung Junior, L., 2006. The effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of corn silage. *J. Dairy Sci.* 89, 3999-4004.
- Konca, Y., Beyzi, S.B., Ayaşan, T., Kaliber, M. & Kiraz, A.B., 2016. The effects of freezing and supplementation of molasses and inoculants on chemical and nutritional composition of sunflower silage. *Asian Australas. J. Ani. Sci.* 29, 965-970.
- Lammers, B.P., Buckmaster, D.R. & Heinrichs, A.J. 1996. A simple method for the analysis of particle size of forage and total mixed rations. *J. Dairy Sci.* 79, 922-928.
- McDonald, P., Henderson, A.R. & Heron, S.J.E., 1991. The biochemistry of silage. 2nd ed. Chalcombe, Southampton, UK.
- Muck, R.E., 2010. Silage microbiology and its control through additives. *Rev. Bras. Zootech.* 39, 183-191.
- Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C. & Kung, L., 2018. Silage review: Recent advances and future uses of silage additives. *J. Dairy Sci.* 101, 3980-4000.
- Paziani, S.F., Nussio, L.G., Loures, R.S.D., Igarasi, S.M., Pedroso, A.F. & Mari, L.J., 2006. Influência do teor de matéria seca e do inoculante bacteriano nas características físicas e químicas da silagem de capim Tanzânia. *Acta Sci. Anim. Sci.* 28, 265-271.
- Santos, E.M., Pereira, O.G., Garcia, R., Ferreira, C.L.L.F., Oliveira, J.S. & Silva, T.C., 2014. Effect of regrowth interval and a microbial inoculant on the fermentation profile and dry matter recovery of guinea grass silages. *J. Dairy Sci.* 97,4423-4432.
- Santos, E.M., Zanine, A.M., Ferreira, D.J., Oliveira, J.S., Pentead, D.C.S. & Pereira, E.O.G., 2008. Inoculante ativado melhora a silagem de capim-tanzânia (*Panicum maximum*). *Arch. Zootec.* 57, 35-42.
- Tabacco, E., Piano, S., Cavallarin, L., Bernardes, T.F. & Borreani, G., 2009. Clostridia spore formation during aerobic deterioration of maize and sorghum silages as influenced by *Lactobacillus buchneri* and *Lactobacillus plantarum* inoculants. *J. Appl. Microbiol.* 107, 1632-1641.
- Tomaz, P.K., Araujo, L.C., Sanches, L.A., Santos-Araujo, S.N., Lima, T.O., Lino, A.D.A. & Ferreira, E.M., 2018. Effect of sward height on the fermentability coefficient and chemical composition of guinea grass silage. *Grass Forage Sci.* 73, 588-598.
- Wilkinson, J.M. & Davies, D.R., 2012. The aerobic stability of silage: Key findings and recent developments. *Grass Forage Sci.* 68, 1-19.
- Zanine, A.M., Bonelli, E.A., Ferreira, D.J., Souza, A.L., Santos, E.M., Pinho, R.M. & Parente, M.O., 2018. Fermentation and chemical composition of guinea grass silage added with wheat meal and *Streptococcus bovis*. *New Zeal. J. Agr Res.* 61, 487-494.
- Zanine, A.M., Bonelli, E.A., Souza, A.L., Ferreira, D.J., Santos, E.M., Ribeiro, M.D., Geron, L.J.V. & Pinho, R.M.A., 2016. Effects of *Streptococcus bovis* isolated from bovine rumen on the fermentation characteristics and nutritive value of Tanzania grass silage. *Sci. World J.* 1-6.