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Chemical composition in soybean silages with inoculant and molasses

Composição bromatológica em silagens de soja com inoculante e melaço

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Abstract

Chemical composition was assessed in soybean silages without any additive (control), with a microbial inoculant (I), with I + molasses (I+M), and with molasses only (M). Soybean plants were harvested at the reproductive 6 (R6) stage and ensiled in 2 kg-capacity PVC laboratory silos. The SIL ALL C4 inoculant produced by Alltech Brazil was used in combination with or without 2.5% molasses added to the natural matter base. A 4 × 6 factorial arrangement (4 additives × 6 fermentation periods) in a completely randomised design with 3 replications was used. The assessed fermentation periods were 1, 3, 7, 14, 28, and 56 days. Excluding the dry matter and crude protein contents it was observed an interaction effect (P<0.05) between the additive and fermentation periods in the silages. It was observed higher average values of dry matter, equal 290.02 g kg^{-1}, and crude protein, equal 151.28 g kg^{-1}, to I+M and M silages, respectively. It was observed lowest values (P<0.05) to neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) contents in I+M silages, equal 180.47 and 125.07 g kg^{-1}, respectively. The addition of inoculant associated or not with molasses improved affect the chemical composition of soybean silages.
Keywords: acid lactic bacteria, additives, nutritive value, pH

Resumo

Avaliou-se a composição bromatológica de silagens de soja sem aditivos (controle), com inoculante microbiano (I), com I + melaço (I+M) e com melaço (M). As plantas de soja foram colhidas no estádio R6 de desenvolvimento e ensiladas em silos laboratoriais de PVC, com capacidade de 2 kg. O inoculante utilizado foi o SIL ALL C4 da Alltech do Brasil associado ou não com 2,5% de melaço, na base da matéria seca. Utilizou-se um esquema fatorial 4 × 6 (4 aditivos × 6 períodos de fermentação) em um delineamento completamente casualizado com 3 repetições. Os períodos de fermentação avaliados foram: 1, 3, 7, 14, 28, e 56 dias. Excluindo os teores de matéria seca e de proteína bruta foi observado efeito (P<0,05) da interação aditivos e período de fermentação sobre as silagens. Observou-se maiores valores médios de matéria seca, de 290,02 g kg^{-1}, e de proteína bruta, de 151,28 g kg^{-1}, para as silagens I+M e M, respectivamente. Foi observado menores valores (P<0,05) de NIDN e de NIDA

nas silagens I+M, de 180,47 and 125,07 g kg^{-1}, respectivamente. A adição de inoculante associada ou não ao melaço altera a composição bromatológica de silagens de soja.
Palavras-chave: aditivo, bactéria ácido lática, pH, valor nutritivo

Introduction

From a historic perspective, leguminous plants were always rated as inadequate for ensiling because they exhibited a high buffer capacity as well as low water-soluble carbohydrates and dry matter content. Besides these fermentation-restrictive characteristics inherent to leguminous plants, soybean further exhibits high ether extract content, which might inhibit bacteria in the ensiled mass; this inhibition affects fermentation, resulting in high silage pH.

For the use of the soybean in the form of silage, one must determine the best moment to realize the harvest, aiming to obtain yield and quality. Although protein content remains slightly variable with advancement in age, later harvesting causes a decrease in forage yield as a function of the shorter cycle[(1)]. In addition, soybean harvest for ensiling might be performed between stages R3 (onset of pod formation) and R7 (onset of maturity)[(2)].

In production systems, the feed quality has limited animal performance. Due to this situation, there has been an increase in the demand for information on bulky foods of higher nutritional value able to meet the nutritional needs of animals of high genetic potential or categories of higher nutritional requirements and reduce production costs, reducing the supplementation of animals with concentrates. The legume silage can be associated with the of the diet of animals, when provided a diet with less crude protein, as is the case of sugar cane and pasture in the dry period of the year. Analyzing diets for Morada Nova sheep the authors[(3)] observed that diets that 20% cane tip silage plus 30 and 60% soy silage supplemented with 20 and 50% of concentrate promoted higher intake of DM (4,2a and 4,4a) and CP (0,84 and 0,85) in % of live weight than diets composed with 20% of cane tip silage plus 80% concentrate.

However, legumes are characterized by high protein content which greatly favors the fermentation carried out by clostridia in the ensiling process. The clostridial fermentation products (concentrations of amines, ammonia and butyric acid) are not as acidic as lactic acid, thus raising the pH of the silage and destabilizing the silage mass, causing a negative effect on the voluntary intake of ruminants (Rooke and Huckfield, 2003; Muck, 1988; Weiss et al, 2003) cited by Coblentz et al.[(4)]. In this sense, legume silages should be produced with additives.

Research on soybean silage is still incipient in Brazil despite the increasing demand for information by local farmers. Therefore, this study aimed at assessing the chemical composition in soybean silages treated with microbial inoculant and molasses.

Material and methods

Soybean plants were grown at the Department of Animal Science of the Federal University of Viçosa, located in Viçosa County, Minas Gerais (20^0 45' S, 42^0 51' W), Brazil.

The DM 339 (Pioneer) soybean variety was sown in November 26th, 2006 in an 800-m^2 area, with 0.7-m spacing between rows and 25 to 30 seeds m^{-1} of sowing density. Soybean plants were hand-harvested using knives when they reached the R6 stage.

After the harvest, the material was cut in a stationary ensiling machine, and additives were applied before ensiling. The treatments were no additive (control), microbial inoculant (I), inoculant and powdered molasses (I+M), and powdered molasses only (M). The inoculant used was SIL ALL C4 (Alltech Brazil, composition: cellulosic enzymes, *Pediococcus acidilactici*, *Lactobacillus plantarum, Enterococcus faecium,* and 80% dextrose. The guaranteed levels of microorganisms were 10 billion CFU g^{-1} *L. plantarum*, 1 billion CFU g^{-1} *P. acidilactici*, and 10 billon CFU g^{-1} *E. faecium*). Following the manufacturer recommendations, the inoculant was applied using a 2-L capacity spray, and 2.5% powdered molasses was added to the natural base matter.

After the application of additives, ensiling was performed in PVC silos, with a diameter of 10 cm and height of 50 cm that included a Bunsen valve for gas release. In each silo was placed 2 kg of fresh forage. A 4 × 6 (4 additives × 6 fermentation periods) factorial scheme was used in a completely randomised design with 3 replications. Silos were opened after each fermentation period (1, 3, 7, 14, 28, and 56 days); samples of approximately 300 g were collected at each period to evaluate the dry matter (DM)(5); organic matter (OM) and ash(5); total nitrogen (TN) was assayed by a Kjeldahl method(6); ether extract (EE) (Soxtherm 2000 S 306 M, Gerhardt; Königswinter, Germany) in Goldfish with ethyl ether at 180 °C for 4 h; neutral detergent insoluble (NDF) was analyzed according to procedures described by Mertens(7), and acid detergent fibre (ADF)(6); neutral detergent fibre corrected for ash and protein(8) (NDFap); acid detergent insoluble N (ADIN) and neutral detergent insoluble N (NDIN) were performed according to Licitra(8) and concentration of sulphuric acid detergent lignin (ADL) was analyzed by soaking the bags containing ADF residue in 12M H_2SO_4 during three hours(6).

The total carbohydrates (TC) content was estimated as: TC = 100 – (%CP + %EE + %ash)(9) and the non-fiber carbohydrates content (NFC) was estimated as: OM – {[NDF - (NDIN × 6.25)] + (N × 6.25) + EE}(10).

The data was submitted to analysis of variance to determine the main effects (inoculant) and, to analyse the unfolding of the interactions, a regression analysis was used, and the means of the treatments were compared by the Tukey test, at a 5% probability level. Both of data was analysed using the SAEG-UFV(11) software version 8.0.

Results

The table 1 describes the chemical composition, pH, and microbial composition of soybean plants before ensiling.

Table 1. Chemical composition (g kg^{-1} of dry matter-DM) and pH in soybean plants before ensiling

Items	Plants[1]		
	Without inoculant		
	0 (Controle)	2	4
DM	237.9	285.2	258.8
OM	923.7	920.7	889.4
Ash	76.3	79.3	110.6
CP[2]	204.6	179.8	185.6
EE	31.9	32.6	32.5
TC	687.2	708.3	671.3
NDF	501.6	453.8	433.1
ADF	263.1	284.5	244.6
Hemicellulosis	238.5	169.3	188.5
Cellulose	263.4	231.5	276.6
Lignin	76.0	51.5	90.9
CNF	185.6	254.5	238.2
SC[3]	65.4	72.1	88.2
LAB	33.1	--	--
ENT	40.7	--	--
MY	29.5	--	--
pH	6.06	--	--
	With inoculant		
	0 (Controle)	2	4
DM	261.4	295.7	251.2
OM	910.0	882.9	891.1
Ash	90.0	117.1	108.9
CP[2]	193.4	173.6	184.3
EE	25.7	22.3	28.4
TC	690.9	687.0	678.4
NDF	435.8	530.7	489.2
ADF	279.5	307.8	244.9
Hemicellulosis	156.3	222.9	244.3
Cellulose	252.9	250.7	255.0
Lignin	88.1	69.9	64.2
CNF	255.1	189.2	298.1
SC[3]	80.3	89.4	85.3

[1]g kg^{-1} of DM, [2]g kg^{-1} of total nitrogen, soluble carbohydrates.

The table 2 contains the chemical composition of soybean silages. Excluding dry matter and crude protein an interaction effect between the additive and fermentation periods it was observed for the variables ($P<0.05$). It was adjusted some regression equations to interaction effect between the additive and fermentation periods to ADIN and ADF variables. Those regression equations are described in the discussion of the results.

Table 2. Chemical composition of soybean silages as a function of additives (A) and fermentation periods (P)

Additives	Fermentation periods (days)							Effect			Standard error
	1	3	7	14	28	56	Average	A	P	AXP	
	Dry Matter (g kg^{-1})							*	*	ns	0.13
Control	265.7	264.6	274.9	273.8	284.8	276.1	272.76				
I	266.3	263.6	271.8	276.4	283.4	269.7	271.87				
I+M	281.1	292.6	294.6	287.1	300.4	284.3	290.02				
M	279.3	269.3	275.5	284.9	291.8	287.0	281.30				
	Crude Protein (g kg^{-1} of DM)							*	*	ns	0.14
Control	169.3	152.4	137.0	152.0	140.1	148.1	149.82				
I	169.4	152.8	138.5	145.8	141.0	147.9	149.23				
I+M	146.4	143.3	134.4	136.3	134.6	138.9	138.98				
M	164.7	153.5	161.1	145.1	142.5	140.8	151.28				
	NDIN (g kg^{-1} of TN)[1]							*	*	*	0.59
Control	296.7b	217.6b	197.3bc	188.0b	148.9c	136.7b	197.53				
I	271.6c	219.9b	167.0c	199.4b	181.6ab	181.1a	207.90				
I+M	226.7d	172.1c	180.9bc	162.0c	162.6bc	178.5a	180.47				
M	330.5a	300.2a	242.4a	235.5a	177.2abc	186.6a	245.40				
	ADIN (g kg^{-1} of TN)							*	*	*	0.30
Control	171.4b	161.2b	127.5bc	116.5c	106.1b	101.3b	130.67				
I	166.3b	129.3c	114.2c	125.2c	125.4a	125.8a	131.03				
I+M	122.6c	112.1d	125.1bc	174.0a	101.0b	115.6a	125.07				
M	184.7a	181.8a	148.2a	138.5b	119.3a	125.7a	149.70				
	EE (g kg^{-1} of DM)							*	ns	*	0.06
Control	87.0a	85.6b	84.0b	87.0ab	98.5a	82.5b	87.43				
I	87.6a	94.1a	92.2ab	93.5ab	88.8b	90.1a	91.05				
I+M	88.7a	93.6a	94.9a	84.1b	84.0b	90.9a	89.37				
M	85.5a	82.9b	87.9b	83.4b	81.6b	91.9a	85.53				
	NDF (g kg^{-1} of DM)							ns	ns	*	0.06
Control	476.6a	468.2a	472.4a	468.6a	474.9a	467.9a	471.43				
I	469.1a	467.8a	475.3a	473.9a	475.5a	473.7a	472.55				
I+M	470.9a	473.3a	463.2a	466.3a	474.0a	465.4a	468.85				
M	468.9a	469.6a	470.2a	477.7a	470.2a	466.8a	470.57				
	ADF (g kg^{-1} of DM)							*	*	*	0.32
Control	277.7a	339.6a	325.2a	282.5a	322.5a	339.5a	314.50				
I	287.4a	334.9a	3251a	293.0a	335.4a	329.0a	317.47				
I+M	263.9b	300.4b	303.5b	267.9b	297.2b	310.8b	288.04				
M	249.3c	286.6c	272.4c	267.1b	299.5b	311.0b	280.98				

Control: Soybean silage without any additive, I: with a microbial inoculant, I+M: with I + powdered molasses and M: with powdered molasses.

[1]TN: total nitrogen.

*significant at a 5% level of significance.

ns: non-significant at a 5% level of significance.

Same letters in the same column do not differ according to a Tukey test at a 5% level of significance.

Discussion

The dry matter content of 265.0 g kg^{-1} of forage before ensiling was close to the 250.0 g kg^{-1} value recommended by McDonald et al.[12] as a necessary condition to minimise effluent losses in the silo and, thus, retain silages nutrients. Assessing soybean forage production and nutritive value in adverse tropical conditions the authors[13] found 267.0 g kg^{-1} DM content in soybean plants harvested at R6 stadium, being these values greater than those found in this study.

Evaluating forage quality of soybean silages not mixed with other crops the authors[14] find values to the DM of 308,0 g kg^{-1} and 458,0 g kg^{-1}, crude protein (CP) content of 181,0 g kg^{-1} and 240,0 g kg^{-1}. All those values are higher than those values find in this study, probably because on that study the authors evaluated soybean plants at R3 to R4 stage.

The decrease in crude protein content observed in silages from the 3rd fermentation day onwards might be explained by the increase in proteolysis as a consequence of the increase the ammonia-N content at the onset of fermentation by presence of enterobacteria at the beginning of fermentation period. Analysing the fermentation profile and the microbial population in soybean silages with inoculant and powder molasses, Rosa et al.[15] observed the enterobacteria only up to the 3rd day of fermentation, besides the effect of the additive and fermentation periods on all variables ($P<0.05$), but this effect was not constant. In that study, on the 56th day of fermentation, the authors found higher ammonia-N values in control (72.5 g kg^{-1}) and I silages (73.8 g kg^{-1}); the latter did not differ among it ($P>0.05$) and on the other hand less ammonia-N values were found in I+M (52.6 g kg^{-1}) and M silages ($P<0.05$) (53.4 g kg^{-1}) with not differ among it ($P>0.05$). In this way, possibly due to the rapid fall of pH, an inhibition of the action of proteolytic bacteria occurs, leading to a lower production of ammonia-N.

According to McDonald et al.[12] the enterobacteria produces ammonia-N from protein structures. Proteolysis during ensiling started with the action of proteolytic enzymes, which resulted in the formation of peptides and amino acids. In addition, bacteria of the genus *Clostridium* and *Enterobacteria*, which develop in environments with high pH[16]. Rosa et al.[15] assessing the fermentation profile and microbial population in soybean silages with inoculant and powdered molasses observed a higher reduction of pH values in the first 10 days of fermentation estimated to I+M, and M silages equal to 0.02316 and 0.01521 units to pH values, respectively and the authors associated those results to fast development of lactic acid bacteria and the lower ammonia-N average values observed to these silages. Thus, reducing the pH of the silage by inoculation may have reduced the development of the undesirable microorganisms, thereby reducing proteolysis.

The lowest values ($P<0.05$) to neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) contents observed to I+M silages, equal 180.47 and 125.07 g kg^{-1}, respectively, is can be explained by association between microbial inoculant and molasses that may have promoted a more efficient transformation of sugars into lactic acid, reducing proteolysis. The lowest ($P<0.05$) neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) contents (g

kg^{-1} of total nitrogen) observed in silages at the 56th day of fermentation might be associated with a lower proteolysis rate by ADIN production[17]. To control and molasses silages were adjusted the following regression equations to ADIN values $\hat{Y}$ = 15,07 – 0,11*X, R^2 = 0,62 and $\hat{Y}$ = 16,82 – 0,102*X, R^2 = 0,58, respectively. Yet, all silages exhibited ADIN values lower than 200 g kg^{-1} of total nitrogen and that according to Van Soest et al.[10], the use of forages with higher ADIN content than this value was restricted due to the reduction in nitrogen availability and dry matter digestibility.

The lower ether extract (82.5 g kg^{-1}) content observed in control silage on the last fermentation day was puzzling because this component was not altered throughout the course of fermentation. Nevertheless, according to McDonald et al.[12], nutrients might have been lost as effluents through lixiviation of some soluble compounds.

The increase of fibrous components in soybean plants after ensiling it must be due to a reduction in the water-soluble carbohydrate content of cells, although this has not been reported in this study. Interestingly, there is contradictory evidence regarding the effect of microbial inoculants on fibrous fractions, and both rise and decline are reported, especially the NDF fraction. The addition of lactobacillus plus enzymes, improved the chemical quality of alfalfa silages, decreasing the NDF content[18]. In the case of *L. buchneri*, it is possible to observe the low values of NDF in sugarcane silages treated with *L. buchneri* compared to the values obtained in the control silage (66.7% and 75.1%, respectively). On the other hand, the use of microbial inoculants did not increase the quality of the fibrous fraction[19,20,21,22,23,24]. Some authors[25,26], investigating the effects of heterofermentative inoculant on the nutritional value of silages, also found that the inoculant did not modify the NDF content in relation to the control treatment.

To the NDF content of silages was observed interaction effect between additive and fermentation period ($P<0.05$). It was observed lower average values, equal to 468.85, to silages with inoculant with molasses (I+M) however no equation fitted the data. The application of the inoculant implied the fall of NDF probably due to the presence of cellulolytic enzymes in this product, which acts on the cellulose contained in the cell wall of the plants, breaking the β-1-4 glycoside bonds releasing sugars[27]. Thus, the inoculant and the molasses proved to be efficient in order to reduce the fibrous fraction and it may be possible to ferment the ensiled mass due to the release of additional sugars, since acid lactic bacteria does not degrade cell wall components[28].

To ADF contents it was observed interaction effect between additives and fermentation periods ($P<0.05$). It was adjusted the following regression equations to ADF values $\hat{Y}$ = 7,5 – 0,019*X, R^2 = 0,79 e $\hat{Y}$ = 26,5 + 0,88*X, R^2 = 0,66 to silages control and inoculant with molasses (I+M), respectively. It was observed lower values to the silages with molasses between 1^{th} and 7^{th} day of fermentation. This fact can be associated to higher water soluble carbohydrates contents in those silages because Van Soest[10] states that the fibrous fraction of the ensiled material can be modified, as a result of the decrease of the soluble carbohydrates, part of the cellulose fraction and the variable degradation of the hemicellulose fraction in the ensiling process. Whereas that the hemicellulose fraction is part of the plant, it implies modifications of the fibrous fraction in the silage. On the other hand, the higher acid detergent insoluble fibre (ADF) contents observed in control and I silages at all fermentation periods were possibly due to a lower hemicellulose content ($P<0.05$). The hemicellulose content was found to be higher in silages compared to plants; this finding is unexpected because hemicellulose usually decreases after ensiling due to acid hydrolysis. However, the reduction of hemicellulose content in silages is can also due to the presence of hemicellulases in the ensiled plant, and the effectiveness of these enzymes significantly varies as a function of the employed source and substrate[29].

Conclusion

The addition of microbial inoculant with or without molasses in soybean silages reduces the proteolysis, producing silages with lower levels of acid detergent insoluble nitrogen. Thus, it can be concluded that the increase in the population of lactic acid bacteria by the addition of inoculant can advantage the fermentation process inside the silo and ensure better characteristics of the silages produced.

References

1. Evangelista AR, Resende PM, Maciel GA. Uso da soja [Glycine max (L.) Merrill] na forma de forragem. Lavras: UFLA, 2003. 36p.

2. Undersanden D, Jarek K, Anderson T, Schneider N, Milligan L. 2007. A guide to making soybean silage. Integrated Pest and Crop Management [Internet]. Available from: <http://www.scielo.br/pdf/abmvz/v70n5/1678-4162-abmvz-70-05-01586.pdf>. Acessed in: 21 mar. 2019.

3. Lima JA, Gavioli ILC, Barbosa CMP, Cunha EA, Gimenes FMA, Bernedt A. Silagem de soja no enriquecimento de dietas composta por silagem de ponta de cana-de-açúcar I - Consumo de nutrientes. In: 48ª REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 2011, Belém. Anais...Belém: SOCIEDADE BRASILEIRA DE ZOOTECNIA, 2011.

4. Coblentz WK, Muck RE, Borchardt MA, Spencer SK, Jokela WE, Bertram MG, Coffey KP. Effects of dairy slurry on silage fermentation characteristics and nutritive value of alfafa. Journal of Dairy Science, v. 97, n. 11, p. 7197–7211, 2014.

5. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official Methods of Analysis, 15th edition. Arlington, VA, USA; 1990. 1140p.

6. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS – AOAC. Official methods of analysis. 16th. ed. Gaithersburg: AOAC International, Washington D.C.; 1997. 1140p.

7. Mertens DR. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of AOAC International. 2002, 85:1217-1240.

8. Licitra G, Hernandez TM, Van Soest PJ. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology. 1996, 57:347-358.

9. Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG, Russel JB. A net carbohydrate and protein system for evaluating cattle diets. Carbohydrate and protein availability. Journal of Animal Science. 1992, 70:3562-3577.

10. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 1991, 74:3583-3597.

11. UNIVERSIDADE FEDERAL DE VIÇOSA – UFV. Sistema de análises estatísticas e genéticas – SAEG, Manual do usuário (versão 8.0). Viçosa: Universidade Federal de Viçosa, MG, Brasil; 2000, 138 p.

12. McDonald P, Henderson AR, Heron SJE. The biochemistry of silage, 2ª. edition, Aberystwyth: Chalcombe Publications; 1991, 340 p.

13. Tobia C, Villalobos E. Producción y valor nutricional del forraje de soya en condiciones tropicales adversas. Agronomía Costarricense. 2004, 28:17-25.

14. Undersanden D, Jarek K, Anderson T, Schneider N, Milligan L. 2013. A guide to making soybean silage. Integrated Pest and Crop Management [Internet]. Available from: <https://fyi.extension.wisc.edu/forage/files/2014/01/SoybeanSilage.pdf>. Accessed in 10 January 2019.

15. Rosa LO, Pereira OG, Ribeiro KG, Valadares Filho SC, Cecon PR. Fermentation profile and microbial population in soybean silages with inoculant and powdered molasses. Arq. Bras. Med. Vet. Zootec., v.70, n.5, p.1586-1594, 2018.

16. Pahlow G, Muck RE, Driehuis F. Microbiology of ensiling. In: BUXTON, D.R.; MUCK, R.E.; HARRISON, J.H. (Eds.) Silage science and technology. 1.ed. Madison: American Society of Agronomy, 2003. p.31-94.

17. Muck R. Inoculant of silage and its effects on silage quality. In: Informational conference with dairy and forage industries. Proceedings... US Dairy forage Research. 1996, 43-52.

18. Rangrab LH, Mühlbach PRF, Berto JL. Silagem da alfafa colhida no início do florescimento e submetido ao emurchecimento e à ação de aditivos biológicos. Efeitos da adição de inoculantes microbianos sobre a composição bromatológica. Revista Brasileira de Zootecnia, Viçosa, v.29, n.2, p.349-356, 2000.

19. Igarasi MS. Controle de perdas na ensilagem de capim Tanzânia (*Panicum maximum* Jacq. Cv. Tanzânia) sob os efeitos do teor de matéria seca, do tamanho de partícula, da estação do ano e da presença do inoculante bacteriano. 2002. 151 f. Dissertação (Mestrado em Agronomia) – Escola Superior Agrícola “Luiz de Queiroz”, Universidade de São Paulo, Piracicaba, 2002. Available from: https://www.teses.usp.br/teses/disponiveis/11/11139/tde-12082002-151258/publico/mauricio.pdf; Accessed in 10 January 2019.

20. Paziani SF. Controle de perdas na ensilagem, desempenho e digestão de nutrientes em bovinos de corte alimentados com rações contendo silagens de capim tanzânia. 2004. 208f. Tese (Doutorado em Agronomia) - Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Piracicaba, 2004. Available from: https://www.teses.usp.br/teses/disponiveis/11/11139/tde-23102006-164429/publico/solidete.pdf. Accessed in 10 January 2019.

21. Magalhães VJA, Rodrigues PHM. Avaliação de inoculante microbiano na composição bromatológica, fermentação e estabilidade aeróbia de silagem pré-seca de alfafa. Revista Brasileira de Zootecnia, Viçosa, v.33, n.1, p.51-59, 2004.

22. Coan RM, Vieira PF, Silveira RN, Reis RA, Magleiros EB, Pereira MS. Inoculante enzimático-bacteriano, composição química e parâmetros fermentativos das silagens dos capins Tanzânia e Mombaça. Revista Brasileira de Zootecnia, Viçosa, v.34, n.2, p.416-424, 2005.

23. Rocha KD, Pereira OG, Valadares Filho SC, Oliveira AP, Pacheco LBB, Chizzotti FHM. Valor nutritivo de silagens de milho (*Zea mays* L.) produzidas com inoculantes enzimo-bacterianos. Revista Brasileira de Zootecnia, Viçosa, v.35, n.2, p.389-395, 2006.

24. Pereira OG, Rocha KD, Ferreira CLLF. Composição química, caracterização e quantificação da população de microrganismos em capim-elefante cv. Cameroon (*Pennisetum purpureum*, Schum.) e suas silagens. Revista Brasileira de Zootecnia, Viçosa, v.36, n.6, p.1742-1750, 2007.

25. Ranjit NK, Kung Jr L. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. Journal of Dairy Science, Savoy, v.83, n.3, p.526-535. 2000.

26. Queiroz OCM. Associação de aditivos microbianos na ensilagem e o desempenho de vacas em lactação recebendo silagem de cana-de-açúcar comparada a um volumoso tradicional. 2006. 122 f. Tese (Doutorado em Agronomia) - Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2006. Available from: http://www.teses.usp.br/teses/disponiveis/11/11139/tde-05102006-142405/publico/OscarQueiroz.pdf. Accessed in 10 January 2019.

27. Beauchemin KA et al. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. Journal of Animal Science, v.81, n.2, p.37-47, 2003. Available from:< http://www.journalofanimalscience.org/content/81/14_suppl_2/E37.full.pdf+html >. Acessed in: 19 april 2019.

28. Muck RE. The role of silage additives in making high quality silage. In: SILAGE PRODUCTION FROM SEED TO ANIMAL, 1993, New York. Proceedings... New York: NRAES, n.67, 1993. p.106-116.

29. Muck RE, Kung Jr L. Effects of silage additives on ensiling. In: SILAGE FIELD TO FEEDBUNK. Pennsylvania. Proceedings... New York: NRAES. 1997, 99:187-199.