University of São Paulo
"Luiz de Queiroz" College of Agriculture

Exogenous fibrolytic enzymes affect the nutritive value and the performance of Nellore bulls when added to corn-based silages

Pedro Augusto Ribeiro Salvo

Thesis presented to obtain the degree of Doctor in Science. Area: Animal Science and Pastures

Piracicaba
2019

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
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1. Xilanase 2. Silagem de grão úmido 3. Silagem de espiga 4. Silagem de planta inteira de milho I. Título

To my parents Antonio and Fátima

To my brother Leonardo

To my girlfriend Viviane

I DEDICATE

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CONTENTS

RESUMO

Enzimas fibrolíticas exógenas afetam o valor nutritivo e o desempenho de touros Nelores quando adicionadas à silagens a base de milho

O uso de silagens em dietas feitas a partir da planta de milho é muito comum no Brasil e no mundo. A silagem de grão úmido e a silagem de planta inteira são amplamente utilizadas por produtores em dietas de bovinos confinados. Outra silagem proveniente da planta de milho que possui potencial para uso é o snaplage, que é composto apenas da espiga do milho, contendo as frações palha, sabugo e grãos. Animais alimentados com dietas compostas com silagem de grão úmido de milho apresentam melhora na eficiência alimentar, devido ao aumento na digestibilidade do amido. Este benefício é dependente de três fatores: o tempo de estocagem, o tamanho de partícula, e o teor de umidade do grão. Esta melhora na digestibilidade do amido acontece em função da solubilização da matrix proteica do endosperma. Entretanto, outra barreira que pode prejudicar a disponibilidade de nutrientes do grão, como o amido, é a parede celular do endosperma. Esta parede celular é composta em sua maioria por arabinoxilanas. Sendo assim, o uso de enzimas fibrolítcas pode se revelar uma boa forma para melhorar a disponibilidade de nutrientes, por meio da solubilização de compostos da parede celular. Além disso, a adição destas enzimas na ensilagem pode otimizar seus efeitos, devido as condições ótimas de pH e de temperatura da silagem para as enzimas. Porém, os resultados de estudos na literatura sobre a adição de enzimas fibrolíticas em diversos tipos de silagens não são esclarecedores. Portanto, o objetivo do primeiro estudo foi avaliar o desempenho de touros da raça Nelore alimentados com dietas contendo silagens de grão úmido de milho e snaplage, com ou sem adição de enzimas fibrolíticas na ensilagem. No segundo estudo o objetivo foi avaliar a composição química, a digestibilidade de nutrientes, e o perfil fermentativo de silagens de planta inteira de milho, quando adicionadas doses crescentes de enzimas fibrolítcas ensiladas por diferentes tempos. Como resultado do primeiro estudo, os animais alimentados com dietas que receberam o tratamento das enzimas fibrolítcas nas silagens, apresentaram menor consumo de matéria seca e maior eficiência alimentar, em comparação as dietas controles. O tratamento com a enzima reduziu o teor de FDN, e aumentou a digestibilidade *in vitro* e *in situ* da matéria seca, da silagem de grão úmido. Além disso, a aplicação da enzima também resultou em maiores concentrações de ácido acético e acetato de etila para as silagens de grão úmido. Não houve diferença entre as dietas. No segundo estudo com silagem de planta inteira, o tratamento com a enzima resultou em maior produção de ácido acético, e menores concentrações de etanol, lactato de etila e acetato de etila. Como conclusão, o uso de enzimas em silagem de grão úmido, melhora o desempenho animal, e em silagem de planta inteira altera o perfil fermentativo.

Palavras-chave: Xilanase, Silagem de grão úmido, Silagem de espiga, Silagem de planta inteira de milho

ABSTRACT

Exogenous fibrolytic enzymes affect the nutritive value and the performance of Nellore bulls when added to corn-based silages

The use of silages made from corn crop is common in Brazil and around the world. The high moisture corn and the whole-plant corn silage are largely used by farmers in feedlot diets. Another silage made from the corn, which seems to have great potential, is the snaplage. The snaplage is composed by the ear of the corn, which contains husk, cob, and grains. Animals fed diets with high moisture corn silages have better feed efficiency, due to the increase of starch digestibility. This increase is dependent on three factors: length of storage, grain particle size, and moisture. The improvement in starch digestibility occurs because of endosperm protein matrix solubilization. However, another barrier that can impair the grain nutrients availability, such as, starch, is the endosperm cell wall. The cell wall is composed mainly by arabinoxylans. Therefore, the application of exogenous fibrolytic enzymes can enhance the availability of the nutrients, by the solubilization of cell wall components. The optimal pH and temperature of the silages might be beneficial for the enzymes to act. The objective of the first study was to evaluate the performance of Nellore bulls fed diets with high moisture corn and snaplage, with or without the addition of exogenous fibrolytic enzymes at ensiling. In the second study, the objective was to evaluate the chemical composition, the nutrient digestibility, and the fermentative profile of whole-plant corn silages added different doses of fibrolytic enzymes, ensiled for different times of storage. As a result of the first study, the bulls fed diets with silages treated with fibrolytic enzymes showed a decreased dry matter intake and an increased feed efficiency. The enzyme treatment decreased the NDF content and increased the DM *in vitro* and *in situ* digestibility of the high moisture corn silage. Moreover, the acetic acid and ethyl acetate concentration was increased for the high moisture corn treated with the enzymes. There was no difference between the diets. In the second study with whole-plant corn silage, the exogenous fibrolytic enzymes addition increased the acetic acid concentration and decreased the concentrations of ethanol, ethyl lactate, and ethyl acetate. In conclusion, the addition of exogenous fibrolytic enzymes in high moisture corn silages improved the animal performance, and in whole-plant corn silages, it affected the fermentative profile.

Keywords: Xylanase, High moisture corn, Snaplage, Whole-plant corn silage

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1. INTRODUCTION

The corn crop is versatile because it allows different parts of the plant to be harvested separately and ensiled. In beef cattle nutrition, high moisture corn (HMC), has been increased as the primary method of grain processing in Brazil. Also, the whole-plant corn silage (WPCS) is primary roughage source in feedlot diets (Millen et al., 2009; Oliveira and Millen, 2014; Pinto and Millen, 2016). In dairy cattle diets, WPCS is by far the main roughage used among the Brazilian farmers (Bernardes and Rêgo, 2014). Another alternative of silage made from corn plant is the snaplage, which consists in harvesting the ear corn (husks, cob, and grains) of the plant using a self-propelled forage harvester combined with a snapper head. With the combination of fiber and grain fraction, snaplage is an economical silage alternative (Mahana, 2008).

The advantage of using HMC in diets is related to the better feed efficiency showed by the animals, given by the increase in starch digestibility (Owens et al. 1997; Zinn et al. 2011). However, to reach this benefit three majors aspects of HMC silage making must be respected: the length of storage; the grain particle size; and the grain moisture (Gomes et al. 2018). The harvesting of high-grain content silages, such as, high moisture corn and snaplage, occurs when the kernel is fully mature, where the starch granules are embedded in a matrix of storage proteins (prolamins), accompanied of cell walls and other minor components, which are necessary to embryo germination. The prolamins in the grain endosperm are hydrophobic zeins, which are classified in four subclasses: α, β, γ, δ (Hoffman et al. 2011). Another nutrient barrier in the endosperm is the cell wall, which is composed mainly of arabinoxylan (Evers and Millar, 2002; Le et al. 2013). To overcome the restriction imposed by the endosperm cell wall, the exogenous fibrolytic enzymes (EFE) might be an effective additive to improve the availability of starch in corn-based silages at ensiling.

The ensiling process represents an optimal environment for the EFE to act, once it has low pH and higher temperatures than the rumen (Adesogan et al. 2014). However, there are inconsistencies when EFE are applied to ensiling of various types of silages (Eun and Beauchemin, 2007). The lack of positive responses of EFE application might be associated with EFE dose, EFE activity composition, the prevailing pH and temperature, the animal performance level, the experimental design, and the fraction and proportion of the diet to which the EFE is applied (Romero et al. 2015).

Therefore, the hypothesis of the thesis was: high moisture corn, snaplage, and whole-plant corn silage represent an environment, which exogenous fibrolytic enzymes could act

properly (optimal temperature and pH), improving the nutritive value of the silages, the fermentative profile, and animal performance.

The objective of the first experiment was to evaluate the performance of Nellore bulls fed diets composed by high moisture corn and snaplage, added or not exogenous fibrolytic enzymes at ensiling. The second trial had the objective to evaluate the effects of doses of exogenous fibrolytic enzymes and different times of storage on the chemical composition, nutrient digestibility and fermentative profile of whole-plant corn silages.

LITERATURE CITED

Adesogan, A, T., Z. X. Ma, J. J. Romero, and K. G. Arriola. 2014. Ruminant Nutrition Symposium: Improving cell wall digestion and animal performance with fibrolytic enzymes. J. Anim. Sci. 92:1317-1330. doi:10.2527/jas.2013-7273

Bernardes, T. F., and A. C. Rêgo. 2014. Study on the practices of silage production and utilization on Brazilian dairy farms. J. Dairy Sci. 97:1852–1861.

Eun, J. S., and K. A. Beauchemin. 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using in vitro fermentation characteristics. Anim. Feed Sci. Technol. 132:298–315.

Evers, T. and S. Millar. 2002. Cereal grain structure and development: some implications for quality. Journal of Cereal Science. 36.3: 261-284. doi:10.1006/jcrs.2002.0435

Gomes, A. L. M., J. L. Bueno, F. A. Jacovaci, D. C. Bolson, C. C. Jobim, and J. L. P. Daniel. 2018. Effects of processing, moisture and length of storage on the fermentative losses and ruminal degradability of reconstituted corn grain silage. Page 500 in Proc. XVIII International Silage Conference. Bonn, Germany.

Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblentz, M. P. Scott, A. L. Bodnar, R. J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465-2474. doi:10.3168/jds.2010-3562.

Le, D. M., P. Fojan, E. Azem, D. Pettersson, and N. R. Pedersen. 2013. Visualization of the anticaging effect of Ronozyme WX xylanase on wheat substrates. Cereal Chemistry, 90(5): 439-444.

Mahanna, B. 2008. Bottom line: Renewed interest in snaplage displayed. Feedstuffs. 80:50. The Miller Publ. Co., Bloomington, MN.

Millen, D. D., R. D. L. Pacheco, M. D. B. Arrigoni, M. L. Galyean, and J. T. Vasconcelos. 2009. A snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. J. Anim. Sci. 87:3427–3439. doi:10.2527/jas.2009-1880

Oliveira, C. A., and D. D. Millen. 2014. Survey of nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. Anim. Feed Sci. Technol. 197: 64–75. doi:10.1016/j.anifeedsci.2014.08.010

Owens, F. N., D. S. W. Secrist, J. Hill, and D. R. Gill. 1997. The effect of grain source and grain processing on performance of feedlot cattle: a review. J. Anim. Sci. 75:868-879.

Pinto, A. C. J., and D. D. Millen. 2016. Situação atual da engorda de bovinos em confinamento e modelos nutricionais em uso. Proc. X Simpósio de Produção de Gado de Corte (Simcorte). (In Portuguese). Federal University of Viçosa, Viçosa- MG, Brazil. 103-120.

Romero, J. J., M. A. Zarate, and A. T. Adesogan. 2015. Effect of the dose of exogenous fibrolytic enzyme preparations on preingestive fiber hydrolysis, ruminal fermentation, and in vitro digestibility of bermudagrass haylage. Journal of dairy science 98.1: 406-417. doi:10.3168/jds.2014-8285

Zinn, R. A., A. Barreras, L. Corona, F. N. Owens, and A. Plascencia. Comparative effects of processing methods on the feeding value of maize in feedlot cattle. Nutr. Res. Rev. 24:183-190, 2011.

2. EXOGENOUS FIBROLYTIC ENZYMES ADDED TO HIGH MOISTURE CORN AND SNAPLAGE AT ENSILING: EFFECTS ON THE PERFORMANCE OF NELLORE BULLS

ABSTRACT

Silages made from corn grains, designated as high moisture corn (HMC), are consensual in the international literature to improve animal feed efficiency, in comparison to dry corn. The addition of high quality whole-plant corn silage (WPCS) and snaplage (SNAP) in the feedlot diets are interesting, because of the contribution of its grain fraction in reducing the addition of other grain sources. Exogenous fibrolytic enzymes (EFE) have been demonstrated using non-ruminant animals to improve the energy of grains feedstuffs, by reducing the encapsulation of the endosperm nutrients created by the grain cell wall. Therefore, the objective of the present study was to evaluate the effects of EFE addition to HMC and SNAP at ensiling, on the silage nutritive value and the performance of Nellore bulls. In this way, the diets were composed by: SNAP + HMC Control (without enzyme); SNAP + HMC EFE (with enzyme); WPCS + HMC Control; WPCS + HMC EFE. In the diets with WPCS, only the HMC had the EFE addition, but there was no interaction between diets and EFE application. Furthermore, there was no difference between diets with SNAP and WPCS on the DMI, ADG and feed efficiency of the Nellore bulls. Regarding the EFE effects, it decreased the DMI (P=0.01), did not alter the ADG, thus improved the feed efficiency of the bulls (P=0.04). Furthermore, the enzymes reduced the NDF content of the HMC (P=0.02), increased the content of acetic acid, and in consequence the ethyl acetate. However, the digestibility (in vitro and in situ) of the HMC silages was only increased for dry matter content. Although the starch and NDF digestibilities were numerically higher in HMC they were not significantly different. No expressive effects were found for the addition of EFE to SNAP silages. The fecal starch was decreased for the EFE application (P=0.05). Therefore, the TDN, NEm, and NEg, calculated from the animal performance, increased (P=0.01) with the addition of the EFE to the silages. In conclusion, bulls fed diets composed by SNAP or WPCS did not affect the intake, weight gain and efficiency, but the addition of EFE at ensiling to silages with high grain proportion could improve animal performance by increasing its energy availability.

Keywords: Feed efficiency, Fermentation, High moisture corn, Snaplage, Xylanase

2.1. INTRODUCTION

The interest in the usage of corn-based silages has been increased in Brazilian feedlots. The number of feedlot consulting nutritionists, which passed to use high moisture corn (HMC) as the primary method of grain processing, increased from zero (Millen et al., 2009), to 3% (Oliveira and Millen, 2014), to 6% (Pinto and Millen, 2016). In the U.S, around 17% of feedlots uses HMC (Samuelson et al. 2016). Moreover, the same pattern occurred for the whole-plant corn silage (WPCS), increasing from 25.8% in 2009, to 27.3% in 2014, to 63,6% in 2016, where it was considered as the primary roughage source. Although snaplage (SNAP) has not been mentioned in the surveys, it seems an economical silage alternative (Mahana, 2008) to improve animal performance (Akins and Shaver, 2014).

Revising the literature about the benefits of HMC over ground corn, in studies conducted in Brazil, it was encountered that the inclusion of ensiled corn kernels in the diets can improve, on average, the feed efficiency of growing/finishing animals in 16.19%, as a result of the reduction of the feed intake in 10.79% (Berndt et al. 2002; Costa et al. 2002; Putrino 2006; Henrique et al. 2007; Silva et al. 2007; Almeida Júnior et al. 2008; Carareto 2011; Caetano et al. 2015; Silva 2015; Tres 2015; Silva 2016). However, these benefits of grain ensiling are based on three major premises (Gomes et al. 2018), the length of storage (Hoffman et al. 2011), the grain particle size (Remond et al. 2004), and moisture (Owens et al. 1997). Furthermore, the harvesting of high moisture corn and snaplage occurs when the kernel is fully mature, which means that the starch granules are embedded in a matrix of storage proteins, accompanied of cell walls and other minor components, which are necessary to embryo germination. The proteins in the grain endosperm are hydrophobic zeins, which are classified in four subclasses: α, β, γ, δ (Hoffman et al. 2011). The cell walls in the endosperm consist mainly of arabinoxylan, which encapsulates nutrients, creating a cage-effect. (Evers and Millar, 2002; Le et al. 2013). According to Kim et al. (2005), the nonstarch polysaccharides (NSP) decrease the nutrient digestibility by entrapping starch and protein, in non-ruminants diets.

The use of exogenous fibrolytic enzymes (EFE), with xylanase as the main activity, is known in the literature to improve in vitro digestion (Phakachoed et al. 2013; Romero et al. 2015) of silages. However, studies have shown inconsistent responses in growth performance when EFE were added to the ruminant feed. Partially, this inconsistency can be attributed to the suboptimal condition of the rumen, 39ºC and pH 6, (Adesogan et al. 2014) where EFE could be less efficient in breaking xylose and cellulose bonds.

Therefore, our hypothesis assumed that exogenous fibrolytic enzymes (EFE) could increase animal performance by increasing digestibility of silages combined with a high percentage of grains, such as high moisture corn and snaplage, by reducing the cage effect of the starch granules, when applied at the ensiling.

2.2. MATERIALS AND METHODS

The performance trial was carried out at the Nutripura's Research Center (Pedra Preta, MT, Brazil) and the feed samples were sent to the Luiz de Queiroz College of Agriculture – University of Sao Paulo, to be analyzed physically and chemically. All the animal procedures were in accordance with the guidelines of the Animal Care and Use Committee of the Luiz de Queiroz College of Agriculture.

2.2.1. Ensiling

Before the beginning of the performance trial, the high moisture corn (HMC), snaplage (SNAP), and whole-plant corn silage (WPCS) were stored for at least 45 days. Corn crop was purchased from a commercial farm near to the experimental beef feedlot. The SNAP and WPCS silages were ensiled in drive-over silos, while HMC was ensiled in Ag-bags, with of 2.74 m of diameter and 60 m of length (Ipesasilo, São Paulo, SP, Brazil). The ensiling period occurred from June 20th to July 4th, 2017. All the silos were made with two repetitions, the first repetition was opened at the beginning of the performance trial (day 0). Then, the storage period at the beginning of the trial for HMC, SNAP, and WPCS was 50, 45 and 55 days, respectively. The second repetition was opened with 118, 126 and 108 days of fermentation, respectively. Each silo of HMC, SNAP, and WPCS had, on average, 125, 121, and 345 tons of material.

At ensiling, HMC and SNAP were treated with an exogenous fibrolytic enzyme complex (EFE) (Rovabio Advance P, Adisseo, Antony, France) at the dose of 100 g per ton of fresh matter. For the EFE application, it was dissolved in chlorine-free water at a ratio of 0.025 g/L, to facilitate the distribution of product on high moisture corn and snaplage. For the HMC, the product was applied by using an onboard device for additives application in the bagger, adjusted to the dose of 100 g/t of fresh matter. In the SNAP, the product was sprayed

and mixed onto the material, using a tractor-mounted sprayer. To ensure the adequate dose, the product was applied according to the batch weight of each truck.

The EFE is composed of two major active enzymes, endo-1,4-β-xylanase (E.C. number 3.2.1.8) and endo-1,3(4)-ß-glucanase (E.C. number 3.2.1.6), obtained from Talaromyces versatilis strains. The dose applied resulted in a concentration of more than 2,500 unities visco (UV) per kg of silage of endo-1,4-β-xylanase, and 1,720 UV of endo-1,3(4)-ß-glucanase. One UV of xylanase or ß-glucanase is defined as the quantity of enzyme required to hydrolyze a standard substrate (wheat arabinoxylan or barley ß-glucan, respectively), reducing the solution viscosity in one unity per minute, at 30 ºC and pH 5.5. The ß-glucanase to xylanase ratio was 0.688:1. Also, a control silo (without Rovabio) was made for both HMC and SNAP. Only the WPCS received no treatment at all. The HMC was harvested with 68% of dry matter (DM), using a combine harvester, and transported to the research center. It was processed right before ensiling, using the roller mill mounted on the bagger machine. The rolls were adjusted to crack grains into 4-5 parts. The SNAP was harvested when it reached 67% of DM, using a self-propelled forage harvester combined with a snapper head. The theoretical length of cut of snaplage was 14 mm with the grain processing at a 1mm roll clearance. Whole-plant corn silage was harvested with 42% of DM, using a self-propelled forage harvester, with a theoretical length of cut of 16 mm at a 1mm roll clearance.

The environment conditions for the ensiling period were: average temperature of 25.5º C (minimum of 18º C and maximum of 33º C) and average rainfall precipitation of 12.5 mm.

2.2.2. Animals and housing

The feeding trial started on August 18th and ended on December 21st of 2017 (122 days). Four hundred and sixty-eight Nellore bulls, 30 months old, were allocated to 16 feedlot pens. The animals were weighed individually, using a hydraulic squeeze chute equipped with scale (Beckhauser Manejo Racional e Produtivo, Paranavaí, PR, Brazil) at the arrival to determine the shrunk body weight, which the mean ± SD was 405 ± 2 kg. Furthermore, at the arrival, the bulls were dewormed and vaccinate against botulism, foot-and-mouth, and carbuncle diseases. Each feedlot pen had 420 m², and it was uncovered, possessing soil floor,

and a feed bunk length of 12 m (approximately 40 cm per animal). During the trial the animals had free access to the water trough.

2.2.3. Diets

2.2.3.1. Adaptation Diets

Initially, all the bulls were adapted to the feedlot for 6 days with a receiving diet, and then to step-up adaptation diets for 21 days, prior to starting the trial. During the adaptation, the diets were altered to decrease the forage:concentrate ratio (Table 1.) In the first week of the adaptation period, the animals received Diet 1, in the second week, Diet 2, and in the last week, they were fed Diet 3. In this phase, control silos and the silos inoculated with EFE were mixed half-to-half, to compose the ingredient snaplage and high moisture corn of the diets.

Table 1. Composition of the adaptation diets (dry matter basis) in the first 21 days

Ingredient, %	Diet 1 (7d)	Diet 2 (7d)	Diet 3 (7d)
Whole-Plant Corn Silage	47.00	37.00	27.00
Snaplage (½ Control and ½ EFE)	32.00	32.00	32.00
High Moisture Corn (½ Control and ½ EFE)	-	10.00	20.00
Soybean Hulls	9.10	9.10	9.10
Soybean Meal	8.30	8.30	8.30
Mineral and vitamin supplement [1]	3.60	3.60	3.60

[1] Mineral and vitamin supplement composition (DM basis): 150 g/kg Urea, 150 g/kg Ca, 4.5 g/kg P, 37 g/kg S, 10 g/kg Mg, 6 g/kg K, 38 g/kg Na, 350 g/kg Cl, 21 mg/kg Co, 250 mg/kg Cu, 6.7 mg/kg Cr, 19 mg/kg I, 620 mg/kg Mn, 4.5 mg/kg Se, 1,050 mg/kg Zn, 45 mg/kg F, 85,000 IU/kg vitamin A, 350 IU/kg vitamin E, 800 mg/kg Monensin. Manufactured by Nutripura, Rondonópolis, Brazil.

2.2.3.2. Experimental Diets

After 21 days of adaptation, the bulls had their water and feed intake restricted for 16 hours, to determine the shrunk body weight. The average shrunk bodyweight of the animals at the beginning of the experimental phase was 420 ± 4 kg. After that, they were randomized into four blocks, according to their weights (heaviest to lightest). Four feedlot pens composed each block. The number of animals per pen ranged from 28 to 30. To create the experimental

diets, the silages were combined as follows: 1) SNAP Control + HMC Control (snaplage without EFE combined with high moisture corn without EFE); 2) SNAP with EFE + HMC with EFE (snaplage added EFE combined with high moisture corn added EFE); 3) WPCS + HMC Control (whole-plant corn silage without EFE combined with high moisture corn without EFE); 4) WPCS + HMC EFE (whole-plant corn silage without EFE combined with high moisture corn added EFE). Although snaplage is considered an intermediate between high-cut corn silage and high moisture shelled corn (Akins and Shaver, 2014), in the current experiment, it was considered as a source of roughage, instead of a source of starch, because of its higher fiber content, showed in previous chemical analysis. The concentrate of the diets was composed of soybean meal, soybean hulls, urea, mineral and vitamin supplement, which contained monensin, and urea (Table 2). The pens within each block were assigned randomly to one of the four treatments, to compose sixteen pens. The diets were formulated to attend the bulls requirements to gain 1.5 kg per day (NASEM, 2016). Also, diets were formulated to reach the same content of NDF from forage (12.58%) and to be iso-protein (13%).

Table 2. Experimental diets composition (dry matter basis)

Ingredient, %	SNAP + HMC[1]		WPCS[2] + HMC	
	Control	EFE	Control	EFE
Whole-plant Corn Silage	-	-	25	25
Snaplage	27.65	27.65	-	-
High Moisture Corn	51.13	51.13	53.18	53.18
Soybean Hulls	12	12	12	12
Soybean Meal	5.42	5.42	6.02	6.02
Mineral and vitamin supplement[3]	3.80	3.80	3.80	3.80
Nutrients, %				
Dry Matter, %FM	72.18	71.79	67.63	66.91
Ash	5.00	5.04	5.28	5.33
Crude Protein	13.56	12.61	12.99	12.75
Ether Extract	3.60	3.66	3.39	3.47
NDF	23.05	22.79	27.19	26.26
rNDF[4]	9.56	10.19	13.16	13.16
Starch	47.43	47.10	42.73	43.93
NFC	48.59	52.57	45.29	50.89

[1] Dose of EFE/animal – 1.05 g/d;

[2] Dose of EFE/animal – 0.72 g/d; WPCS – not added EFE;

[3] Mineral and vitamin supplement composition (DM basis): 250 g/kg Urea, 150 g/kg Ca, 4.5 g/kg P, 37 g/kg S, 10 g/kg Mg, 6 g/kg K, 38 g/kg Na, 350 g/kg Cl, 21 mg/kg Co, 250 mg/kg Cu, 6.7 mg/kg Cr, 19 mg/kg I, 620 mg/kg Mn, 4.5 mg/kg Se, 1,050 mg/kg Zn, 45 mg/kg F, 85,000 IU/kg vitamin A, 350 IU/kg vitamin E, 800 mg/kg Monensin . Manufactured by Nutripura, Rondonópolis, Brazil.

[4] NDF from roughage.

2.2.4. Feeding and Animal Performance

During the experiment, the animals were fed twice daily, 0800 and 1500. The feed was delivered to the animals using a pull-type wagon (model 3120, Kuhn, Saverne, France), equipped with horizontal auger and a digital scale (± 2 kg). First, the concentrate was pre-mixed in the wagon (1 minute), and after that, the silages were added and mixed for 5 more minutes. To monitor the feed intake daily, it was used a feed bunk score. Every morning, the

target was score 1, which meant, at least 300 grams of DM per animal as orts, per day (3% of orts). Once per week, samples of the silages and all the ingredients were collected and dried overnight at 105º C to correct the DM of the diets offered to the animals. Until stabilize the feed intake, the feed bunk was monitored at night to ensure enough feed till the next day. The orts were removed from the feed bunk, whenever it was needed (old feed or soaked by rain). After removal, the orts were weighed and sampled for DM, and then discounted from the quantity offered to that feedlot pen on the day before. After the last day of the experiment, the orts were weighed e sampled for DM, to calculate the feed intake of the period.

At the end of the experiment (day 95) another weighing occurred. The animals of blocks 1, 2, 3 and 4 were weighed shrunk on December 18th, 19th, 20th, and 21st, respectively. The last weighing was used to calculate the average daily gain (ADG) of the entire trial. The TDN, NEm, and Neg of the diets were calculated using mean values of the observed shrunk BW, DMI, and ADG of the bulls in each pen (Zinn and Shen, 1998). Those same energy variables were estimated for the HMC and SNAP silages, with or without the EFE. This estimation was proceeded by subtracting the observed energy of the diets (as mentioned above) from the energy of the diets without the energetic contribution of the HMC and SNAP. For this, the TDN value of each ingredient was estimated using the equation of Weiss et al. (1992).

From December 18th to 21st, during weighing and before slaughtering, the carcass quality of the animals was evaluated by using ultrasound scanning technology, approved by the Ultrasound Guidelines Councill (Pleasantville, IA, USA). The measurements in the animal were made using the Aloka 500 ultrasound scanner (Aloka Co, Tokyo, Japan). The post-analysis of the data was integrated using the software BIA (Design Genes Technologies Brasil, Presidente Prudente, SP, Brazil). The Nellore bulls had their ribeye area, fat thickness and marbling measured using this technology. For the ribeye area and back fat thickness measurements, the ultrasound probe was placed between the 12th and 13th ribs, above the longissimus dorsi muscle. Regarding the marbling, the probe was placed between the 11th and 13th ribs. Before each measurement, the animal was contained properly, and soy oil was applied at the skin surface to better conduct the ultrasound waves. Posteriorly, the bulls were shipped to a near commercial slaughterhouse (JBS S.A., Pedra Preta, MT, Brazil) and the hot carcass weight (HCW) was determined by summing up the two carcass halves.

2.2.5. Feces Collection

During the first half of the experimental period, from day 57 to 59, and from day 101 to 103 of the second half, the feces of the bulls were collected, in order to estimate the concentration of starch in feces. Ten fresh fecal spots were sampled directly from the ground, avoiding dirt, of different animals to compose one sample per pen per day (Sartec 2018). The collection time in the three consecutive days followed the sequence: d1 – 0700; d2 – 1200; d3 1800. Samples from the three days were composite, resulting in one sample per pen per period (first half or second half). Right after the collection, the samples were dried to a constant weight at 60 ºC in an air-forced oven. The samples were ground until pass 1-mm screen of a Wiley mill (Arthur H. Thomas, Philadelphia, PA).

2.2.6. Chemical and Physical Analysis

During the experimental period, once per week samples of silages and ingredients were collected and subsequently frozen. The collected samples were dried in a forced-air oven for 72 h at 55ºC and ground to pass through a 1 mm mesh screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Subsamples were analyzed for DM, ether extract (EE), and ash according to the Association of Official Analytical Chemistry (AOAC) (1990; methods 934.01, 920.39 and 924.05, respectively). The NDF was analyzed according to Mertens (2002), using amylase and sodium sulfite for the silages. The nitrogen was analyzed by the Dumas method 990.03 (AOAC, 2006), using a nitrogen analyzer (FP-2000A, Leco Corp., St. Joseph, MI, USA). Crude protein was obtained by using the factor 6.25 × N. The soluble protein was estimated from the difference between the total nitrogen of the samples and the insoluble nitrogen after the application of the borate-phosphate buffer solution (Krishnamoorthy et al., 1982). The NFC was calculated according to Hall (2000) calculated as NFC = 100 – (CP + EE + ash + NDF). For the silages, soluble carbohydrates were extracted using an 80% ethanol solution (Hall, 2000) and the starch content was analyzed using the enzymatic hydrolysis method, according to Hall (2009).

High moisture corn, snaplage and whole-plant corn silage subsamples were weighed, 25g, and added 225 g of deionized water and mixed for 4 min at 152 rpm using an automatic agitator. The extract was filtered with 3 layers of cheesecloth and the pH was measured (DM 20 pH meter, Digimed Analítica, SP, Brazil). Then, it was centrifuged at 10,000 × g for 15

min at 4ºC, and the lactic acid (Price, 1969) and the concentrations of VFA were quantified. The concentrations of VFA, alcohols, and esters were analyzed using a gas chromatographer with a mass detector (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, PA, USA; 60 m, 0.25 mm, i.d., 0.25 m). The DM content was corrected for the volatile compounds according to the equation proposed by Weissbach (2009).

In order to separate the grain fraction from the stover in SNAP and WPCS, a hydrodynamic separation method was applied (Savoie et al. 2004). The grains were weighed (250g), dried at 60 ºC in an air-forced oven for 72h, and then analyzed for particle size distribution using a Ro-Tap Shaker (Bertel Ltda., Caieiras, SP, Brazil) equipped with 9 sieves with nominal square apertures of 9.50, 6.70, 4.75, 3.35, 2.36, 1.70, 1.18, and 0.59-mm, the bottom pan.

Sorting behavior was measured once per week according to the method of Leornardi and Armentano (2003). The roughage silages (SNAP and WPCS), as well the diets and the orts had the particle size distribution measured using 3 sieves (19, 8, 4 mm, and the bottom pan), of the Penn State Particle Size separator, according to the procedure described by Maulfair et al. (2011). The physically effective factor (pef>4) was obtained through the sum of the fraction retained on the sieves with the aperture 19, 8 and 4 mm.

2.2.7. Silage Digestibility

The subsamples of HMC, SNAP, and WPCS silages were submitted to *in situ* and *in vitro* digestibility techniques. For the in situ, 15 g of the samples were placed into 10×20 cm woven bags (R1020 Forage Bag, ANKOM Technology, Macedon, NY, USA). According to the producer, the porosity of the bag was 50 ± 10 micron. The ratio of a sample size to free bag surface area was 37.5 mg/cm². Each bag was tied 1 cm below the top with rubber bands and clips were used to attach it to a chain. To permit an adequate degradation, the chain with the bags were placed in the ventral sac of the rumen for 6 and 12 h for HMC, and 24 and 48h for both SNAP and WPCS. The bags were removed simultaneously. Two cannulated dry cows (Holstein) fed 55% (DM) of corn silage and 45% (DM) concentrate, were used to incubate the silages. Each cow received a replicate of the samples and blank bags were added to correct the weight of the bag tare weight. After the removal, all the bags were washed 5 times using a washing machine to remove adherent feed particles and bacteria. The bags were

dried for 48h at 60ºC and then weighted to calculate de DM digestion. The residues of the bags were analyzed for NDF and starch content, as described previously, to estimate the respective digestibilities.

The in vitro true digestibility (IVTD) of the silages was performed using F57 Filter Bags and the DAISY II Incubator (ANKOM Technology, Macedon, NY, USA). The rumen inoculum was collected from a fistulated Nellore bull, fed a diet composed of 10% (DM) of sugarcane bagasse and 90% (DM) of concentrate. The bags were incubated for 48h. To remove microbial debris and any remaining soluble fractions, the bags were placed in TE-149 Fiber Analyzer (Tecnal Equipamentos, Piracicaba, Brazil) with neutral detergent solution.

2.2.8. Statistical analysis

The statistical design for the animal performance was the randomized complete blocks, with factorial treatment structure, two diets (snaplage + high moisture corn or whole-plant corn silage + high moisture corn) and two treatments (control or EFE). The data were analyzed using the PROC MIXED procedure of SAS, with random effects for block, as follows the model structure: $Yijk = \mu + Bi + Dj + Tk + DTjk + eijk$; where: μ = overall mean; Bi = random effect of blocks (i =1,2,3,4); Dj = fixed effect of diet (j = SNAP, WPCS); Tk = fixed effect of EFE (k = Control, EFE); DTjk = interaction between diet and EFE; eijk = residual error. The experimental unit considered was the pen for all performance variables.

The completely randomized design was applied to the chemical, physical, and digestibility analyses. The days of the collection were used as repeated measures within the model. The model used was: $Yij = \mu + Ti + Dj + TDij + eij$; in which: μ = overall mean; Ti = fixed effect of EFE i; Dj = fixed effect of day of collection j (repeated measure) ; DTij = interaction between EFE i and day of collection j; eij = residual error. The best covariance structure for the repeated measure was defined by the smallest value for Akaike's information criterion among autoregressive, compound symmetry or unstructured. For the in situ digestibility, cows were added to the model as blocks (random effect). Means were considered statistically significant when $P \leq 0.05$ and tendency when $P > 0.05 \leq 0.10$.

2.3. RESULTS

Tables 3 and 4 present the results of the chemical composition, digestibility and physical characteristics of HMC and SNAP, respectively. Comparing the treatments (Control and EFE) inside HMC, the application of the EFE, reduced (P = 0.02) the NDF content in 22.55%. The other chemicals nutrients (starch, CP, soluble protein, EE and ash) were not affected by the enzyme (P > 0.05). Regarding the in vitro and in situ digestibility, the EFE affected only the DM digestion. In the in vitro analysis, the treatment with the enzyme added during the ensiling, improved (P = 0.05) the true digestibility of DM in 1.26% in the HMC. Furthermore, there was a trend (P = 0.10) in favor to the EFE, to enhance the in situ DM digestibility, incubated for 6h, in 2.23%, but not for 12h of rumen incubation. On the contrary, there was no statistical difference for the NDF and starch digestibility, just some numerical differences. A measurement to evaluate how processed was the corn kernel of the silages was described as the percentage of grains below 4.75 mm (Dias Junior et al. 2016). There was no statistical difference for this variable, between the control and EFE treatment. In Table 4, are described the characteristics related to SNAP silages. The EFE did not affect the chemical composition of this silage. However, the in situ NDF digestibility (24h), showed a tendency (P = 0.08), in which the enzyme treatment reduced it in 23.06%. For the SNAP in Table 4, are presented the results of the particle size distribution of the silages, using the Penn State Particle Separator methodology (PSPS). The SNAP silage inoculated with EFE, trended (P = 0.10) to show fewer particles retained above the sieve of 19 mm of aperture. The retention in all the other sieves was not different (P > 0.05). The mean particle length (MPL) of the silages were not affected. Also, the percentage of grains below 4.75 mm did not differ for the EFE treatment.

Table 3. Chemical composition of the high moisture corn silages

Nutrient (DM basis)	Control	EFE[1]	SEM	P-value
Starch, %	69.4	71.4	1.72	0.43
NDF, %	7.36	5.70	0.317	0.02
CP, %	7.88	7.38	0.676	0.61
Soluble Protein CP, % CP	78.6	80.4	3.63	0.73
EE, %	4.83	4.94	0.070	0.27
Ash, %	1.28	1.17	0.095	0.43
Digestibility *in vitro* and *in situ* (DM basis)				
IVTD[2], %	95.6	96.8	0.37	0.05
DMD 6h, %	91.7	93.8	0.80	0.10
DMD 12h, %	94.2	94.7	0.52	0.39
NDFD 6h, %	13.4	8.89	2.95	0.31
NDFD 12h, %	22.9	18.7	2.17	0.17
StarchD 6h, %	95.1	96.4	1.83	0.62
StarchD 12h, %	97.2	97.3	0.46	0.78
Grain processing				
Grains <4.75mm, %	77.2	84.1	3.39	0.18

[1] EFE – Exogenous fibrolytic enzyme (Rovabio Advance P, Adisseo, Antony, France).

[2] *In vitro* true digestibility of DM.

Table 4. Chemical composition of the snaplage silages

Nutrient (DM basis)	Control	EFE[1]	SEM	P-value
Starch, %	41.6	39.7	1.87	0.50
NDF, %	34.6	36.9	3.06	0.61
CP, %	7.27	7.26	0.296	0.98
Soluble Protein CP, %CP	48.3	44.7	7.87	0.75
EE, %	3.43	3.40	0.073	0.74
Ash, %	2.12	2.42	0.169	0.23
Digestibility *in vitro* and *in situ* (DM basis)				
IVTD[2], %	79.8	77.4	1.67	0.34
DMD, 24h, %	75.3	68.4	5.22	0.36
DMD, 48h, %	86.6	83.8	1.87	0.31
NDFD, 24h, %	37.1	28.5	3.23	0.08
NDFD, 48h, %	62.0	60.9	1.33	0.55
StarchD, 24h, %	82.8	77.1	6.59	0.55
StarchD, 48h, %	92.0	88.8	1.64	0.19
PSPS[3]				
19 mm, %FM retained	5.00	4.75	0.090	0.10
8 mm, %FM retained	27.6	26.7	1.25	0.62
4 mm, %FM retained	41.2	43.4	1.45	0.30
Botton pan, %FM retained	26.5	25.5	2.70	0.80
MPL[4], mm	5.78	5.80	0.237	0.97
pef >4mm[5], %	74.6	73.5	2.74	0.78
Grain processing, %				
Grains <4.75mm, %	77.1	76.3	1.62	0.75

[1] EFE – Exogenous fibrolytic enzyme (Rovabio Advance P, Adisseo, Antony, France).

[2] *In vitro* true digestibility of DM.

[3] PSPS – Penn state particle separator.

[4] MPL - Mean particle length.

[5] pef - Physically effective factor.

In Table 5, are shown the data regarding the particle stratification of the experimental diets. The diets were considered as four different treatments, thus, possessing no individual effects among them. The percentages of fresh matter retained above 19-mm and 8-mm sieves (PSPS) were different ($P < 0.05$) between the diets. The diets composed by WPCS+HMC had more particles retained above 19 mm, about 94%, than the diets with SNAP+HMC. The same

response occurred for the percentage of particles retained above 8 mm. Regardless of the EFE inoculation in silages, WPCS+HMC retained more particles (66% more) above 8 mm. The diet SNAP+HMC Control presented more particles retained on the 4-mm sieve than the other diets (P = 0.01). The mean of particles smaller than 4 mm (bottom pan retention) was greater for the SNAP+HMC EFE treatment (P = 0.01). The WPCS+HMC Control diet had on average 62.94% of the particles retained above 4 mm (pef>4mm; P = 0.01), the mean particle size of this diet also was greater (5.26 mm).

Table 5. Physical characteristics of the experimental diets, using the Penn State Particle Separator

	SNAP + HMC[1]		WPCS + HMC[1]			
Item	Control	EFE	Control	EFE	SEM	P-value
19 mm, %FM retained	2.50b	2.73b	5.07a	5.07a	0.545	0.01
8 mm, %FM retained	17.3b	16.3b	29.4a	26.2a	1.46	<0.0001
4 mm, %FM retained	34.5a	31.5ab	28.8ab	26.7b	1.45	0.01
Botton pan, %FM retained	46.1b	49.3ab	37.1b	41.8b	1.79	0.01
MPL[2], mm	4.26b	4.03b	5.26a	4.88ab	0.143	0.01
pef >4mm[3], %	53.9b	50.7b	62.9a	58.1ab	1.79	0.01

[1] SNAP - Snaplage; HMC – High moisture corn; WPCS – Whole-plant corn silage; EFE – Exogenous fibrolytic enzyme (Rovabio Advance P, Adisseo, Antony, France).

[2] MPL - Mean particle length.

[3] pef - Physically effective factor.

The sorting index of the bulls is shown in Table 6. For large particles, above 19 mm, and small particles, below 4 mm, there was no intake preference in favor or against these particles by the bulls fed the experimental diets. However, for mean particles (below 19 mm and above 4 mm), there was an interaction between the diet and enzyme effects (P = 0.02). The animals, which received the diet SNAP+HMC EFE, preferred to consume median particles (101.70), more than the other treatments.

Table 6. Sorting index and of the bulls fed experimental diets

	SNAP + HMC[1]		WPCS + HMC[1]			P-value		
Item	Control	EFE	Control	EFE	SEM	Diet	Enzyme	D×E[2]
> 19mm	100.5	100.6	100.7	100.3	0.20	0.67	0.49	0.32
19 - 4mm	100.8b	101.7a	100.8bc	100.4c	0.24	0.01	0.26	0.02
<4mm	98.3	98.2	98.5	99.4	0.44	0.12	0.33	0.27

[1] SNAP - Snaplage; HMC – High moisture corn; WPCS – Whole-plant corn silage; EFE – Exogenous fibrolytic enzyme (Rovabio Advance P, Adisseo, Antony, France).

[2] D×E – Interaction diet × enzyme.

The characteristics related to the silage fermentation are presented in Table 7 for the HMC, and in Table 8 for the SNAP silages. According to Table 7, the DM of the HMC silages, with EFE or not, corrected for the volatile compounds of the silages were not statistically different (P = 0.50). The absence of difference in the DM between control and EFE silages resulted in values not different (P>0.10) for the mains variables related to fermentation (i.e. pH, soluble carbohydrates and lactic acid). The ester ethyl acetate was increased by 57.14%, when the EFE was applied to the HMC silages (P = 0.0397). Despite the non-statistical significance (P = 0.1360), one of the precursors of the ethyl acetate, the acetic acid, was numerically greater for the enzyme treatment (0.12% vs. 0.07%). The other components were not altered by EFE application. Regarding the fermentation pattern of the snaplage silages presented in Table 8, the DM corrected, pH, soluble carbohydrates, lactic acid, and the majority of the other acids, alcohols, and esters were not different for the treatment SNAP EFE. However, acetic acid showed a trend (P = 0.08) to be affected by the enzyme treatment. Uncommonly, the acid acetic content of the EFE snaplage was smaller than the control (0.11% vs. 0.16%). The chemical and physical characteristics, as well as the digestibility and fermentative profile of the whole-plant corn silage (WPCS), are presented separately in the appendix (Table 11).

Table 7. Fermentative profile of high moisture corn silages

Item	Control	EFE[1]	SEM	P-value
DM corr[2], % AF	68.6	67.7	1.00	0.50
pH	4.37	4.28	0.051	0.33
WSC[3], %	1.06	1.37	0.166	0.27
Lactic acid, %	0.53	0.56	0.043	0.66
Acetic acid , %	0.07	0.12	0.021	0.14
Ethanol, %	0.07	0.07	0.008	0.77
1,2-Propanediol, mg/kg	115.4	631.6	339.41	0.30
1-Propanol, mg/kg	39.7	35.6	15.29	0.85
2,3-Butanediol, mg/kg	7.67	6.50	0.640	0.22
Ethyl lactate, mg/kg	18.2	19.7	2.91	0.72
Propionic acid, mg/kg	18.2	15.1	4.02	0.60
Butyric acid, mg/kg	3.58	2.33	1.256	0.49
Ethyl acetate, mg/kg	1.75	2.75	0.306	0.04

[1] EFE – Exogenous fibrolytic enzyme (Rovabio Advance P, Adisseo, Antony, France).

[2] DM corr – Dry matter corrected for silage volatile compounds, using Weissbach (2009) equation.

[3] WSC – Water soluble carbohydrates.

Table 8. Fermentative profile of snaplage silages

Item	Control	EFE[1]	SEM	P-value
DM corr[2], % AF	67.0	68.6	1.73	0.51
pH	4.40	4.40	0.086	0.99
WSC[3], %	1.52	1.47	0.029	0.36
Lactic acid, %	0.40	0.32	0.082	0.52
Acetic acid , %	0.16	0.11	0.017	0.08
Ethanol, %	0.03	0.02	0.003	0.18
1,2-Propanediol, mg/kg	299.4	152.7	64.50	0.13
1-Propanol, mg/kg	113.7	62.2	27.80	0.22
2,3-Butanediol, mg/kg	17.2	17.5	3.14	0.94
Propionic acid, mg/kg	42.2	33.1	5.81	0.29
Ethyl lactate, mg/kg	7.08	5.75	0.636	0.16
Butyric acid, mg/kg	3.33	2.33	0.576	0.24
Ethyl acetate, mg/kg	1.50	1.33	0.205	0.58

[1] EFE – Exogenous fibrolytic enzyme (Rovabio Advance P, Adisseo, Antony, France).

[2] DM corr – Dry matter corrected for silage volatile compounds, using Weissbach (2009) equation.

[3] WSC – Water soluble carbohydrates.

The outcomes related to the animal performance and carcass characteristics of the total experimental period (27 to 122 days), are shown in Table 9. The initial BW of the animals (day 27) did not differ between treatments and the standard error of the mean was 22.9 kg. Regarding the final body weight (122d), also there was no difference between the diets and enzyme ($P > 0.10$).

Table 9. Performance and carcass characteristics of Nellore bulls fed diets with high moisture corn and snaplage, inoculated with EFE

	SNAP + HMC[2]		WPCS + HMC[2]			P-value		
Item	Control	EFE	Control	EFE	SEM	Diet	Enzyme	D×E[3]
Initial BW, kg	423	420	420	419	22.9	0.45	0.31	0.66
Final BW, kg	571	569	568	570	19.0	0.87	0.97	0.63
ADG, kg	1.48	1.52	1.52	1.54	0.045	0.53	0.53	0.92
DMI, kg/day	9.51	9.10	9.58	9.23	0.305	0.35	0.01	0.77
Feed efficiency	0.1568	0.1678	0.1593	0.1670	0.0088	0.83	0.04	0.70
Hot carcass weight, kg	319	320	320	318	13.1	0.91	0.91	0.62
Carcass yield, %	55.8	56.1	56.2	55.8	0.61	0.89	0.82	0.34
Ribeye area, cm²	82.8	84.3	84.4	84.1	2.60	0.55	0.61	0.48
Ribeye area ratio[1]	0.488	0.489	0.491	0.490	0.0031	0.38	0.93	0.85
Marbling, score	3.48	3.48	3.40	3.46	0.072	0.51	0.65	0.70
Back fat thickness, mm	4.70	4.71	4.86	4.79	0.213	0.22	0.76	0.66

[1] Ribeye area ratio - ribeye area height × width.

[2] SNAP - Snaplage; HMC – High moisture corn; WPCS – Whole-plant corn silage; EFE – Exogenous fibrolytic enzyme (Rovabio Advance P, Adisseo, Antony, France).

[3] D×E – Interaction diet × enzyme.

The differences in Table 9, were noticed for the feeding-related characteristics (DMI and feed efficiency) for the trial period. The DMI of the bulls was affected by the EFE only. Comparing the diets, there was no difference (P = 0.35) between SNAP and WPCS for DMI. The EFE application reduced (P = 0.01) the DMI in 3.98% but did not affect the ADG (P = 0.53). As a result, the feed efficiency was higher (P = 0.04) in 5.91% for diets with EFE. The SNAP and WPCS diets did not alter the ADG (P = 0.53) and, consequently, the feed efficiency (P =0.83) of the bulls.

The data related to carcass evaluation such as: hot carcass weight, carcass yield, ribeye area, ribeye area ratio, marbling, and fat thickness, were not affected by the treatments (P > 0.10).

The data regarding the energy fractioning of the diets are presented in Table 10. The bulls, which were fed the diet with SNAP silage, consumed more starch (265 g) than diets composed with WPCS (P = 0.01). Numerically (P = 0.11), the feces of the animals fed SNAP diets had 13.82% more starch content than diets with WPCS. The addition of EFE in the silages tended to reduce (P = 0.06) the starch intake in 95 g, and reduced (P= 0.05) the starch content of the feces in 15.17%. Regarding the TDN of the experimental diets, the diet factor

had no effect, but the enzyme increased it (P=0.01) by 3.54%. Likewise, the EFE addition in the silages, increased (P=0.01) the NEm in 4.36% and NEg in 5.77% of the diets (P=0.01).

Table 10. Fecal starch and observed energy of the experimental diets

	Snap + HMC[1]		WPCS + HMC[1]			P-value		
Item	Control	EFE	Control	EFE	SEM	Diet	Enzyme	D×E[2]
Starch intake, kg	4.44	4.29	4.12	4.08	0.14	0.01	0.06	0.24
Fecal Starch, %	5.47	4.91	5.08	4.04	0.37	0.11	0.05	0.53
TDN, %[3]	80.5	83.5	80.6	83.3	1.08	0.94	0.01	0.83
NEm, Mcal/kg[3]	1.95	2.04	1.95	2.03	0.03	1.00	0.01	0.87
NEg, Mcal/kg[3]	1.30	1.38	1.30	1.37	0.03	0.96	0.01	0.81

[1] SNAP - Snaplage; HMC – High moisture corn; WPCS – Whole-plant corn silage; EFE – Exogenous fibrolytic enzyme (Rovabio Advance P, Adisseo, Antony, France).

[2] D×E – Interaction diet × enzyme.

[3] Estimated using the equations of Zinn and Shen (1998).

2.4. DISCUSSION

The presented results are prone to partially refute the null hypothesis, accepting that the EFE can affect the animal performance by reducing the NDF content, only of the high moisture corn silage, improving the digestibility of dry matter, thus reducing the dry matter intake, which leads to an improvement in feed efficiency.

Feeding diets with SNAP and WPCS, which resulted in different contents of NDF from roughage (rNDF) did not affect the animal performance. The possible reason for this to occur was the different NDF digestibility content between SNAP and WPCS. Both WPCS diets had 13.16% (DM basis) of rNDF, in contrast of 9.56% of SNAP control and 10.19% of SNAP EFE diets. According to Caetano et al. (2015), approximately 13% of NDF from roughage (rNDF) from sugarcane silage, was necessary to result in optimal values of DMI, which enhanced ADG, HCW, and final BW, when Nellore bulls fed HMC compared with finely ground corn. However, in the current study, the absence of result in the animal performance, may be attributed to the lower digestibility of the NDF (less 23.85% in 24h-NDF digestibility), for the SNAP silages than the WPCS. These reduction in SNAP silages is related to greater amount of less digestible components, such as cob and husks (Klopfenstein et al., 1987; Petzel et al. 2019). Although the mean particle size and physically effective

factor of the WPCS was higher than the SNAP, the lower digestibility of the SNAP silages may resulted in similar chewing activity (Corrêa et al. 2003; Sá Neto et al. 2014), though it was not measured. The Nellore bulls can sort particles in favor of roughage, especially when the diets have higher contents of concentrates (Caetano et al., 2015). This may explain why the animals fed SNAP+HMC EFE could select in favor of particles between 19 and 4mm, but it did not reflect in the performance. Regarding the combination between SNAP plus HMC or WPCS plus HMC, there was no evidence of associative effect between the less processed and immature grains of WPCS in combination with HMC grains in the diets (Stock and Erickson, 2006).

About the EFE application to ensiling, this treatment indeed altered the performance of the animals. The bulls fed EFE had feed intake decreased, the same ADG, thus, greater feed efficiency than those fed control diets. This could be caused by the reduction in the "cage effect", given by the cell wall in the endosperm (Evers and Millar, 2002; Le et al. 2013), where the nutrients, such as starch, could be more available for fermentation in the silo or in the rumen. It seems, once the prolamins of the endosperm are hydrolyzed by the plant and bacterial enzymes (Junges et al., 2017), which results in soluble protein content to increase, the next barrier of starch digestion is the cell wall of the endosperm. However, Zahiroddini et al. (2004), found that EFE in combination with LAB inoculant applied in barley silage at ensiling, increased the ADG and the feed efficiency of steers, by hydrolyzing the fiber content, not just of the grain, but of the whole silage.

When applied in the HMC silages, the EFE reduced the NDF content, increasing the IVTD and in situ DM digestibility at 6h of incubation. Eun et al. (2007) stated that the ratio between endoglucanase and xylanase is essential to improve the digestibility, and it should be more than 0.4:1. The ratio provided by the EFE in the current study was 0.69:1. According to Romero et al. (2016), the treatment, which provided the high xylanolytic capacity, increased the DMI in early-lactation dairy cows, when applied directly onto TMR, composed by 35% of corn silage and 10% of bermudagrass silage (DM basis). However, the EFE application did not improve nutrients digestibility in the same study. The increase in the digestibility was associated with the availability of more soluble carbohydrates generated by the hydrolysis of the arabinoxylan chains.

Another product, which indicate the fiber fermentation in HMC was the acetic acid and its derivative ester, ethyl acetate, which increased for the EFE treatment. According to the study of Fred et al. (1919) and Dehghani et al. (2012), the increase in acetic acid could be associated with the fermentation of xylans in the silo. Using doses of xylanase in sugarcane

silage, Del Valle et al. (2019), also found greater acetic acid content in intermediate levels of the fibrolytic enzyme (185 mg/kg DM). The ethyl acetate is formed during the fermentation of silages by the combination of ethanol and acetic acid, but it is more dependent of the alcohol than the carboxylic acid concentration (Weiss et al. 2016). Since the ethanol concentration remained indistinctly between the EFE and control, the acetic acid concentration was major factor to increase the ethyl acetate in present study.

Although the dry matter digestibility was different statistically, surprisingly the starch and NDF in situ digestibility differences were only numerical in the HMC with fibrolytic enzymes. The increase in the grain starch digestion was expected, because according to Watson (1987), the breakage of corneous endosperm (flint grain hybrids), using dry-milling process, occurs along the cell walls, as a result of the strength of the protein matrix. Thus, the endosperm cell wall would represent a barrier to starch digestion. The NDF fraction of the corn grain is encountered mostly in the pericarp and in the endosperm. The pericarp comprises about 51% of grain fiber, being the starchy endosperm, aleurone endosperm, and germ responsible for 12%, 15%, and 16%, respectively, of the fiber grain content. The hemicellulose responds for 70% of the NDF in the grains (Watson, 1987) and xylose and arabinose account for 90-95% of hemicellulose (Oomiya and Imazoto, 1982). Therefore, the EFE used in the current experiment, having the major activities of xylanase, could hydrolyze the hemicellulose, liberating xylan monomers to be fermented in the silo. However, the resistant NDF would result in a less digestible fraction for the rumen bacteria. The results of the experiments with pigs, which used the similar EFE to present study, showed an increase in the energy of the corn-based or wheat-based diets (Cozannet et al. 2018). In agreement to that, the fecal starch content of the bulls fed EFE was smaller and the TDN, NEm, and NEg of the treated diets were greater than the control. The fecal starch is a valuable tool to estimate the starch digestion in feedlots (Zinn et al. 2002).

On the contrary, these benefits were not observed, when the EFE was applied in the SNAP silages. This occurred probably because of the starch content, about less 44%, in comparison to HMC silages, since the same dose (100 g/ton of FM) was used for both HMC and SNAP silages, and the major effect was expected in the grain fraction. Besides grains, cob, husks, and some of the upper plant material is also presented in snaplage (Nigon et al. 2016). The corn cob composition is 41.2% of cellulose, 36.0% of hemicellulose, where only 30% of it is expressed as xylan, and 6.1% of lignin (Bagby and Widstrom, 1987). The only effect of the EFE on SNAP silages regards the digestibility of NDF after 24 h of incubation, which there was a tendency to reduce it. In agreement, Lynch et al. (2015), applied EFE to

corn silages at ensiling and also presented a reduction of the NDF digestibility at 24h of ruminal incubation. The authors claimed that the EFE acts on compounds that are more susceptible to be hydrolyzed during fermentation, leaving only the resistant fraction of the fiber. Conversely, the starch content presented in the SNAP were much less than expected. Comparing the performance of dairy cows fed diets with high moisture corn against diets with snaplage, Akins and Shaver (2014) presented that the value of starch content for snaplage was about 61%. In the present study, the starch values found were 41.6% and 39.7%, for control and EFE snaplage, respectively. According to Mahana (2008), one of the concerns of snaplage usage is the variation that can occur from one operation to another. The risk to dilute the energy content of snaplage is higher, since fibrous fractions, such as leaves above the ear, are susceptible to be included in the material. Therefore, the addition of higher doses of fibrolytic enzymes may be necessary to show the same benefits, when other sources of fibers (cob and husks) are included in grain silages. Furthermore, the lack of studies using snaplage in animal performance makes it worthwhile to be investigated.

2.5. CONCLUSION

The addition of EFE had effect only in HMC, where it was effective to improve the nutrient utilization by the Nellore bulls, thus enhancing the feed efficiency, by reducing the intake. Moreover, substituting whole-plant corn silages for snaplage in the diets did not affect the characteristics related to animal performance and carcass traits.

LITERATURE CITED

Adesogan, A, T., Z. X. Ma, J. J. Romero, and K. G. Arriola. 2014. Ruminant Nutrition Symposium: Improving cell wall digestion and animal performance with fibrolytic enzymes. J. Anim. Sci. 92:1317-1330. doi:10.2527/jas.2013-7273

Akins, M. S., and R. D. Shaver. 2014. Effect of corn snaplage on lactation performance by dairy cows. The Professional Animal Scientist 30 (Vol.1):86-92. doi:10.15232/S1080-7446(15)30088-7

Almeida Júnior, G. A., C. Costa, S. M. R. Carvalho, P. Persichetti Júnior, and A. Panichi. 2008. Desempenho de bezerros holandeses alimentados após o desaleitamento com silagem de grãos úmidos ou grãos secos de milho ou sorgo. (In Portuguese) Rev. Bras. Zoot, 37(1): 148-156. doi:10.1590/S1516-35982008000100022

AOAC – Association of Official Analytical Chemistry. 1990. Official methods of analyses. 15th ed. AOAC International, Arlington, VA, USA.

AOAC – Association of Official Analytical Chemistry. 2006. Official methods of analysis, 18th ed. [Revised] Association of Official Analytical Chemists. Washington, DC.

Berndt, A., W. Henrique, D. P. D. Lanna, P. R. Leme, and G. F. Alleoni. 2002. Milho úmido, bagaço de cana e silagem de milho em dietas de alto teor de concentrado: 2. composição corporal e taxas de deposição dos tecidos. Rev. Bras. Zoot, 31(n. 5): 2105-2112. doi: 10.1590/S1516-35982002000800026

Caetano, M., R. S. Goulart, S. L. Silva, J. S. Drouillard, P. R. Leme, and D. P. Lanna. 2015. Effect of flint corn processing method and roughage level on finishing performance of Nellore-based cattle. J. Anim. Sci. 93:4023– 4033. doi:10.2527/jas.2015-9051

Carareto, R. 2011. Fontes de nitrogênio, níveis de forragem e métodos de processamento de milho em rações para tourinhos da raça Nelore terminados em confinamento. (In Portuguese). Diss., University of São Paulo, Piracicaba, Brazil.

Corrêa, C. E. S., M. N. Pereira, S. G. Oliveira, and M. H. Ramos. 2003. Performance of Holstein cows fed sugarcane or corn silages of different grain texture. Sci. Agric. 60:621–629.

Costa, C., M. B. Arrigoni, A. C. Silveira, and H. N.Oliveira. 2002. Desempenho de bovinos superprecoces alimentados com silagem de milho ou feno de aveia e grãos de milho ensilados ou secos.(In Portuguese). Acta Sci, 24: 1175-1183. doi:10.4025/actascianimsci.v24i0.2585

Cozannet, P., Lawlor, P. G., Leterme, P., Devillard, E., Geraert, P. A., Rouffineau, F., and Preynat, A. (2018). Reducing BW loss during lactation in sows: a meta-analysis on the use of a nonstarch polysaccharide-hydrolyzing enzyme supplement. Journal of animal science, 96(7), 2777-2788.

Dehghani, M. R., Weisbjerg, M. R., Hvelplund, T., and Kristensen, N. B. (2012). Effect of enzyme addition to forage at ensiling on silage chemical composition and NDF degradation characteristics. Livestock science, 150(1-3), 51-58.

Del Valle, T. A., Antonio, G., Zenatti, T. F., Campana, M., Zilio, E. M. C., Ghizzi, L. G., ... and de Morais, J. P. G. (2018). Effects of xylanase on the fermentation profile and chemical composition of sugarcane silage. The Journal of Agricultural Science, 156(9), 1123-1129.

Dias Junior, G. S., Ferraretto, L. F., Salvati, G. G. S., de Resende, L. C., Hoffman, P. C., Pereira, M. N., and Shaver, R. D. (2016). Relationship between processing score and kernel-fraction particle size in whole-plant corn silage. Journal of dairy science, 99(4), 2719-2729.

Eun, J-S., K. A. Beauchemin, and H. Schulze. 2007. Use of exogenous fibrolytic enzymes to enhance in vitro fermentation of alfalfa hay and corn silage. Journal of Dairy Science 90.3: 1440-1451. doi:10.3168/jds.S0022-0302(07)71629-6

Evers, Tony, and Sam Millar. 2002. Cereal grain structure and development: some implications for quality. Journal of cereal science. 36.3: 261-284. doi:10.1006/jcrs.2002.0435

Fred EB, W.H. Peterson, and A. Davenport 1919. Acid fermentation of xylose. Journal of Biological Chemistry 39, 347–384. FALTA O DOI

Gomes, A. L. M., J. L. Bueno, F. A. Jacovaci, D. C. Bolson, C. C. Jobim, and J. L. P. Daniel. 2018. Effects of processing, moisture and length of storage on the fermentative losses and ruminal degradability of reconstituted corn grain silage. Page 500 in Proc. XVIII International Silage Conference. Bonn, Germany.

Hall, M.B. 2009. Determination of Starch, Including Maltooligosaccharides, in Animal Feeds: Comparison of Methods and a Method Recommended for AOAC Collaborative Study. Journal of Association of Official Analytical Chemists International. 92:42-49.

Henrique, W., J. A. Beltrame Filho, P. R. Leme, D. P. D. Lanna, G. F. Alleoni, J. L. V. Coutinho Filho, and A. A. M. Sampaio. 2007. Avaliação da silagem de grãos de milho úmido com diferentes volumosos para tourinhos em terminação: desempenho e

características de carcaça. (In Portuguese). Rev. Bras. Zoot, 36(1): 183-190. Doi:10.1590/S1516-35982007000100022

Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblentz, M. P. Scott, A. L. Bodnar, R. J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465-2474. doi:10.3168/jds.2010-3562.

Junges, D., G. Morais, M. H. F. Spoto, P. S. Santos, A. T. Adesogan, L. G. Nussio, and J. L. P. Daniel. 2017. Short communication: Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. J. Dairy Sci. 100:9048-9051. https://doi.org/10.3168/jds.2017-12943

Kim, J. C., Simmins, P. H., Mullan, B. P., and Pluske, J. R. (2005). The digestible energy value of wheat for pigs, with special reference to the post-weaned animal. Animal Feed Science and Technology, 122(3-4), 257-287.

Krishnamoorthy, U., Muscato, T. V., Sniffen, C. J., and Van Soest, P. J. (1982). Nitrogen fractions in selected feedstuffs. Journal of Dairy Science, 65(2), 217-225.

Le, D. M., Fojan, P., Azem, E., Pettersson, D., and Pedersen, N. R. 2013. Visualization of the anticaging effect of Ronozyme WX xylanase on wheat substrates. Cereal Chemistry, 90(5), 439-444.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557. doi:10.3168/jds.S0022-0302(03)73634-0

Lynch, J. P., J. Baah, and K. A. Beauchemin. 2015. Conservation, fiber digestibility, and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant. J. Dairy Sci. 98:1214–1224. doi:10.3168/jds.2014-8768

Mahanna, B. 2008. Bottom line: Renewed interest in snaplage displayed. Feedstuffs. 80:50. The Miller Publ. Co., Bloomington, MN.

Maulfair, D.D., M. Fustini, and A.J. Heinrichs. 2011. Effect of varying total mixed ration particle size on rumen digesta and fecal particle size and digestibility in lactating dairy cows J. Dairy Sci. 94:3527-3536. doi:10.3168/jds.2010-3718

Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85:1217-1240.

Millen, D. D., R. D. L. Pacheco, M. D. B. Arrigoni, M. L. Galyean, and J. T. Vasconcelos. 2009. A snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. J. Anim. Sci. 87:3427–3439. doi:10.2527/jas.2009-1880

National Academies of Sciences, Engineering, and Medicine, NASCEM, 2016. Nutrient requirements of beef cattle: Eight Revised Edition. Washington, DC: The National Academies Press. doi.org/10.17226/19014.

Oliveira, C. A., and D. D. Millen. 2014. Survey of nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. Anim. Feed Sci. Technol. 197: 64–75. doi:10.1016/j.anifeedsci.2014.08.010

Owens, F. N., D. S. W. Secrist, J. Hill, and D. R. Gill. 1997. The effect of grain source and grain processing on performance of feedlot cattle: a review. J. Anim. Sci. 75:868-879.

Phakachoed, N., W. Suksombat, D. Colombatto, and K. A. Beauchemin. 2013. Use of fibrolytic enzymes additives to enhance in vitro ruminal fermentation of corn silage. Livestock science, 157(1):100-112.

Pinto, A. C. J., and D. D. Millen. 2016. Situação atual da engorda de bovinos em confinamento e modelos nutricionais em uso. Proc. X Simpósio de Produção de Gado de Corte (Simcorte). (In Portuguese). Federal University of Viçosa, Viçosa- MG, Brazil. 103-120.

Pryce, J. D. 1969. A modification of Barker-Summerson method for determination of lactic acid. Analyst 94:1151-1152.

Putrino, S. M. 2006. Composição corporal, exigências de energia e proteína para ganho e composição da carne de novilhos Nelore alimentados com dietas com milho grão seco ou úmido contendo gordura não degradável no rúmen. (In Portuguese). Diss. University of São Paulo, Pirassununga, Brazil.

Rémond, D., J. I. Cabrera-Estrada, M. Champion, B. Chauveau, R. Coudure, and C. Poncet. 2004. Effect of corn particle size on site and extent of starch digestion in lactating dairy cows. J. Dairy Sci. 87:1389–1399. doi:10.3168/jds.S0022-0302(04)73288-9

Romero, J. J., M. A. Zarate, and A. T. Adesogan. 2015. Effect of the dose of exogenous fibrolytic enzyme preparations on preingestive fiber hydrolysis, ruminal fermentation, and in vitro digestibility of bermudagrass haylage. Journal of dairy science 98.1: 406-417. doi:10.3168/jds.2014-8285

Romero, J. J., Macias, E. G., Ma, Z. X., Martins, R. M., Staples, C. R., Beauchemin, K. A., and Adesogan, A. T. (2016). Improving the performance of dairy cattle with a xylanase-rich exogenous enzyme preparation. Journal of dairy science, 99(5), 3486-3496.

Sá Neto, A., Bispo, A. W., Junges, D., Bercht, A. K., Zopollatto, M., Daniel, J. L. P., and Nussio, L.G. 2014. Exchanging physically effective neutral detergent fiber does not affect chewing activity and performance of late-lactation dairy cows fed corn and sugarcane silages. J. Dairy Sci. 97:7012-7020. doi: 10.3168/jds.2013-7856.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Loest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey. J. Anim. Sci. 94:2648–2663. doi:10.2527/jas.2016-0282

Sartec. 2007. Fecal starch protocol. Processing parameters determine the efficiency of starch (grain) utilization by cattle on a finishing ration. Accessed Jul. 21, 2017. https://www.sartec.com/wp-content/uploads/2018/08/fecal-starch-procedure.pdf.

Savoie, P., K. J. Shinners, and B. N. Binversie. 2004. Hydrodynamic separation of grain and stover components in corn silage. Appl. Biochem. Biotechnol. 113-116:41-54. doi:10.1007/978-1-59259-837-3_5

Silva, M. R. H. 2015. Processamento e ensilagem no valor nutritivo de grãos de milho para novilhos em confinamento. (In Portuguese). Diss. State University of Maringa, Maringa, Brazil.

Silva, N. C. 2016. Características das silagens de grãos de milho influenciadas pela reidratação e pela inoculação com L. buchneri sobre o desempenho de bovinos de corte confinados. (In Portuguese). Diss. State University Paulista “Júlio De Mesquita Filho”, Jaboticabal, Brasil.

Silva, S. L., P. R. Leme, S. M. Putrino, A. C. Valinote, J. C. M. Nogueira Filho, and D. P. D. Lanna. 2007. Milho grão seco ou úmido com sais de cálcio de ácidos graxos para novilhos Nelore em confinamento. (In portuguese.) R. Bras. Zootec. 36:1426–1434. doi:10.1590/S1516-35982007000600028.

Stock, R. A. and G. E. Erickson. 2006. Associative effects and management-combinations of processed grains. In: Cattle Grain Processing Symposium. 2006. Proceedings… Tulsa: Oklahoma State University.

Tres, T. T. 2015. Inclusão de okara na qualidade da silagem de grãos de milho reidratados e na alimentação de ruminantes.(In Portuguese). Diss. State University of Maringa, Maringa, Brazil.

Weiss, K., B. Kroschewski, and H. Auerbach. 2016. Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. J. Dairy Sci. 99:8053–8069. doi:10.3168/jds.2015-10323

Weiss, W. P., H. R. Conrad, and N. R. St Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed Sci. Technol. 39:95–110. doi:10.1016/0377-8401(92)90034-4

Weissbach, F. 2009. Correction of dry matter content of silages used as substrate for biogas production. Pages 483-484 in Proceedings of the 15th International Silage Conference, US Dairy Forage Research Center, Madison, WI.

Yang, W. Z., and K. A. Beauchemin. 2006. Increasing the physically effective fiber content of dairy cows diets may lower efficiency of feed use. J. Dairy Sci. 89:2694-2704. doi:10.3168/jds.S0022-0302(06)72345-1

Zahiroddini, H., Baaha, J., Absalom, W., and McAllister,T. A. 2004. Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. Anim. Feed Sci. Technol. 117:317–330. doi:10.1016/j.anifeedsci.2004.08.013

Zinn, R. A., and Y. Shen. 1998. An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feedlot calves. J. Anim. Sci. 76:1280–1289. doi:10.2527/1998.7651280x

Zinn, R. A., F. N. Owens, and R. A. Ware. 2002. Flaking corn: processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. J. Anim. Sci. 80:1145–1156. doi:10.2527/2002.8051145x

APPENDIX 1: WHOLE-PLANT CORN SILAGES CHARACTERISTICS

The characteristics of whole-plant corn silage regarding the chemical composition, nutrient digestibility, fermentative profile, particle size distribution on PSPS and grain processing are presented in Table 11.

Table 11. Data of the whole-plant corn silage (WPCS) mean ± SD

Nutrient (DM basis)	Mean	SD
Starch, %	24.7	4.34
NDF, %	46.6	1.70
CP, %	6.75	0.56
EE, %	2.51	0.28
Ash, %	3.20	0.33
Soluble protein CP, % of CP	48.8	11.55
Digestibility *in vitro* and *in situ* (DM basis)		
IVTD[1], %	70.7	2.82
DMD, 24h, %	70.2	1.23
DMD, 48h, %	81.4	1.35
NDFD, 24h, %	43.2	1.42
NDFD, 48h, %	64.8	2.21
StarchD, 24h, %	77.8	2.12
StarchD, 48h, %	86.5	1.18
Fermentative profile (DM basis)		
DM corr[2], % AF	48.1	6.04
pH	4.28	0.12
WSC[3], %	1.86	0.19
Lactic Acid, %	0.77	0.29
Acetic acid , %	0.26	0.08
Ethanol, %	0.06	0.02
1-Propanol, mg/kg	135.9	145.86
2,3-Butanediol, mg/kg	23.4	13.19
Propionic acid, mg/kg	47.6	30.88
Ethyl lactate, mg/kg	13.4	6.76
Butyric acid, mg/kg	4.85	5.18
Ethyl acetate, mg/kg	3.00	2.17

Grain processing		
Grains <4.75mm, %	54.9	7.63
PSPS[4]		
19 mm, %FM retained	9.54	1.80
8 mm, %FM retained	55.7	4.43
4 mm, %FM retained	21.8	2.89
Botton pan, %FM retained	13.0	2.44
MPL[5], mm	8.96	0.68
pef>4[6], %	87.00	2.44

[1] *In vitro* true digestibility of DM.

[2] DM corr – Dry Matter corrected for silage volatile compounds, using Weissbach (2009) equation.

[3] WSC – Water soluble carbohydrates.

[4] PSPS – Penn state particle separator.

[5] MPL - Mean particle length.

[6] pef - Physically effective factor.

3. EXOGENOUS FIBROLYTIC ENZYMES AND STORAGE TIME AFFECT THE NUTRITIVE VALUE AND THE FERMENTATION PROFILE OF CORN SILAGES

ABSTRACT

The corn silage is used throughout the world as the main roughage in dairy cows diets. One of the ways to improve the nutritive value of corn silage is applying exogenous fibrolytic enzymes (EFE) at ensiling, where it can solubilize the NDF content, allowing more substrate to be fermented in the silo and more nutrient to be digested in the rumen. Also, the time of storage can influence the nutritive value of corn silages. Therefore, the objective of the present study was to evaluate the nutritive value and the fermentative profile of corn silages added EFE doses at ensiling, with different lengths of storage. The study was elaborated as a completely randomized design with a factorial arrangement of treatment, 4 EFE doses × 3 times of storage, and 4 replicates per treatment The EFE treatments were: control (without EFE addition); E100 (100 g/ton of DM); E150 (150 g/ton of DM); and E200 (200 g/ton of DM). The times of storage were 30, 60 and 90 days. Regarding the chemical composition, the time of storage decreased ($P<0.01$) the DM content, from 30 to 90 days, but increased the NDF content ($P=0.01$), NDF digestibility ($P<0.01$), and CP content ($P=0.01$) from 30 to 60 days. At the fermentative profile, the time of storage, increased ($P<0.01$) the DM losses and the butyric acid concentration ($P=0.01$), from 30 to 90 days. However, ethanol, ethyl lactate, and ethyl acetate concentrations were decreased ($P<0.01$), as the length of storage, increased. The EFE treatments E150 and E200 also decreased ($P<0.01$) the corn silage DM content. Although ethanol concentration of all the EFE doses was smaller ($P<0.01$) than the control, only the doses E150 and E200 reduced the ethyl lactate concentration ($P<0.01$), and ethyl acetate concentration was reduced solely by the highest dose (E200). The interactions between EFE doses and times of storage occurred for: starch digestibility ($P=0.01$), EE ($P=0.01$), pH ($P=0.05$), water-soluble carbohydrates (WSC; $P=0.01$), lactic ($P=0.01$), acetic ($P=0.01$), propionic acid ($P=0.01$), 1,2 - propanediol ($P=0.01$), 2,3 - butanediol ($P=0.01$), 1 - propanol ($P<0.01$) . In conclusion, the addition of EFE in corn silages did not affect the chemical composition, but it increased the acetic acid content, without increase the DM losses during fermentation, resulting in a decrease of ethanol, ethyl lactate, and ethyl acetate concentration. Also, the time of storage affected the chemical composition and the fermentative profile of corn silages.

Keywords: Xylanase, Length of storage, Nutrient digestibility, Fermentation

3.1. INTRODUCTION

The corn silage is the most roughage used in diets of dairy cows in Brazil (Bernardes and Rêgo, 2014) and worldwide (Ferraretto et al. 2018). Moreover, any improvement in corn

silage nutritive value would result in a greater impact on milk production and the reduction of diets costs (Hatfield et al. 1999). In this way, the use of exogenous fibrolytic enzymes (EFE) in corn silages have been studied, once the forage digestibility is a limiting factor to the intake of digestible energy of dairy cows (Beauchemin et al. 2003) and EFE can catalyze the depolymerization of forage fiber, which is the main barrier to nutrient availability (Meale et al., 2014). The application of EFE at ensiling can optimize the effects of the enzyme's activity, since silages present low pH and relative higher temperatures than rumen (Adesogan et al. 2014), and cellulases and xylanases could promote the production of water-soluble carbohydrates (WSC) from fiber hydrolysis, which could result in an increase of lactic acid content (Higginbotham et al., 1994). However, studies have shown inconsistencies in the use of EFE, when applied at ensiling (Eun and Beauchemin, 2007). One of the major factors that contribute to the EFE positive effect in the silage nutritive value is the dose rate application (Eun et al. 2007). Although the major fractions of the corn silages are fibrous components (stem, leaves, husks, and cob), high-quality corn silages have a considerable amount of grains. These grains represent a potential fraction where the EFE can act to improve the silage quality, because the cell walls in the endosperm, which also encapsulates the nutrients, such as starch, consist mainly of arabinoxylan. (Evers and Millar, 2002; Le et al. 2013). Besides the EFE application, the length of storage also can affect the nutritive value of silages (Der Bedrosian et al. 2012; Weinberg and Chen, 2013).

Therefore, we hypothesized that exogenous fibrolytic enzymes (EFE) could solubilize the fiber components, as well as, endosperm cell walls of grains, then allowing more substrate to be fermented, altering the fermentative profile, reducing the protection of the nutrients and enhancing the nutrient availability, according to the dose applied and the time of storage.

3.2. MATERIALS AND METHODS

3.2.1. Ensiling

The corn hybrid (Pioneer P2866H) was grown at the Luiz de Queiroz College of Agriculture – University of Sao Paulo, Piracicaba, Sao Paulo, Brazil. The corn crop was harvested manually in September of 2018 at half of the milk line. It was chopped in a stationary mill (Trapp, Jaraguá do Sul, SC, Brazil) adjusted to reach the mean particle size of 10 mm.

The chemical composition (means ± SD) of the corn plant with the treatments before ensiling was: 31.47 ± 0.56 of DM; 52.77 ± 2.66 of NDF; 9.08 ± 0.18 of CP; 2.65 ± 0.20 of EE; 71.07 ± 1.47 of DM digestibility; 60.80 ± 2.83 of NDF digestibility; 4.89 ± 0.22 of pH. Four 7-kg replicated subsamples of the corn plant were treated by hand-spraying the exogenous fibrolytic enzyme (Rovabio Advance L2, Adisseo, Commentry, France), onto the material. The exogenous fibrolytic enzyme (EFE), in liquid form, was composed of two major active enzymes, endo-1,4-β-xylanase (E.C. number 3.2.1.8) and endo-1,3(4)-ß-glucanase (E.C. number 3.2.1.6), obtained from Talaromyces versatilis strains, as declared by the manufacturer. The guaranteed concentration of the enzymes was 12,500 UV/mL (1.8%) of xylanase and 8,600 UV/mL (1.2%) of ß-glucanase. The product also contained sorbitol (25.2%) and potassium sorbate (0.11%). One UV of xylanase or ß-glucanase is defined as the quantity of enzyme required to hydrolyze the substrate (wheat arabinoxylan or barley ß-glucan, respectively), reducing the solution viscosity in one unity per minute, at 30 ºC and pH 5.5. The endo-ß-glucanase to xylanase ratio of the product was 0.688:1. The doses applied were 100 (E100), 150 (E150), and 200 (E200) g of the product per ton of DM of the ground corn plant. Each dose was applied randomly to one of the four subsamples. The quantity of product was chosen accordingly to previous studies that used the commercial EFE at the non-ruminant feed. All the doses were diluted in 100 mL of deionized water, to facilitate the distribution onto the forage. The control silage was added 100 mL of deionized water, exclusively. The silos were vacuum-sealed bags, made of low-density polyethylene, with the dimension of 250 × 350 mm with 0.20 mm of thickness (ZPP Embalagens, Santa Rita do Passa Quatro, SP, Brazil). Approximately 500 g of forage was placed in each bag. To each bag was added one of the four doses and it was stored at room temperature for 30, 60 and 90 days, and each combination of dose × time of storage had four replicates, totaling 48 experimental units. The mini-silos were weighted right after sealing and before each opening time to determine the DM losses, according to Jobim et al. (2007).

3.2.2. Chemical Analysis

After 30, 60 or 90 days of ensiling silos were opened and samples were collected from each silo and, subsequently, frozen. The collected samples were dried in a forced-air oven for 72 h at 55ºC and ground to pass through a 1 mm mesh screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Next, subsamples were analyzed for DM content and

concentration of ether extract (EE), and ash according to the Association of Official Analytical Chemistry (AOAC, 1990). The NDF content was analyzed according to Van Soest et al. (1991) with heat-stable α-amylase and sodium sulfite, using a fiber analyzer (Marconi, Piracicaba, SP, Brazil). The nitrogen was analyzed by the Dumas method (AOAC, 2006), using a nitrogen analyzer (FP-2000A, Leco Corp., St. Joseph, MI, USA). Crude protein (CP) was obtained by using the factor 6.25 × N %. Samples were also analyzed for water-soluble carbohydrates (WSC), submitted to 4 hours of extraction using an 80% ethanol solution, as described by the phenol-sulfuric acid assay (Hall, 2000). The starch content was analyzed using the enzymatic hydrolysis method according to Hall (2009) and the concentrations of WSC and starch were determined using a spectrophotometer (Janway 6305, Marconi, Piracicaba, SP, Brazil), with the absorbance set at 490 nm for WSC and 510 nm for starch.

Subsamples of 25g of corn silage were mixed with 225 g of deionized water for 4 min at 152 rpm using a stomacher (Nova Ética, Vargem Grande Paulista, SP, Brazil). The extract was filtered through 3 layers of cheesecloth and the pH was measured using a DM 20 pH meter (Digimed Analítica, SP, Brazil). Then, the extract was centrifuged at 10,000 × g for 15 min at 4ºC. The lactic acid was determined on the filtrate according to the method of Pryce (1969), using the spectrophotometer at a wavelength of 565 nm. The concentrations of VFAs, alcohols, and esters were analyzed using a gas chromatographer with a mass detector GCMS QP 2010 plus (Shimadzu, Kyoto, Japan) using a capillary column Stabilwax (cross bond carbowax polyethylene glycol), of 60 m with 0.25 mm of diameter (Restek, Bellefonte, PA, USA). The DM content was corrected for the volatile compounds according to equation proposed by Weissbach (2009): DMcorr (% as fed) = DM (% as fed) + 0.08 × lactic acid (% as fed) + 0.77 × 1,2-propanediol (% as fed) + 0.87 × 2,3-butanediol (% as fed) + 0.95 × VFA´s (% as fed) + esters (% as fed) + alcohols (% as fed).

3.2.3. Silage Digestibility

The subsamples were submitted to in situ digestibility. Approximately 15 g of the samples were placed into 10×20 cm woven bags (R1020 Forage Bag, ANKOM Technology, Macedon, NY, USA). The samples were not dried or processed, and it was degraded in the rumen as it was fed (Huntington and Givens, 1997). According to the producer, the porosity of the bag was 50 ± 10 micron. The ratio of sample size to free bag surface area was 37.5 mg/cm². Each bag was tied 1 cm below of the top with rubber bands and clips were used to

attach it to a chain. To allow an adequate degradation, the chain with the bags were placed in the ventral sac of the rumen for 48h. All the bags were removed simultaneously. Two fistulated dry cows (Holstein) fed 55% (DM) of corn silage and 45% (DM) concentrate, were used to incubate the silages. Each cow received a replicate of the samples and blank bags were added to correct for the weight of the bag tare weight. After they were removed from the rumen, all the bags were placed into an ice bucket and washed using a washing machine to remove adherent feed particles and bacteria. The bags were dried for 48h at 60ºC and then weighted to calculate de DM digestion. The residues of the bags were analyzed for NDF and starch content, as previously described, to estimate the respective digestibilities. The incubation into rumen-cannulated cows was approved according to the animal welfare committee of the University of São Paulo, protocol number: 2017.5442.11.4.

3.2.4. Statistical Analyses

The data were analyzed as a completely randomized design with a factorial arrangement of treatment, 4 doses × 3 times of storage, and 4 replicates per treatment, using the MIXED procedure of SAS 9.4 software (SAS Studio). The model used was: $Yij = \mu + Di + Tj + DTij + eij$; in which: μ = overall mean; Di = fixed effect of dose i; Tj = fixed effect of time of storage j (repeated measure); DTij = interaction between dose i and time of storage j; eij = residual error. The best covariance structure for the repeated measure was defined by the smallest value for corrected Akaike's information criterion among variance components first-order autoregressive, compound symmetry or unstructured. For the 48h-in situ digestibility assay, cows were included in the model as blocks with random effect. The residue was used to establish degrees of freedom for the tests of fixed effects of the model. The Tukey's honest significant difference test was used to compare the effect of dose, time, and the interaction between dose and time of storage. Means were considered statistically significant when $P \leq 0.05$ and tendency when $P > 0.05 \leq 0.10$.

3.3. RESULTS

In Table 12 are presented the characteristics related to the chemical composition and the nutrient digestibility of the corn silages treated with doses of EFE, ensiled for 30, 60 and 90 days. The DM content was lower ($P<0.01$) for E150 and E200 compared to control and

E100. Considering the length of storage, the DM content decreased (P<0.01), as the time increased from 30 to 90 days. The DM digestibility was not affected by the enzyme treatment, but it tended (P=0.10) to be higher at 60 days than 30 and 90 days.

For the NDF content, only the storage time differed significantly (P=0.01), where there was an increase from 30 to 60 days of storage. The same pattern occurred for the NDF digestibility, which 60 days of storage increased (P<0.01) the NDF digestibility in comparison to 30 days. There was a tendency for dose × time interaction (P=0.08), where the dose E150 at 60 days and the doses E150 and E200 at 90 days showed to increase NDF digestibility compared to control.

The starch content was not affected by the enzyme treatment or by the storage time (P>0.10). For the starch digestibility, there was an interaction between doses of EFE and storage times (P=0.01). The enzyme did not affect the starch digestibility at 30 days, but at 60 days of storage, E100 showed higher starch digestion than control. At 90 days, starch digestibility increased numerically with the application of the EFE dose, in comparison to the control.

The CP was affected (P=0.01) only by the length of storage, silages had higher CP at 60 and 90 days than at 30 days. There was an interaction between doses and times of storage (P=0.01) for EE content. The EE content was higher in E200 than in control at 30 days, but not at 60 and 90 days.

Table 12. Chemical composition and 48-h in situ digestibility (DM basis) of corn silages treated with exogenous fibrolytic enzyme (EFE) at 0, 100, 150 and 200 g/ ton of DM ensiled for 30, 60 and 90 days

	DM	DMD[1]	NDF	NDFD[1]	Starch	StarchD	CP	EE
	% AF	%	%	%	%	%	%	%
30d								
Control	32.0	70.3	51.8	55.1	31.8	76.2^{ab}	9.44	2.01^{d}
E100	31.3	71.4	49.0	54.4	33.0	72.0^{ab}	9.64	2.09^{cd}
E150	31.0	72.2	49.0	55.9	31.2	79.1^{ab}	9.67	2.26^{bcd}
E200	31.0	71.5	48.5	55.5	33.4	79.3^{ab}	9.55	2.38^{abc}
60d								
Control	31.7	71.6	56.8	60.9	31.2	70.6^{b}	9.85	2.50^{ab}
E100	31.5	73.3	52.0	60.8	35.1	82.3^{a}	9.81	2.50^{ab}
E150	30.0	73.5	54.5	62.3	31.1	80.3^{ab}	10.26	2.41^{abc}
E200	30.3	72.2	50.8	56.4	32.5	77.3^{ab}	10.09	2.34^{abcd}
90d								
Control	30.5	70.3	51.0	57.2	30.4	71.2^{b}	10.00	2.32^{abcd}
E100	30.7	69.9	51.8	55.9	31.8	76.5^{ab}	10.26	2.50^{ab}
E150	29.3	72.4	51.5	60.8	32.7	78.6^{ab}	10.64	2.38^{abc}
E200	29.8	73.1	52.5	60.2	32.9	79.6^{ab}	10.14	2.60^{a}
EFE doses								
Control	31.4^{a}	70.8	53.2	57.7	31.2	72.6	9.76	2.28
E100	31.1^{a}	71.5	50.9	57.0	33.3	76.9	9.90	2.36
E150	30.1^{b}	72.7	51.7	59.6	31.7	79.4	10.19	2.35
E200	30.3^{b}	72.3	50.6	57.4	32.9	78.7	9.93	2.44
Storage times								
30d	31.3^{a}	71.4	49.6^{b}	55.2^{b}	32.4	76.7	9.58^{b}	2.19
60d	30.9^{b}	72.6	53.5^{a}	60.1^{a}	32.5	77.6	10.00^{a}	2.44
90d	30.1^{c}	71.4	51.7^{ab}	58.5^{ab}	32.0	76.5	10.26^{a}	2.45
SEM[2]	0.002	0.02	0.008	0.02	1.43	0.04	0.232	0.066
P-value								
Dose	<0.01	0.22	0.12	0.06	0.24	0.09	0.17	0.05
Time	<0.01	0.1	0.01	<0.01	0.86	0.64	0.01	<0.01
D×T[3]	0.19	0.47	0.22	0.08	0.65	0.01	0.91	0.01

[a–d] Means in a column with different superscripts differed (P < 0.05).
[1]DMD – DM digestibility; NDFD – NDF digestibility.
[2]SEM – Standard error of means.
[3]D×T -Interaction between dose and time.

Table 13 presents the results of the principal characteristics related to silage fermentation measured in this study (DM corr, DM losses, pH, soluble carbohydrates, lactic acid, acetic acid, propionic acid, and butyric acid).

The correction of DM content to the volatile compounds tended to follow the same pattern of the uncorrected DM content, in which E150 and E200 had greater DM content than control and E100 ($P<0.01$). The DM corr was reduced from 60 days to 90 days of storage ($P<0.01$).

The DM losses were not affected by enzyme treatment, but it was affected by the storage time ($P<0.01$). There were a 2-fold increase DM losses from 30 to 60 days, and a 1.50-fold, from 60 to 90 days of storage. The pH of all corn silages, independent of enzyme treatment was lowest ($P=0.05$) at 60 days of storage.

The soluble WSC content also presented an interaction ($P=0.01$), in which E150 had higher WSC content than control at 60d.

For the lactic acid, there was an interaction between doses and storage times ($P=0.01$), where the dose E150 had higher lactic acid content than the control, within the time of 60 days of storage.

The acetic acid content was higher in silages treated with E150 and E200 compared to control at all the three times of storage ($P=0.01$). The increase in acetic acid concentration was more pronounced at 90 days.

There was an interaction for propionic acid content ($P=0.01$), its concentration was lower in E200 than in control, E100, and E150 at 90 days of storage, with its concentration increasing over time. Butyric acid content increased over time ($P=0.01$).

Table 13. Fermentation characteristics (DM basis) of corn silages treated with exogenous fibrolytic enzyme (EFE) at 0, 100, 150 and 200 g/ ton of DM ensiled for 30, 60 and 90 days

	DM Corr[1] % AF	DM Losses %	pH	WSC %	Lactic acid %	Acetic acid %	Propionic acid mg/kg	Butyric acid mg/kg
30d								
Control	32.7	0.92	4.26^{a}	4.01^{abc}	2.87^{abcd}	0.68^{f}	80.8^{d}	15.0
E100	31.9	4.01	4.32^{a}	3.58^{bc}	2.87^{abcd}	1.13^{e}	90.5^{d}	15.3
E150	31.5	1.99	4.21^{ab}	4.16^{abc}	3.53^{abc}	1.03^{e}	85.8^{d}	11.5
E200	31.6	2.34	4.19^{ab}	3.94^{bc}	4.25^{a}	1.26^{de}	101.3^{d}	10.8
60d								
Control	32.3	3.25	4.00^{c}	3.33^{c}	2.65^{bcde}	1.22^{de}	150.8^{cd}	20.8
E100	32.2	4.09	4.00^{c}	4.05^{abc}	3.30^{abc}	1.55^{cd}	195.5^{cd}	9.0
E150	31.0	5.38	4.00^{c}	5.53^{a}	4.29^{a}	1.82^{bc}	421.3^{c}	15.8
E200	30.9	6.02	4.09^{bc}	4.16^{abc}	4.07^{ab}	1.70^{bc}	304.5^{cd}	19.0
90d								
Control	31.4	6.02	4.22^{ab}	4.25^{abc}	1.74^{de}	1.63^{bcd}	1106.5^{a}	23.5
E100	31.5	6.75	4.17^{ab}	4.76^{ab}	1.13^{e}	2.00^{b}	1058.8^{a}	15.8
E150	30.2	8.52	4.16^{abc}	4.25^{abc}	2.10^{cde}	2.46^{a}	1070.5^{a}	29.0
E200	30.8	6.90	4.19^{ab}	4.54^{abc}	1.56^{de}	2.65^{a}	728.0^{b}	15.0
EFE doses								
Control	32.1^{a}	3.40	4.16	3.86	2.42	1.18	446.0	19.8
E100	31.9^{a}	4.95	4.16	4.13	2.43	1.56	448.3	13.3
E150	30.9^{b}	5.30	4.12	4.65	3.31	1.77	525.8	18.8
E200	31.1^{b}	5.09	4.16	4.21	3.29	1.87	377.9	14.9
Storage times								
30d	31.9^{a}	2.32^{c}	4.25	3.92	3.38	1.03	89.6	13.1^{b}
60d	31.6^{a}	4.69^{b}	4.02	4.27	3.58	1.57	268.0	16.1^{ab}
90d	31.0^{b}	7.05^{a}	4.19	4.45	1.63	2.19	990.9	20.8^{a}
SEM[2]	0.27	0.884	0.032	0.294	0.303	0.083	51.35	3.34
P-value								
Dose	<0.01	0.21	0.4199	0.02	0.01	<0.01	0.07	0.19
Time	<0.01	<0.01	<0.01	0.05	<0.01	<0.01	<0.01	0.01
D×T[3]	0.30	0.26	0.05	0.01	0.01	0.01	0.01	0.14

$^{a–f}$ Means in a column with different superscripts differed (P < 0.05).
[1]DM corr – Dry Matter corrected for silage volatile compounds, using Weissbach (2009) equation
[2]SEM – Standard error of means.
[3]D×T -Interaction between dose and time.

The alcohols and esters concentrations in the corn silages treated with increased doses of EFE are presented in Table 14. The ethanol content was lower in enzyme-treated silages than in control, and the lowest concentration of ethanol was observed for E200 dose ($P<0.01$) The ethanol concentration decreased over the time of storage ($P<0.01$).

Besides ethanol, are presented in Table 14, the alcohols: 1,2-propanediol, 2,3-butanediol, and 1-propanol. The dose E200 showed numerically the greatest value (1873 mg/kg) at 30 days of storage for the 1,2-propanediol ($P=0.01$), and at the 90 days, the dose of E100 exhibited numerically the smallest value (796 mg/kg). At 60 days, E150 had higher 2,3-butanediol content than control ($P=0.01$). At 60 days, E150 had a higher concentration of 1-propanol, but at 90 days of storage, the highest value was found in the control treatment ($P<0.01$).

The derivative esters of the combination of ethanol and lactic acid or ethanol and acetic acid, ethyl lactate, and ethyl acetate, respectively, were affected by the doses and the days of storage. In comparison to the control, the ethyl lactate and ethyl acetate concentrations were decreased by the highest EFE dose ($P<0.01$ and $P=0.01$, respectively). Regarding the time, ethyl lactate concentration was gradually decreased from 30 to 90 days ($P<0.01$). The ethyl acetate content decreased from 30 to 60 days and remained constant from 60 to 90 days of storage ($P<0.01$).

Table 14. Concentrations of alcohols and esters (DM basis) in corn silages treated with exogenous fibrolytic enzyme (EFE) at 0, 100, 150 and 200 g/ ton of DM ensiled for 30, 60 and 90 days

	Ethanol	Propanediol	Butanediol	Propanol	Ethyl lactate	Ethyl acetate
	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
30d						
Control	0.140	690^{b}	131^{d}	2.75^{b}	18.75	17.68
E100	0.090	1152^{ab}	189^{d}	4.50^{b}	15.31	9.67
E150	0.092	1194^{ab}	166^{d}	2.50^{b}	15.50	14.00
E200	0.055	1873^{a}	188^{d}	4.25^{b}	14.50	11.50
60d						
Control	0.065	1111^{ab}	169^{d}	27.75^{b}	16.50	5.00
E100	0.042	1429^{ab}	241^{bcd}	30.00^{b}	14.25	6.00
E150	0.045	1587^{ab}	347^{abc}	83.75^{a}	14.75	6.75
E200	0.010	1474^{ab}	229^{cd}	19.50^{b}	5.00	1.50
90d						
Control	0.042	1281^{ab}	496^{a}	100.09^{a}	12.50	9.00
E100	0.010	1480^{ab}	424^{a}	30.50^{b}	6.50	5.25
E150	0.005	796^{b}	384^{ab}	12.00^{b}	3.50	3.75
E200	0.002	1010^{ab}	333^{abc}	13.25^{b}	3.00	3.75
EFE doses						
Control	0.082^{a}	1027	265	43.53	15.92^{a}	10.56^{a}
E100	0.047^{b}	1354	285	21.67	12.02^{ab}	6.97^{ab}
E150	0.047^{b}	1192	299	32.75	11.25^{bc}	8.17^{ab}
E200	0.022^{c}	1452	250	12.33	7.50^{c}	5.58^{b}
Storage times						
30d	0.094^{a}	1227	168	3.50	16.02^{a}	13.21^{a}
60d	0.041^{b}	1400	247	40.25	12.63^{b}	4.81^{b}
90d	0.015^{c}	1142	409	38.96	6.38^{c}	5.44^{b}
SEM	0.0104	199.2	28.9	7.796	1.885	1.455
P-value						
Dose	<0.01	0.1	0.18	<0.01	<0.01	0.01
Time	<0.01	0.16	<0.01	<0.01	<0.01	<0.01
D×T	0.44	0.01	0.01	<0.01	0.12	0.16

$^{a–d}$ Means in a column with different superscripts differed (P < 0.05).

[1]SEM – Standard error of means.

[2]D×T -Interaction between dose and time.

3.4. DISCUSSION

The application of increased doses of exogenous fibrolytic enzyme did not affect the major aspects related to the chemical composition and the digestion of the corn silages, but it clearly changed the fermentative profile. Furthermore, the length of storage also affected some variables related to the nutritive value and the fermentation parameters.

3.4.1. Effects of the length of storage on the chemical composition and nutrient digestibility

Store corn silages for different times might affect its nutrient composition and digestibility. According to Weinberg and Chen (2013), the DM content of corn silages decreased constantly over time, from fresh cut to 12 months of storage, in about 10.5%. In the present study, the reduction of the DM content from 30 to 90 days of storage, although statistically significant, was small (3.83%). However, Weinberg and Chen (2013) attributed this reduction of DM to the hydrolysis of hemicelluloses, which did not occur in the current study.

The DM digestibility was not affected by the time in the present study, but Weinberg and Chen (2013) showed a tendency of DM digestibility to decrease along the time in wheat silages. However, Der Bedrosian et al. (2012), found that DM content of corn silages, harvested at two different maturities (32% and 41% of DM), tended to increase over time. In agreement to Weinberg and Chen (2013), the decrease in the DM content was associated with the increase of DM losses along the time, with the concomitant production of acetic acid (heterolactic fermentation), which may result in the production of carbon dioxide (Driehuis et al., 1999).

Although Morrison (1979) reported that the hemicellulose can be hydrolyzed along the time of storage in perennial ryegrass, the NDF content of the current study, in fact, increased from 30 to 60 days. Sanderson (1993) reported that the NDF concentration of sorghum silages was greater at 160 than 30 days of ensiling. Moreover, Weinberg and Chen (2013), found the same pattern of NDF to increase over the time for wheat silages at milk stage, but not for corn silages. However, Der Bedrosian et al. (2012) did not find any response in NDF content over a year of storage for two corn hybrids.

Regarding the NDF digestibility, Sanderson (1993) also reported an increase in sorghum silages, but in the study of Weinberg and Chen (2013), the NDF digestibility of corn silage and wheat silage, at the flowering stage, decreased as the time of storage was increased.

The starch content of brown mid-rib hybrid remained constant, but of the normal corn hybrid slightly decreased over the time in the study of Der Bedrosian et al. (2012), which not occurred for the normal hybrid in the present study. In the same manner, the starch digestibility was not affected by the storage time, but Der Bedrosian et al. (2012) showed that it increased in different hybrids at different maturities.

The increase in CP might reflect on the solubilization of other compounds during the fermentation since the nutrients were evaluated as centesimal composition.

The increase of EE content over time could also be related to reduced hydrolysis of fatty acids during silage fermentation, but fatty acids hydrolysis can occur in the first days of ensiling (Han and Zhou et al. 2013).

3.4.2. Effects of EFE doses on the chemical composition and nutrient digestibility

The effect of EFE doses also changed some variables related to the chemical composition and digestibility of the corn silages. The decrease in the DM content, as the dose was augmented, was accompanied by a numerical increase of the DM losses. Spoelstra et al. (1992), Sheperd and Kung (1996), and Ying et al. 2017, also showed a decrease of the DM content in corn silages treated with EFE. These losses might be attributed to the formation of acetic acid, as will be discussed later. On the contrary, other studies did not find differences in DM content when EFE was applied in corn silages at ensiling (Higginbotham et al., 1994; Colombatto et al., 2004; Lynch et al., 2015).

In comparison to the length of storage, the EFE, also, did not affect the DM digestibility, but it tended to alter the NDF and starch digestibility. Regarding the NDF content and digestibility, the effects were not clear, since only the highest dose (200 g/ton of DM) reduced both at 60 days of storage. However, the majority of studies where EFE was applied to corn silages at ensiling, are in concordance about the reduction of the NDF content (Spoelstra et al., 1992; Higginbotham et al., 1994; Sheperd and Kung, 1996, Colombatto et al., 2004, Lynch et al. 2015; Ying et al. 2017). The studies previously mentioned attributed the NDF content reduction to the degradation of the cell wall carbohydrates of the corn silage. The NDF digestibility was also reduced in the study of Lynch et al. (2015), at 24h of ruminal

incubation. The authors claimed that the easily digestible components of the fiber fraction are more susceptible to be hydrolyzed during the silage fermentation. However, the increase in NDF digestibility at 90 days, for the doses of E150 and E200, are more similar to the results of Sheperd and Kung (1996) and Ying et al. (2017), even though no differences for NDF content were found to these two treatments at the referred time of storage. Digestibility in vitro studies have shown that the use of EFE, as a ruminal feed additive, is effective to increase the DM or NDF digestibility (Eun and Beauchemin, 2007), but the optimal ratio between endoglucanase and xylanase to improve the digestibility should be more than 0.4:1 (Eun et al. 2007).

The explanation for the increase in starch digestibility could be related to the hydrolysis of the cell wall endosperm (Evers and Millar, 2002), which could facilitate the access of the enzymes of the total digestive tract to the starch granules (Le et al. 2013).

The CP increase at 60 days might be the result of less proteolysis with higher doses of EFE, which is a result of well-preserved silages either achieved with the application of inoculants or fibrolytic enzymes (Sheperd et al. 1995; Nadeau et al. 2000).

The EE content increase in 30 days storage may be attributed to the less oxidation of fatty acids in those silages (Khan et al. 2009).

3.4.3. Effects of the time of storage on fermentative profile

Lengths of ensiling also affect the fermentative profile of the corn silages. Although there was no difference for WSC content, the pH of the corn silages dropped from 30 to 60 days and raised again at 90 days. Since the main responsible to maintaining the pH of silage low is the lactic acid (pKa = 3.86), its reduction at 90 days resulted in a higher pH.

The higher concentration of acetic acid in silages stored for long periods of time is a consequence of the lactic acid conversion to equimolar parts of acetic acid and 1,2 – propanediol (Oude Elferink et al., 2001).

Moreover, the present study showed that the percentage of lactic acid reduction (55%) from 60 to 90 days is in accordance with the increase in percentage of acetic acid (39%). Even though the 1,2 – propanediol concentration did not increase over time, it could have been metabolized to other components, such as propionic acid and 1-propanol, by strains of Lactobacillus diolivorans (Krooneman et al., 2002).

The increase in propionic acid over time can possibly be explained by this conversion, but the same did not occur for 1-propanol concentration. The increase in acetic acid was accompanied by DM losses along the time of storage, which is the result of carbon dioxide production (Driehuis et al., 1999) due to the anaerobic degradation of lactic acid (Oude Elferink et al., 2001).

Because acetic acid has antifungal proprieties (Kleinschmit et al., 2005; Tabacco et al., 2011), the decline in ethanol concentration through the time is probably a result of the inhibition of yeast population (Moon, 1983), which can lead to a greater aerobic stability (Muck et al. 2018). These are only speculations since we did not evaluate the microbial population numbers or measured the aerobic stability of the corn silages.

Furthermore, the decrease of the esters content formed by the combination of ethanol with acetic or lactic acid, ethyl acetate, and ethyl lactate, respectively, was caused by the reduction of the ethanol concentration over the time, once the esterification process seems to be more linked with the alcohol than the carboxylic acid (Weiss et al. 2016). However, the magnitude of the reduction of esters was below of the proposed by the equation of Weiss and Auerbach (2012), where the difference in 0.5% DM of ethanol would result in a difference of 100 mg/kg of ethyl esters.

The increase of butyric acid from 60 to 90 days and 2,3 – butanediol from 30 to 90 days of storage, suggests a possible presence of proteolytic microorganisms such as clostridia (Muck 2010; Siemerink et al., 2011). However, the butyric acid concentration is far below the threshold (0.5% of DM) to consider clostridial fermentation. Furthermore, as mentioned earlier, the crude protein, in fact, was greater for longer times of storage (90 d).

3.4.4. Effects of EFE on fermentative profile

The application of EFE doses changed some majors and minors aspects related to the fermentative profile of corn silages. The WSC concentration did not change among the treatments, with the exception of the dose E150 at 60 days of storage, which had the greatest value. At 30 days of storage, the decrease in pH was accompanied by an increase in the lactic acid content, as the doses increased. Although the same pattern of increase in lactic acid content at 60 days was observed, the pH remained low for all the treatments at this time of storage.

Higginbotham et al. (1994) evaluating the addition of EFE (cellulase and xylanase) to corn silage at ensiling, found at 30 d of storage an increase in lactic acid content and no differences in pH between the treated and untreated silages. Although the authors did not find a significant increase of the water-soluble carbohydrates, they justified that the increase of the lactic acid content was a consequence of the NDF solubilization. Usually, changes in pH or in the lactic acid may not occur because the substrate in corn silages could be not a limiting factor to fermentation (Sheperd and Kung, 1996).

The EFE application increased the acetic acid content, at the three times of storage (30, 60 and 90 days). As a consequence, there was a decline in ethanol production. The main active compound of the product applied in the silages was the xylanase, therefore it was expected that more xylose monomers would be released after the hemicellulose hydrolysis. The fermentation of xylose, when it is free from the hemicellulose, occurs rapidly in the silo by anaerobic facultative bacteria, and the main products are acetic acid and lactic acid (Fred et al., 1919). Supporting this idea, Dehghani et al. (2012) found that a mixture of glucanase and xylanase increased the acetic acid concentration when the enzymes were applied in corn stover silage. Furthermore, Del Valle et al. (2012) presented a quadratic effect, with the highest acetic acid concentration found at the dose of 185 mg/kg DM of xylanase in sugarcane silages. Since the fermentation of xylose produces equimolar amounts of acetic and lactic acid (Wood 1961), the further increase in acetic acid content might be consequence of the lactic acid conversion into acetic acid (Chamberlain 1988).

Furthermore, the decline in ethanol concentration might be attributed to the antifungal effect of the acetic acid (Kleinschmit et al., 2005; Tabacco et al., 2011), which also led to a reduction of its derivatives esters. Even though during the acetic acid formation in silages, by xylose fermentation or lactic acid formation, there is the release of carbon dioxide, as an end-product (Shaw et al. 2008; Oude Elferink et al., 2001), the EFE application did not result in an increase of DM losses in corn silages.

Furthermore, the increase in acetic acid content did not reflect in an increase of 1,2 – propanediol concentration, in contradiction to the proposed pathway of Oude Elferink et al. (2001). Moreover, the changes in propionic acid and 1 – propanol did not follow the acetic acid production pattern.

3.5. CONCLUSION

The EFE application and the time of storage markedly increased the acetic acid content, which resulted in a reduction of the ethanol concentration, probably as a consequence of yeasts inhibition, but DM losses were increased over time. Even though the effect of EFE on the nutritive value was not clear, the time of storage altered the chemical composition (NDF, CP, and EE), the NDF digestibility, also the acids, alcohols, and esters of corn silages.

LITERATURE CITED

Adesogan, A. T., Z. X. Ma, J. J. Romero, and K. G. Arriola. 2014. Improving cell wall digestion and animal performance with fibrolytic enzymes. J. Anim. Sci. 92:1317–1330.

AOAC – Association of Official Analytical Chemistry. 1990. Official methods of analyses. 15th ed. AOAC International, Arlington, VA, USA.

AOAC – Association of Official Analytical Chemistry. 2006. Official methods of analysis, 18th ed. [Revised] Association of Official Analytical Chemists. Washington, DC.

Beauchemin, K. A., D. Colombatto, D. P. Morgavi, and W. Z. Yang. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81 (Suppl. 2E): 37–E47.

Bernardes, T. F., and A. C. Rêgo. 2014. Study on the practices of silage production and utilization on Brazilian dairy farms. J. Dairy Sci. 97:1852–1861.

Chamberlain, D. G. 1988. Effect of added glucose and xylose on the fermentation of perennial ryegrass silage inoculated with Lactobacillus plantarum. Journal of the Science of Food and Agriculture 46.2: 129-138.

Colombatto, D., F. L. Mould, M. K. Bhat, R. H. Phipps, and E. Owen. 2004. In vitro evaluation of fibrolytic enzymes as additives for maize (Zea mays L.) silage: I. Effects of ensiling temperature, enzyme source and addition level. Animal Feed Science and Technology, 111(1-4):111-128.

Dehghani, M. R., M. R. Weisbjerg, T. Hvelplund, and N. B. Kristensen. 2012. Effect of enzyme addition to forage at ensiling on silage chemical composition and NDF degradation characteristics. Livestock science, 150(1-3), 51-58.

Del Valle, T. A., Antonio, G., Zenatti, T. F., Campana, M., Zilio, E. M. C., Ghizzi, L. G., ... and de Morais, J. P. G. (2018). Effects of xylanase on the fermentation profile and chemical composition of sugarcane silage. The Journal of Agricultural Science, 156(9), 1123-1129.

Der Bedrosian, M. C., K. E. Nestor Jr, and L. Kung Jr. 2012. The effects of hybrid, maturity, and length of storage on the composition and nutritive value of corn silage. Journal of Dairy Science, 95(9): 5115-5126.

Driehuis, F., S. O. Elferink, and S. F. Spoelstra. 1999. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with Lactobacillus buchneri inhibits yeast growth and improves aerobic stability. Journal of applied microbiology, 87(4): 583-594.

Evers, T., and S. Millar. 2002. Cereal grain structure and development: some implications for quality. Journal of cereal science. 36.3: 261-284.

Eun, J. S., and K. A. Beauchemin. 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using in vitro fermentation characteristics. Anim. Feed Sci. Technol. 132:298–315.

Eun, J. S., K. A. Beauchemin, and H. Schulze. 2007. Use of exogenous fibrolytic enzymes to enhance in vitro fermentation of alfalfa hay and corn silage. J. Dairy Sci. 90:1440–1451.

Ferraretto, L. F., R. D. Shaver, and B. D. Luck. 2018. Silage review: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting. J. Dairy Sci. 101:3937–3951.

Fred E. B., W. H. Peterson, and A. Davenport. 1919. Acid fermentation of xylose. Journal of Biological Chemistry 39, 347–384.

Hall, M. B. 2000. Calculation of non-structural carbohydrate content of feeds that contain non-protein nitrogen. University of Florida, A-25 (Bulletin 339).

Hall, M. B. 2009. Determination of Starch, Including Maltooligosaccharides, in Animal Feeds: Comparison of Methods and a Method Recommended for AOAC Collaborative Study. Journal of Association of Official Analytical Chemists International. 92:42-49.

Han, L., and H. Zhou. 2013. Effects of ensiling processes and antioxidants on fatty acid concentrations and compositions in corn silages. Journal of animal science and biotechnology, 4(1): 48.

Hatfield, R. D., J. Ralph, and J. H. Grabber. 1999. Cell wall structural foundations: Molecular basis for improving forage digestibilities. Crop Sci. 39:27–37.

Higginbotham, G. E., S. C. Mueller, C. A. Collar, and T. A. Schultz. 1994. Effects of an enzyme addition on corn silage fermentation. Prof. Anim. Sci. 10: 163–168.

Huntington, J. A., and D. I. Givens. 1995. The in situ technique for studying the rumen degradation of feeds: a review of the procedure. Nutricional Abstracts and Reviews. 65:63-93.

Jobim, C. C., L. G. Nussio, R. A. Reis, and P. Schmidt. 2007. Avanços metodológicos na avaliação da qualidade da forragem conservada. Revista Brasileira de Zootecnia, 101-119.

Khan, N. A., J. W. Cone, and W. H. Hendriks. 2009. Stability of fatty acids in grass and maize silages after exposure to air during the feed out period. Animal Feed Science and Technology, 154(3-4): 183-192.

Kleinschmit D. H., R. J. Schmidt, and L. Kung. 2005. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. J Dairy Sci 88: 2130–2139.

Krooneman, J., F. Faber, A. C. Alderkamp, S. J. H. W. Oude Elferink, F. Driehuis, I. Cleenwerck, J. Swings, J. C. Gottschal, and M. Vancanneyt. 2002. Lactobacilllus diolivorans sp. Nov., a 1,2 propanediol-degrading bacterium isolated from aerobically stable maize silage. Int. J. Syst. Evol. Microbiol. 52:639–646.

Le, D. M., Fojan, P., Azem, E., Pettersson, D., and Pedersen, N. R. 2013. Visualization of the anticaging effect of Ronozyme WX xylanase on wheat substrates. Cereal Chemistry, 90(5), 439-444.

Lynch, J. P., J. Baah, and K. A. Beauchemin. 2015. Conservation, fiber digestibility, and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant. Journal of dairy science 98.2: 1214-1224.

Meale, S. J., K. A. Beauchemin, A. N. Hristov, A. V. Chaves, and T. A. McAllister. 2014. Opportunities and challenges in using exogenous enzymes to improve ruminant production. J. Anim. Sci. 92:427–442.

Morrison, I. M. 1979. Changes in the cell wall components of laboratory silages and the effect of various additives on these changes. The Journal of Agricultural Science, 93(3): 581-586.

Moon, N.J. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. Journal of Applied Bacteriology 55: 454–460.

Muck, R. E. 2010. Silage microbiology and its control through additives. Revista Brasileira de Zootecnia, 39: 183-191.

Muck, R. E., E. M. G. Nadeau, T. A. McAllister, F. E. Contreras-Govea, M. C. Santos, and L. Kung Jr. 2018. Silage review: recent advances and future uses of silage additives. Journal of dairy science, 101(5): 3980-4000.

Nadeau, E. M. G., J. R. Russell, and D. R. Buxton. 2000. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant and formic acid for lambs. J. Anim. Sci. 78:2980–2989.

Oude Elferink, S. J. W. H., J. Krooneman, J. C. Gottschal, S. F. Spoelstra, F. Faber, and F. Driehuis. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by Lactobacillus buchneri. Appl. Environ. Microbiol. 67:125–132.

Pryce, J. D. 1969. A modification of Barker-Summerson method for determination of lactic acid. Analyst 94:1151-1152.

Sanderson, M.A. 1993. Aerobic stability and in vitro fiber digestibility of microbially inoculated corn and sorghum silages. Journal of Animal Science 71: 505–514.

Shaw, A. J., F. E. Jenney Jr, M. W. Adams, and L. R. Lynd. 2008. End-product pathways in the xylose fermenting bacterium, Thermoanaerobacterium saccharolyticum. Enzyme and microbial technology, 42(6): 453-458.

Sheperd, A.C., and L. Kung Jr. 1996. Effects of an enzyme additive on composition of corn silage ensiled at various stages of maturity. J. Dairy Sci. 79: 1767–1773.

Sheperd A. C., M. Maslank, D. Quinn, L. Kung. 1995. Additives containing bacteria and enzymes for alfalfa silage. J Dairy Sci 78: 565–572.

Siemerink, M. A., W. Kuit, A. M. L. Contreras, G. Eggink, J. Van der Oost, and S. W. Kengen. 2011. D-2, 3-butanediol production due to heterologous expression of an acetoin reductase in Clostridium acetobutylicum. Appl. Environ. Microbiol., 77(8): 2582-2588.

Spoelstra, S. F., P. G. Van Wikselaar, and B. Harder. 1992. The effects of ensiling whole crop maize with a multi-enzyme preparation on the chemical composition of the resultant silages. J. Sci. Food Agric. 60: 223–228.

Tabacco, E., S. Piano, A. Revello-Chion, and G. Borreani. 2011. Effect of Lactobacillus buchneri LN4637 and Lactobacillus buchneri LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. J. Dairy Sci. 94:5589–5598.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds. S0022-0302(91)78551–2

Weinberg, Z. G., and Y. Chen. 2013. Effects of storage period on the composition of whole crop wheat and corn silages. Animal Feed Science and Technology 185.3-4: 196-200.

Weiss, K., and H. Auerbach. 2012. Occurrence of volatile organic compounds and ethanol in different types of silages. Pages 128–129 in Proc. XVI Int. Silage Conf., Hämeenlinna, Finland. K. Kuop-pala, M. Rinne, and A. Vanhatalo, ed. MTT Agrifood Research Finland, University of Helsinki, Helsinki, Finland.

Weiss, K., B. Kroschewski, and H. Auerbach. 2016. Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. J. Dairy Sci. 99:8053–8069.

Weissbach, F. 2009. Correction of dry matter content of silages used as substrate for biogas production. Pages 483-484 in Proceedings of the 15th International Silage Conference, US Dairy Forage Research Center, Madison, WI.

Wood, W. A. 1961. Fermentation of carbohydrates and related compounds. The Bacteria. (2): 572-601.

Ying, H. L., N. Borjigin, and Z. Yu. 2017. Effect of inoculants and fibrolytic enzymes on the fermentation characteristics, in vitro digestibility and aflatoxins accumulation of whole-crop corn silage. Grassland science, 63(2): 69-78.