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Nutritional composition and ruminal degradability of corn silage (*Zea mays* L.) with addition of glycerin in silage

Composição nutricional e degradabilidade ruminal da silagem de milho (*Zea mays* L.) com adição de glicerina na ensilagem

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Abstract

The objective was to study the corn silage with addition of 0, 5, 10, 15 and 20% glycerin, ensiled in experimental silos of PVC. At the opening of the silos there were evaluated the chemical composition, *in vitro* digestibility of dry matter (IVDDM) and cell wall (IVDCW) and aerobic stability through measures of temperature and pH of silage at 0, 24, 48, 72, 96 and 120 hours. *In situ* degradability of dry matter, crude protein and neutral detergent fiber of silages were evaluated in fistulated cattle using samples incubated at 0, 2, 4, 6, 12, 24, 48 and 96 hours. The experimental design was completely randomized design and statistical analyzes were performed using Bayesian inference. Increases were observed in DM, mineral matter (MM), non-fibrous carbohydrates (NFC) and reductions for neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and ether extract (EE) as the inclusion of glycerin was higher. The content of total digestible nutrients (TDN) estimated in treatments with 5, 10, 15 and 20% glycerin, were superior to the control ($P<0,05$) range of 2.13, 8.95, 10.33 and 13.13% respectively. The temperature and pH of the silage with 10 and 15 and 20% glycerin remained lower than those levels ($P<0.05$) measured during silage and 20% glycerin showed high stability after 120hours. The IVDDM and IVDCW in corn silages with 15 and 20% were higher than the other ($P<0,05$). The effective degradability of DM fractions in passage rates of 2, 5 and 8% / h was better in silage with 15% and 20% glycerin. Glycerin has proved to be a great additive for corn silage being able to enrich the energy density, improving the degradability of DM and maintaining the aerobic stability of silage.
Key words: Digestibility of dry matter, energy, aerobic stability, glycerol, pH, temperature

Resumo

Objetivou-se estudar a silagem de milho ensilada com 0, 5, 10, 15 e 20% de glicerina, em silos experimentais de PVC. Foi avaliada a estabilidade aeróbia por medidas de pH e temperatura da massa ensilada (TME) às 0, 24, 48, 72, 96 e 120h. Foi avaliada a composição química e a digestibilidade *in vitro* da matéria seca (DIVMS) e da parede celular (DIVPC). Em três bovinos fistulados avaliou-se a degradabilidade *in situ* da matéria seca (MS), e a porcentagem de desaparecimento da proteína bruta (PB) e da fibra em detergente neutro (FDN) incubadas em 0, 2, 6, 12, 24, 48, 72 e 96h. O delineamento experimental foi inteiramente casualisado e as análises estatísticas realizadas por Inferência Bayesiana.

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Foram observados aumentos de MS, CNF, matéria mineral e a redução de FDN, PB, fibra em detergente ácido e extrato etéreo à medida que se aumentou a inclusão da glicerina. O NDT estimado nas silagens com 5, 10, 15 e 20% de glicerina, foi superior ao controle (P<0,05) variando de 2,13; 8,95; 10,33 e 13,13%, respectivamente. O pH e a TME das silagens com 10, 15 e 20% de glicerina se mantiveram inferiores (P<0,05) aos com 0 e 5% de glicerina no período avaliado, destacando-se a silagem com 20% de glicerina com maior estabilidade até 120h. A DIVMS e DIVPC nas silagens com 15 e 20% foram superiores (P<0,05) as com 0, 5 e 10% de glicerina. A degradabilidade efetiva da MS nas taxas de passagem de 2, 5 e 8%/h foram melhores na silagem com 15 e 20% de glicerina (P<0,05). A glicerina demonstrou ser um aditivo capaz de enriquecer a densidade energética, melhorar a degradabilidade ruminal da MS e manter a estabilidade aeróbia nas silagens de milho.

Palavras-chave: Digestibilidade da matéria seca, energia, estabilidade aeróbia, glicerol, pH, temperatura

Introduction

Forage conservation is an effective strategy used by the productive chains of beef and dairy cattle, not only to meet the demands of cattle in times of drought and winter but also to improve the energy density of diets reflecting improvements in productivity. Corn silage, widespread among milk producers, is more and more present in the properties of beef cattle, used for supplementation of the whole herd, even in the stages of rearing, growing and fattening.

Corn is one of the most used plants for silage for its productivity and adaptation to practically all kinds of climates and soils, and also for its great dry matter and soluble carbohydrates content which provide ideal conditions for its conservation in the form of silage (ALMEIDA, 2000) producing quality food and good acceptance by animals. Corn silage has high energy value when compared to other materials with strategic roughage for cows with high milk production.

Dairy cows with high milk production in early lactation are not able to consume enough energy leading them to the mobilization of body reserves in an attempt to overcome this deficit. However, the mobilization of the adipose tissue in excess can cause metabolic disorders such as ketosis and fatty liver (NRC, 2001).

By reducing the DMI during the transition period, a diet with high energy density and protein is necessary to meet the demands of this period and reduce the risk of metabolic disorders (NRC, 2001), even with the reduced food consumption.

To formulate a high energy density diet, the use of readily fermentable carbohydrates is necessary to provide an increase to the formation of propionate, stimulating the production of insulin to reduce the mobilization of the tissue, minimizing the metabolic disorders, common during this period (GRUMMER, 1995). However, providing very high amounts of concentrates to cows in early lactation can cause problems such as reduced fat in the milk, acidosis, and depression in the fiber digestibility and a decrease in the dry matter intake (VAN SOEST, 1994).

Fat supplements have been used in diets for lactating animals with the goals of increasing milk production and reduce body fat mobilization. Most food used in ruminant feeding contains low proportions of lipids, with values ranging from 1 to 4% of DM (VAN SOEST, 1994).

Palmquist and Jenkins (1980) suggested that the inclusion of lipids in the diet of ruminants be limited up to 5% of the total of DM, whereas rumen microorganisms do not possess physiological mechanisms to digest them efficiently. One of the main deleterious effects of the inclusion of high concentrations of lipids is the reduction in the ruminal fiber digestion (WETTSTEIN; MACHMÜLLER; KREUZER, 2000).To minimize these effects, it has been suggested the use of ruminally inert fats that can increase energy intake without adversely affecting ruminal digestibility of the dietary fiber (NOCEK, 1995).

A good alternative to fat and readily fermentable carbohydrates to increase the energy density of the ruminants diet can be glycerin. Südekum (2008) proposed that glycerol could replace rapidly fermentable carbohydrate and the sweet taste can improve the ingestion of diets with less palatability containing, for example, extensively fermented silages. The same author estimated in approximately 9.5 MJ / kg (2.27 Mcal / kg) of net energy for lactation.

Abo El-Nor et al. (2010), did not observe changes in the pH of artificial fermenters in *in vitro* trials, in which corn was replaced with glycerol in the diet at 0, 36, 72 and 108 g / kg DM. This is another important feature of glycerin that possibly ranks it in the class of rapidly fermentable carbohydrate of high energy content without starch that does not cause a decrease in the ruminal pH, something common to starchy carbohydrates.

The research on the use of biodiesel from glycerin as a component in animal feed has advanced greatly, due to the high production of biodiesel and glycerin surplus, being more recent its addition in the silage.

Even with optimal nutritional characteristics of corn, the way the ensiling process is performed can change its nutritional value due to losses which occur during the fermentation process and the usage phase after opening the silo.

In the usage phase, the ensiled material is exposed to oxygen which facilitates the action of spoilage microorganisms, reducing soluble sugars and lactic acid, which results in increased pH, reduced digestibility and energy content (JOBIM; GONÇALVES, 2003). Martins et al. (2014), when assessing the corn and sunflower silages with 0, 15, 30 and 45% of glycerin did not observe differences in pH at the opening of the silages.

Additional studies on nutritional value, aerobic stability, dry matter losses, and fermentation characteristics of silages with added glycerin from biodiesel are needed to enable better use of it in ruminant feeding. This research aimed to study the effects of adding five levels (0, 5, 10, 15 and 20%) of semipurified glycerin to corn silage, evaluating the chemical-bromatological composition and aerobic stability of the silages.

Material and methods

The experiment was conducted at the Experimental Farm of Iguatemi, State University of Maringá (UEM), Paraná, located at 23 º 25' south latitude, and 51 º 57' longitude west of Greenwich and 550 m of altitude and chemical analyzes were performed at the Laboratory of Animal Nutrition, Department of Animal Science at UEM (LANA) between October 2011 and May 2012.

The corn, Coodetec-CD 250 RR hybrid, was planted with density of 60,000 plants / ha and with fertilization according to soil analysis.

The cutting was performed with JF 92Z10 silage cutter machine, regulated to average particle size of 10 mm. After cutting, on 16/03/2011, the material was taken to a covered shed, cemented and covered with plastic tarp where it was distributed into five piles. Then the semi-purified glycerin from biodiesel was added in the proportion of 0, 5, 10, 15 and 20% (based on fresh weight) and manually homogenized.

The material was ensiled in experimental PVC silos with 20 cm in diameter and 40 cm in height, compressed to the specific mass of 600 kg/m^3 and then covered with a polypropylene plastic tarp and sealed with tape.

Four replications were performed for each level of glycerin inclusion, totaling 20 experimental silos, which we restored in a shed for 68 days.

The medium purity glycerin, from the production of biodiesel from soybean oil was acquired at the company TCS-Meridional (Ponta Grossa PR) and analyzed by the Institute of Technology of Paraná - Biofuels Division (Tecpar), Curitiba. The chemical composition of the glycerin used in the silage contained: 82.54% of glycerol, 10.23% of water, 120ppm of methanol, 1.3% of sodium, 7.4% of ashes and pH of 5.8.

After a period of 68 days of storage, the silos were opened and 15 cm from the top was discarded. A 1kg sample was collected from each silo and dried in a drying oven with forced ventilation at 55 º C for 72h and grounded in mesh sieve with 2 mm in a "Willey" type of mill, then and stored in hermetic plastic pots, identified for further analysis.

The remaining corn silage in the silos was exposed to air, covered with tulle type fabric, where the values of potential hydrogen (pH) and temperature of the ensiled mass (TEM) was read. The pH and TEM measurements were performed at the opening of the silo and then every 24 hours at times: 0, 24, 48, 72, 96 and 120h to assess the aerobic stability of the material. The pH was measured using a bench top digital pH meter from aqueous extract of the silage (CHERNEY; CHERNEY, 2003). The TEM of the silage exposed to air was measured with a digital thermometer with a penetration probe, Gulterm 180® (Gulton, São Paulo), positioned in the geometric center of the forage mass of each experimental silo. The ambient temperature, taken as reference, was recorded at the same time as the pH and TEM measurements.

In the samples the content of the dry matter (DM), crude protein (CP), ether extract (EE), mineral matter (MM) AOAC (1990), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined (VAN SOEST; ROBERTSON; LEWIS, 1991). The *in vitro* digestibility of the dry matter (IVDDM) and *in vitro* digestibility of the cell wall (IVDCW) were also determined. The IVDCW was calculated by the difference between the amount of incubated NDF and the residue left after determining the NDF of the incubated material.

For the IVDDM and IVDCW determination, approximately 0.25g of the sample were weighed and placed in sealed bags (F57) previously washed with acetone and then incubated at 39.5 ° C, using an *in vitro* incubator, TE-150 (Tecnal®), according to Goering and Van Soest (1970).The inoculant was obtained from castrated Holstein bovine, fitted with rumen cannula receiving diet based on corn silage.

The experimental design was completely randomized and statistical analyzes of variables were performed by means of Bayesian inference described in Rossi (2011). It was considered that the response (Y_i) follows Normal distribution, that is, $Y_i \sim N(\mu_i, \sigma_i^2)$, $i = 1, 2, ..., n_j$, for *j*-th treatments for each μ_i and σ_i^2 *a priori* non-informative distributions were considered, respectively, $\mu_i \sim N(0.10^6)$ e $\sigma_i^2 \sim Gamma(10^3, 10^3)$. Multiple comparisons were performed between the *a posteriori* distributions of the averages. Considering the 5% level of significance, treatments whose credibility intervals for the mean differences did not include zero were not significant. For the linear regression $Y_i = \beta_0 + \beta_1 Treatment + \varepsilon_i$, assuming $\varepsilon_i \sim N(0.\sigma^2)$, the same specifications applied in the *a priori* distributions were used for the parameters.

The posteriori marginal distributions for all parameters were obtained through the *BRugs* package program R (R Development Core Team, 2012). 510,000 values were generated in a MCMC (Monte Carlo Markov Chain) process and observing a discard sample period of 10,000 initial values. Thus, the final sample taken in leapsofsize10 contains 50,000 generated values. The convergence of the chains was verified by the Heidelberger and Welch (1983) criterion in the *coda* package from R package.

Three castrated Holstein steers, fitted with rumen cannula and an average weight of 420 ± 30kg were used to determine the ruminal degradability of the DM, the disappearance percentage of CP and NDF. The animals were adapted with sugarcane silage and concentrate for ten days on a diet that had roughage / concentrate ratio of 70:30 (VANZANT; COCHRAN; TITGEMEYER, 1998).

The ruminal degradability of the DM of the silage and its respective degradation rates were estimated by the *in situ* technique, using nylon bags measuring 10 cm x 20 cm, with pore diameters of

53 microns, as recommended by Vanzant, Cochran and Titgemeyer (1998). Approximately 6 g samples were placed in each bag, closed and tied with rubber band. On incubation days, the bags were attached to a stainless steel cylindrical bar (600 g) suspended by a 60 cm chain that was attached to a nylon rope tied out of the ruminal fistula.

Incubation times were 0, 2, 6, 12, 24, 48, 72 and 96 and the nylon bags were placed in the rumen in order to achieve the desired times, being removed all at once. After being removed from the rumen, the nylon bags were immersed in ice water to stop microbial fermentation. Then they were washed in running water for 10 min. together with the nylon bags referring to time 0 of incubation. After these procedures, all nylon bags were placed in a forced air drying oven at 55 º C for 72hand later weighed to obtain the weight of the dry residue. Next the NDF (VAN SOEST; ROBERTSON; LEWIS, 1991) and CP (AOAC, 1990) content of the dry residue were analyzed and the disappearance percentage of the NDF and crude protein.

The DM degradability of corn silage was adjusted by nonlinear regression, which predicts the potential degradability (y = PD) of food through the model proposed by Mehrez and Ørskov (1977), as follows:

Equation (1): $y_{ijk} = a_k + b_k(1 - e^{-c_{ik} t_j})$,

i-animal : 1, 2, ... , N;

j-time : 1, 2, ... , J;

k-treatment : 1, 2, ... , K;

y is the percentage of the degraded nutrient after time *t* (in hours);

a is the curve intercept or the soluble fraction of the material in the nylon bag;

b is the potentially degradable fraction of the material in the nylon bag after time 0;

c *i*s the fractional rate constant of degradation of the potentially degradable fraction b;

t is the incubation time in the rumen, in hours.

To estimate the effective degradability (ED) the Ørskov and McDonald (1979) model was used:

Equation (2): $DE = a + \frac{bc}{c + k^*}$,

In which k * is the passage rate of solids in the rumen, whose value was set at 2, 5 and 8% per hour.

The modeling follows the Bayesian approach (ROSSI et al., 2010; ROSSI, 2011), it was considered that the observations follow Normal distribution, that is, $y_i \sim N\left(f(t_i);\sigma_e^2\right)$, in which *f (ti) the nonlinear function is* proposed in (1).

For the parameters a and b*, a priori* non-informative normal distributions were considered, that is, *a, b* ~ N(0,10^6) and for c, a Gamma distribution, also not informative, restricted to the interval (0,1), that is: *c* ~ Gama (10^3,10^3)$I_{(0,1)}$.

For $\sigma^2_{e,}$ a gamma distribution was assumed, i.e.σ^2_e ~ Gamma (10^3,10^3). *A posteriori* marginal distributions were obtained through the *BRugs* package program R (R DEVELOPMENT CORE TEAM, 2012).

For each parameter, 310,000 values were generated in a MCMC (Markov Chain Monte Carlo) process, considering a sampling period discard of 10,000 initial values. Thus, the final sample obtained in leaps at every 30 values, contains 10,000 generated values. The chains convergence was verified by the *coda* package of program R, by the Heidelberger and Welch (1983) criteria.

To compare treatments, multiple comparisons were made between the *a posteriori* distributions of the averages of the parameters of interest. It was considered as different, at the level of 5% of significance, the treatments whose credibility intervals for the mean differences did not include zero.

Results and Discussion

In assessing the aerobic stability of the silage, it was found that the average temperatures of the silage with 10, 15 and 20% of glycerin remained lower (P <0.05) than those with 0 and 5% of glycerin, highlighting the level with 20% of glycerin which remained stable during the evaluation period (Figure 1).The average temperature of the ensiled mass of the corn silage control was 1.98 ° C higher than the average temperature of the corn silage with 20% of glycerin. These results indicate that, possibly, there was reduced action of common spoilage microorganisms during air exposure (JOBIM; GONÇALVES, 2003) and therefore less heat production in the ensiled mass.

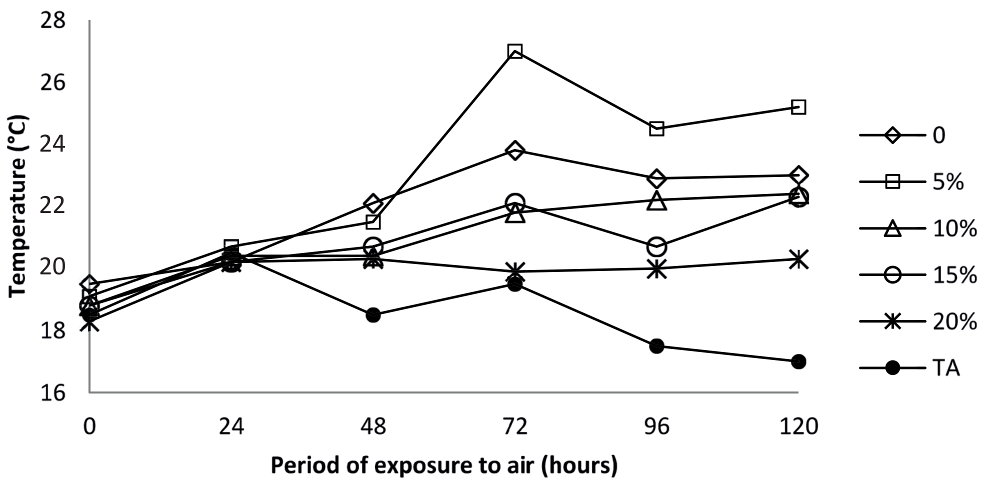
Table 1. Bayesian estimates for average and standard deviation of temperature and pH of corn silage with and without glycerin, at 0, 24, 48, 72, 96 and 120h.

Item	Glycerin level				
	0	5%	10%	15%	20%
TEM (ºC)	21.72a	21.52a	20.04b	20.05b	19.74b
(standard deviation)	0.5103	0.6952	0.3137	0.2340	0.2552
pH	4.803a	3.990ab	3.810b	3.702b	3.582b
(standard deviation)	0.4298	0.3016	0.1286	0.0946	0.0741

a, b, c Averages followed by different letters are statistically different by Bayesian comparisons (P <0.05). pH= potential hydrogen. TEM= temperature of the ensiled mass.

There was an increase of the silages temperature of similar magnitude up to 24 hours of exposure to air (Figure 1). From this point, the control silage and those with the addition of 5% of glycerin showed higher heating rate compared to the other treatments. However the corn silage with the addition of 20% of glycerin remained with stable temperature during the observed period of 120h.

Figure 1. Temperature curves of corn silage exposed to air for levels 0, 5, 10, 15 and 20% of glycerin and ambient temperature curve (AT) measured at 0, 24, 48, 72, 96 and 120h.



The average pH values also remained lower (P< 0.05) in the corn silage with 10, 15 and 20% of glycerin, compared to the control, however without difference in the level of 5% of glycerin.

Silages with 10, 15 and 20% maintained the pH lower than the control and the one with 5% of glycerin, possibly due to lower activity of aerobic organisms which invariably cause pH elevation as a result of lactic acid consumption. According to McDonald, Henderson and Heron (1991), the lactic acid degradation causes an increase in the silage pH, which, in turn, causes the growth of many other spoilage organisms.

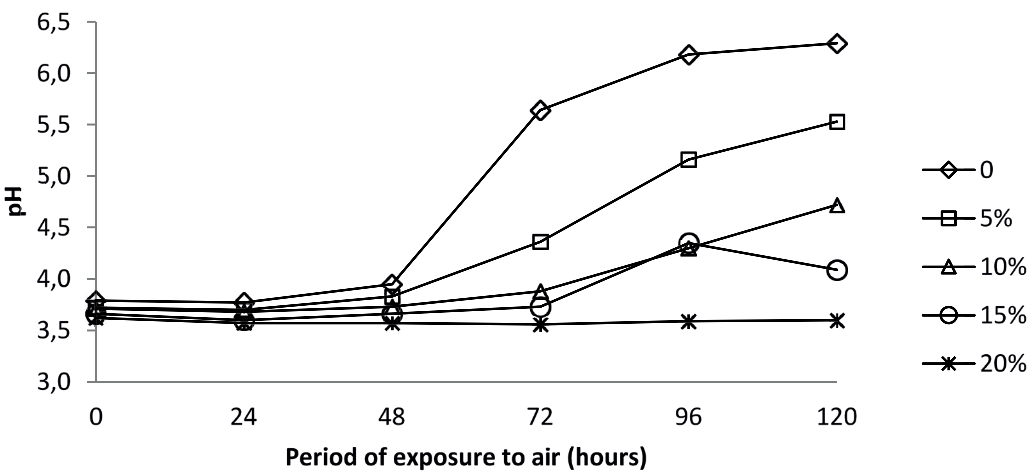
The result is consistent with the observed in the temperature of the silage which also showed less variation (Figure 1). This occurred, possibly because there has been less action of yeasts and less use of lactic acid, maintaining the low pH during the evaluated period. Yeasts are facultative anaerobic microorganisms in aerobic conditions, many species degrade the lactic acid into carbon dioxide (CO^2) and water (H_2O) (MCDONALD; HENDERSON; HERON, 1991).

Figure 2 shows that the control silage and those with the addition of 5% of glycerin demonstrated severe increase in pH after 48h of exposure to air. The pH increase at the levels of 10 and 15% of glycerol was only observed after 72h, and the silage with 20% of glycerin kept low pH until the sixth day of observation.

It is possible to infer that the silages with 10, 15 and 20% of glycerin when exposed to air, have lower losses of soluble sugars and lactic acid and, therefore, smaller changes in digestibility and energy content.

The results found in this study indicate that the corn silages with 10, 15 and 20% of glycerin were efficient in maintaining the pH more acid and the aerobic stability of the ensiled material after opening. This behavior is might occur since the glycerin does not constitute a good substrate for the growth of yeasts, fungi and other spoilage organisms of the silage.

Figure 2. pH Curves of corn silage exposed to air at the levels of 0, 5, 10, 15 and 20% of glycerin observed at 0, 24, 48, 72, 96 and 120h.



It was observed, in the chemical composition of the corn silages with various levels of glycerin (Table 2) that the levels of DM, NFC, TDN and MM increased with the addition of increasing levels of glycerin. On the other hand, the crude protein, neutral detergent fiber, acid detergent fiber and ether extract contents decreased. These results are similar to those found by Martins et al. (2014) that observed increase of dry matter, non-fiber carbohydrates and total digestible nutrients and reduction of crude protein levels, neutral detergent fiber and acid fiber, due to the addition of glycerin (0, 15, 30 and 45%) in the corn silage.

The variation in the DM content was observed by the addition of the glycerin which has a density of 1,2613 g cm-3 (200C) and its hygroscopic properties (IUPAC, 1997) connect to the water molecules of the ensiled material, increasing the density of the silage.

The CP content decreased (P <0.05) as the levels of glycerin increased (Table 2).This fact occurred due to a dilutive effect on the CP content by the absence of proteins and low occurrence of nitrogen compounds in the glycerin. The same dilutive effect can be observed in the NDF, ADF and EE content lower than the control (P <0.05) by the absence of fibers and low lipid content of the semi-purified glycerin.

The MM contents increased (P <0.05) with the inclusion of glycerin, by the ashes (7.4%) and sodium (1.3%) contents of the glycerin used in this experiment. The mineral content of the glycerin is derived from residues in the transesterification and purification process of the glycerin from biodiesel with sodium methylate and bases. Semi-purified glycerin contains mineral elements such as phosphorus, sulfur, magnesium, calcium and sodium which may be used as substrate for rumen microorganisms during fermentation processes (THOMPSON; HE, 2006).

Table 2. Bayesian estimates for the chemical composition averages of the corn silage with 0, 5, 10, 15 and 20% of glycerin.

Glycerin level	Averages (%)							
	DM	CP	NDF	ADF	EE	MM	NFC*	TDN**
0	31.59c	6.15a	52.60a	30.32a	2.54a	3.13c	35.99a	76.54a
5%	34.36c	5.39a	47.74a	29.44a	1.99bc	4.21b	40.83a	78.17a
10%	36.84b	5.09a	44.49ab	23.32b	2.01b	4.26ab	44.21c	83.39b
15%	39.96ab	4.51b	38.51bc	20.88c	1.71bc	4.63a	50.64bc	84.45b
20%	42.78a	4.30b	36.29c	19.19c	1.48c	4.43ab	53.54b	86.59c

a, b, c Averages followed by different letters are statistically different by Bayesian comparisons (P <0.05). Linear regression: Dry Matter (DM: y = 31.52 + 0.56 T; R^2 = 0.92); Crude Protein (CP y = 5.68 to 0.07T; R^2 = 0.45); Neutral Detergent Fiber (NDF y = 52.30 -0.84T; R^2 = 0.70); Acid Detergent Fiber (ADF: y = 30.79 - 0.62T; R^2 = 0.78); Ether Extract (EE: y = 2.43 -0.48T; R^2 = 0.65); Mineral Matter (MM: y = 3.52 + 0.06T; R^2= 0.54); Non-fiber Carbohydrates (NFC: y = 36.06 + 0.90T; R^2 = 0.74) and Total Digestible Nutrients (TDN: y = 76.55 + 0.53T; R^2 = 0.65). * Estimated NFC, calculated by the equation NFC = 100 - (CP + EE + NDF + MM) (MERTENS, 1997); ** Estimated TDN, calculated using the equation: TDN (%) IVDDM = + (1.25 * EE) – MM, according to Van Soest (1994).

The NFC contents varied from 13.39; 22.83 and 40.64 to 48.66% for the levels 5, 10, 15 and 20% of glycerin, respectively, compared to the control. It is observed that there was an enrichment of the roughage in readily fermentable carbohydrates, as the inclusion of glycerin was higher, offsetting to some extent the losses that occur during ensiling and exposure to oxygen after opening the silo. Martins et al. (2014) when evaluating levels of inclusion of glycerin in corn silage observed that the NFC also increased linearly with values of 36.2; 44.8; 53.4 and 62.8% respectively.

In the present study, an increase (P <0.05) in the energy density of corn silage was found in which the estimated TDN varied increasingly (Table 2).Comparing the variation percentage of the estimated TDN content of the control silos to those with 5, 10, 15 and 20% of glycerin, the surplus differences of TDN were 2.13, 8.95, 10.33 and 13.13%, respectively.

The DM, NFC and TDN increments added to a reduction of NDF and ADF by dilution can be considered positive because they give the opportunity to correct the nutritional values of the ensiled material offsetting possible losses of quality in the ensiling processes, enabling glycerin as an important additive able to enrich the ensiled material.

The IVDDM results for the silage got higher according to the addition of glycerin and significant (P <0.05) for the levels of 10, 15 and 20% compared to the control level and the ones with 5% of glycerin, however, the best result was observed in the material with 20% of glycerin. There was no difference (P> 0.05) between levels 10 and 15% neither between the control silage and the ones with 5% of glycerin (Table 3).

Villalba et al. (2014) evaluating the ruminal kinetics of the rations with different levels of glycerin inclusion (0, 30, 60, 90, 120, 150, 180, 210 and 240 g kg^{-1} of DM) concluded that the *in vitro* digestibility of dry matte, organic matter and fiber increased linearly with the addition of glycerin, which agrees with the results of this study.

Table 3. Bayesian estimates of the averages for the *in vitro* digestibility of dry matter (IVDDM) and *in vitro* digestibility of the cell wall (IVDCW) of the corn silages with 0, 5, 10, 15 and 20% of glycerin.

Digestibility	Averages (%)				
	0	5%	10%	15%	20%
IVDDM (%)	74.49c	79.89c	85.13b	86.95ab	89.18a
IVDCW (%)	26.65c	32.39bc	4 0.19ab	39.35b	46.72a

a, b, c Averages followed by different letters are statistically different by Bayesian comparisons (p <0.05). IVDDM = *in vitro* digestibility of dry matter; IVDCW = *in vitro* digestibility of the cell wall; linear regression (IVDDM: $y = 77.04 + 0.65T$; $R^2 = 0.75$); (IVDCW: $y = 27.63 + 0.94T$; $R^2 = 0.71$).

It can be interpreted that the results for IVDDM increased due to the inclusion of glycerin which has high solubility and digestibility, however it cannot be said that there was improvement in the digestibility of fiber or cellular content of the ensiled corn.

The higher the level of glycerin, the lower the pH remained in the ensiled mass (Table 1). This data indicates that acid hydrolysis of the cell wall of the corn silage with glycerin might have occurred, thus influencing the positive response of the silages with glycerin for IVDDM.

The percentage increase of readily degradable portion in the rumen (***a***) was significant (P <0.05) as the level of glycerin in silage was higher (Table 4).

When comparing the control treatment with the one with 20% of glycerin, an increase of 54.5% is observed in the readily degradable portion (***a***), possibly due to high degradability and solubility of glycerin. The insoluble fraction but potentially degradable (***b***) was also reduced (P <0.05) as the level of glycerin increased in the silage.

Aroeira, Lopes and Dayrel (1996) reported that effective degradability (ED) of any food can be considered as the energy digested in the rumen, so the ingestion of food with increased degradability of DM, CP and fibers provided greater energy to microorganisms.

Table 4. Bayesian estimates averages for the model parameters of the effective degradability of the dry matter of the ensiled corn silage with levels of glycerin and passage rates of 2, 5 and 8% / hour.

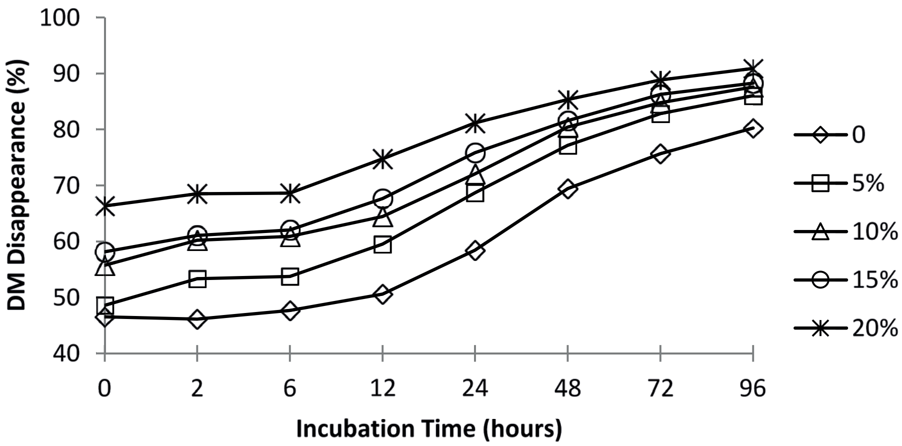
Glycerin level	Parameters						
	***a**(%)*	***b**(%)*	***c**(%/h)*	ED**k**=2%	ED**k**=5%	ED**k**=8%	σ_e
0	42.80e	49.44a	1.61d	64.89d	54.98d	51.21b	2.93
5%	49.04d	44.14b	2.04c	71.31c	61.88c	58.07b	2.43
10%	56.80c	34.69c	2.37b	75.56b	67.98b	64.76b	2.05
15%	62.93b	35.97c	1.71d	79.73ba	72.54ba	69.69a	4.29
20%	66.13a	25.61d	3.24a	81.94a	76.20a	68.99a	1.62

a, b, c, d, e Distinctive letters indicate significant differences between the treatment averages, through Bayesian comparisons in the level of 95% of credibility. a = readily degradable portion b = insoluble fraction but potentially degradable c = constant rate of degradability of fraction b / h. ED = effective degradability of the dry matter. k = is the rate of passage of solids in the rumen whose value was set at 2, 5 and 8% per hour. sd = standard deviation error.

The effective degradability of the DM at 2, 5 and 8% / h were higher in corn silages with glycerin in the readily soluble fraction, that even with high passage rate had higher degradability than in the control (P <0.05) at the levels of 15 and 20% of inclusion. This is possibly due from the increased presence of glycerin. The data in this experiment show that the inclusion of 20% had better effective degradability of dry matter at 2, 5 and 8%,percentage of readily degradable portion (***a***) and reduction of the insoluble fraction, but potentially degradable (***b***) combined with the best constant rate of the fraction degradability ***b%*** / h.

In Figure 3, it is observed that the disappearance of the dry matter was higher as the levels of glycerin increased, even in the first hours of incubation, especially at the level of 20% of glycerin, which reached the highest level compared to the other sat the time of 96h of incubation and the control with slower disappearance among treatments.

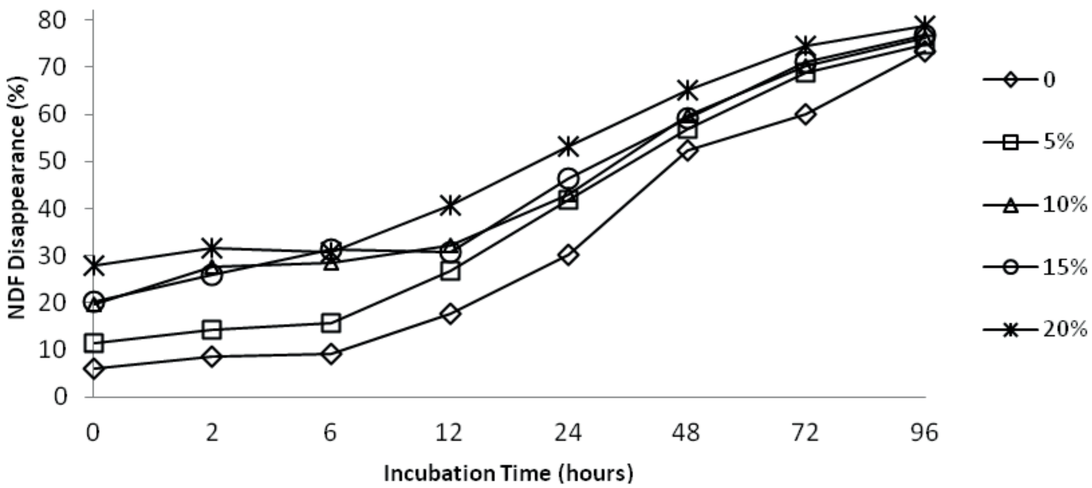
Figure 3. Disappearance curves of dry matter of corn silage, for levels of 0, 5, 10, 15 and 20% of glycerin incubated at 0, 2, 6, 12, 24, 48, 72 and 96 hours.



Possibly, the glycerin contained in the silage with 5, 10, 15, and 20% provided higher solubility in the rumen. This difference in the rate of disappearance between corn silages with and without glycerin can be an important factor to influence animal consumption, increasing the rate of passage and food utilization by animals as observed in this trial.

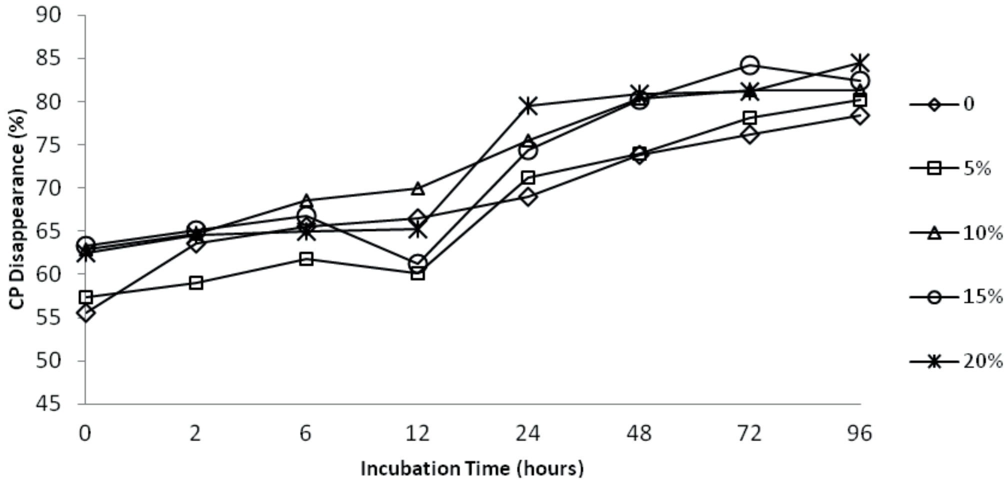
The disappearance curve of the NDF fraction (Figure 4) shows that the behavior of corn silage with glycerin was different from the rate of disappearance of the control silage. The corn silages with the addition of 10, 15 and 20% of glycerin at time 0 showed disappearance of the NDF fraction higher than expected, since the tendency at this time is of values close to 0, this is possibly related to the granulometry of the sample. Up to 6 hours of incubation the rate of disappearance of the control silage and the ones with 5% of glycerin differed slightly, remaining lower than the others until the time of 96h.

Figure 4. Disappearance curves of neutral detergent fiber (NDF) of the corn silage, for the levels 0, 5, 10, 15, and 20% of glycerin after incubation at 0, 2, 6, 12, 24, 48, 72 and 96h.



The NDF disappearance from the silage with 20% of glycerin increased starting from 6 hours of incubation and the levels of 10 and 15% increased after 12h of incubation, maintaining it higher than the control silage and those with 5% glycerin. Possibly the glycerin contained in the silages with 10, 15 and 20% of glycerin provided major input of energy to the rumen microbiota enhancing NDF degradation. This difference between the corn silages with and without glycerin can be an important factor to influence the increase in the DM intake, a result also observed for the rate of passage (Table 4).

Figure 5. Disappearance curves for crude protein (CP) of corn silage, for levels of 0, 5, 10, 15, and 20% glycerin after incubation at 0, 2, 6, 12, 24, 48, 72 and 96h.



The disappearance curve of the CP fraction (Figure 5) shows wide dispersion up to 12 h of incubation. All corn silage, equally had, at time 0, high disappearance rate, values that should also be close to 0, which can be related to granulometry and starch residues of the grains. The high rate of CP disappearance (Figure 5) from the silage could favor high concentration of ammonia in the rumen, especially for corn silages with 10, 15 and 20% which had approximately 60% of CP missing in the first 2h.

The CP disappearance did not differ (P> 0.05) among silages, with a tendency for greater disappearance in treatments with 15 and 20% of glycerin among the incubation times from 24 to 96h.

Conclusion

Improvement in the chemical-bromatological composition of corn silage with the addition of glycerin was observed, with increased *in vitro* digestibility of forage. The use of glycerin as additive in the corn silage, at levels of 10, 15 and 20% improved aerobic stability of the silage.

This study was approved by the Ethics Committee on Animal Use in Experimentation –CEAE of the State University of Maringa PR UEM on 04/03/2009, No 009/2009 and 020/2009 approval.

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