Nutritional additives in high moisture corn silage

Moyses Calixto Junior¹, Clóves Cabreira Jobim¹, Milene Puntel Osmari¹, Tamara Tais Tres¹

¹ Universidade Estadual de Maringá, Centro de Ciências Agrárias, Departamento de Zootecnia, Av. Colombo, 5970, Zona 7, CEP 87020-900, Maringá-PR, Brasil. E-mail: moyses@potensal.com.br; ccjobim@uem.br; mileneosmari@yahoo.com.br; tamaratais@zootecnista.com.br

ABSTRACT

Because of the high total digestible nutrient and dry matter (DM) digestibility contents, high moisture silage may be an alternative to maximize animal performance. Thus, the objective of this study was to evaluate the chemical composition, ruminal degradability and aerobic stability of high moisture corn silage (pure corn grain silage and soybean-, sunflower- or urea-enriched silages). Plastic barrels with capacity of 200 kg were used as experimental silos, which remained closed for nine months. The experimental design was completely randomized with four replications. After opening the silos, the chemical-bromatological composition and the quality of silages were determined. The addition of soybean or sunflower increased the crude protein content (CP) in 48.2% on average, and the neutral detergent fiber content in 60%, but decreased the starch content when compared to pure high moisture corn silage. Moreover, additions decreased the effective DM and CP degradability. Addition of urea decreased by 30% DM losses and improved the chemical composition and aerobic stability of the silage. In general, additives improved the chemical composition without affecting the silage conservation quality.

Key words: buffering capacity; sunflower; ammonia nitrogen; soybean; urea

RESUMO

Por apresentarem elevados teores de nutrientes digestíveis totais e digestibilidade da matéria seca (MS), as silagens de grãos úmidos podem ser uma alternativa para maximizar o desempenho dos animais. Desta forma, o objetivo foi avaliar a composição química, a degradabilidade ruminal e a estabilidade em aerobiose de silagens de grãos úmidos de milho (silagem de grãos de milho exclusivo e as ensiladas com adição de soja crua, de girassol ou de ureia). Foram utilizados como silos experimentais, tonéis de plástico com capacidade para 200 kg, que permaneceram vedados por nove meses. O delineamento experimental utilizado foi o inteiramente casualizado, com quatro repetições cada. Após a abertura dos silos foram determinadas as composições químico-bromatológicas e de qualidade das silagens. A adição de grãos de soja ou de girassol aumentaram 48,2%, em média, o teor de proteína bruta (PB) e em 60% o teor de fibra em detergente neutro das silagens, porém reduziu o teor de amido, em relação a silagem de grãos úmidos de milho puro. Além disso, diminuíram a degradabilidade efetiva da MS e da PB. A adição de ureia diminuiu em 30% as perdas de MS e melhorou a composição química e a estabilidade em aerobiose da silagem. De maneira geral, os aditivos melhoraram a composição químico-bromatológica, sem prejudicar a qualidade de conservação das silagens.

Palavras-chave: capacidade tampão; girassol; nitrogênio amoniacal; soja; ureia
Introduction

The cattle feeding, especially in feedlot, deserves special attention because this is the aspect responsible for the major part of production costs (Jobim et al., 2007). The use of silage is advantageous because it allows the preservation of feed quality and its consequent use throughout the year or during periods of forage restriction.

In this context, high moisture silage can significantly contribute to improving animal productivity; the economic importance of this item as a feed constituent is justified by its high total digestible nutrient content (TDN > 80%) and dry matter digestibility (DM) (Jobim et al., 2010a). Furthermore, grain silage can be a practical and economical way to store grain for use as concentrate in different animal production systems.

Among the high moisture silages, the one produced from corn stands out because this is the most used silage. Jobim et al. (2008) suggest the use of nutritional additives in high moisture corn silage production to increase the nutritional value, especially the content of crude protein (CP) and energy.

Among the additives that can be added to the corn grain silage are soybean, sunflower and urea. Ground soybean grain (*Glycine max* Merrill) has 38% of CP on average, 18% of oil and around 7% of fiber (Valadares Filho et al., 2006) and its use can improve the protein quantity and fermentation quality during ensiling (Jobim et al., 2010b).

The use of sunflower (*Helianthus annuus* L.) stands out as a good alternative in animal feeding because it presents high DM production, high ether extract (51.3%) and CP content (15.9%) (Jobim et al., 2008), besides presenting a good drought resistance. Thus, because of its high nutritional value, high moisture corn silage can result in less need of concentrate feed to ruminants supply.

Another way to enrich the high moisture corn silage is to add urea to improve the nutritional value of silage, since the grain does not have high protein content (Jobim et al., 2008).

The objective of the experiment was to evaluate the conservation and nutritional characteristics of high moisture corn silage with soybean, sunflower or urea as nutritional additives.

Material and Methods

The experiment was conducted at the State University of Maringá (UEM), on Arenito Campus, in Cidade Gaúcha, PR, and the Experimental Farm of Iguatemi, also part of the UEM.

Corn planting (hybrid DKB 747) was conducted in the Arenito Campus in Cidade Gaúcha-PR in soil classified as Caiuá sandstone and pedological cover made up of red latosol (Cunha & Nobrega, 1998). Fertilization at planting consisted in the application of 380 kg of NPK 08-30-16 chemical formulation, then 100 kg of urea in coverage, at 23 days after plant emergence. The harvest took place 121 days after planting, when corn had reached nearly 68% of DM, which is the appropriate point for grain silage (Jobim et al., 2008). After harvesting, the corn was threshed in a stationary threshing machine connected to the tractor power plug and was subsequently ground and sieved through 8 mm sieves.

Three nutritional additives mixed with the high moisture ground corn at the moment of ensiling were evaluated, arranged in a completely randomized design with four replications. Treatments were organized as follows: high moisture silage (HMCS); HMCS + 20% soybean (HMCS-S); HMCS + 20% sunflower (HMCS-SF) and HMCS +1% urea (HMCSU). Mixtures were calculated based on natural material.

Soybean or sunflower grains were added at the moment of processing corn grains, while urea was added later, according to the treatments. Once processed, the grains were placed in a feed mixer with capacity of500 kg for better homogenization. While filling and compacting experimental silos, a bacterial inoculant (Lalsil milho®, *Lallemand Animal Nutrition*) was added in all treatments at a dosage of 100 g/20 t of high moisture grain.

Silages were stored in plastic barrels with capacity of 200 kg each one. After compacting and proper sealing, the barrels were stored for nine months, and after this period the silos were opened for evaluation.

After opening the silos, silage samples (approximately 0.6 kg) were dried in a forced air oven (55 °C for 72 h) and after, were ground in sieves with 1mm of mesh for further chemical analysis. The DM, CP and ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), ammonia nitrogen and starch, were obtained according Detmann et al. (2012). The pH values were determined according to Cherney & Cherney (2003), while buffering capacity (BC) followed the Playne & McDonald (1966) method. Total losses of silages were determined according to Jobim et al. (2007), and aerobic stability evaluation was performed as described by Kung Jr. et al. (2000). The experimental design used in the aerobic stability evaluation was completely randomized, with four replications, in a split plot scheme, where the plots corresponded to treatments and the subplots, to the time of evaluation.

For rumen degradation evaluation, four male Holstein bovine animals, with 420 kg live weight on average and with ruminal cannula were used. The animals were adapted to feed for five days before ruminal incubation and they were fed twice a day (8:00 and 16:00 h). The diet was composed of corn silage (ad libitum intake), as forage, and concentrate based on corn and soybean meal (4.0 kg day-1).

Ruminal degradability of DM and CP, of the incubated silages, and their respective degradation rates were estimated by the technique of *in situ* nylon bag in the same way as described by Jobim et al. (2008). Nylon bags (Ankom Bar Diamond, Inc., Parma Idaho - USA) were used for ruminal incubation. The bags dimensions were 10 cm x 20 cm with pore diameter of 53 microns. Approximately 7 g samples (DM basis) were placed in each bag, closed and tied with an elastic rubber. The incubation times used were 0; 2; 6; 12; 24; 48 and 72 h. After removal, the bags were washed in running water and subsequently rinsed only with water in a washing machine in five cycles of 1 minute each.

The bags with samples of the time 0 were placed in a water bath at 39 °C for 60 min and then washed. After washed, all bags were dried in a forced ventilation oven at 55 °C for 72 h. The percentage of disappearance of DM and CP by incubation time was calculated by the proportion of orts in the bags after ruminal incubation.
Data from the rumen disappearance of nutrients, effective degradability of DM and CP were evaluated following the methodology described by Jobim et al. (2008). The results were arranged in a randomized block design with three replications. Results were submitted to ANOVA and Tukey test at 5% probability (SAS, 2001).

### Results and Discussion

Silage enriched with sunflower or soybeans had higher DM content (P < 0.05) than high moisture corn silage and high moisture grain silage + urea (Table 1). However, the average of DM content of these silages was close to 70%, regarded as suitable for the process conservation of ensiled corn grains.

High moisture corn silage + sunflower grain had higher DM content compared to other silages, which can be explained by the high DM content (94%) of sunflower added to the silage.

In the study carried out by Jobim et al. (2008), DM levels observed for soybean and sunflower were 91.7 and 94.9%, respectively, causing an increase in the DM content of the silage. Jobim et al. (2010b) evaluated the soybean inclusion levels in high moisture corn silage and observed a gradual increase in DM concentration (66 to 74%), being proportional to the increase of soybean in the silages. However, they observed no negative effect of higher DM content in the silage fermentation.

High moisture corn silage + soybean showed higher values of CP than the other evaluated silages (P < 0.05), with protein content 43% higher than the control silage. Soybeans usually have high levels of CP and when associated with silage they can meet the requirements of ruminants and promote adequate levels of production.

The crude protein content of silage + urea was similar to silage + sunflower, but superior to high moisture corn silage (P < 0.05), corroborating with Silva et al. (2014) who stated that urea can improve the nutritional value of silage by increasing the CP content. This can increase the rumen microbial growth, improve the use of fiber and increase forage digestibility.

In any case, the protein composition of high moisture corn silage produced can be considered satisfactory, since literature data show values of 6.96% to 10.1% (Itavo et al., 2006; Jobim et al., 2008). Changes in the protein content of high moisture corn silages are affected by the difference between hybrids, harvest time and contamination with corn cob (Mendes et al., 2015).

The inclusion of soybean or sunflower increased NDF levels (Table 1) at 3.5 and 5.5 percentage units, respectively, compared to high moisture corn silage (P < 0.05). However, when urea was added, the NDF value was similar to high moisture corn silage, precisely because the additive is a source of N only.

The ADF content was higher (P < 0.05) when soybean or sunflower was added to the high moisture corn silage (Table 1) due to the high ADF content of the grains added to the silages (corn, 2.8%; soybeans, 10.2% and sunflower, 19.4%, respectively). However, the high moisture corn silage, and soybean-enriched and urea-enriched silage were equivalent, with average values of 3.43% of ADF. In general, low ADF values correspond to the observed for high moisture grain silages, since fibrous portions of the plant such as stem, leaves and cobs are not included.

As for EE content, soybean-enriched and sunflower-enriched silages had greater (P < 0.05) content than the others, as a reflection of the high EE content that these grains usually present (Table 1). These silages showed EE concentrations above 6-7% of DM, which is considered the limit for the nutrient intake by ruminants. High levels of EE in the diet can decrease fiber digestion due to the possibility of fat involve feed particles, preventing the digestion on these surfaces, resulting in decreased DM intake (Van Soest, 1994). However, it should be considered that the silage represents the fibrous fraction of the diet, and for this reason, it is essential to monitor the EE content of concentrate fraction, so that at the end, the diet presents EE values lower than 7% of DM.

The starch content in high moisture corn silage was 66.7% higher (P < 0.05) than soybean-enriched and sunflower-enriched silages and 7% higher than urea-enriched silage (Table 1). The lowest concentration of starch in the silage with soybeans and sunflowers can be attributed to the low content of this component in the grains used, causing dilution of this nutrient due to the increase of other compounds present in these grains.

The soluble fraction (a) of silage, which comprises a combination of preserved material of the original forage and fermentation end products, had higher values for soybean-enriched and urea-enriched silages (Table 2). This corroborates with Diaz et al. (2013) who observed the addition of ammonia improved the soluble carbohydrates accumulation in the silage.

The reduction in the fraction “a” of silage (DM) with sunflower may have occurred due to the high EE content of sunflower, which made the solubilization of some components of DM difficult. This result corroborates with Jobim et al. (2008) who found that the inclusion of 20% of sunflower may have altered the rumen fermentation pattern, directing to the responses observed.

The fraction of DM potentially degradable in the rumen (b) shows the additives effect (P < 0.05). High moisture corn silage

### Table 1. Average dry matter (DM, %), crude protein (CP, %), neutral detergent fiber (NDF, %), acid detergent fiber (ADF, %), ether extract (EE %) and starch of high moisture corn silages according to the additive used

| Silages          | DM (%) | CP (%) | NDF (%) | ADF (%) | EE (%) | Starch |
|------------------|--------|--------|---------|---------|--------|--------|
| HMCS             | 63.05  | 11.2   | 7.5     | 2.5     | 4.6    | 68.44  |
| HMCSS            | 69.37  | 19.7   | 11.0    | 4.7     | 8.3    | 45.82  |
| HMCSSF           | 72.53  | 13.5   | 13.0    | 7.0     | 14.4   | 45.42  |
| *HSU             | 63.76  | 16.0   | 9.5     | 3.1     | 4.2    | 63.58  |
| wCV              | 1.5    | 6.6    | 9.8     | 23.1    | 12.7   | 1.92   |

HMCS = high moisture corn silage; HMCSS = high moisture corn silage + soybean; HMCSSF = high moisture corn silage + sunflower; HMCSU = high moisture corn silage + urea. *CV = Coefficient of variation. Values followed by different letters in the same column differ according to Tukey test (P < 0.05)
showed higher degradation rate (b), which can be explained by the smaller fraction “a”. However, sunflower-enriched and urea-enriched silages showed intermediate values, without significant difference between them (Table 2).

The differences observed between degradation rates of the fraction “b” can be attributed to differences in the silages chemical composition, especially the values of the NDF fraction: high moisture corn silage (7.5%), soybean-enriched silage (11.0%), sunflower-enriched silage (13.0%) and urea-enriched silage (9.5%). Although NDF values are considered low in relation to the fiber fraction, they can influence the degradation rate of the potentially degradable fraction in the rumen (b).

The degradation rate “c” of DM was different between silages; it was higher for urea-enriched silage, followed by high moisture corn silage and sunflower-enriched corn silage, and the rate was lower in soybean-enriched silage.

According to Silva et al. (2014), urea-enriched and ground corn silages have higher ED, probably because they have higher soluble fraction, since urea is 100% degradable and corn may present high ED as demonstrated in the present study (Table 2).

High moisture corn silage and urea-enriched silage had higher fraction “a” of the CP while soybean-enriched and sunflower-enriched silage had lower results and different from each other (Table 2). The fraction “a” result of the CP from urea-enriched silage is attributed to the high solubility of the nitrogen product.

According to Huhtanen (2013), plant and microorganism enzymes cause proteolysis and protein deamination during silage fermentation. This, in turn, may increase the rumen degradability of protein, expressed in larger values of the fraction “a”. However, the non-ammoniacal nitrogen could be a better substrate for rumen microorganisms than ammoniacal nitrogen. Soybean-enriched silage had the lowest fraction “a” to CP degradability, and also showed less N-NH₃ content (Figure 1), which may indicate lower proteolysis in the silo in the case of soybean addition.

Conversely, for the potentially degradable protein fraction in the rumen (b), soybean-enriched or sunflower-enriched silage showed higher values (P < 0.05; Table 2) as a result of lower values of the fraction “a”. Thus, addition of soybean or sunflower to high moisture corn silage resulted in slower degradation rate compared to other silages, possibly due to higher EE content (Table 1).

![Figure 1. Buffering capacity (m.eq NaOH/100 g DM), pH and ammonianal nitrogen (N-NH₃/N_NA) of high moisture corn silage (HMCS), high moisture corn silage + soybean (HMCSS), high moisture corn silage + sunflower (HMCSF), high moisture corn silage + urea (HMSCU)](image-url)
Because of urea’s high solubility, the “ED” for CP was higher for the silage that received this additive, similar (P > 0.05) to high moisture corn silage, in the different passage rates (2; 5 and 8% h⁻¹). Soybean and sunflower addition led to lower “ED” values in relation to silage without additives in all passage rates. The reduction in ED may be the result of the proteic profile of sunflower and soybean, and of the increase in the EE content of these silages, which corroborates the results obtained by Jobim et al. (2008).

In relation to buffering capacity (BC) and pH (Figure 1), urea-enriched silage was the one with the highest BC (5.00 m.eq NaOH/100 g DM), followed by soybean-enriched silage. The alkaline environment generated by the N degradation during the ensiling process, with hydrolysis of urea to ammonia by urease enzyme, may lead to high resistance to lowering the pH.

On the other hand, the lower BC values of high moisture corn silage and the silage with sunflower (2.80 and 1.96 m.eq NaOH/100 g DM, respectively) show the low buffering capacity of these feeds.

The BC is an important parameter for silage conservation quality, not only in the storage phase, but with indirect effects in the usage phase. When the plant has a high BC, the speed of pH lowering is slow and, therefore, losses in silage fermentation are higher, reducing the quality of the silage (Jobim & Nussio, 2013).

When silage is used, a rapid increase in pH leads to the appearance of undesirable microorganisms with active metabolism that produce heat and end up consuming the nutrients contained in the silage. The BC values observed in high moisture corn silages are considered low when compared to those observed in grasses and legumes, but the larger BC values favored higher pH values. Literature data have shown mean values of 31 m.eq of NaOH/100 g of DM for corn silage (Oliveira et al., 2011), and 70.55 to 80.70 m.eq of NaOH/100 g of DM for legumes such as soybean (Dias et al., 2010).

The pH values of grain silages had little variability, with values from 4.05 to 4.40 (Figure 1). High moisture corn silage had lower values (4.05) when compared to others silages. The higher pH value of enriched silages may be explained by the higher amount of protein compounds due to addition of soybean, sunflower or urea. These have a negative influence in pH lowering by raising the BC, as these are variables that have directly proportional behavior, corroborating with Jobim & Nussio (2013).

Additives had an effect on the ammoniacal nitrogen (N-NH₃/Nₜₐ₅) of the evaluated silages. Values varied from 0.40 to 0.88% and the average value was 0.72% in relation to the total N (Figure 1). Soybean-enriched and sunflower-enriched silage had lower values (0.40% and 0.77%, respectively) than high moisture corn silage and urea-enriched silage, with values between 0.84 and 0.88% in relation to the total N, respectively.

The addition of urea resulted in higher N-NH₃/Nₜₐ₅ value in the silage, because besides being a non-protein nitrogen source, urea is also active in fermentative dynamics, changing the pH of the forage mass. However, the N-NH₃ values obtained in this study are considered low (Itavo et al., 2006; Morais et al., 2012), indicating low activity of enzymes and proteolytic microorganisms. Clostridial microorganisms are the ones that have greater proteolytic activity in silages. However, because of the high dry matter content of corn grains and the sensitivity to osmotic pressure (water activity) of these bacteria, it can be inferred that the proteolytic activity is small, especially in silage with soybeans and silage with sunflower, where the DM content is higher (Table 1).

As N-NH₃ is the product of clostridial fermentation, high values indicate intense proteolysis with consequent decline in the nutritional value of the ensiled material (Pereira et al., 2007). N-NH₃/Nₜₐ₅ values observed in this study are below those recorded by Itavo et al. (2006) and Morais et al. (2012), who observed values of 1.32% and 1.20%, respectively, but without the inclusion of soybean and/or sunflower. In a study conducted by Jobim et al. (2010b) where different levels of soybean addition (10, 20, 30 and 40%) to high moisture corn silage were evaluated, the average value of 1.21 of N-NH₃ was observed, which is higher than that observed in the present study.

Silage aerobic stability (Table 3) was not influenced by the additives (P > 0.05); an increase of 2 °C in relation to the room temperature was observed only after 41 h of exposure to air. Under these conditions, urea-enriched silage reached a maximum temperature of 25.7 °C after 89 h of exposure to air, which is the lower temperature compared to other silages, showing more aerobic stability.

Allen et al. (2003) explain that silages treated with ammonia have lower temperature, lower DM losses and lower pH than untreated silages, promoting greater aerobic stability, as ammonia is toxic to filamentous fungi and yeasts, thus proving able to reduce DM and carbohydrates losses.

There was an effect of additives on the pH during exposure to air; the high moisture corn silage reached a maximum of 5.6 at 192 h of aeration. High moisture corn silage showed greater deterioration speed as evident by greater DM losses during the period of exposure to air (50.4%), high temperature value of the mass (260.0 °C) and high pH values (5.6). Total losses in the period of aerobic stability evaluation (9 days) are considered high for the evaluated silages (39.3% on average) (Table 3). These losses are primarily composed of gases (CO₂) as a result of the action of decomposing microorganisms, particularly yeast.

| Variable* | HMCS | HMCSS | HMCSFF | HMCSU |
|-----------|------|-------|--------|-------|
| N² H 2 °C | 41   | 41    | 41     | 41    |
| Tmax     | 26.0  | 26.7  | 26.4   | 25.7  |
| N° H Tmax | 89   | 96    | 120    | 89    |
| N° D Tmax | 7    | 6     | 6      | 6     |
| pH Max   | 5.6  | 4.5   | 4.7    | 4.5   |
| N° H PH Max | 192  | 192   | 216    | 192   |
| N° D PH Max | 8    | 8     | 9      | 8     |
| DML (%)  | 50.4 | 29.8  | 41.3   | 35.7  |

Values followed by the same letters, in the line, do not differ from each other (P > 0.05). *)N° H 2 °C = number of hours to increase the temperature by 2 °C above the room temperature; Tmax = maximum temperature reached by the mass (°C); N° H Tmax = number of hours to increase the temperature by 2 °C; N° D Tmax = number of days to reach maximum temperature; pH Max = maximum pH reached; N° H pH Max = number of hours to reach the maximum pH; N° D pH Max = number of days to reach the maximum pH; DML = dry matter loss in 216 h.
Jobim et al. (2008) found a mean value of 12.26% of DM losses. However, the room temperatures which silages were exposed showed no significant changes during the evaluation period and they were lower than the ones found in the present study.

The deteriorated material may cause economic losses of nutrients, intake depression or the production of undesirable compounds, with consequent decrease in animal production. Thus, preventing aerobic deterioration of silage may increase the production efficiency of a farm (Kung Jr., 2010).

Silages that exhibit restricted fermentation are generally unstable when exposed to air because of the insufficient amounts of acid, especially acetic acid, that are produced to inhibit secondary microbial growth. The higher BC of the soybean-enriched silage (Figure 1) may have caused increased production of acids during fermentation, leading to lower DM losses (29.8%) during the evaluation of aerobic stability and also lower proteolysis, as evidenced by the lower N-NH₃ content.

After opening the silos, silages had low temperature, reaching a value close to 22 °C in 65 h of exposure to air (Figure 2). An increase in the temperature of all silages was seen after 72 h under aerobic conditions, until reaching 25.7 °C in 216 h after exposure to air. It was observed that silages had lower temperature than the ambient, indicating low microorganism activity. However, silages showed high stability, with similar behavior throughout the period of exposure to air.

On the first day after opening the silos, at the beginning of the deterioration process (Figure 2), high moisture corn silage showed low pH (4.06) compared to other silages. In this day, pH values were 4.30; 4.25 and 4.34 for soybean-enriched, sunflower-enriched and urea-enriched silage, respectively. In the period between the first and the sixth day, when silages reached 137 h of aerobic exposure, there was an increase in the pH value for all evaluated silages.

Significant increase in pH was observed in high moisture corn silage from the sixth day onwards (137 h), reaching a maximum of 5.57 after 192 h of aerobic exposure. This may be a result of volatilization and consumption of organic acids (lactic acid) by microorganisms as energy source for growth during the process of deterioration (Kung Jr., 2010). Thus, increases in the silage pH indicate deterioration and reduced aerobic stability. The other silages had lower pH variations and changes during the total period of exposure to air (216 h), which shows greater aerobic stability.

**Conclusions**

The addition of soybean, sunflower or urea into high moisture corn silage increased crude protein, neutral detergent fiber and ether extract of silages and reduced the starch percentage, but without compromising the quality of silage conservation.

The use of soybean or urea increased the buffering capacity of the silage and promoted higher pH, without negatively affecting silage stability.

The additives also improved aerobic stability and decreased DM losses by nearly 30%, a highly positive factor in the use of silages.

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