Scientific Paper

Effect of the incorporation of green manures of legumes and of microbial inoculation on the quality of *Zea mays* L. silages

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Abstract

Objective: To evaluate two managements of incorporation of green manure from four legumes and the use of microbial inoculants on the quality of *Zea mays* L silage in the Colombian dry tropic.

Materials and Methods: The experiment was conducted at the Motilonia Research Center, located in the Cesar Valley, Colombia. A randomized block design was applied, with split-plot arrangement. The integration of the factors season of incorporation of manure, type of green manure (four legumes, control without incorporation of the legume and control with nitrogen fertilization) and use or not of microbial inoculation of the silage, were evaluated as treatments. The dry matter, neutral detergent fiber and acid detergent fiber, crude protein, *in vitro* digestibility, pH, ammoniacal nitrogen and concentration of lactic, acetic and butyric acid, were determined.

Results: There was no effect of the incorporation season on silage quality. Higher content of dry matter (31,9 and 30,7 %), crude protein (8,9 and 8,0 %) and IVDMD (86,0 and 58,9 %) was found in the silo inoculated with regards to the non-inoculated one. In the parameters of fermentation, lower value of pH (3,6 and 4,0), NH_3 (38 and 4,4 %), acetic acid (1,9 and 2,4 %), butyric (0,3 and 0,4 %) and increase of lactic acid (7,7 and 6,25) were noted in the inoculated silage compared with the non-inoculated one.

Conclusions: Independently from the incorporation season and from the legume used as green manure, the inoculation of the silages improved the nutritional quality and their fermentation parameters.

Keywords: inoculation, forage legumes, nutritional value, silos

Introduction

Animal husbandry in the Colombian dry tropic is drastically affected by the dry periods that occur during the year (December-March and June-October), in which there is marked decrease of the forage production for feeding ruminants. As alternative to this situation, preserved feeds are produced, such as *Zea mays* L silage, which, because of depending on fertilization and irrigation, is a high-cost technology, which cannot be afforded by all farmers (Mojica-Rodríguez *et al.,* 2019).

As sustainable production strategy to cover the production of preserved forages, the use of green manures emerges, which has among its benefits the reduction of soil erosion, improvement of soil quality and increase of biodiversity in the cultivation area (Castro *et al.,* 2017), effects that have incidence on the production of the next crop. Nevertheless, the works conducted with green manure in different zones have been developed from the perspective of *Z. mays* as grain (Droppelmann *et al.,* 2017), and not as forage. Thus, it is necessary to study about the use of green manures for the production of forage crops aimed at silage, with the use of already evaluated technologies, which have as purpose improving the fermentation conditions of the silo.

Fischler and Wortmann (1999), in east Uganda, used *Crotalaria ochroleuca* L, *Mucuna pruriens* (L) DC, *Lablab purpureus*, *Canavalia ensiformis* (L) DC as green manures, and achieved higher grain yields of *Z. mays* from 50 to 60 % higher compared with the yields without green manure. In Kenya, after incorporating *M. pruriens, C. ensiformis, C. ochroleuca* and *L. purpureus*, the grain production of *Z. mays* increased from 35 to 100 % compared with the application of nitrogen fertilizer (Kinyua *et al.,* 2019).

In other studies, with the incorporation of legumes the production of *Z. mays* has increased up to 18 % over the control and even up to 10 % with the use

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of nitrogen fertilization (Scotta *et al.,* 2018). A successful example of the integration of green manure to the production of forage crops for animal feeding is the work carried out by CIAT (2008) in Nicaragua, with *Canavalia brasiliensis* (Mart). ex. Benth, in an intercropped planting system of *Z. mays*-Canavalia and grazing of harvest residues of *Z. mays*, where increase was obtained of milk production (10-15 %) and of the *Z. mays* grain (15-20 %). Besides the research focused on the production of *Z. mays* for silage production, studies have been conducted in which the improvement of the fermentation parameters through bacterial inoculants is documented (Castillo-Jiménez *et al.,* 2009), where an adequate decrease of pH (Tobia and Villalobos, 2004), low levels of NH_3 (Forouzmand *et al.,* 2005), increase in the production of lactic acid and decrease of the acetic and butyric acids (Blajman *et al.,* 2018), stand out. In some cases improvement of nutritional quality, specifically of protein, has also been reported (Espinoza-Guerra *et al.,* 2017).

From the fact that forages for conservation, produced with the use of green manure as source of nutrients could be included in livestock feeding systems during the forage scarcity periods, the use of microbial inoculants is added as an improvement factor of the preserved material. Thus, the objective of this study was to evaluate two managements of incorporation of green manure from four legumes and the use of microbial inoculants on the quality of *Z. mays* silage in the Colombian dry tropic.

Materials and Methods

Location and climate. The experiment was conducted at the Motilonia Research Center of AGROSAVIA, in the Cesar Valley micro-region, located at 10° 11' North latitude and 73° 15' West longitude, at 160 m.a.s.l. The mean annual temperature of this micro-region is 29 ºC and the average annual rainfall of 1 360 mm. The Cesar Valley is framed in the agroecological zone of the Colombian dry tropic.

Treatment and experimental design. The treatments with silage were included in a complete randomized block design, with split-plot arrangement. The main plot was the season, with an area of 608 m^2 (32 x 19 m), where the 20-m^2 subplots (4 x 5 m) were included with the legumes used as green manure and the respective controls (without the incorporation of the legume and nitrogen fertilization) and the subplot, with inclusion or not of inoculant in the silage. Each one of them had three replicas. Two main plots and six subplots were established, with three replicas for each experimental unit (36). The treatments are described below:

- Plot 1. Green manure sown at the beginning of the rains of the first semester was used and was incorporated at the end of the rains of that semester; *Z. mays* was planted at the beginning of the rains of the second semester. Subplots: 1) *C. brasiliensis* Mart. ex. Benth*,* 2) *Vigna unguiculata* (L.) Walp, 3) *Clitoria ternatea* L*,* 4) *L. purpureus* L, 5) control with application of 50 kg N ha^{-1} and 6) control without incorporation of legume.
- Plot 2. The green manure, planted at the end of the rains of the first semester was used and was incorporated at the end of the dry season, followed by the sowing of *Z. mays* at the beginning of the rains of the second semester. Subplots: 1) *C. brasiliensis* 2) *V. unguiculata* 3) *C. ternatea* 4) *L. purpureus* 5) control with application of 50 kg N ha^{-1} and 6) control without incorporation of legume.

Silage elaboration. In each subplot the forage of the indicator crop was harvested and six micro-silos were elaborated in 2-kg PVC tube. From them, three were inoculated, and three were left without inoculation. Inoculation was done with lactic-acid bacteria, in a concentration of 1,0 x 10^{15} CFU/g of forage at the moment of preparation of the micro-silos.

Variables measured in the silage. In the micro-silos (three per treatment) the nutritional quality was determined, after 60 days of fermentation of the forage. The following variables were measured in lyophilized samples: moisture (AOAC, 2016), ash, neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest *et al*. (1991); cell content by difference, crude protein (CP) by the Kjeldahl method (AOAC, 2016); *in vitro* digestibility according to Tilley and Terry (1963), pH with potentiometer (AOAC, 2016), ammoniacal nitrogen (AOAC, 2016); concentration of lactic, acetic and butyric acid by gas chromatography (Peters *et al*., 1989).

Statistical analysis. The response variables (nutritional quality of the silage) were subject to a variance analysis, after verifying the normality, independence and homogeneity assumptions. The differences among means were determined through Tukey's mean comparison tests with the program SAS®, version 9.4 (SAS Institute Inc., 2013). According to the utilized statistical design, when interaction appeared among the factors (season-legume-inoculation), the results were shown graphically. For the individual analysis of the factors tables were used.

Results and Discussion

The DM content was not affected by season or by the incorporated legume. It was higher in the treatments with inoculation ($p < 0,05$); it reached a value of 31,9 % and 30,7 % without inoculation (table 1).

Filya (2003) reported that the presence of inoculants of lactic-acid bacteria improved the stability of DM in silages of *Z. mays* and *Sorghum bicolor* (L.) Moench. This can be explained by the direct effect of bacteria on the activity of yeasts during the fermentative process, by decreasing their survival and inhibiting their growth (Reyes-Gutiérrez *et al.,* 2018).

Figure 1 shows the CP content, when a significant interaction appeared ($p < 0,05$) of the legume and the inoculation. The highest CP values were found in the treatments where the silage was inoculated. The CP ($p < 0,05$) was higher in the treatments of 0 nitrogen and *C. ternatea* (9,1 %) with inoculation, and the lowest values were found with *C. ternatea* and 0 nitrogen without inoculation (7,1 and 7,0 %). This could respond to the fact that the inoculation with lactobacilli accelerates the initial fermentation rate of lactic acid, reduces pH and generates a reduction in degradation (proteolysis) and loss of protein during the fermentation process (Silva *et al.,* 2017; Ertekin and Kızılşimşek, 2019).

The CP contents of this study are found in the range obtained by Villa *et al*. (2010), who reported between 8,1 and 9,0 % of CP. Nevertheless, they were higher than those reported by Lajús *et al.* (2020), who referred CP values of 6,5-7,5 %.

Table 1. Nutritional quality in the *Z. mays* silo, according to the studied factors (%).

Treatment	DM	CP	NDF	ADF	IVDMD
Season					
1	31,1	8,5	60,2	43,6	59,6
2	31,5	8,5	60,0	43,2	59,4
SE ±	0,349	0,004	0,095	0,110	0,886
P - value	0,009	0,849	0,553	0,385	0,562
Legume					
0 N	31,6	8,1b	59,5b	43,5	58,6
50 kg N	31,2	8,7a	61,4a	43,5	60,6
C. brasiliensis	31,5	8,9a	59,3b	43,6	59,3
C. ternatea	31,2	8,1b	59,4b	43,3	58,8
L. purpureus	31,6	8,7a	61,4^{a}	43,4	60,5
V. unguiculata	30,9	8,9a	59,4b	43,3	59,2
SE ±	0495	0,164	2,731	1,367	3,960
P - value	0,039	< 0,0001	0,002	0,998	0,024
Inoculation					
Without inoculant	30,7	8,1	61,0	43,6	59,0
With inoculant	31,9	9,0	59,2	43,3	60,0
SE ±	0,360	0,134	2,437	3,144	2,781
P - value	< 0,0001	< 0,0001	< 0,0001	0,497	0,013
Season x legume	NS	NS	S	S	NS
Season x inoculation	NS	NS	NS	NS	NS
Legume x inoculation	NS	S	S	NS	NS
Season x legume x inoculation	NS	NS	S	NS	NS

Means followed by different letters in the same column differ for $p < 0,05$
Season 1: green manure, sown at the beginning of the rains of the first semester and incorporation at the end of the rains of that semester.
Season 2: green manure, sown at the end of the rains of the first semester and incorporation at the end of the dry season.

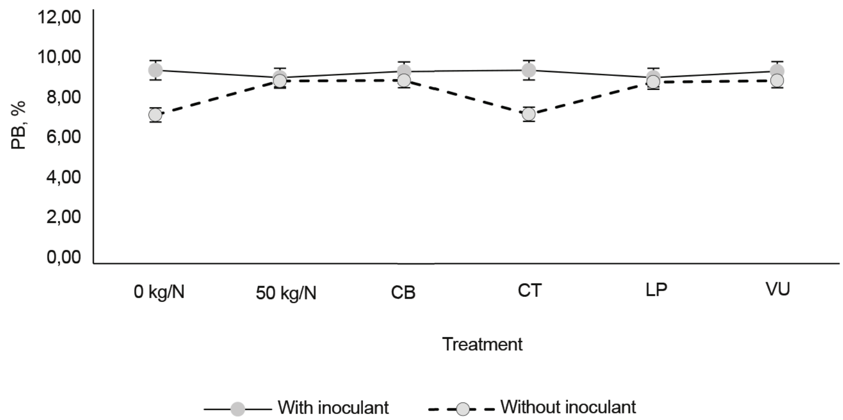

Figure 1. Interaction legume and inoculation for the protein content in *Z. mays* silages.
CB: *C. brasiliensis*, CT: *C. ternatea*, LP: *L. purpureus* and VU: *V. unguiculata*
p < 0,05

Besides the reduction in proteolysis, in this work the direct effect of inoculation on the protein content was observed, as described by Espinoza-Guerra *et al.* (2017), who found an increase from 6,5 to 10,2 % in *Z. mays* in the inoculated material. This increase is associated, in some cases, to the increase in microbial biomass due to the effect of inoculation (Miranda-Yuquilema *et al.,* 2017). However, in other studies with the same species no improvements were shown in the protein values related to the inoculation of the silage (Lajús *et al.,* 2020).

It has been documented that besides the use of inoculants, influences the inclusion of legumes, because an increase of CP from 8,5 to 10 % was found in *Z. mays* silos with the inclusion of *V. unguiculata* or *L. purpureus* (La-Guardia-Nave and Corbin, 2018).

For NDF there was significant interaction (p < 0,05) among season, type of legume and inoculation (fig. 2). Higher NDF content was observed in the treatment of *V. unguiculata* without inoculant (61,7 %) with regards to the treatment of *V. unguiculata* with inoculation (57,1 %).

Significant interaction was shown between season and type of legume (p < 0,05). The highest content was recorded in the treatments with chemical fertilization (50 kg N) in season 1 (61,7 %) and *L. purpureus* in 2 (61,7 %), and the lowest one in *V. unguiculata* (57,4 %) in season 2 (fig. 3).

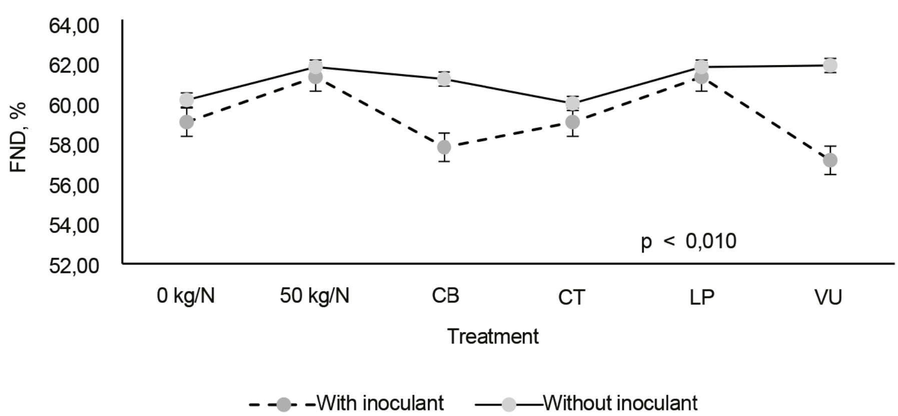

Figure 2. Interaction legume and inoculation for the NDF content in *Z. mays* silages.
CB: *C. brasiliensis*, CT: *C. ternatea*, LP: *L. purpureus* and VU: *V. unguiculata*
p < 0,05

The NDF value was associated to the three evaluated factors. The interaction of the legume with the inoculant was representative, where there was lower content in the treatments with the inoculation and the ones from incorporated legumes.

Phelan *et al*. (2015) state that the inclusion of legumes as green manure reduces the NDF content in grass crops, due to the N fertilization rate. The information found about the interaction inoculation and NDF content is diverse, and it mentions from the decrease due to the use of inoculants (Mier-Quiroz, 2009) to its increase (Castillo-Jiménez *et al.,* 2009).

The NDF contents recorded in this study are over the values reported by Silva *et al.* (2018) in *Z. mays* without inoculation (31,8-44,1 %), and those obtained by Lajús *et al.* (2020). Likewise, they exceed the ones declared by Silva *et al.* (2018) with inoculation.

Additionally, it should be taken into consideration that the NDF content can vary associated to other factors, such as harvest age, particle size, environmental conditions and relation between number of ears and *Z. mays* variety (Gallo *et al*., 2016).

In the ADF content, the response associated to inoculation was not so clear, and turned out to be significant (fig. 4) due to the effect of season and the incorporated legume ($p < 0,05$). This is contrary to the report by other studies, where there was direct effect of the use of inoculants in *Z. mays* silages, with an increase in the ADF content due to inoculation (Cubero *et al*., 2010).

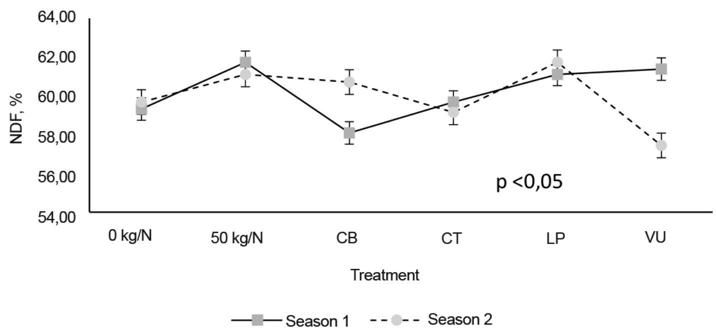

Figure 3. Interaction season and inoculation for the NDF in *Z. mays* silages.
CB: *C. brasiliensis*, CT: *C. ternatea*, LP: *L. purpureus* and VU: *V. unguiculata*
$p < 0,05$

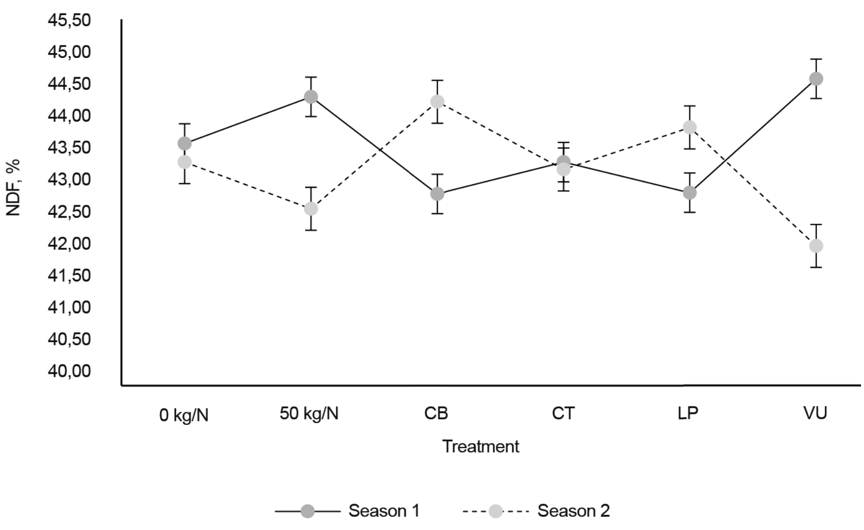

Figure 4. Interaction season and inoculation for the ADF content in *Z. mays* silage.
CB: *C. brasiliensis*, CT: *C. ternatea*, LP: *L. purpureus* and VU: *V. unguiculata*
$p < 0,05$

The highest ADF content was recorded in the treatment of *V. unguiculata* in season 1 (44,6 %), and the lowest one in the same species, but in season 2 (42,1 %). The recorded ranges in this study (43,3-43,6 %) are over the ones reported by Villa *et al*. (2010) and Skonieski (2017) for *Z. mays* silo.

Regarding the IVDMD, a direct increase associated to inoculation was shown. This coincides with the report by Espinoza-Guerra *et al*. (2017) in *Z. mays* silo. However, even with the increase in IVDMD, the values of this study were lower than those recorded by Villa *et al*. (2010) in *Z. mays* (64,3-65,5%) at 56 days of age, in different warm climate zones in Colombia. They are also below those reported by Jiménez *et al*. (2005) in the association *C. brasiliensis* and *Z. mays* (63,2 %).

In this case, it should be taken into consideration that the inoculation can affect NDF, ADF and IVDMD, which is favorable for the preservation of the quality of the ensiled material, because it prevents that the degradation of DM occurs and, thus, the degradation of the fiber components at silo level, because they will be source of nutrients for the rumen microorganisms, where the fiber degradation process should be carried out as factor of production of volatile fatty acids (Kung *et al*., 2018).

Regarding the IVDMD, there was direct effect of inoculation ($p < 0,05$), independently from the season and the incorporated legume. The highest IVDMD was obtained in the plots with inoculation (60,0 %) with regards to the ones that were not inoculated (59,0 %). This can be related to a faster drop of pH, which decreases the survival of clostridial microorganisms. This prevents the solubilization and degradation of soluble nutrients, during the initial stage of silage (Kung *et al.,* 2018).

It should be considered that the silage process does not improve the nutritional quality of the original material (Vanegas-Ruiz and Codero-Ahiman, 2019). Thus, the use of additives should be considered as an alternative to optimize the silage process and, in some cases, increase the nutritional value and fermentation parameters (Muck *et al.,* 2018). Yet, as occurred in this study, Kung *et al.* (2008) found increase (10-15 %) in the DM content of Z. mays after the inoculation process, with higher values than the ones reported for *Z. mays* without ensiling (32,9-33,5 %); this is related to the use of micro-silo, which does not allow the output of effluents.

On the other hand, the pH of the silo showed interaction of the season and the incorporated legume ($p < 0,05$). The lowest pH appeared in the treatment *C. brasiliensis* in season 1 (3,72) with regards to *C. brasiliensis* in 2 (3,92). There was no effect on pH associated to season, but there was effect related to inoculation, where the pH decreased (fig. 5).

Generally, the pH range in this study was in correspondence with the report by Silva *et al*. (2018) and Fernandes *et al*. (2019) for adequate silage.

Low and adequate pH values allow to infer a dominance in the production of lactic acid and its benefit for ruminant feeding, because under normal feeding conditions the lactic acid from the forage turns into propionic in the rumen (Kung *et al*., 2018). The low pH value indicates an adequate preservation of the material, which is added to the beneficial effect of inoculation on the fermentation

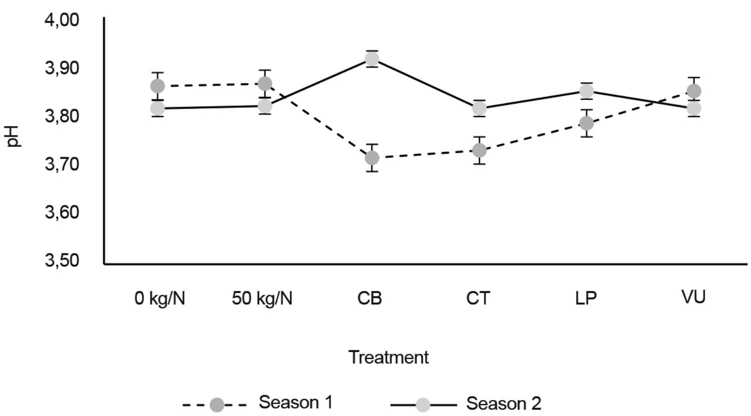

Figura 5. Interacción época y leguminosa para el pH en silos de *Z. mays*.
CB: *C. brasiliensis*, CT: *C. ternatea*, LP: *L. purpureus* and VU: *V. unguiculata*
$p < 0,05$

and fast decrease of pH (Jankowska *et al*., 2017). This can reduce the losses due to anaerobic decomposition and prevent the growth of undesired microorganisms (Gómez-Guarrola *et al*., 2015).

Regarding NH_3, there was interaction of the season and the legume ($p < 0,05$). The highest NH_3 content was recorded in the treatment *C. ternatea* in season 2 (3,6 %) with regards to 0 N in season 2 (3,8 %). It could be observed that inoculation decreased the NH_3 content, independently from the season of incorporation and the legume (fig. 6).

In this study, the NH_3 range (NH_3/NT) is found in the interval reported as desirable (< 7,0) for corn silage (Kung *et al.,* 2018). It is also in accordance with the report by López-Herrera and Briceño-Arguedas (2017) in mixed silage of *V. unguiculata* and *Z. mays* (3,9 %), in which a decrease of NH_3 occurs associated to the use of inoculants (Gallo *et al*., 2018).

In general, it can be said that the NH_3 contents in this study were adequate, given that an increase of NH_3 over 12 % indicates increase of the proteolytic activity and loss of protein of the silo (Gang *et al*., 2020).

Regarding the content of volatile fatty acids (VFAs), for the lactic, acetic and butyric acid, there was no effect of season or the incorporated legume or interaction of the factors. Differences associated to the inoculation of the silage ($p < 0,05$) were observed.

With higher contents of lactic acid in the treatments with inoculation (7,8 %) with regards to those in which there was no inoculation (6,3 %), the acetic acid content decreased due to its effect (2,0 *vs* 2,5 %). In butyric acid a content of 0,4 % was observed in the treatments without inoculation, with regards to 0,31 % which was obtained with inoculation (table 2).

It was proven that with the use of inoculants the production of lactic acid was favored and there was lower production of acetic and butyric acid, as indicators of adequate fermentation, according to the ideal parameters in the content of acids for a *Z. mays* silo (lactic: 4-7 %, acetic: 1-3 % and butyric: < 0,5 %) (Weiss *et al*., 2016; Silva *et al*., 2018).

The highest production of lactic acid due to the inoculation directly favored the decrease of pH, as reported in other studies (Blajman *et al*., 2018; Rabelo *et al*., 2018). This process is also accompanied by the decrease of acetic, butyric acids and NH_3 (Chen *et al*., 2017).

Conclusions

Independently from the incorporation season and the legume used as green manure, the inoculation of the silages improved the nutritional quality and their fermentation parameters.

Interaction of the factors season and legume species was shown, only for the variables NH_3 and pH. In turn, the inoculation of *Z. mays* silage increased the DM, CP contents and IVDMD, with decrease of the NDF values in the final product.

Acknowledgements

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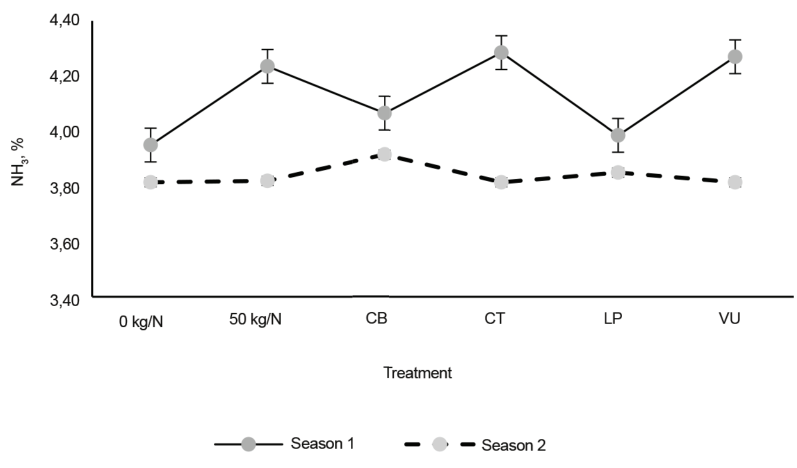

Figure 6. Interaction season and legume for NH3 (% Nt) in *Z. mays* silos.
CB: *C. brasiliensis,* CT: *C. ternatea*, LP: *L. purpureus* and VU: *V. unguiculata*
p < 0,05

Table 2. Characterization of fermentation in the *Z. mays* silo, regarding the studied factors.

Treatment	pH	NH_3	Lactic	Acetic	Butyric
Season					
1	3,8	4,1	7,0	2,2	0,3
2	3,8	4,1	7,0	2,2	0,3
SE ±	0,025	0,059	0,115	0,055	0,004
P - value	0,161	0,348	0,750	0,930	0,571
Legume					
0 N	3,8	4,0	6,9	2,2	0,3
50 N	3,8	4,3	7,0	2,2	0,3
C. brasiliensis	3,8	4,0	7,0	2,2	0,34
C. ternatea	3,8	4,1	7,1	2,2	0,3
L. purpureus	3,8	4,1	7,1	2,3	0,3
V. unguiculata	3,8	4,1	7,0	2,2	0,3
SE ±	0,006	0,056	0,136	0,037	0,004
P - value	0,704	0,191	0,556	0,938	0,636
Inoculation					
Without inoculant	4,0	4,4	6,3	2,5	0,4
With inoculant	3,6	3,8	7,8	2,0	0,3
SE ±	0,012	0,073	0,0131	0,034	0,004
P - value	< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001
Season x Legume	S	S	NS	NS	NS
Season x inoculation	NS	NS	NS	NS	NS
Legume x inoculation	NS	NS	NS	NS	NS
Season x legume x inoculation	NS	NS	NS	NS	NS

Means followed by different letters in the same column differ for p <0,05
Season 1: green manure, sown at the beginning of the rains of the first semester and incorporation at the end of the rains of that semester.
Season 2: green manure, sown at the end of the rains of the first semester and incorporation at the end of the dry season.

Authors' contribution

- Edwin Castro-Rincón. Conducted the research, wrote the original draft and did the analysis of the manuscript.
- Andrea Milena Sierra-Alarcón. Conducted the research and the data processing.
- José Edwin Mojica-Rodríguez. Conducted the research, wrote the original draft and did the analysis of the manuscript.
- Juan Evangelista Carulla-Fornaguera. Did the design of the methodology, supervision, revision and edition.
- Carlos Lascano-Aguilar. Conducted the design of the methodology and created the models.

Conflict of interests

The authors declare that there is no conflict of interests among them.

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