PRODUCTIVITY AND NUTRITIONAL VALUE OF CORN FOR SILAGE SUBJECTED TO THE APPLICATION OF PLANT BIOSTIMULANTS

PRODUTIVIDADE E VALOR NUTRICIONAL DO MILHO PARA SILAGEM SUBMETIDO A APLICAÇÃO DE BIOESTIMULANTES VEGETAIS

PRODUCTIVIDAD Y VALOR NUTRICIONAL DEL MAÍZ PARA ENSILAJE SOMETIDO A LA APLICACIÓN DE BIOESTIMULANTES VEGETALES

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ABSTRACT

The growing demand for food drives the search for more efficient and sustainable agricultural practices. Among them, the use of plant biostimulants has consolidated as a viable alternative to increase crop productivity and favor cultivation under adverse conditions. This study aimed to evaluate the isolated and combined effects of plant biostimulants on the agronomic characteristics and fermentative quality of maize silage. The experiment was carried out from March to July 2023 in the experimental area of the Forage and Pasture Sector at the State University of Southwest Bahia (UESB), in a randomized block design. The FEROZ VIP3 maize hybrid was subjected to *Azospirillum brasilense*, exogenous phytohormones, or their combination, in addition to a control treatment. At 79 days after planting, plants were harvested to determine total yield, morphological fractions, and nutritional value, as well as whole-plant silage quality. Exogenous phytohormones increased total forage yield by 10.94%, ear yield by 17.59%, and leaf yield by 25.52% compared to the control. Significant increases ($p<0.05$) were observed for leaf dry matter (21.5%) and total yield (15.3%) with A. brasilense or phytohormones. Combined use raised mineral matter, crude protein, and non-fibrous carbohydrate contents, while reducing fiber and lignin, without impairing fermentative quality. It is concluded that the isolated or combined application of biostimulants increases productivity and improves the bromatological characteristics of the evaluated cultivar.

Keywords: *Azospirillum brasilense*. Biofertilizers. Exogenous Phytohormones.

RESUMO

A crescente demanda por alimentos estimula a busca por práticas agrícolas mais eficientes e sustentáveis. Neste cenário, o uso de bioestimulantes vegetais se apresenta como alternativa viável para aumentar a produtividade das culturas e favorecer o cultivo em condições adversas. Este estudo teve como objetivo avaliar os efeitos isolados e combinados de bioestimulantes vegetais sobre as características agronômicas e a qualidade fermentativa da silagem de milho. O experimento foi conduzido entre março e julho de 2023, na área experimental do Setor de Forragicultura e Pastagem da Universidade Estadual do Sudoeste da Bahia (UESB), em delineamento em blocos casualizados. Utilizou-se o milho híbrido feroz VIP3, submetido à aplicação de *Azospirillum brasilense*, fitormônios exógenos ou à combinação de ambos, e um tratamento controle adicional. Aos 79 dias após o plantio, as plantas foram colhidas para determinação da produtividade total, das frações morfológicas e do valor nutritivo, além da análise da silagem da planta inteira. A aplicação de fitormônios exógenos elevou a produção de forragem total em 10,94%, de espigas em 17,59% e de folhas em 25,52% em relação ao controle. Houve aumento significativo ($p<0,05$) de 21,5% na massa seca de folhas e de 15,3% na produção total com *A. brasilense* ou fitormônios, comparado à ausência de bioestimulantes. O uso combinado elevou os teores de matéria mineral, proteína bruta e carboidratos não fibrosos, e reduziu fibras e lignina, sem prejudicar a qualidade fermentativa. Conclui-se que a aplicação isolada ou combinada dos bioestimulantes incrementa a produtividade e melhora as características bromatológicas da cultivar avaliada.

Palavras-chave: *Azospirillum brasilense*. Biofertilizantes. Fitormônios Exógenos.

RESUMEN

La creciente demanda de alimentos impulsa la búsqueda de prácticas agrícolas más eficientes y sostenibles. Entre ellas, el uso de bioestimulantes vegetales se ha consolidado como una alternativa viable para aumentar la productividad de los cultivos y favorecer su desarrollo en condiciones adversas. Este estudio tuvo como objetivo evaluar los efectos aislados y combinados de bioestimulantes vegetales sobre las características agronómicas

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y la calidad fermentativa del ensilaje de maíz. El experimento se llevó a cabo entre marzo y julio de 2023 en el área experimental del Sector de Forrajes y Pasturas de la Universidad Estatal del Sudoeste de Bahía (UESB), en un diseño de bloques al azar. El híbrido de maíz FEROZ VIP3 fue sometido a la aplicación de *Azospirillum brasilense*, fitohormonas exógenas o su combinación, además de un tratamiento control. A los 79 días después de la siembra, las plantas fueron cosechadas para determinar el rendimiento total, las fracciones morfológicas y el valor nutritivo, así como la calidad del ensilaje de planta entera. Las fitohormonas exógenas incrementaron el rendimiento total de forraje en 10,94%, de espigas en 17,59% y de hojas en 25,52% en comparación con el control. Se observaron aumentos significativos ($p<0,05$) en la materia seca de hojas (21,5%) y en el rendimiento total (15,3%) con *A. brasilense* o fitohormonas. El uso combinado aumentó los contenidos de materia mineral, proteína bruta y carbohidratos no fibrosos, reduciendo las fibras y la lignina, sin afectar la calidad fermentativa. Se concluye que la aplicación aislada o combinada de bioestimulantes incrementa la productividad y mejora las características bromatológicas de la cultivar evaluada.

Palabras clave: *Azospirillum brasilense*. Biofertilizantes. Fitohormonas Exógenas.

1 INTRODUCTION

The growing demand for food has led to the search for efficient and sustainable agricultural practices. Among such practices, the use of plant biostimulants has been consolidated as a viable alternative to increase crop productivity and favor their cultivation in adverse situations. Plant biostimulants are biological or synthetic products, which, when applied to the soil or plants, promote the growth and health of crops, and are related to different mechanisms, such as biological nitrogen fixation, nutrient release, systemic resistance, and the production and regulation of phytohormones (Du Jardim, 2015).

This approach has shown significance in the cultivation of maize (*Zea mays L*.), widely produced and consumed in the world by humans and animals. Recent studies have shown that the application of biostimulants, from the class of beneficial bacteria (Chen et al., 2021) and exogenous phytohormones (Thomé et al., 2023), can provide significant benefits to crops, including improvements in nutrient absorption efficiency, resistance to abiotic stress, in addition to stimulating root development and increasing corn crop productivity.

According to a study carried out by Hungria *et al*., (2010) increases in corn grain yield were identified when inoculated with *Azospirillum brasilense strains*, in the proportion of 26% in relation to the crop in the absence of the bacterium. In studies on the exogenous application of phytohormones combined with the inoculation of plant growth-promoting microorganisms, Andrade et al., (2024) found increases of 21% for the production of biomass of Basilisk grass, which represents a potentiation of physiological mechanisms, resulting in greater development for grasses.

In addition to the productive benefits, the use of biostimulants in corn cultivation can improve the nutritional value, favoring the supply of higher quality silage, especially in periods of food scarcity. Studies demonstrate the possibility of increasing the concentration of essential micronutrients, leading to an increase in the concentration of amino acids, carbohydrates, and proteins (Ocwa et al., 2024), which directly reflects on the bromatological composition of the ensiled material, and can increase the crude protein content, dry matter digestibility, and nutrients required by ruminants.

Thus, this study aimed to investigate the isolated and combined effects of plant biostimulants on the agronomic characteristics and fermentative quality of corn silage.

2 METHODOLOGY

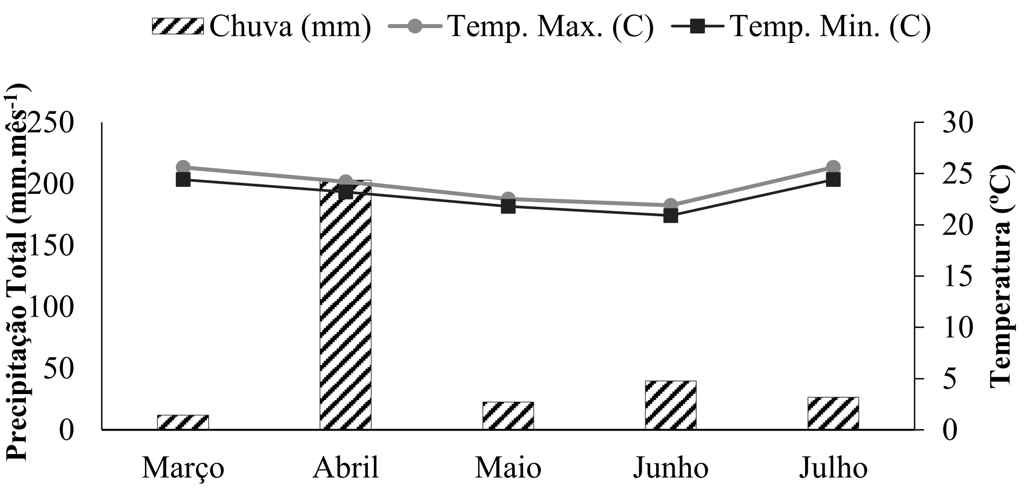
2.1 EXPERIMENTAL DETAILS

The experiment was carried out in the experimental area of the Forage and Pasture Sector of the State University of Southwest Bahia (UESB), Juvino Oliveira campus, located in Itapetinga, BA, between the months of March and July 2023. The municipality of Itapetinga is located in the Central-South Bahia Mesoregion, with geographic coordinates 15º38'46" south latitude and 15º24' west longitude, at an altitude of 280 meters.

The region is characterized by having a tropical climate with a dry season in winter. The rainfall index varies between 1400 and 1600 mm per year and the temperature throughout the year is between 20º C and 28ºC, with a minimum of not less than 18º C, according to the Köppen-Geiger classification. The data on the maximum, average and minimum temperatures of the experimental period were collected at the meteorological station of the National Institute of Meteorology (Figure 1).

Figure 1

Monthly average rainfall (mm) and maximum and minimum temperatures (ºC) during the experimental period



Source: INMET (2023)

2.2 EXPERIMENTAL DESIGN AND TREATMENTS

The experiment was conducted in a randomized block design, consisting of four treatments with five replications, totaling twenty experimental units. The treatments consisted of the evaluation of the hybrid corn ferocious VIP3, submitted to four treatments, being (i) Control: absence of biostimulant; (ii): Inoculation of *Azospirillum brasilense* at a dosage of 300 ml $^{ha-1}$, with a concentration of 2x108 CFU/ml of the AbV5 and AbV6 strains; (iii) FIT: application of exogenous phytohormones at a dosage of 500 ml.ha-1 composed of Kinetin (0.09 g.L-1), Gibberellic Acid (g.L-1) and Indolebutyric Acid (0.05 g.L-1); (iv) AZO+FIT: combined application.

2.3 SOIL PREPARATION, PLANTING AND APPLICATION PROTOCOLS

An area of 476 m² was used, which was demarcated into 20 experimental units with dimensions of 3.2 m × 5 m. After demarcation, soil samples were collected from 0 to 20 cm deep for chemical and physical analysis. The samples were sent to the Laboratory of the Department of Agricultural Engineering and Soils of the State University of Southwest Bahia (UESB), Vitória da Conquista Campus. The results of the analysis are presented in Table 1.

Table 1

Chemical analysis of the soil of the experimental area

ph	mg/dm3				.... CMOLC/DM3 of soil................................							%	g/dm3
(H2O)	P	K+	Ca2+	Mg2+	Al3+	H+	Na+	S.B1	T2	T3	V4	m5	M.O6
6,4	26	0,41	2,6	1,0	0,0	1,5	-	4,0	4,0	5,5	73	0	14

SB: Sum of bases; T: Effective Cation Exchange Capacity; T: Cation exchange capacity at pH 7; V: Saturation by bases; m: Aluminum Saturation; MO: Organic matter.

After plowing and harrowing, sowing and fertilization were carried out. Sowing was carried out manually on March 22, 2023, and thinning was done eight days after planting to obtain a density of 62500 plants.ha-1 with spacing of 0.80 m between plants and 0.20 m between rows. Fertilization was carried out in accordance with the recommendations of the Soil Fertility Commission of the State of Minas Gerais – CFSEMG (Álvarez Ribeiro, 1999), applied in the total area. At sowing time, 20 kg of N $^{ha-1}$ in the form of urea and 70 kg of P2O5 $^{ha-1}$ in the form of simple superphosphate were applied. For top dressing, 40 kg of K2O ha-1 in the form of potassium chloride and 180 kg of N $^{ha-1}$ in the form of urea were applied, divided into three applications, in the V4, V6 and V8 stages.

The application of biostimulants was carried out at the phenological stage V6 27 days after sowing. For each experimental unit, the preparation of the mixture was carried out based on a volume equivalent to 200 L $^{ha-1}$, which corresponded to 320 mL per plot. The solution was prepared individually for each treatment, before application, with application performed on the leaves of the plants, at 4 pm, with manual sprayer.

2.4 VARIABLES ANALYZED

At seventy-nine days after planting (30% dry matter), five plants were cut in each experimental unit, at the level of 20 cm from the soil, then the collected material was separated and weighed into leaves, stem and ear, to obtain the production of green mass of each structural component and of the total production (kg.ha-1). Subsequently, pre-drying was carried out in a forced air circulation oven at 55°C until constant weight to obtain the values of dry mass (kg.ha-1).

After pre-drying and weighing, the plants were ground in a knife mill with a 1 mm sieve and submitted to bromatological analysis to determine the dry matter contents (DM; Method INCT-CA G-003/1), mineral matter (MM; Method INCT-CA M-001/1), ether extract (EE; Method INCT-CA G-004/1), crude protein (CP; INCT-CA N-001/2), neutral detergent fiber (NDF; Method INCTCA F002/2), acid detergent fibre (FDA; Method INCT-CA F-004/2) and lignin (Method INCT-CA F-005/1) according to the techniques described by Detmann et al. (2021). To determine the contents of non-fiber carbohydrates (NFC) and total carbohydrates (TC), the equations proposed by Sniffen et al. were used. (1992).

To evaluate the chemical composition and fermentative quality of the silage, the remaining plants of the plots were cut into a 2 cm particle and ensiled in PVC silos 40 cm high by 10 cm in diameter, with lids and Bunsen valve and mesh to separate the sand from the ensiled material. It has the capacity to store 2.2 to 2.6 kg of forage in each mini silo, equivalent to a compaction density of 850 kg.m-3, being weighed and stored for a period of 36 days. After the storage period, the values of gas losses (PG), effluent losses (PE) and dry matter recovery (RMS) were determined according to equations described by Jobim et al. (2007). While the evaluation of pH after opening silage according to the methodology of Cherney & Cherney (2003). A subsample of 200g was taken from each mini silo for bromatological analyses, which were carried out in the same way as those described for the samples of five plants.

2.3 STATISTICAL ANALYSIS

The data were submitted to analysis of variance by the F test, and, when significant, the means were compared by Tukey's test at 5% probability, using the PROC MIXED of the statistical program SAS® OnDemand for Academics.

3 RESULTS AND DISCUSSION

The variables total green mass production of corn, ear production and leaf production showed a significant difference ($p < 0.05$) among the biostimulants tested (Table 2), in which the application of exogenous phytohormones was responsible for the highest averages found. Evidencing its effectiveness in regulating plant growth, which becomes an efficient strategy to increase the productive potential of corn.

Table 2

Green mass production of maize morphological components in response to the application of plant biostimulants

Item (kg. ha-1)	Biostimulants				CV	*P* value
	CON	AZO	FIT	AZO+FIT		
Ear	1293.00b	1370.61ab	1520.63a	1258.81b	10,10	0,046
Thatch	1758.88a	1025.02a	1743.82a	1693.84a	14,73	0,971
Leaf	8325.05b	8875.00b	1045.00a	9437.25ab	9,47	0,014
Total	3884.45b	2360.62ab	4309.42a	3896.34ab	11,02	0,036

COM: Control; AZO: *Azospirillum brasilense;* FIT: Exogenous phytohormones; CV: Coefficient of variation. Means followed by different letters in the line differ from each other by Tukey's test (P<0.05).

Thomé et al., 2023 find positive effects of the combined use of auxin, gibberellin and cytokinin in corn crops, these authors found that exogenous application increased grain production by 345 kg.ha-1, in addition to positively affecting growth characteristics. In the present study, the application of exogenous phytohormones provided increases of 10.94% in total forage, 17.59% for ear and 25.52% for leaf, in relation to the treatment without biostimulants.

For the production of total dry matter, a significant difference was identified only for leaf and total production (Table 2). The application of exogenous phytohormones provided significant increases ($p<0.05$), corresponding to a percentage of 21.5% for dry leaf mass, while inoculation with *Azospirillum brasilense* or exogenous phytohormones provided an increase of 15.3% for total production, based on the treatment with the absence of biostimulants.

Table 3

Dry matter production of morphological components and total forage of hybrid corn FEROZ VIP3 in response to the application of plant biostimulants

Item (kg.ha-1)	Biostimulants				CV	P value
	CON	AZO	FIT	AZO+FIT		
Ear	6243.04a	7093.24a	7237.42a	6034.75a	19,61	0,541
Thatch	3554.42a	3583.15 to	3931.76a	3387.25a	11,11	0,136
Leaf	2093.07b	2336.21ab	2543.49a	2362.47ab	9,32	0,045
Total	11890.42b	13039.25a	13711.47a	11783.89ab	9,93	0,037

COM: Control; AZO: *Azospirillum brasilense;* FIT: Exogenous phytohormones; CV: Coefficient of variation. Means followed by different letters in the line differ from each other by Tukey's test (P<0.05).

The results of the response to the isolated application of plant biostimulants, of the class of organic substances or beneficial microorganisms, favor the morphological development of maize, promoting greater accumulation of dry biomass with a higher proportion of leaves. The productive increases that were found in this study may be related to biological nitrogen fixation by inoculation of *Azospirillum brasilense strains*, which has the ability to partially supply the nitrogen requirement to plants (Fukami *et al*., 2018) and by hormonal signaling via exogenous supply, capable of acting in the division and elongation of tissues (Calvo *et al*., 2014).

For the plant's bromatological composition, the content of dry matter, total carbohydrates and organic matter did not show significant difference (p>0.05) between the treatments. The content of mineral matter, crude protein and non-fiber carbohydrates had a higher average for the combined use of biostimulants, as well as reduced levels of fiber and lignin (Table 4).

Table 4

Chemical-bromatological composition of maize, plant, in response to the application of plant biostimulants

Item	Biostimulants				CV	P value
	CON	AZO	FIT	AZO+FIT		
MS (%)	28,59	30,31	28,36	27,72	7,47	0,311
MM (% MS)	6.04b	6.26b	6.30b	6.97a	4,93	0,003
MO (% MS)	93,95	93,73	93,94	93,47	0,62	0,543
EE (% MS)	2.76b	2.39b	3.46a	2.43b	14,00	0,003
NDF (% MS)	55.45a	52.43bc	54.27b	51.66c	2,86	0,008
FDA (%MS)	30,32a	28.61b	29.48ab	25,03c	3,12	<0.001
LIG (% MS)	4.25a	3.22c	3.80b	3.95ab	5,74	<0.001
PB (% DM)	9.57b	9.60b	9.59b	10.33a	3,10	0,004
CT (% MS)	81,71	81,43	81,02	80,94	1,71	0,796
CNF (% MS)	25.61c	29.80b	26.85c	32.35a	4,53	<0.001

CON: Control; AZO: *Azospirillum brasilense;* FIT: Exogenous phytohormones; CV: Coefficient of variation; MS: dry matter; MM: mineral matter; MO: organic matter; EE: ethereal extract; NDF: neutral detergent fiber; FDA: acid detergent fiber; LIG: lignin; CP: crude protein; CT: total carbohydrates; CNF: non-fiber carbohydrates. Means followed by different letters in the line differ from each other by Tukey's test (P<0.05).

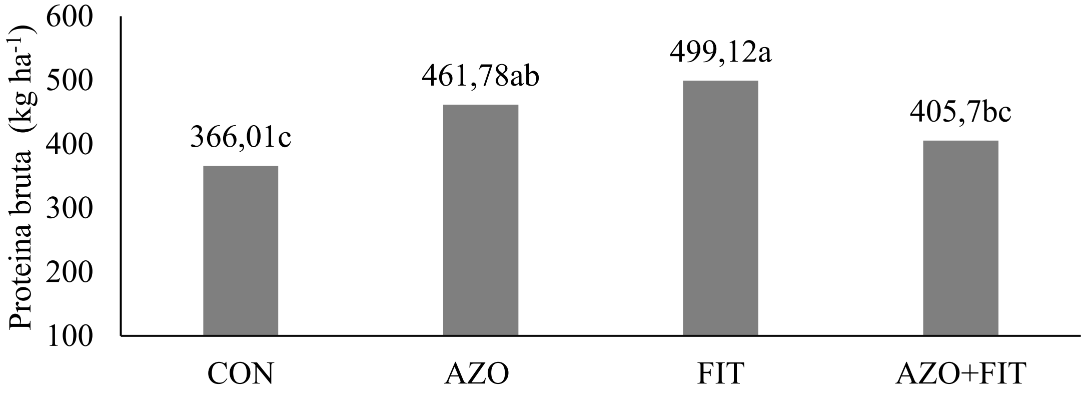
The biostimulants used in our study are able to trigger physiological responses in plant development, providing better assimilation of nutrients, which favors plant growth in addition to increasing its nutritional intake. With emphasis on the combined application of biostimulants, by promoting a significant increase in the mineral content in plants, being corroborated by Bashan and de-Bashan (2010) and Thomé *et al*. (2023), which highlight the potential of the bacterium *Azospirillum brasilense* and the hormones cytokinin, auxin and gibberellin in favor the absorption of nutrients essential to plant development.

In addition, the application of plant biostimulants resulted in lower NDF and ADF values, indicating better fiber quality, which consequently caused a significant increase in non-fiber carbohydrates, possibly due to the increase in soluble sugars, which contributes to greater energy availability and increased nutritional value of forage (Hungria et al., 2010).

There was a significant difference ($p<0.05$) for the crude protein production of the leaf fraction, in which the biostimulants applied alone presented higher averages, compared to plants in the control group or those that received the combination of biostimulants (Figure 2).

Figure 2

Crude protein production (kg.ha-1) of corn leaf in response to the application of plant biostimulants



Leite et al. (2019) corroborate the results of increased nitrogen intake in leaves of mombaça grass inoculated with *Azospirillum* spp., the authors identified that the evaluated species obtained a 12% increase in the percentage of leaf nitrogen with inoculation, while in our study this value was 25%. Still on nitrogen intake due to the use of biostimulants, phytohormones of the cytokinin class optimize nutrient uptake, such as nitrogen, which increases the nutritional status of the plant, favoring its growth (Taiz et al. 2017).

The ensiling process promoted significant changes in the chemical-bromatological composition of corn (Table 5).

Table 5

Chemical-bromatological composition of corn silage in response to the application of plant biostimulants

Item	Biostimulants				CV	P value
	CON	AZO	FIT	AZO+FIT		
MS (%)	27,83	27,37	27,83	27,89	5,42	0,940
MM (% MS)	5.22b	5.71a	5.64a	5.39a	4,98	<0.001
EE (% MS)	1,61	1,67	1,19	1,21	22,65	0,064
NDF (% MS)	51,11	50,75	48,91	51,15	3,52	0,220
FDA (%MS)	27,89	28,07	26,63	27,61	4,55	0,318
LIG (% MS)	5.10A	5.47a	4.00b	3.87b	10,12	0,003
PB (% DM)	7.99c	9.96b	10.01b	10.89a	9,06	0,412

CON: Control; AZO: *Azospirillum brasilense;* FIT: Exogenous phytohormones; CV: Coefficient of variation; MS: dry matter; MM: mineral matter; EE: ethereal extract; NDF: neutral detergent fiber; FDA: acid detergent fiber; LIG: lignin; CP: crude protein; Means followed by different letters in the line differ from each other by Tukey's test ($P<0.05$).

After the ensiling process, a reduction in the fibrous fractions, NDF and ADF, as well as an increase in the MM contents, was observed, possibly due to the concentration of minerals resulting from the partial degradation of the forage in the active fermentation phase (Weinberg & Muck, 1996). The CP content also increased after the opening period, probably due to the proportional reduction of soluble carbohydrates, concentrating the nitrogen fraction. It is an indication of an adequate fermentation process of the silage, given the preservation and, in some cases, the slight increase in the nutritional quality of the forage, especially by the reduction of fibrous fractions.

The chemical-bromatological analysis of the silage shows that the use of plant biostimulants contributes to the improvement of forage quality, reflected in lower fiber contents and higher concentration of crude protein. After the storage period, it was observed that the treatments with biostimulants maintained these characteristics, with a moderate increase in the concentration of crude protein. This effect may be related to the presence of soluble carbohydrates, which act as a substrate for the development of microorganisms beneficial to the fermentation process, which are capable of promoting rapid pH stabilization, reduction of fermentative losses and partial degradation of fibrous constituents, which contributes to silage quality (Jobim et al., 2007).

The parameters losses due to gases, effluents, RMS and pH of corn silage in response to foliar application of biostimulants did not show significant difference for the treatments studied (Table 6).

Table 6

pH values and losses of corn silage in response to the application of plant biostimulants

Item	Biostimulants				CV	P value
	CON	AZO	FIT	AZO+FIT		
ph	3,93	3,88	3,87	3,86	1,35	0,235
Gas losses	0,02	0,03	0,02	0,02	16,37	0,140
Effluent losses	0,14	0,12	0,09	0,15	31,79	0,189
Dry matter recovery	117,92	12,95	130,09	119,03	7,75	0,192

CON: Control; AZO: *Azospirillum brasilense;* FIT: Exogenous phytohormones; CV: Coefficient of variation; PG: Gas losses; PE: Effluent losses; RMS: Dry matter recovery.

Despite the absence of significant difference, the fermentation quality indices indicate that there was no loss of the nutritive value of the silage. For the pH values, the mean found in our experiment was 3.8, which is in accordance with the recommendations of McDonald *et al*. (1991), as an indication of adequate fermentation without significant losses during the process.

4 CONCLUSION

The application of plant biostimulants, with *A. brasiliense* and exogenous phytohormones, alone or in combination, increases productivity and provides improvements in the bromatological characteristics of hybrid corn FEROZ VIP, without causing reductions in the fermentative quality parameters of the silage.

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