INCLUSION OF CRUSHED CORN GRAINS AND CITRUS PULP IMPROVES THE QUALITY OF SOYBEAN SILAGE[1]

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ABSTRACT: We aimed to evaluate the effect of the inclusion of corn and citrus pulp as additives in soybean silage made in plastic (PVC) tubes. The experimental design was completely randomized, with a 2x4 factorial arrangement consisting of two additive groups (corn and citrus pulp) and four inclusion levels (no inclusion, 7, 14 and 21%). The experiment was conducted at the Forage Sector of the Lageado Experimental Farm, Faculty of Veterinary Medicine and Animal Science, UNESP, Botucatu, SP, and chemical composition analysis was carried out at the Laboratory of Bromatology. The soybean variety used was BMX Potência RR. Mechanical harvest occurred at the R7 phenological stage, at a height of 15 cm, using a harvester equipped with a two-row platform. The material was chopped into medium particles of 3 to 6 mm. After 45 days, the silages were analyzed for dry matter, crude protein (CP), total digestible nutrients, pH, ethereal extract, neutral detergent fiber, acid detergent fiber, lignin, total dry matter loss, and total carbohydrates (CHOS). The inclusion of corn (21%) and citrus pulp (14 and 21%) reduced CP values (P<0.05). Control silage contained less CHOS (P<0.05). The inclusion of corn meal is better than citrus pulp to improve the values of soybean silage.

Keywords: Corn, Soybean silage, Soybean, Citrus pulp.

INCLUSÃO DE GRÃOS DE MILHO MOÍDO E POLPA CÍTRICA MELHORA A QUALIDADE DA SILAGEM DE SOJA

RESUMO: Objetivamos avaliar o efeito da inclusão de milho e polpa cítrica como aditivo em silagem de soja em tubos plásticos de PVC. O delineamento experimental foi inteiramente casualizado, com arranjo fatorial 2x4, dois grupos aditivos (milho e polpa cítrica) e quatro níveis de inclusão (sem inclusão, 7, 14 e 21%). O experimento foi conduzido no Setor Forrageiro da Fazenda Experimental Lageado da Faculdade de Medicina Veterinária e Zootecnia da UNESP de Botucatu - SP e nas análises de composição química realizadas no laboratório de Bromatologia. A variedade de soja utilizada foi a BMX Potência RR e a colheita mecânica ocorreu no estádio fenológico R7, na altura de 15 cm, utilizando-se um Harvester equipado com plataforma de duas fileiras, com o material cortado em partículas médias entre 3 e 6 mm. Após 45 dias, as silagens foram analisadas quanto à matéria seca (MS), proteína bruta (PB), nutrientes digestíveis totais (NDT), pH, extrato etéreo (EE), fibra em detergente neutro (FDN), fibra em detergente ácido (FDA), lignina (LIG), perda total de matéria seca (PMS) e carboidratos totais (CHOS). A inclusão de milho (21%) e polpa cítrica (14 e 21%) reduziu os valores de PB (P <0,05). A silagem sem inclusão apresentou menor quantidade de carboidratos totais (P <0,05). A inclusão de fubá é melhor que a polpa cítrica para melhorar os valores da silagem de soja.

Palavras chave: Milho, Silagem de soja, Soja, Polpa cítrica.

INTRODUCTION

Soybean (*Glycine max* L. Merrill) has been one of the agricultural crops with the largest growth in Brazil in recent decades (Freitas, 2011). Significant increases in productivity were observed with the improvement of management practices and the use of varieties that are better adapted to the edaphoclimatic conditions. Traditionally used as bran, there has been growing interest in soybean silage as ruminant feed in several countries, including Brazil (Coutinho et al., 2017). In Brazil, forage availability varies during the year due to climatic conditions, which can increase production in summer and reduce it in winter. Thus, there is a need for preserving forage to supplement the animals during periods of low pasture production. Supplementation with soybean silage is a potential alternative to decrease the use of concentrates (Evangelista et al., 2003).

The use of silage as bulk feed during the dry season is a common practice among cattle farmers. The most suitable crops for ensiling are corn and sorghum, which provide good quality silage without the use of additives. Among forages, corn (*Zea mays*) is the most widely used because it provides high levels of essential soluble carbohydrates, that accelerates the fermentation process and presents high yield per hectare. The use of legume silages such as soybeans has been shown to be an interesting alternative to produce bulk feeds in some production systems, especially those in which protein demand is high (Barros et al., 2017). The advantages of using whole-plant soybean silage include reducing the amount of grain in the diet, crop rotation, a 2.5 times higher protein yield per kg of dry matter, high level of total digestible nutrients of corn, and a cost reduction of up to 30% (De Córdova Gobetti, 2011). It should only be considered details such as mass yield, since small cultivars will result in low productivity per area, to choose the most appropriate cultivar (Evangelista et al., 2003).

The nutritional value of soybean is good, but the high moisture at harvest time, high buffering power and low soluble carbohydrate and ethereal extract content are characteristics that negatively affect the process of fermentation of the ensiled mass by favoring high pH after ensiling (Barros et al., 2017). Other carbohydrate sources can be used to help in the fermentation process, for example citrus pulp. Citrus pulp is an alternative food with a high content of dry matter and soluble carbohydrates, in addition to its high capacity to absorb water, which favors the desirable fermentation of the ensiled mass (Lima et al., 2017).

We found no studies in the literature to elucidate the effects of citrus pulp added to soybean silage. Therefore, the objective of this work was to evaluate the effects of corn and citrus pulp, added at different proportions (no inclusion, 7, 14 and 21%), on the quality and fermentation of whole-plant soybean silage.

MATERIAL AND METHODS

Experimental site

The study was conducted at the Lageado Experimental Farm of FMVZ, Unesp, Botucatu, SP (22°51'2.97" S and 48°25'28.79" W, altitude of 777 meters). According to the classification of Köppen, the predominant climate in the region is the Cwa type, which is characterized by a high-altitude tropical climate, with dry winters and rainy summers. The experimental area comprises 1.82 ha.

Soybean cultivar

The BMX soybean cultivar Potência RR (Maturation group: Semiprecoce - 6.6) with an indeterminate growth habit destined for whole-plant silage was used. Mechanical harvesting of the silage mass was carried out at a height of 10-15 cm using a two-row platform with reduced spacing (0.45 to 0.55 m between rows) coupled to a forage harvester model JF C-120 (12 knives). The material was chopped into medium particles of 3 to 6 mm. The soybean plants were harvested at the R7 phenological stage (onset of grain maturation and 50% yellowish leaves).

Experimental silo, management and sample collection

The experimental silos (PVC pipe) measured 10 cm in diameter and 30 cm in length and were properly sealed. The chopped fodder was placed inside each tube after the additive of the specific treatment (no inclusion, 7, 14 and 21%

of corn meal inclusion, and 7, 14, and 21% of citrus pulp inclusion) was added in layers with the aid of a press for compacting of up to 650 kg/m^3 (± 1.5 kg per tube).

After 45 days of storage at room temperature in the shadow, the experimental silos were weighed to determine dry matter losses and the top cover was removed. The surface layer (7.5 cm) was removed and the remainder was homogenized. After the whole sample was obtained, the tube was weighed and the weight of the same set before ensiling was subtracted.

Each sample was divided into two portions. The first portion was stored in a paper bag for drying in a forced ventilation oven at 55° C for subsequent bromatological analysis and analysis of fresh forage. The second portion was stored in a plastic bag and frozen immediately at -20° C for preparation of an aqueous extract for pH analysis.

Chemical analysis

At the time of ensiling and after 45 days of storage, forage samples of approximately 300 g were obtained and divided into two sub-samples. The first sub-sample was weighed and oven dried at 105° C for dry matter (DM) determination (AOAC, 1995). The dried and ground samples were used for bromatological analyses to obtain crude protein (CP), ethereal extract (EE), acid detergent fiber (ADF), lignin (H_2SO_4, 72% w/w), neutral detergent fiber (NDF) and ashes according to AOAC methods (1995).

The second sub-sample was used to prepare the aqueous extract to determine soluble carbohydrates (Maharjan et al., 2018), and to determine the buffering power. The pH values of the solution were obtained with a digital potentiometer and the buffering power was determined (Playne and McDonald, 1966).

The analyses were carried out at the Laboratory of Bromatology, Department of Animal Nutrition and Improvement, Faculty of Veterinary Medicine and Animal Science at Júlio de Mesquita Filho University (FMVZ - UNESP), Botucatu, Brazil.

Statistical analysis

The experimental design was completely randomized, with two additive groups (corn and citrus pulp) and four inclusion levels (no inclusion, 7, 14 and 21%). The results were submitted to analysis of variance (ANOVA) using the PROC REG procedure of the SAS software, version 9.3 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

The average bromatological composition (DM basis) of whole-plant soybean with addition of 0%, 7%, 14% and 21% of corn meal and citrus pulp as additives is shown in Table 1 and Table 2, respectively, for pre-ensiled material and in Table 3 (corn meal) and Table 4 (citrus pulp) for post-ensiled material.

With respect to the pre-ensiled material, no effects (P>0.05) of the level of inclusion were found for corn meal or citrus pulp on chemical composition, except for percentages of TDM, CP, EE, pH, NDF and ADF for corn meal inclusion, and TDM, CP and EE for citrus pulp inclusion, with the observation of linear effects. A positive linear effect (R^2 = 0.99) on TDM was found for both corn meal and citrus pulp inclusion. Corn meal (R^2 = 0.89) and citrus pulp (R^2 = 0.53) exerted a negative linear effect on CP. There was a negative linear effect of both inclusions on EE (R^2 = 0.74). In addition, there was a negative linear effect of corn meal inclusion on pH (R^2 = 0.73), NDF (R^2 = 0.67), and ADF (R^2 = 0.81).

Regarding the silage, no effect (P>0.05) was only found for TDN. There was a quadratic

Table 1 - Bromatological composition of pre-ensiled whole-plant soybean with no inclusion (control treatment) and 7%, 14% and 21% of corn meal inclusion, on a dry matter basis.

	Control	Corn meal			P value
		7%	14%	21%	
TDM[1] (%)	30.13	32.77	35.87	39.90	0.003
CP[2] (%)	15.91	14.66	14.48	13.34	<0.001
TDN[3] (%)	75.40	76.48	74.71	76.51	0.5049
pH	6.28	6.23	6.25	6.22	<0.001
EE[4] (%)	8.41	7.62	6.07	5.16	<0.001
NDF[5] (%)	46.29	42.98	43.42	37.64	0.001
ADF[6] (%)	33.02	30.46	30.04	29.44	<0.001
LIG[7] (%)	7.85	7.13	7.17	6.14	0.2423

[1]Total dry matter (TDM), [2]crude protein (CP), [3]total digestible nutrients (TDN), [4]ethereal extract (EE), [5]neutral detergent fiber (NDF), [6]acid detergent Fiber (ADF), [7]lignin (LIG). (P<0.05).

Table 2 - Bromatological composition of pre-ensiled whole-plant soybean with no inclusion (control treatment) and 7%, 14% and 21% of citrus pulp inclusion, on a dry matter basis.

	Control	Citrus pulp			SEM	P value
		7%	14%	21%		
TDM[1] (%)	30.13	33.25	36.32	39.65	0.3187	<0.001
CP[2] (%)	15.91	14.79	12.36	12.62	0.2736	0.007
TDN[3] (%)	75.40	71.24	72.60	70.53	0.0570	0.5438
pH	6.28	6.34	6.09	6.29	1.0889	0.6705
EE[4] (%)	8.41	6.14	4.84	5.04	0.5796	0.003
NDF[5] (%)	46.29	47.05	42.79	46.52	0.1220	0.8202
ADF[6] (%)	33.02	34.89	30.21	32.92	0.2871	0.5221
LIG[7] (%)	7.85	8.67	7.38	8.45	0.7784	0.9488

[1]Total dry matter (TDM), [2]crude protein (CP), [3]total digestible nutrients (TDN), [4]ethereal extract (EE), [5]neutral detergent fiber (NDF), [6]acid detergent Fiber (ADF), [7]lignin (LIG). Means in the same row followed by different lowercase letters differ from each other by the Tukey test (P<0.05).

effect of corn meal inclusion on TDM (maximum of 38.63% at 27.54% of inclusion) and a positive linear effect of citrus pulp inclusion. Regarding CP, corn meal exerted a quadratic effect (maximum of 18.39% at 1.44% of inclusion) and citrus pulp a negative linear effect (R^2 = 0.99). A quadratic effect on EE was observed for both corn meal (minimum of 12.77% at 17.16% of inclusion) and citrus pulp (maximum of 15.50% at 6.85% of inclusion). There was a quadratic effect of corn meal (minimum of 4.08 at 18.46% of inclusion) and citrus pulp (minimum of 4.08 at 16.76% of inclusion) on pH. Corn meal exerted a negative linear effect on NDF (R^2 = 0.94) and citrus pulp a quadratic effect (minimum of 45.18% at 18.73% of inclusion). There was a negative linear effect of corn meal (R^2 = 0.94) and a quadratic effect of citrus pulp (minimum of 45.18% at 18.73% of inclusion) on ADF. Corn meal exerted a quadratic effect on lignin (maximum of 9.30% at 6.37% of inclusion).

DISCUSSION

For pre-ensiled material with increased inclusion levels of corn meal (Figure 1) or citrus pulp (Figure 2) at the time of ensiling, the DM content of the silages increased, following the same pattern as reported by Rodrigues et al. (2005) who found a positive linear effect on DM with increasing inclusion of citrus pulp

Table 3 - Bromatological composition of post-ensiled whole-plant soybean with no inclusion (control treatment) and 7%, 14% and 21% of corn meal inclusion, on a dry matter basis

	Control	Corn meal			SEM	P value
		7%	14%	21%		
TDM[1] (%)	32.58	36.28	36.38	38.60	0.7495	<0.001
CP[2] (%)	18.39	18.20	17.60	16.30	0.3125	<0.001
TDN[3] (%)	82.41	81.81	80.11	80.93	1.250	0.2640
pH	4.86	4.40	4.11	4.11	0.0806	<0.001
EE[4] (%)	14.80	14.06	12.32	13.06	0.3723	0.009
NDF[5] (%)	51.82	50.19	42.41	34.25	0.7748	<0.001
ADF[6] (%)	35.48	33.60	28.60	22.71	0.5858	<0.001
LIG[7] (%)	8.75	9.06	8.65	5.98	0.4042	<0.001
Effluent[8]	0.71	1.42	1.07	1.07	0.2300	0,5674
Gas production	2.12	2.29	2.25	2.13	0.1625	0.7973

[1]Total dry matter (TDM), [2]crude protein (CP), [3]total digestible nutrients (TDN), [4]ethereal extract (EE), [5]neutral detergent fiber (NDF), [6]acid detergent fiber (ADF), [7]lignin (LIG), [8]kg/ton of organic matter, [9]percentage of dry matter. Means in the same row followed by different letters differ from each other by the Tukey test (P<0.05).

Table 4. Bromatological composition of post-ensiled whole-plant soybean with no inclusion (control treatment) and 7%, 14% and 21% of citrus pulp inclusion, on a dry matter basis

	Control	Citrus pulp			SEM	P value
		7%	14%	21%		
TDM[1] (%)	32.58	31.68	31.89	40.32	0.7495	0.0030
CP[2] (%)	18.39	17.50	15.87	14.82	0.3125	<0.001
TDN[3] (%)	82.41	78.96	81.44	82.32	1.250	0.5049
pH	4.86	4.28	4.15	4.10	0.0806	<0.001
EE[4] (%)	14.80	14.58	14.76	12.53	0.3723	0.0022
NDF[5] (%)	51.82	47.15	46.15	46.07	0.7748	<0.001
ADF[6] (%)	35.48	32.90	31.03	30.31	0.5858	<0.001
LIG[7] (%)	8.75	8.61	7.51	7.81	0.4042	0.2423
Effluent[8]	0.71	0.71	1.07	0.00	0.2300	0.2293
Gas Production	2.12	2.09	1.80	1.81	0.1625	0.0226

[1]Total dry matter (TDM), [2]crude protein (CP), [3]total digestible nutrients (TDN), [4]ethereal extract (EE), [5]neutral detergent fiber (NDF), [6]acid detergent fiber (ADF), [7]lignin (LIG), [8]kg/ton of organic matter, [9]percentage of dry matter. Means in the same row followed by different letters differ from each other by the Tukey test (P<0.05).

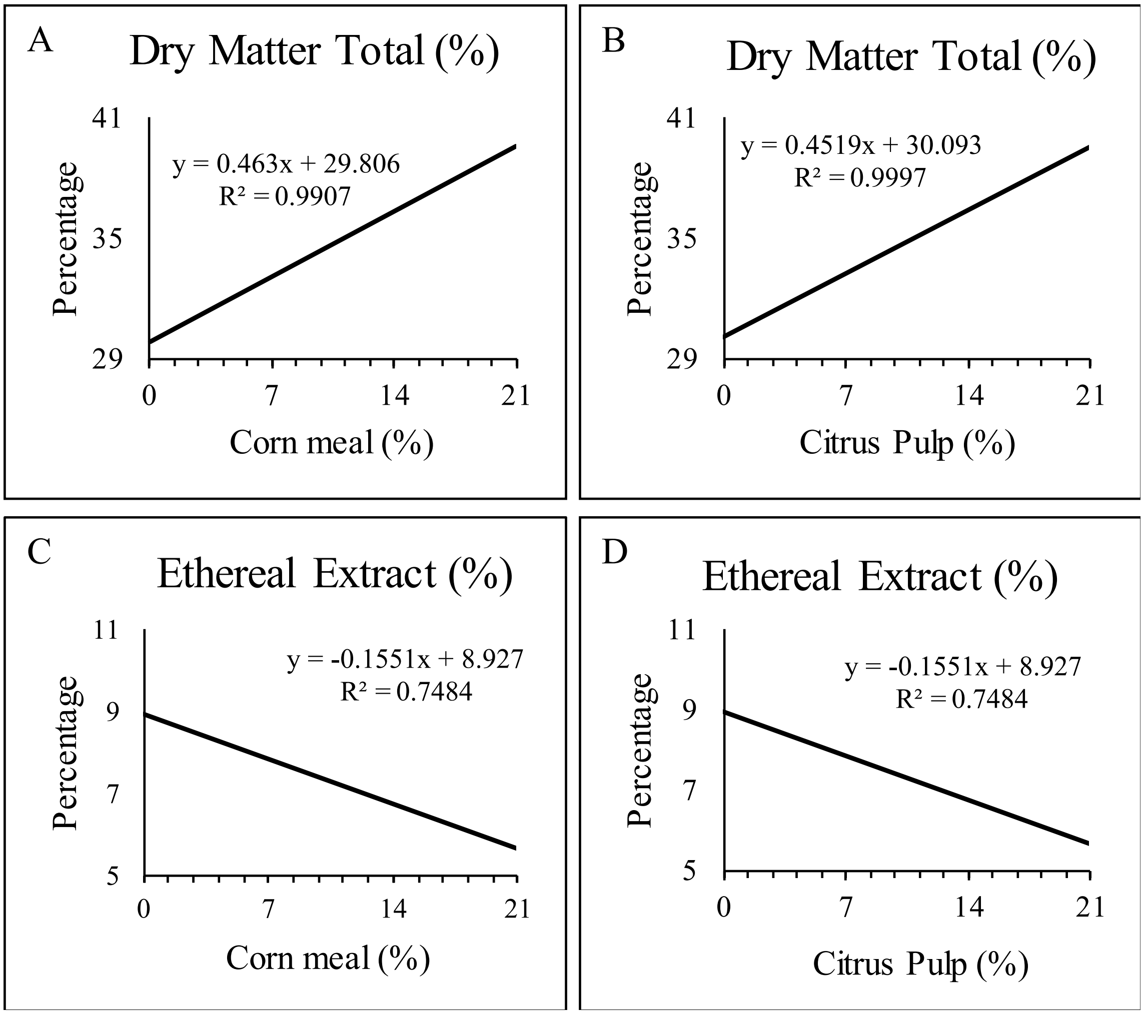

Figure 1 - (%) and ethereal extract (%) of soybean silage with corn meal (A and B) and citrus pulp (C and D) inclusion.

in elephant grass silage. The amounts of DM in silages are within the range of 30 to 40%, necessary to obtain quality silage. A negative linear effect was observed for EE, which can be considered good since high levels of dietary EE can limit DM intake due to high fat concentration and may negatively influence fiber digestibility (Barros et al., 2017).

Regarding the analysis of the chemical composition of the silage, inclusion of ground corn showed a positive quadratic effect (Fig 3A) (R^2 = 0.91) and inclusion of citrus pulp showed positive linear effect (Fig 4A) on TDM. High moisture crops, such as the whole-plant soybean, are prone to poor fermentation and nutrient loss due to excessive effluent production, which is also a potential contaminant of the environment (Silva et al., 2017). The mixture of corn meal or citrus pulp with soybean silage favored the absorption of moisture and consequently nutrient preservation in the silage.

A high DM content is also favorable to the animal because of the two types of limitations that can occur in ruminants (Owens and Basalan, 2016). The first is chemical, because of high levels of nutrients or energy in the diet, usually in feedlot animals. The other is physical, which occurs by filling the rumen completely. Thus, animals consuming high amounts of DM have greater nutrient levels than those consuming small amounts of DM (Arnold, 2017). Bulk feed containing excess water limits the intake of nutrients and impairs animal development (Manni et al., 2016; Huuskonen et al., 2017).

In prediction equations proposed to quantify effluent production in silages, TDM values

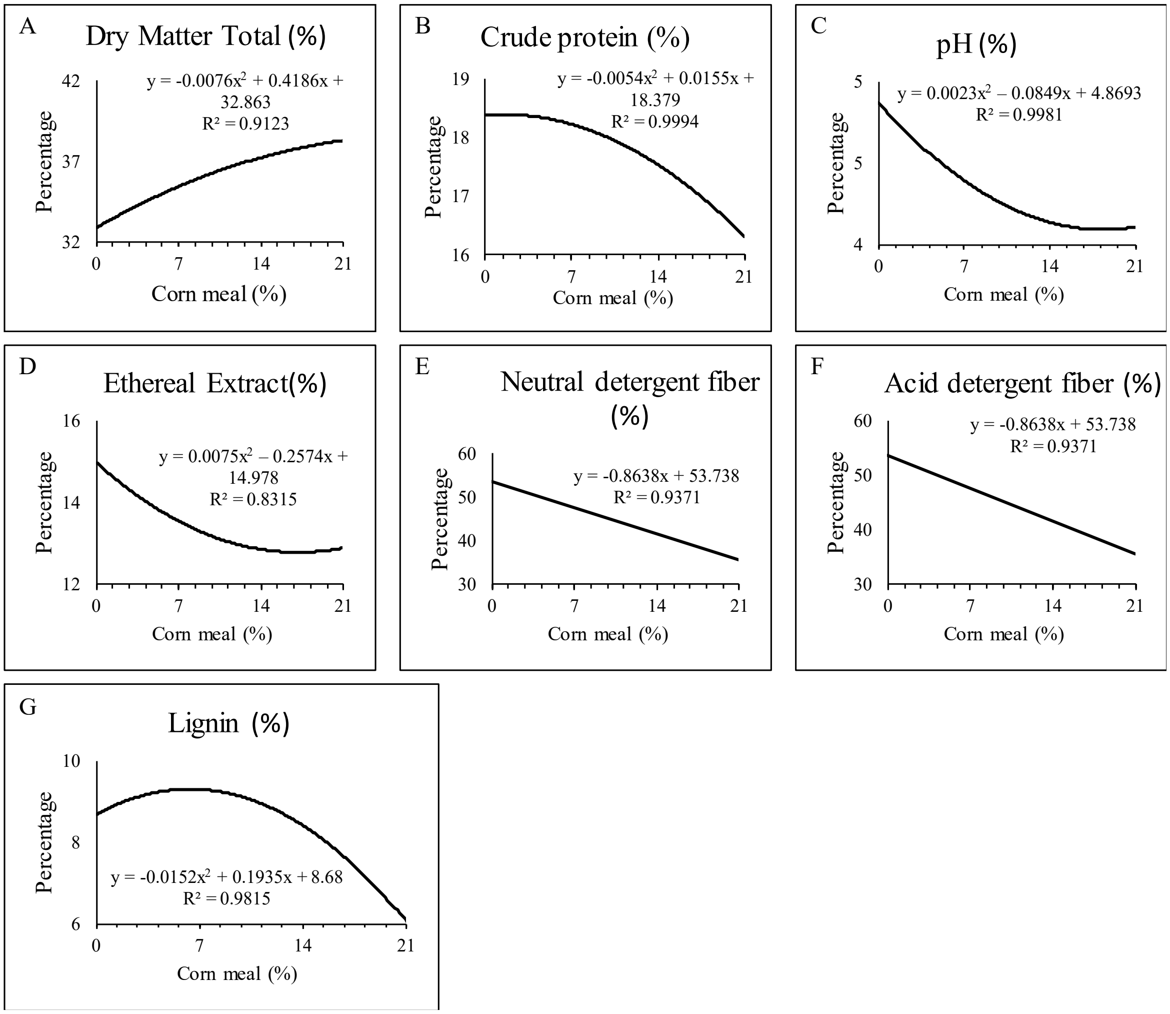

Figure 2 - Total dry matter (%), crude protein (%), pH, ethereal extract (%), neutral detergent fiber (%), acid detergent fiber (%), and lignin (%) of soybean silage with corn meal inclusion.

between 28.5 and 30.7% would be required to eliminate effluent production (Tomaz et al., 2018). On the other hand, even at high TDM levels, our silages with higher inclusion of citrus pulp had lower effluent production when compared to control (0.71 kg/ton of organic matter). These findings demonstrate that the inclusion of 21% of citrus pulp, in addition to increasing silage DM (40.32%), effectively reduces effluent production (0.00 kg/ton of organic matter) and, consequently, the impact and environmental pollution usually caused by silage production.

There was a quadratic effect of corn meal (maximum of 18.39% at 1.44% of inclusion) and a negative linear effect of citrus pulp ($R^2 = 0.99$) on CP. A protein level above 7% is necessary for good functioning of the rumen (Coelho da Silva and Leão, 1979) to ensure the best use of carbohydrates. Corn meal and citrus pulp are foods with lower levels of protein (Valadares Filho et al., 2018). Even so, the inclusion of these additives in soybean silage provided protein silage above the traditional corn silage (7.5%), which favors the lower inclusion of a protein source in the total diet when soybean silage is used.

Silages with a pH less than 4.6 for a DM content between 26 and 35% can be classified as excellent (Ojeda and Wernwli, 1990). In this study, the optimum pH was observed with 18.46% of corn meal inclusion (pH = 4.08) and 16.76% of citrus pulp inclusion (pH = 4.08) in soybean silage. In legume silages, pH values stabilize at higher levels because of the high buffering capacity, high CP and EE content

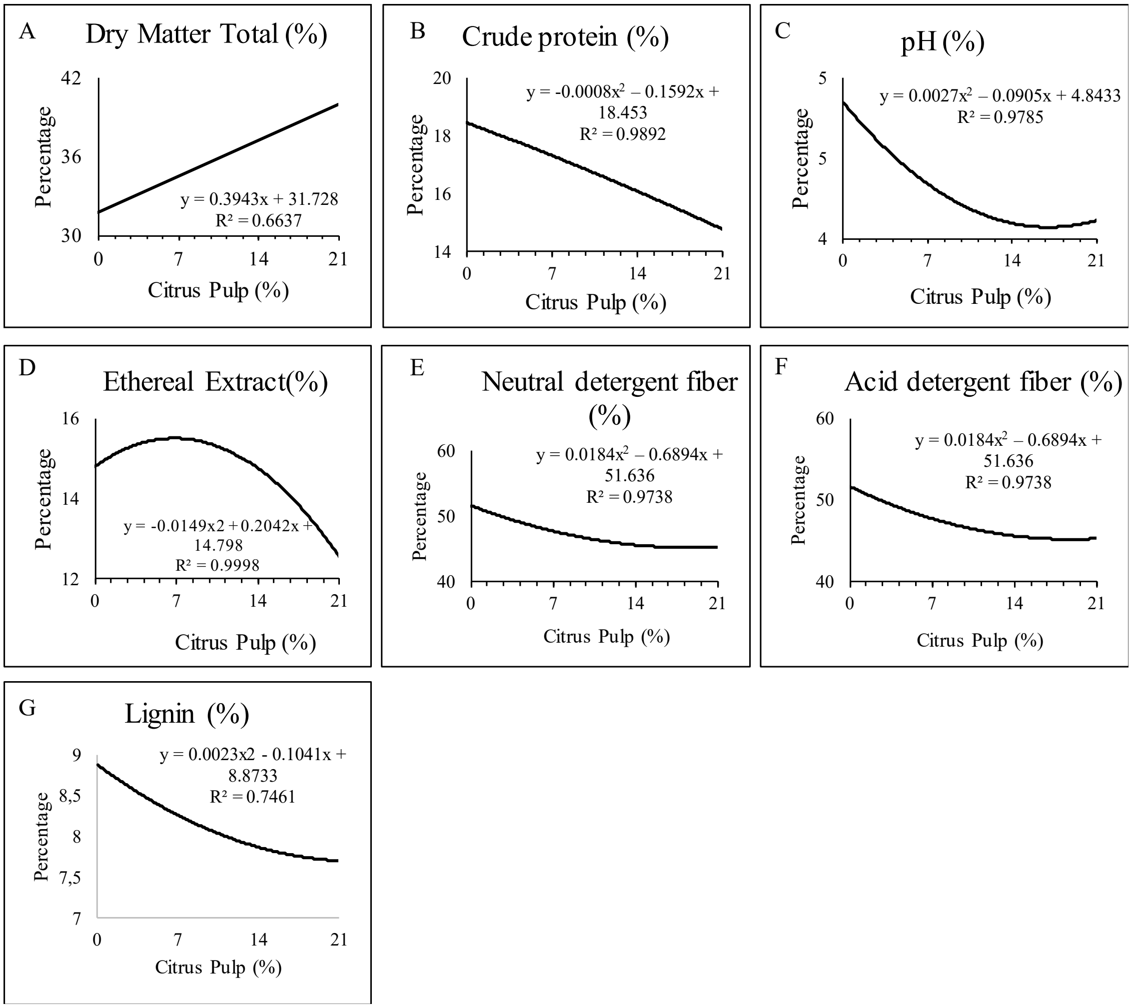

Figure 3 - Total dry matter (%), crude protein (%), pH, ethereal extract (%), neutral detergent fiber (%), acid detergent fiber (%), and lignin (%) of soybean silage with citrus pulp inclusion.

and low soluble carbohydrate content of these silages, which prevent an immediate reduction in pH.

The levels of EE in the silage were different for each inclusion process. Corn meal inclusion resulted in a negative quadratic effect ($R^2 = 0.83$) and citrus pulp inclusion in a positive quadratic effect ($R^2 = 0.99$). This probably occurred due to losses in the fermentation process of the silage containing corn meal, which favored the reduction in EE levels. According to the NRC (2016), beef cattle should be offered up to 6% EE in the diet. However, the inclusion of soybean silage in ruminant diets should be based on EE levels in the total mix.

An increase in the fat levels of animal feed leads to a decrease in fiber digestibility (Owens and Basalan, 2016) and a consequent reduction in rumen motility and an increase in bacterial proliferation. Increasing the supply of dietary lipids can provide up to 2.25 times more energy than supplying carbohydrate and protein to the animals (Arrigoni et al., 2016). This is the greatest advantage of the use of soybean silage compared to other silages.

The NDF and ADF values exhibited the same declining pattern both for inclusion of corn meal (negative linear effect) and for inclusion of citrus pulp (negative quadratic effect). For the data analyzed, the NDF values guarantee the good amount of fiber required for the animal at different levels of inclusion. NDF values above 60% impair rumen motility and delay the digestibility of ingested fiber. The results of this experiment show a decrease in NDF and ADF as the inclusion of ground corn

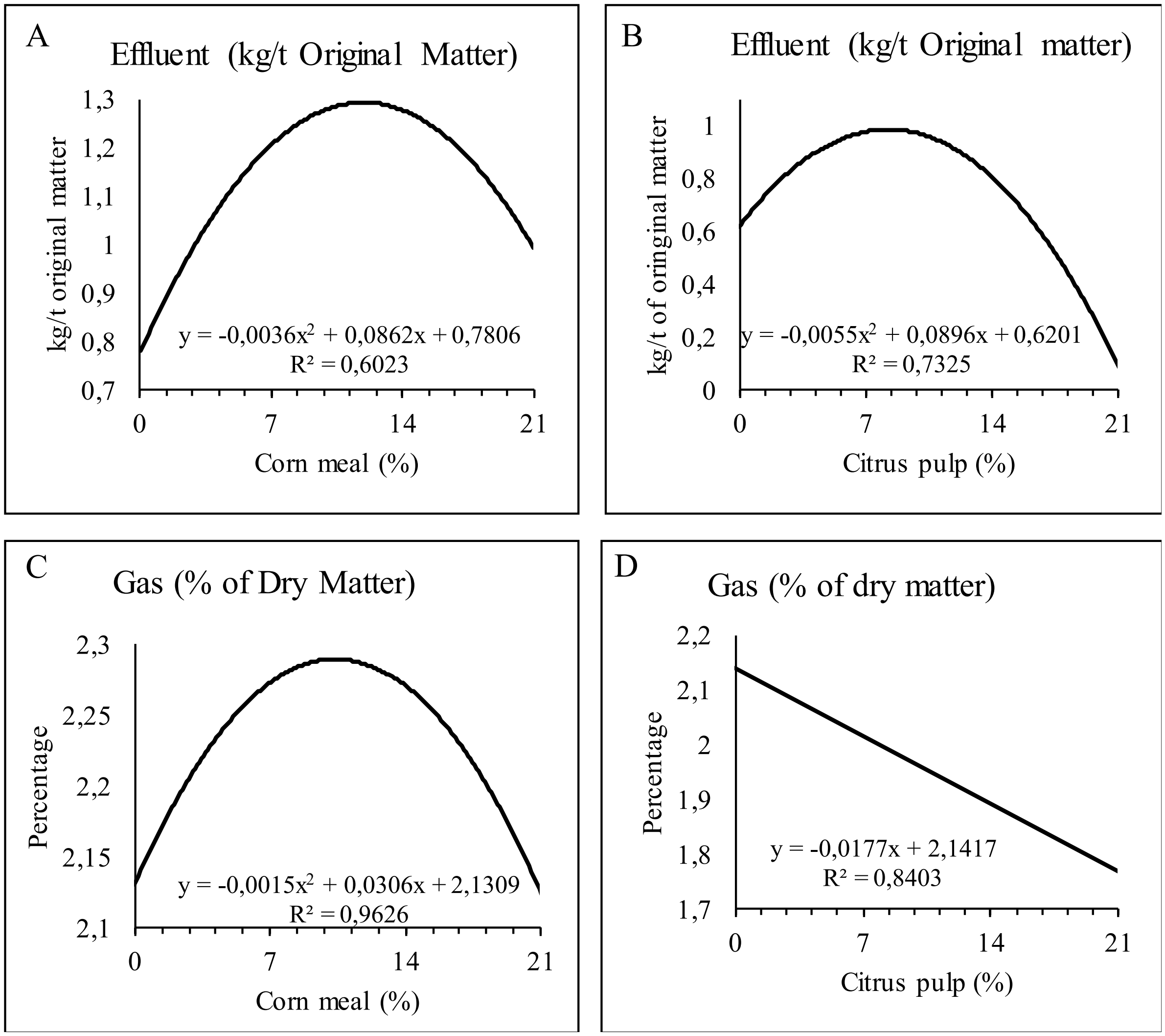

Figure 4 - Effluent (kg/ton of organic matter) and gas production (% of dry matter) of soybean silage with corn meal or citrus pulp inclusion.

or citrus pulp increases. FDA levels greater than 40% of DM are found only in plants of advanced physiological age (Dias et al., 2010).

Lignin is the second most abundant biological material on the planet, exceeded only by cellulose, and accounts for 15-25% of the dry weight of woody plants. In our work, there was a positive quadratic effect for inclusion of ground corn and a negative quadratic effect for inclusion of citrus pulp. These results indicate the higher lignification of milled corn, possibly due to the advanced stage of development at the time of harvest.

Lignin is one of the main obstacles to fiber digestion and limits the nutritive value of silage. However, this characteristic, which is important for plant survival, is often responsible for energy limitation when ingested by ruminants (Van Soest, 2018). Lignin is the most important limiting factor of digestibility (Maciel et al., 2012) because its accumulation reduces digestible energy, causing the incomplete use of cellulose and hemicellulose by the animals. Lignin, NDF and FDA should not be evaluated separately when digestibility limitation is determined.

CONCLUSION

The inclusion of corn meal and citrus pulp improves the pH of the silage, reduces EE levels, and increases the DM content of soybean silage. The inclusion of corn meal provides a higher percentage of protein compared to citrus pulp, while the amount of TDN remains the same in both inclusions. Based on the results, the inclusion of corn meal as an additive promotes better quality of soybean silage after silage opening.

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