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Common bean residue as additive in sugarcane silage[1]

Resíduo do feijão comum como aditivo em silagem de cana-de-açúcar

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ABSTRACT - The sugarcane has a high content of soluble carbohydrates, thereby resulting in many losses during its fermentation. Thus, it is necessary to use an additive that reduces the losses in the nutritional value of the forage during the ensiling process. The objective was to evaluate the use of common bean residue during the sugarcane ensiling process over the silage nutritional quality and fermentation characteristics. Were used 20 silos made of PVC pipes, in which the residue of crude and previously ground common bean processing (*Phaseolus vulgaris* L.) were added to newly chopped sugarcane in the proportions of 0; 50; 100 and 150 g kg^{-1} of fresh weight. A randomized design was used and the regression analysis was performed according to the level of common bean residue in silages. The losses from the ensiling process, the aerobic stability of the material, and the silage chemical composition, pH, NH_3-N and *in vitro* dry matter digestibility were analyzed. The inclusion of increasing levels of common bean residue until 150 g kg^{-1} improved the quality of silages, since there was a reduction in gaseous losses, improvement in food composition and *in vitro* dry matter digestibility, without impairing the silages pH. The common bean residue can be used as additive in sugarcane silage until 150 g kg^{-1} fresh weight for improving silage fermentation characteristics and dry matter digestibility.

Key words: Aerobic stability. Chemical composition. Fermentation. pH.

RESUMO - Objetivou-se avaliar o efeito da adição do resíduo do feijão comum (*Phaseolus vulgaris* L.) durante o processo de ensilagem da cana-de-açúcar sobre as características fermentativas e qualidade nutricional da silagem. Foram utilizados 20 silos experimentais feitos com canos de PVC em que o resíduo do feijão previamente moído foi adicionado à cana-de-açúcar recém-picada, nas proporções de 0; 50; 100 e 150 g kg^{-1} do peso fresco da forragem. Adotou-se o delineamento inteiramente casualizado e foram realizadas análises de regressão em função dos níveis de resíduo na silagem. Foram analisadas as perdas resultantes do processo de ensilagem, a estabilidade aeróbica do material, composição química, pH, N-NH_3 e digestibilidade *in vitro* da matéria seca das silagens. A inclusão dos níveis crescentes do resíduo até o nível de 150 g kg^{-1} melhorou a qualidade das silagens obtidas, uma vez que houve redução nas perdas na forma de gases, melhoria na composição química e na digestibilidade *in vitro* da matéria seca, sem haver comprometimento do pH das silagens. O resíduo do feijão comum pode ser utilizado como aditivo em silagens de cana-de-açúcar até 150 g kg^{-1} do peso fresco ensilado por melhorar as características fermentativas da silagem e a digestibilidade da matéria seca.

Palavras-chave: Composição química. Estabilidade aeróbia. Fermentação. pH.

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INTRODUCTION

The high potential production of dry matter and energy per unit area arouses the interest of producers to use the sugarcane (*Saccharum* spp.) in ruminant feed. However, the use of forage *in natura* has a very high operating cost due to the need for daily hand labor. Furthermore, its adoption is limited in larger herds as a result of logistical constraints related to the daily cutoff management. The silage is presented as an alternative process to the daily cutting process to supply the forage *in natura* form (SÁ NETO *et al*., 2013).

The ensiling of sugarcane is an excellent operational solution. However, it results in increased production of ethanol due to the high content of non-fibrous carbohydrates, represented almost exclusively by soluble carbohydrates, which favors the yeast proliferation during the fermentation process (DANIEL *et al.*, 2013). An intense growth of yeast can result in high dry matter loss and reduction in the quality of the feed (CARVALHO *et al.*, 2014). Thus, one should consider the possibility of using additives during ensiling that favor fermentation, improve the nutritional value of the silage mass and allow greater aerobic stability after opening the silo.

For ensiling of sugarcane, the better additive would be the product with a high content of dry matter (DM), avoiding both the losses of nutrients arising from the proliferation of yeast which results in significant alcoholic fermentation and the possible formation of effluents. According to Santos, Pinho and Bezerra (2012), absorbing additives most often used during the practice of ensiling tropical grasses are wheat bran, ground corn, citrus pulp and coffee rusk as well as dried fruit waste.

The additive choice should fall on that with effectively control of quantitative and qualitative losses during fermentation and aerobic exposure, taking into account the low cost of the product. The residue from the processing of common bean (*Phaseolus vulgaris* L.), composed mostly of broken and defective beans, is a good option for its chemical composition regarding the crude protein (236.4 g kg^{-1} DM) and total digestible nutrients (726.9 g kg^{-1} DM) (VALADARES FILHO *et al*., 2016). In addition, the grinding of the residue results in a powdery material with high absorption capacity.

In this study we aimed to evaluate the inclusion of residue from the processing of the common bean (*Phaseolus vulgaris* L.) as additive during ensiling of sugarcane (*Saccharum* spp.) on the quality of the silage mass.

MATERIALS AND METHODS

The filling of experimental silos was performed at the Academic Unit of Garanhuns at the Federal Rural University of Pernambuco (UAG-UFRPE), as well as processing and storage of samples. Laboratory analyses were performed at the Laboratory for Animal Nutrition (LANA-UAG), the headquarters of UFRPE in Recife-PE and the Center for Agricultural Sciences UFPB in Areia-PB.

We used a variety of sugarcane RB 867515 aged 12 months, with chemical composition shown in Table 1, which was manually harvested during the dry season, peeling it to remove the dried leaves, as it is typically done when this fodder is provided *in natura* to animals. Disintegration was carried out in a stationary chopper regulated to cut the material into particles of about 2 cm in length.

The residue from common bean (*Phaseolus vulgaris* L.) consisted of straw waste arising from the bean pod and damaged grain of types: integers (crushed, wrinkled, stained, without pellicle and others), broken, from the mixture of black and red cultivars, with no predominance of any given variety, with chemical composition shown in Table 1. The material obtained from commercial grain processing was previously ground and added to the sugarcane freshly chopped at the ratios of 0; 50; 100 and 150 g kg^{-1} of fresh weight, representing 0; 130; 240 and 330 g kg^{-1} of dry matter of the mixtures, respectively, with chemical composition shown in Table 2.

The experimental design was completely randomized with four treatments (levels 0; 130; 240 and 330 g kg^{-1} DM) and five repetitions for each treatment, totaling 20 silos made with PVC pipes (60 cm height and 30 cm in diameter), which were kept at room temperature until the time of opening. Initially, each silo was filled with a layer of dry sand of known weight for retention and subsequent quantification of effluent. In each silo, were placed approximately 23 kg of material, in order to obtain approximate density of 550 kg m^{-3}. Compression is performed manually with the aid of wooden socket and mass trampling. After the forage accommodation inside the silo, they were sealed with plastic lids provided with a *Bunsen* valve to exhaust gases, which were sealed with silicone glue and tape.

After 300 days of storage, the silos were opened and the entire content was removed and placed on plastic canvas for homogenization. After this procedure, samples were taken from each experimental unit. A sample was used to extract the juice by means of a hydraulic press, approximately 50 ml of silage juice has been used to quantify the pH in digital potentiometer, previously calibrated with buffer solutions of pH 4.0 and 7.0.

Table 1 - Chemical composition of the common bean residue and sugarcane used during ensiling

Item	Common bean residue	Sugarcane
Dry matter, g kg^{-1} FW[1]	896.2	319.7
Ash[2]	55.8	22.7
Crude protein[2]	235.6	22.6
Neutral detergent insoluble nitrogen[3]	357.9	277.6
Acid detergent insoluble nitrogen[3]	177.8	177.1
Ether extract[2]	14.8	9.6
Neutral detergent fiber[2]	247.9	509.5
Acid detergent fiber[2]	168.9	340.3
Hemicellulose[2]	58.0	169.2

[1]FW = fresh weight; [2] g kg^{-1} dry matter; [3] g kg^{-1} total nitrogen

Table 2 - Chemical composition of mixtures sugarcane and common bean residue before ensiling

Item	Residue level (g kg^{-1} DM)			
	0	130	240	330
Dry matter, g kg^{-1} FW[1]	319.7	348.5	377.3	406.1
Ash[2]	22.7	26.0	25.8	27.8
Crude protein[2]	22.6	33.2	43.9	54.5
Ether extract[2]	9.6	9.8	10.1	10.4
Neutral detergent fiber[2]	509.5	471.9	444.1	425.3
Acid detergent fiber[2]	340.3	316.6	301.3	293.4
Hemicellulose[2]	169.2	155.3	142.8	131.9
Non fibrous carbohydrates[2]	435.6	459.1	476.1	482.0

[1]FW = fresh weight; [2]g kg^{-1} dry matter

Another sample was weighed, wrapped in paper bags and kept in a forced air oven at 55 ºC for 72 hours to obtain the pre-dry matter. It was subsequently processed in Willey mill with sieve of 1 mm and stored in plastic containers for subsequent chemical analysis.

The dry matter (DM) contents were determined by drying in oven at 105 °C for 24 h (AOAC, 1995), the ash was quantified after firing the samples at 600 °C for 3 hours (AOAC, 1990), while the organic matter (OM) was obtained by the difference (AOAC 1990). Nitrogen content was determined by the Kjeldahl method (AOAC, 1995), and the crude protein content calculated by the 6.25xN factor. The ether extract was determined by extraction in diethyl ether (AOAC, 1990). The contents of neutral detergent insoluble fiber (NDF) and acid detergent insoluble fiber (ADF) were determined according to the methodology described by Mertens (2002). The hemicellulose (HEMI) was obtained from the equation: HEMI = NDF - ADF. The total carbohydrates (CHO) were estimated by equation (CHO) = 100 - (CP + EE + MM), while the content of non-fiber carbohydrates (NFC) were estimated by the difference between total carbohydrates and NDF, all expressed in g kg^{-1} DM. The total digestible nutrients (TDN) were calculated according to the equation TDN = IVDMD + (1.25 x EE) - MM, proposed by Van Soest (1994). The digestible energy was estimated as DE (Mcal kg^{-1}) = 0.04409 x % TDN and metabolizable energy (ME) estimated by the conversion of DE (DE x 0.82).

The *in vitro* dry matter digestibility (IVDMD) was determined according to Holden (1999), using the artificial rumen developed by TECNAL®. The ammonium nitrogen compared to the total nitrogen was determined according to the method of Preston (1986).

Three other samples were used to estimate the aerobic stability. Each sample (approximately 1 kg of silage) was packaged in plastic buckets with a capacity of two liters. The buckets were placed in a room under controlled temperature, with average of 24 ± 1 °C. Were carried out the temperature monitoring of the silages and the environment over a period of 240 hours via thermometers with mercury bulbs at 12 hours intervals on the first day and 24 hours intervals in the others, totaling 12 measurements of each sample. For the stability calculation, time taken in hours for the forage mass increase in 2.0 °C the environment temperature was used (KUNG JÚNIOR *et al*., 2000). After the exposure period of silage to air, the juice of mass was extracted for measurement of pH after stability using digital potentiometer.

Losses by the formation of effluents captured in the sand layer at the bottom silos were estimated using the formula: EP = (WBE - WAE) / FoNM x 1000, where: EP = effluents production (effluent kg t^{-1} of natural matter ensiled), WBE = weight of the whole silo/sand before ensiling (kg), WAE = weight of the whole silo/sand after emptying (kg), FoNM = forage natural mass (kg), as proposed by Siqueira *et al*. (2010).

The gaseous losses were evaluated according to the methodology proposed by Siqueira *et al*. (2010). To obtain the net weight of the material contained in each silo at the early and late experiment, the following equation was used: GL = (IW – FW)/IDM x 100 where: GL = gas losses (g kg^{-1} DM), IW = initial weight of the silo at the time of ensiling (kg), FW = final weight of the silo at the opening (kg), IDM = initial dry matter ensiled.

Dry matter recovery was calculated following the formula DMR (%) = [(FoNM x FoDM)/(SiNM x SiDM)] x 100; where FoNM: forage natural mass (kg) at the time of ensiling; FoDM: Forage dry matter (g kg^{-1} NM) at the time of ensiling; SiNM: silage natural mass (kg) in the opening of the silo; SiDM: silage dry matter (g kg^{-1} NM) in the silo opening, according to Jobim *et al*. (2007).

The data was subjected to variance and regression analysis (PROC REG) according to the level of common bean residue, considering the level of 0.05 probability for type I error, using the Statistical Analysis System (SAS, 2001) software.

RESULTS AND DISCUSSION

There were losses in the dry matter for all silages with average losses of 28.5% compared to the material before ensiling. These losses are associated with decreased cellular content, primarily soluble carbohydrates during fermentation (Table 2 and 3). Common bean residue addition increased ($P<0.05$) the contents of DM, crude protein (CP), non-fiber carbohydrates (NFC) and the *in vitro* dry matter digestibility (IVDMD) of silages (Table 3). On the other hand, according to Santos *et al*. (2015) ensiling sugarcane without additives results in alcoholic fermentation from the growth of yeasts and this results in a loss of nutritional value as the total concentration of sugars and sucrose decline.

The highest DM content of the silages containing common bean residue can be attributed to its high DM content. The addition of 240 and 330 g kg-1 DM of the common bean residue afforded to obtain silages with DM content above 25%, demonstrating the effectiveness of the additive used to reduce the DM losses. In contrast, the lowest DM content in the silage without common bean residue addition can be attributed to low DM of sugarcane.

Regarding the components of the fiber fraction of the silages, there was decreased linear response ($P<0.05$) for NDF, ADF and hemicellulose with the common bean residue addition (Table 3). The effect on the hemicellulose fraction meant 3.78 g kg^{-1} DM hemicellulose reduction for each unit of common bean residue. The silage with adding the common bean residue showed lower concentration of NDF (551.9 g kg^{-1}DM) compared to silages without addition (795 g kg^{-1} DM), meaning reduction of 24.3 percentage units. The higher NDF in silages without additive can be attributed to its lower content NFC, as shown in Table 3. The addition of common bean residue decreased NFC losses, being observed losses of 65.8% in the silages without additive, relative to initial NFC concentration. On the other hand, in the silages with 330 g kg^{-1} DM common bean residue addition, these losses amounted to only 21.7%, that means a reduction of 44.1% in NFC losses with the common bean residue addition. Furthermore, the NDF of the sugarcane was already high, which contributed to the higher NDF proportion of silage without additive.

Ferreira *et al*. (2007) explained that during the fermentation process, there is a reduction of the soluble carbohydrates content used by the microorganisms present in the silage, and consequently there is DM content reduction, thus leading to increased percentage of cell wall constituents which are not used by microorganisms for their metabolism. Sugarcane silages made without additives often result in materials with high fiber content, due to the lack of yeasts inhibition that are most responsible for the reduction of cellular content in these silages (LOPES; EVANGELISTA, 2010). The increased soluble carbohydrates accompanied by the reduced NDF in silages with common bean residue shows up as a very promising result and of great interest to nutritionists, since the high NDF concentration are generally found in sugarcane silages relate negatively with DM intake, due to poor fiber quality (SIQUEIRA *et al*., 2012).

The common bean residue positively influenced ($P<0.05$) CP content of the ensiled material, resulting in 2.46 g kg^{-1} DM increase in the protein content for each

Table 3 - Chemical composition and digestibility of sugarcane silages enriched with common bean residue

Item	Residue level (g kg^{-1} DM)				MSE	Equation	R²
	0	130	240	330			
DM[1]	233.0	244.0	273.0	286.0	1.2	$\hat{Y}$= 229.4 + 1.70x	78.22
OM[2]	971.0	969.0	971.0	967.1	0.2	$\hat{Y}$ = 969.5	-
Ash[2]	29.0	31.0	29.0	32.8	0.2	$\hat{Y}$ = 30.45	-
CP[2]	26.0	67.0	87.0	108.5	0.7	$\hat{Y}$ = 29.22 + 2.46x	95.72
NDIN[3]	558.0	287.0	251.0	243.6	7.1	$\hat{Y}$ = 552.61 – 25.40x + 0.49x²	94.49
ADIN[3]	314.0	255.0	232.0	198.4	5.0	$\hat{Y}$ = 249.85	-
EE[2]	17.0	18.0	17.0	23.5	0.7	$\hat{Y}$ = 18.87	-
NDF[2]	795.0	696.0	594.0	551.9	1.9	$\hat{Y}$ = 792.09 – 7.60x	96.30
ADF[2]	531.0	470.0	408.0	387.2	1.4	$\hat{Y}$ = 527.84 – 4.51x	94.64
HEMI[2]	259.0	227.0	192.0	171.3	1.6	$\hat{Y}$ = 247.62 – 3.78x	90.39
NFC[2]	149.0	212.0	305.0	324.2	2.3	$\hat{Y}$ = 148.35 + 5.66x	91.26
TC[2]	928.0	884.0	866.0	835.0	1.0	$\hat{Y}$ = 925.71 – 2.70x	92.55
IVDMD[4]	369.0	381.0	464.0	584.8	4.0	$\hat{Y}$ = 367.30 + 0.642x	84.44
ME[5]	1.3	1.4	1.6	2.1	0.1	$\hat{Y}$ = 1.229e0.0014x	87.67

[1]g kg^{-1} fresh weight; [2] g kg^{-1} dry matter; [3] g kg^{-1} total nitrogen; [4] g kg^{-1}; [5] Mcal kg^{-1}; DM = dry matter; OM = organic matter; PB = crude protein; NDIN = neutral detergent insoluble nitrogen; ADIN = acid detergent insoluble nitrogen; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; HEMI = hemicellulose; NFC = non-fibrous carbohydrates; TC = total carbohydrates and IVDMD = *in vitro* dry matter digestibility; ME = metabolizable energy; MSE = mean standard error; R² = determination coefficient

unit of additive. This contribution is quite significant since the low protein content of the sugarcane is a major limitation to their use in ruminant feed. The increase in the concentration of CP provided by the addition of common bean residue, can favor the microorganisms population growth in the rumen of animals fed with these silages. Despite this increase in protein concentration of silages, the ADIN (unavailable N fraction) was not influenced (P>0.05). This may be indicative of a good standard fermentation of silage, since the increase of this fraction reduces the digestibility of protein.

The inclusion of the common bean residue reduced NDIN concentration (P<0.05). This fraction represents the N associated with the cell wall, presenting slower degradation rate, thus, silages with higher NDIN proportion can limit the intake of this food and its ability to provide nutrients (FREITAS *et al*., 2006). Therefore, the reduced concentration of this fraction can improve the nutritional value of these silages.

The common bean residue influenced (P<0.05) the total carbohydrates and non-fiber carbohydrates (Table 3). The reduction in total carbohydrates is explained by the increased CP concentrations with the inclusion of common bean residue. The increase of non-fiber carbohydrates was due to the reduction of neutral detergent fiber, promoted by additive inclusion.

The *in vitro* dry matter digestibility showed a linear effect, which leads to the inference that the additive improves the dry matter digestibility. The cell wall constituents present in sugarcane show low rate of degradation by rumen microorganisms (SIQUEIRA *et al*., 2012), whereas the common bean residue has constituents with high digestibility, which helped to improve the silage IVDMD. The increase in the constituents of higher digestibility improved nutritional quality of the silage, resulting in increase (P<0.05) in energy value. With the addition of 330 g kg^{-1} DM of common bean residue there was an increase of 0.8 Mcal kg^{-1} DM in the silages metabolizable energy, which means 61.5% increase in energy value, in relation to silages without additive.

There was no effect (P>0.05) of the common bean residue addition on the loss by effluents (Table 4). The values obtained (33.5 kg t^{-1}; MSE = 16.4) were similar to those observed by Santos, Nussio and Mourão (2008), they also observed losses of 31.3 kg t^{-1} of FW (MSE = 16.1) for sugarcane silages without additive. The addition of common bean residue decreased the gaseous losses (P<0.05) in the silages (Table 4). The largest loss observed in silage without additive should be associated with higher moisture content of the silage that resulted in higher activity of epiphytic yeasts, which convert sugars and lactate into ethanol, CO_2 and water (BERNARDES *et al*., 2007).

Table 4 - Losses from effluent, losses from gases and dry matter recovery of sugarcane silages enriched with common bean residue

Item	Residue level (g kg^{-1} DM)				MSE^3	Equation	R^2
	0	130	240	330			
Effluents, kg t^{-1} FW[1]	35.5	31.0	39.3	28.3	16.4	Ŷ = 33.51	-
Gases, g kg^{-1} DM[2]	190.0	169.0	165.0	119.0	1.8	Ŷ = 19.430-0.190x	64.24
DM recovery, %	76.9	79.4	91.1	88.0	4.6	Ŷ = 83.85	-

[1]FW = fresh weight; [2]DM = dry matter; [3]MSE = mean standard error; R² = determination coefficient

The lower fiber content and higher DM in silages, associated with lower losses recorded in the silages with the additive, confirm that the common bean residue was effective in reducing the losses of soluble carbohydrates (SC) in silages, considering that NFC in sugarcane is represented almost exclusively by sucrose (SANTOS *et al*., 2015).

The pH at the time of opening the silos and after 240 hours of exposure to air was influenced ($P<0.05$) by common bean residue, increasing linearly (Table 5). However, it is observed that the pH values for all silages are within the range considered by Muck (2010) as ideal (less than 4.5). Therefore, it can be inferred that the increased CP content of the silage had no effect on lowering the pH during fermentation. However, Schmidt, Mari and Nussio (2007) emphasized that in substrates with high soluble carbohydrates, such as sugarcane, pH is not inhibitory to yeasts that may develop under these environments below pH 3.5. For this reason, losses resulting from the fermentation process tend to increase overtime, because the yeast type microorganisms are not inhibited with decreasing pH.

The inclusion of increasing levels of common bean residue resulted in increased pH after 240 hours. When the silo is opened and the silage is exposed to air, aerobic microorganisms begin growth, having organic acids, such as lactic acid, as energy source (MUCK, 2010). This is what determines the silages pH increase when exposed to air.

The increased pH can also be explained by the greater amount of remaining water-soluble carbohydrates in silage with additive, serving as a substrate for the development of aerobic microorganisms in silage, making it more prone to heating and deterioration during the post-opening period (AMARAL *et al*., 2009). In this study the addition of common bean residue resulted in higher concentration of NFC and consequently higher proportion of residual soluble carbohydrates.

Regarding the ammonia nitrogen (Table 5), there was also the influence of the additive, which linearly increased ($P<0.05$) the amounts of NH_3-N due to increased protein in silages with the inclusion of common bean residue. However, the increased CP content of the silage at the studied levels was not enough for forming NH_3-N in quantities that impaired the lowering pH of the silage, which is quite satisfactory. Concentrations of ammonia nitrogen found in evaluated silages were lower than the 11 - 12% recommended by Lopes and Evangelista (2010) as maximum for good standard fermentation of silages. The results also indicate that no excessive degradation of NH_3-N, protein to the levels studied, allowing greater availability of true dietary protein for use by rumen microrganisms.

The temperature of silages after opening the silos increased linearly ($P<0.05$) with the common bean residue inclusion (Table 5). Therefore, the common bean residue decreased the aerobic stability of silages. Mohd-Setapar, Abd-Talib and Aziz (2012) concluded that the temperature

Table 5 - Ammonia nitrogen, pH in the silo opening and after 240 hours and aerobic stability of sugarcane silage enriched with common bean residue

Item	Residue level (g kg^{-1} DM)				MSE	Equation	R^2
	0	130	240	330			
Ammonia nitrogen[1]	22.0	37.0	49.0	55.0	0.6	Ŷ = 29.21+2.46x	95.72
pH in the opening	3.5	3.7	3.7	3.8	0.1	Ŷ = 3.495+0.010x	63.42
pH after 240 hours[2]	3.4	4.3	5.2	5.2	0.8	Ŷ = 3.551+0.057x	53.90
Aerobic stability[3]	177.1	104.7	95.5	62.5	5.1	Ŷ = 181.74-28.696x	58.82

1FW = fresh weight; 2DM = dry matter; 3MSE = mean standard error; R² = determination coefficient of ensiling process will affect the quality of silage due to the additive used in the silage. One of the causes for the aerobic deterioration is the presence of fermenting yeast in silage, which use sugars and lactate in their metabolism (TABACCO *et al*., 2009). As the silage containing common bean residue resulted in higher concentration of residual SC, the deterioration of such silages was faster.

CONCLUSIONS

1. The common bean residue addition during ensiling of sugarcane promotes silage with superior nutritional quality compared with the silage without additive, verified by increased *in vitro* digestibility, higher final crude protein concentration and non-fibrous carbohydrates and lower NDF of ensiled mass;
2. The additive use decreases losses by gas formation, which can be added to the amount of 150 g kg^{-1} ensiled fresh weight.

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