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Nutritive value of sugarcane silages added with increasing levels of acetic acid

Valor nutricional de silagens de cana-de-açúcar aditivadas com níveis crescentes de ácido acético

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Abstract

The objective of this study was to evaluate the effect of adding acetic acid during ensiling on fermentation quality and nutritive value of sugarcane (*Saccharum officinarum*). The treatments consisted of sugarcane silages of IAC 86-2480 variety added with five inclusion levels of glacial acetic acid (0, 15, 30, 45 and 60 g kg^{-1} in the dry matter basis). A completely randomized design with five treatments and six replicates was used. The glacial acetic acid had a pH of 2.9 (0.1M). The pH of the sugarcane silage reduced by 0.07 units for each 10 g kg^{-1} of acetic acid added *($P < 0.01$)*. The $N\text{-}NH_3$ concentration in the ensiled mass was not affected by treatments, averaging 4.5 g kg^{-1} in the dry matter basis ($P = 0.91$). The means for effluent losses were adjusted to the quadratic regression model *($P < 0.01$)*. The yeast population reduced by 0.44 log CFU g^{-1} of silage for each 10 g kg^{-1} of acetic acid added ($P < 0.01$). The addition of acetic acid to sugarcane silages reduces the fermentation losses and the yeast population, besides improving the nutritive value of silages in levels from 15 g kg^{-1} of dry matter.
Key words: pH. Fermentative profile. Yeasts. Dry matter. Degradability.

Resumo

Objetivou-se com este estudo avaliar o efeito da adição de ácido acético durante a ensilagem na qualidade fermentativa e valor nutritivo de silagem de cana-de-açúcar (*Saccharaum officinarum*). Os tratamentos consistiram de silagem de cana-de-açúcar variedade IAC 86-2480 com cinco doses de ácido acético glacial (0, 15, 30, 45 e 60 g kg^{-1} na base da matéria seca). Foi utilizado delineamento inteiramente

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casualizado com cinco tratamentos e seis repetições. O pH do ácido acético glacial foi de 2,9 (0.1M). O ph da silagem de cana-de-açúcar reduziu 0,07 unidades para cada 10 g kg^{-1} de ácido acético adicionado *(P < 0,01)*. A concentração de N-NH_3 na massa ensilada não foi afetada pelos tratamentos, com média de 4,5 g kg^{-1} na matéria seca *(P = 0,91)*. As médias para perdas por efluente se ajustaram ao modelo de regressão quadrático *(P < 0,01)*. A população de leveduras reduziu 0,44 log UFC g^{-1} na silagem para cada 10 g kg^{-1} de ácido acético adicionado *(P < 0,01)*. A adição de ácido acético em silagens de cana-de-açúcar reduz as perdas fermentativas e a população de leveduras, além de melhorar o valor nutritivo de silagens na dose de 15 g kg^{-1} na matéria seca.
Palavras-chave: Ph. Perfil fermentativo. Leveduras. Matéria seca. Degradabilidade.

Introduction

In many tropical countries, sugarcane (*Saccharum officinarum* L.) is used as an important feed source for ruminants due to its high productivity per area (25-40 t ha^{-1} of dry matter [DM]) (ÁVILA et al., 2009; DANIEL et al., 2013; RIGUEIRA et al., 2017). Furthermore, the sugarcane is harvested in the winter and has a high concentration of soluble carbohydrates (>400 g kg^{-1} of DM) compared to other cultures (ÁVILA et al., 2009). Sugarcane is fed as silage in diets for beef cattle and dairy cows in Brazil (FREITAS et al., 2006; MILLEN et al., 2009; BERNARDES; RÊGO, 2014) due to the difficulty in harvesting this forage source on a daily basis (ROTH et al., 2016; RIGUEIRA et al., 2017). Moreover, the silage is a useful alternative to prevent total loss of the forage in cases of accidental fire or severe frosts (ROTH et al., 2016).

However, sugarcane silage is characterized by the development of yeasts, which can ferment soluble carbohydrates to alcohol. It results in high DM losses (> 300 g kg^{-1}), besides reducing the nutritive value of silages (ALLI et al., 1982; RIGUEIRA et al., 2017), aerobic stability and intake by animals (KUNG; STANLEY, 1982). Several studies have tested the use of additives during the ensiling of sugarcane in order to reduce the yeast population and increase the population of lactic acid bacteria (CAVALI et al., 2010), aiming at increasing the competition between these microorganisms and then reducing DM losses (RABELO et al., 2014) and ethanol production (SIQUEIRA et al., 2011; ROTH et al., 2016). Danner et al. (2003) reported that the use of acetic acid in corn silage as an additive was effective in inhibiting the development of filamentous fungi and yeasts. It occurs because acetic acid at a pH lower than the pKa (4.73) remains in its nonionic (non-dissociated) form, and the membrane of fungi and yeasts becomes permeable to the acid, which enters the cell via passive transport (DAVIDSON, 1997). Inside the cell, the acid is dissociated ($RCOO^- + H^+$) due to the pH being close to 7.0, thus releasing H^+ ions. It reduces the intracellular pH of fungi and yeasts by modifying their metabolism (MOON, 1983). Microorganisms must eliminate H^+ ions for maintaining a constant intracellular acidity; then, they lose energy in the process, which slows their growth and leads to cell death (MOON, 1983; DAVIDSON, 1997). Schmidt and Kung Junior (2010) found that the application of *Lactobacillus buchneri* 40788 isolated or associated with *Pediococcus pentosaceus* (4×10^5 CFU g^{-1} and 1×10^5 CFU g^{-1} of corn) during whole-plant corn ensiling process increases the concentration of acetic acid and reduces the population of yeasts in the ensiled mass. For barley straw silage (*Hordeum vulgare* L.), Qiu et al. (2014) found that the application of 3 g kg^{-1} of acetic acid in the dry matter improved both fermentation quality and aerobic stability. However, when it comes to sugarcane silage, more information is needed on the best application level of acetic acid and its effects on the fermentation profile and nutritive value.

The objective of this study was to evaluate the effect of adding acetic acid during ensiling on fermentation quality and nutritive value of sugarcane silage.

Materials and Methods

The experiment was carried out in the Center of Agricultural Sciences of the State University of Montes Claros, Janaúba *Campus* (latitude 15°52'38" S, longitude 43°20'05" W). The average annual rainfall at the location is 800 mm, with an average annual temperature of 28 °C and relative humidity of approximately 65%. According to the Köppen classification, the predominant climate in the region is Aw (ANTUNES, 1994).

The treatments consisted of sugarcane (*Saccharum officinarum*) silages of IAC 86-2480 variety added with five inclusion levels of glacial acetic acid (0, 15, 30, 45 and 60 g kg^{-1} in the dry matter basis). A completely randomized design with five treatments and six replicates was used.

The forage was harvested on June 29, 2016, from pre-selected areas at the UNIMONTES Experimental Farm after 12 months of the last cut. The sugarcane was cut manually and later chopped using a forage chopper coupled to an electric motor; the chopping knives were set to cut the forage to a particle size of 2 cm. The forage was harvested from five piles, and the acetic acid was added according to the experimental levels and homogenized before ensiling.

Experimental PVC silos of known weight and measuring 40 cm in length and 10 cm in diameter were used to store the silage. The bottom of the silos contained 10 cm of dry sand that was separated from the forage by foam to allow the measurement of effluents. After complete homogenization, the resultant material was deposited into the silos and compacted using a wooden plunger. The silage density (550 kg of dry matter m^{-3}) was quantified for each treatment, and approximately 3 kg of the chopped fresh forage was ensiled as recommended by Ruppel et al. (1995). After filling with forage, the silos were closed with PVC lids, fitted with Bunsen-type valves, sealed with adhesive tape and weighed.

The silos were stored at room temperature on the Laboratory of Food Analysis of UNIMONTES and were opened 60 days after the ensiling. Samples were collected from the middle of each silo after discarding the silage at the top, where fungi were present, and then were pre-dried in an oven (samples) with forced ventilation at 55 °C to constant weight. Subsequently, a portion of the pre-dried material was ground in a Willey mill to pass a 1 mm screen for chemical composition analysis. The remaining samples were ground in a Willey mill to pass a 5 mm screen for the *in situ* degradability assay. The ground material was stored in properly identified plastic bags. The silage fermentation analyses were performed as follows: pH was determined using a digital potentiometer according to the methodology described by Silva and Queiroz (2006), and ammonia nitrogen concentration, expressed as total nitrogen ($N\text{-}NH_3$, g kg^{-1} DM), was analyzed using approximately 25 g of silage, as proposed by Bolsen et al. (1992). The effluent losses were obtained using the following equation: E = (Wop - SWen)/(GRME) x 1000, where: E: effluent production (kg ton^{-1} of green mass); Wop: set weight (full bucket + lid + wet sand + bag) at silo opening (kg); SWen: set weight (empty bucket + lid + dry sand + bag) at the ensiling (kg); GRME: green forage mass ensiled (kg).

The losses through gases were calculated as suggested by (JOBIM et al., 2007): G = {[(Wen - SWen)* DMen] - [(Wop-SWen)*DMop] x 100} / [(Wen - SWen)* DMen], where: G represents the gas losses (g kg^{-1} DM); Wen is the weight of the full bucket (kg) at the ensiling; SWen is the set weight (empty bucket + lid + dry sand + bag) (kg) at the ensiling; DMen is the forage dry matter content (%) at the ensiling; Wop is the weight of the full bucket (kg) at the opening; DMop is the forage dry matter content (%) at the opening.

The pre-dried forage was analyzed for dry matter (DM, 934.01), ash (942.05), ether extract (EE; 920.39), and crude protein (CP, 978.04) as described by the AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the sequential method according to

procedures described by Robertson and Van Soest (1981) using a TECNAL® TE-149 fiber analyzer (Piracicaba, SP, Brazil). Cellulose was solubilized in sulfuric acid (72%), and the lignin content was obtained from the resulting difference in weight (GOERING; VAN SOEST, 1970). The contents of neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined according to Licitra et al. (1996), and the levels of neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) were obtained by multiplying the NDIN and ADIN by 6.25.

The estimation of the indigestible dry matter and indigestible NDF (iNDF) was performed using the nylon bag technique, as proposed by Ørskov and McDonald (1979), according to the recommendations of Nocek (1988). The nylon bags were preheated in an oven with forced air ventilation for 24 hours at 55° C and then weighed to obtain the empty bag weight. The samples were weighed and placed in the nylon bags before sealing. The samples were put in the bags according to a ratio of 20 mg DM/cm^2 of surface (NOCEK, 1988). The nylon bags were attached to a nylon cord and placed within the rumen of a fistulated adult cattle. The incubation period lasted 144 hours; after this period, the bags were collected from the rumen and washed in running water, soon after undergoing a drying process. Dry matter (DM) was determined in an oven at 55 °C for 72 hours. The obtained residue was used to perform the NDF analyzes, following the methodologies described by Robertson and Van Soest (1981).

Microbiological analysis was performed for the quantification of yeasts at the Laboratory of Microbiology of UNIMONTES. A sample of 25 g of sugarcane silage was weighed and diluted in 225 mL of peptone water at 0.1%. Serial dilutions (10^{-1} to 10^{-7}) were made and placed on Petri dishes containing potato dextrose agar (Difco) acidified at pH 3.5 and then incubated in aerobiosis for 72 hours. Subsequenyl, the colonies were counted. Table 1 shows the chemical composition of sugarcane *in natura* used in the experiment.

Table 1. Chemical composition of sugarcane at the ensiling.

Item	Sugarcane *in natura* (g kg^{-1} DM)
Dry matter	300.8
Ash	106.2
Crude protein	27.6
Neutral detergent fiber	332.2
Acid detergent fiber	176.2
Ether extract	18.9
Lignin	132.5

DM - Dry matter

The collected data were submitted to analysis of variance, and when the result of the "F" test was significant, the acetic acid inclusion levels were subjected to regression analysis by fitting data to linear, quadratic and cubic models. For all statistical procedures, $\alpha = 0.05$ was set as the maximum acceptable level of type III error. If two models were significant (e.g., linear and quadratic), the model with the highest degree polynomial was chosen.

Results

The pH of the sugarcane silage reduced by 0.07 units for each 10 g kg^{-1} of acetic acid added (P

<0.01). The pH variation between the control silage and the highest level (60 g kg^{-1} of dry matter; OM) reached 11.20% (Table 2). However, the N-NH_3 concentration in the ensiled mass was not affected by treatments, averaging 4.5 g kg^{-1} of DM (P = 0.91).

Table 2. Fermentation profile of sugarcane silages added with increasing levels of acetic acid.

Item	Inclusion levels of acetic acid (g kg^{-1} DM)					SEM	P-value
	0	15	30	45	60		
pH[1]	3.39	3.28	3.02	3.04	3.01	0.03	<0.01
N-NH_3, g kg^{-1} DM	4.2	4.9	4.7	4.5	4.4	0.1	0.91
Effluent loss[2], kg t^{-1}	38.47	29.92	29.92	33.17	34.55	7.1	<0.01
Gas loss[3], g kg^{-1} DM	78.4	67.4	67.4	55.6	51.4	3.3	<0.01
Yeasts[4], log CFU g^{-1}	4.79	5.02	5.02	3.15	2.38	0.3	<0.01

pH - potential of hydrogen; N-NH_3 - ammonia nitrogen; DM - dry matter; SEM - standard error of the mean; *P*- probability; [1]$\hat{Y}$=3.35 - 0.07*X, R^2= 0.82; [2]$\hat{Y}$=37.45 - 4.82*X + 0.75X^2, R^2= 0.80; [3]$\hat{Y}$=77.2 - 0.43X, R^2= 0.93; [4]$\hat{Y}$=5.4 - 0.44X, R^2= 0.75

The means for effluent losses were adjusted to the quadratic regression model, reaching its minimum at 32.1 g $kg^{-1}$of OM (P <0.01). Gas losses decreased by 4.3 g kg^{-1} of DM for each 10 g kg^{-1} of acetic acid added during the ensiling of sugarcane (P < 0.01). Similarly, the yeast population reduced (P < 0.01) by 0.44 log colony forming unit (CFU) for each 10 g kg^{-1} of acetic acid added (P < 0.01). The variation in yeast population from the lowest to the highest inclusion level of acetic acid during the ensiling reached 50.31%.

The contents of DM (P < 0.01), non-fibrous carbohydrates (NFC; P < 0.01), total digestible nutrients (TDN; P < 0.01) and DM digestibility (DMD; P <0 .01) increased linearly with increasing levels of acetic acid in sugarcane silage (Table 3), increasing 9.5, 19.7, 9.9 and 8.0 g kg^{-1}, respectively.

Table 3. Nutritive value of sugarcane silages added with increasing levels of acetic acid.

Item (g kg^{-1} DM)	Inclusion levels of acetic acid (g kg^{-1} DM)					SEM	P-value
	0	15	30	45	60		
Dry matter[1]	221.4	227.1	236.8	250.1	239.2	3.3	<0.01
Ash	47.9	55.9	51.3	51.1	49.0	3.0	0.42
Crude protein	56.5	44.0	55.4	58.1	59.8	5.0	0.93
Neutral detergente fiber[2]	562.2	543.2	489.8	465.6	448.6	10.7	<0.01
Acid detergente fiber [3]	314.5	289.3	255.2	244.5	234.1	7.7	<0.01
Lignin[4]	197.3	190.1	170.6	163.4	158.2	4.6	<0.01
Non-fibrous carbohydrates[5]	303.2	316.7	373.4	394.9	412.5	12.1	<0.01
Total digestible nutrients[6]	549.6	558.0	585.8	596.5	605.3	5.4	<0.01
Potentially digestible DM[7]	670.0	684.9	695.4	702.8	721.3	4.2	<0.01
Potentially digestible NDF	539.7	533.8	556.9	554.6	555.6	10.7	0.44

DM - dry matter; DM - dry matter; SEM - standard error of the mean; *P*- probability;[1]$\hat{Y}$= 218.9 + 0.95*X - 0.09*X^2, R^2= 0.82; [2]$\hat{Y}$=562.9 - 2.03*X, R^2= 0.93; [3]$\hat{Y}$= 308.6 - 1.37*X, R^2= 0.94; [4]$\hat{Y}$= 196.9 - 0.69X, R^2=0.97; [5]$\hat{Y}$= 300.8 + 1.97*X, R^2= 0.95; [6]$\hat{Y}$= 549.0 + 0.99*X, R^2=0.96; [7]$\hat{Y}$= 670.7 + 0.80*X, R^2= 0.98

The contents of ash (P = 0.42), crude protein (CP; P = 0.93) and potentially digestible fibrous fraction (DFND; P = 0.44) were not affected by the inclusion of acetic acid during the ensiling, averaging 51.0, 54.7 and 548.0 g kg^{-1}, respectively. Reductions of 2.03, 1.3 and 0.69 percentage points for each 10 g kg^{-1} of acetic acid added to the sugarcane silage were observed for neutral detergent fiber (NDF; P < 0.01), acid detergent fiber (ADF, P < 0.01) and lignin (P < 0.01), respectively.

Discussion

The use of acetic acid during the ensiling of sugarcane reduced the pH values from 3.39 in the control silage to 3.01 in the highest application level (60 g kg^{-1}), which is related to the low pH (2.9) of acetic acid. This acid in the ionized form releases H^+ in the medium, consequently reducing the pH of the ensiled mass. However, acetic acid is inefficient in reducing the pH of the silage compared to lactic acid (SCHMIDT; KUNG JUNIOR, 2010). The microbial activity might also influence the pH of the ensiled mass because some microorganisms reduce the pH of the medium through the natural production of acids, especially the lactic acid (DANIEL et al., 2013).

Roth et al. (2016) reported pH values below 3.5 in sugarcane silage, which is similar to those observed in this study (mean pH of 3.39 in the control silage). It indicates that the pH values in sugarcane silages are lower than that of corn (SILVA et al., 2018) and sorghum silages (above 4, MOURA et al., 2017), mainly due to the high content of soluble carbohydrates (sucrose), allowing for high production of dry acids by fermenting microorganisms (DANIEL et al., 2013). According to McDonald et al. (1991), well-conserved silages must have pH values lower than 4.2, which allows inferring that all silages are within the recommended range.

The $N\text{-}NH_3$ concentration in the silages was not affected by the addition of acetic acid, averaging 4.5 g kg^{-1} of DM. The values found are desirable in well-conserved silages. Increasing ammonia nitrogen concentration may be associated with a gradual reduction in the conservation efficiency of the ensiled material, indicating proteolysis by undesirable fermenting microorganisms (SANTOS et al., 2012). According to McDonald et al. (1991), silages with $N\text{-}NH_3$ concentrations below 100 g kg^{-1} are well preserved. Due to the low protein content in sugarcane, the silages had low levels of ammoniacal nitrogen, which is mostly diluted in the produced effluents.

The lowest effluent loss (29.70 kg t^{-1}) was observed at 32.1 g kg^{-1} of acetic acid in the dry matter. Effluent losses are influenced by the DM content of the material to be ensiled, compaction level and type of fermentation that occurred. Although the DM content and compaction level have been standardized in the experiment, the effect of acetic acid on effluent losses is possibly due to variations in fermentative populations, especially undesirable microorganisms such as clostridia (DANNER et al., 2003) and yeast (SCHMIDT; KUNG JUNIOR, 2010).

Gas losses from the ensiled sugarcane mass decreased with the inclusion of acetic acid. According to Muck (1988), silo gas forms as a result of the secondary fermentation by enterobacteria, clostridia bacteria, and aerobic microorganisms, which usually grow in high pH media. It would explain the decrease in gas losses, since the addition of acetic acid during the ensiling process inhibited the growth of microorganisms by maintaining the pH value at less than 32.8 g kg^{-1} among treatments.

The yeast population in the sugarcane silage was reduced from 4.79 to 2.38 log CFU g^{-1} of silage with the inclusion of acetic acid. It suggests that the acetic acid acted on the microbial metabolism, leading to cellular energy exhaustion, thus affecting phosphorus absorption and the activity of glycolytic enzymes. Moreover, the addition of acetic acid during the ensiling reduced the intracellular pH, leading to higher ATP consumption for removing

H^+ ions within the cells after their dissociation (DAVIDSON, 1997). Siqueira et al. (2011) evaluated *in natura* or burned sugarcane silages with or without *L. buchneri,* and reported a yeast concentration of 4.85 log CFU g^{-1} of *in natura* silage without inoculant.

Yeasts in silages are known to convert forage sugars into ethanol, carbon dioxide, and water. Therefore, it is reasonable to reduce gas losses by showing the importance of acetic acid during the ensiling of sugarcane. In order to increase the levels of acetic acid in the ensiled mass, several studies (SIQUEIRA et al., 2011; SILVA et al., 2018) suggest the use of inoculants such as *L. buchneri*; however, Siqueira et al. (2011) reported that the results are still contradictory in sugarcane silages due to the influence of factors such as substrate, moisture, initial population, among others. In silages of common species such as sorghum and rehydrated corn, the natural production of acetic acid by bacteria reaches 2.25% (MOURA et al., 2017) and 3.6% of dry matter (SILVA et al., 2018), respectively. However, in sugar cane silages, the population of acetic acid-producing bacteria tends to be smaller due to substrate competition with yeasts. Thus, the production of acetic acid by the remaining bacteria is generally not sufficient (SIQUEIRA et al., 2011) and then the addition of acetic acid in the silage becomes an alternative for controlling the population of yeasts in the silage.

Regarding the nutritive value, the DM content increased from 221.4 g kg^{-1} in the control treatment to 239.2 g kg^{-1} in the silage added with 60 g kg^{-1} of acetic acid. This effect is probably associated with decreased water production during the fermentation process of the silage due to the effect of acetic acid on the epiphytic microflora of silage. According to McDonald et al. (1991), the production of water resulting from fermentative pathways contributes to DM losses and varies according to the type of fermentative microorganisms. The DM content of the silage is directly associated with the fermentative quality and losses during fermentation. In tropical forages, Jobim and Nussio (2013) reported that the DM contents at the ensiling should vary between 280 to 400 g kg^{-1} for ensuring proper fermentation in the silo and avoiding losses by effluents. The DM content of sugarcane silage at the ensiling (mean of 308.0 g kg^{-1}, Table 1) was within the recommended by Jobim and Nussio (2013). Roth et al. (2016) ensiled sugarcane with a DM content of 270.0 g kg^{-1} one day after accidental burning and did not verify an effect on the fermentation profile. Thus, the results verified in this study confirmed that the losses by effluents are related to the presence of undesirable microorganisms (ROTH et al., 2016).

The NDF, ADF, and lignin contents of the silages decreased with increasing levels of acetic acid. This result may be related to the higher proportion of non-fibrous carbohydrates in silages added with acetic acid due to the smaller population of yeasts, which would degrade the sugars as mentioned by Siqueira et al. (2011). When inoculating silages with *L. buchneri*, Qiu et al. (2014) reported increases in the concentration of acetic acid but did not observe an effect on NDF content. According to them, the dose of inoculant used is the likely explanation for the lack of effect. When not controlled by the concentration of acetic acid in sugarcane silage, the yeasts dominate the fermentation process, since their growth is not inhibited by the pH in the range of 2 to 8 (SIQUEIRA et al., 2011). Yeast fermentation will yield ethanol, carbonic gas, water, and ATP, generating losses of DM and, consequently, providing proportional increases of the fibrous fractions. It justifies the result of these fractions in this study for silages without acetic acid.

The DMD increased with the inclusion of acetic acid, with gains of 80 g kg^{-1} for each 10 g kg^{-1} of acetic acid added. This increase is associated with the lower fibrous fractions in silages (NDF and ADF) due to the decrease of the lignin fraction as a result of the higher proportions of soluble carbohydrates in the silages that received the additive. It allowed maintaining a higher proportion of DM content compared to the fibrous fractions.

After 60 days of silage storage, the potentially digestible DM increased by 711 g kg^{-1} with the inclusion of acetic acid at the ensiling. This result is associated with a higher concentration of non-fibrous carbohydrates with the inclusion of acetic acid, which is more digestible by the ruminal microorganisms compared to the fibrous carbohydrates.

Conclusion

The addition of acetic acid to sugarcane silages reduces the fermentation losses and the yeast population, besides improving the nutritive value of silages in levels from 15 g kg^{-1} of dry matter.

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