Effects of microbial inoculants and by-product from amino acids production on fermentation, chemical composition and aerobic stability of corn silage

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SUMMARY

In recent years, the use of microbial inoculants has been increasing in Brazil, although the results have been conflicting. This study was conducted to evaluate the effects of adding a by-product from amino acid production or biological inoculants on fermentative characteristics, chemical composition and aerobic stability of corn silage. The treatments were: Control (control silage without additive); BAP (silage with addition of 1% of by-product from monosodium glutamate amino acid production); Pionner (silage with addition of bacterial inoculant Pioneer 1174®, containing *S. faecium* and *L. plantarum*) and Mercosil (silage with addition of bacterial inoculant Mercosil Mais 11C33®, containing *L. buchneri, L. plantarum* and *E. faecium*). Silages were produced in laboratory silos, constituted of plastic buckets with suitable lids for sealing. Silos were opened after 60 days of ensiling. The contents of DM, WSC and IVDMD were not affected ($p>0.05$) by any of the additives. The BAP treatment increased ($p<0.05$) CP content of corn silage in 23.0%, resulting in a drop ($p<0.05$) of 36.3% in ADIN content. Moreover, BAP treatment decreased ($p<0.05$) the concentrations of ADF and NDF, while Mercosil decreased ADF and NIDA contents. The additives promoted adequate fermentation pattern according to pH values (3.48 to 3.54) and ammonia nitrogen concentration (11.07 to 13.30% of total N) except that treatment with BAP increased $N\text{-}NH_3$ in 88.30%. Low fermentation losses were observed in the treatments Control, BAP and Pioneer when compared to Mercosil, so that Mercosil treatment increased in 49.70% acetic acid concentration when compared to Control group. The additives had no effect on the other fermentation characteristics (concentration of ethanol, propionic, butyric and lactic acids) or aerobic stability. In conclusion, the additive BAP can be added to corn silage to improve only its chemical composition. However, Mercosil and Pioneer produced limited effects on improving the fermentation profile.

Efeitos de inoculantes microbianos e do subproduto da produção de aminoácidos sobre a fermentação, composição química e estabilidade aeróbia da silagem de milho

Palavras chave adicionais

Conservação.
Deterioração.
pH.
Temperatura.

RESUMO

O uso de inoculantes microbianos no Brasil vem aumentando nos últimos anos, embora com resultados conflitantes. Objetivou-se avaliar os efeitos da adição de um subproduto da produção de aminoácidos ou inoculantes microbianos sobre as características fermentativas, bromatológicas e estabilidade aeróbia da silagem de milho. Os tratamentos foram: Controle (silagem de milho sem aditivo), SPA (silagem de milho com adição de 1% de subproduto da produção de aminoácidos), Pioneer (silagem com inoculante bacteriano Pioneer 1174®, contendo *S. faecium* e *L. plantarum*) e Mercosil (silagem com inoculante bacteriano Mercosil Mais 11C33®, contendo *L. buchneri, L. plantarum* e *E. faecium*). Os teores de MS, CHOs e DIVMS não foram alterados ($p>0,05$) por nenhum dos aditivos. O tratamento com o SPA aumentou ($p<0,05$) o teor de PB da silagem de milho em 23,0%, resultando em queda ($p<0,05$) de 36,3% nos teores de NIDA. Ainda, o SPA diminuiu ($p<0,05$) as concentrações de FDA e de FDN, sendo que o inoculante Mercosil diminuiu os teores de NIDA e FDA. Os aditivos promoveram adequado padrão de fermentação frente aos valores de pH (3,48 a 3,54) e de nitrogênio amoniacal (11,1 a 13,3% do N-total) excetuando o tratamento com SPA que aumentou o $N\text{-}NH_3$ em 88,3%. As menores perdas fermentativas foram observadas nas silagens controle, SPA e no Pioneer em relação ao Mercosil, sendo que o Mercosil aumentou em 49,7% a concentração de ácido acético em relação ao grupo Controle. Os aditivos não tiveram nenhum outro efeito sobre as características fermentativas (etanol, ácidos propiônico, butírico e lático) ou de estabilidade aeróbia. Concluiu-se que o aditivo SPA pode ser adicionada à silagem de milho para melhorar somente sua composição bromatológica. Mercosil e Pioneer tiveram limitados efeitos em melhorar as características fermentativas da silagem.

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INTRODUCTION

Silage is a good alternative for feed conservation and can be composed by grasses, legumes, grains, whole grains, tubers, feed industry residues and any other material that has a free sugar component required for fermentation (Pinto *et al.*, 2007).

When corn plant is used for ensilage, has several characteristics that makes it different from other grasses usually used for this process. Its widespread cultivation, high production per area, high energetic value and its adequate content of dry matter and soluble carbohydrates, combined to low buffering capacity, are great incentives to indicate this plant as the best choice for ensilage (Ramos *et al.*, 2002). The use of additives aims to improve dry matter recovery of conserved forage and mainly optimize fermentative process as well as minimize losses during ensilage (Rodrigues *et al.*, 2004). The use of microbiological additives in silage has been subject to several researches with the objective to inhibit aerobic microorganism's growth (especially those associated to aerobic stability such as yeasts, *Listeria* and others), to inhibit undesirable anaerobic microorganism's growth, such as enterobacter and clostridium, and to inhibit protease and deaminase activities of the plant and the microorganism.

Microbial inoculants commonly used as additives include homofermentative and heterofermentative bacteria, as well as, the combination of them. Homofermentative strains are characterized by faster fermentation rate, lower proteolysis, higher lactic acid production, lower contents of acetate and butyrate, lower content of ethanol and higher recovery of energy and dry matter. Lactic acid and glucose are used as substrates by heterofermentative strains for acetic and propionic acid production, which are effective in fungi and yeast control under low pH conditions (Zopolatto *et al.*, 2009).

The success in the use of microbiological additives in silages depends on the ability of inoculated bacteria to quickly grow in ensiled forage mass, on the presence of adequate substrate and on the inoculated bacteria population in relation to epiphytic population of the forage. Therefore, the use of microbial additives consists in an important resource, once it contributes to enzymatic proteolysis reduction, as a result of the quick pH reduction of ensiled mass, promoting great quantities of lactic acid production, which represents the possibility of higher recovery of ensiled dry matter (Henderson, 1993).

Although the effects of several microbial additives have been tested, it is necessary to study the use of new residues or industry by-product in order to reduce animal feeding costs and improve performance. As a rich in nitrogen content product, the addition of by-product from amino acid production (BPA) can increase the ammonia nitrogen content of the ensiled mass, controlling fungi and yeast growth which results in lower ethanol production. As information about by-products from amino acids production are scarce, this trial was designed to evaluate the effects of adding microbial inoculants and by-product from amino acids production on nutritive composition, fermentation profile and aerobic stability of corn silage.

MATERIAL AND METHODS

Making of silages

The trial was carried out at Department of Animal Nutrition and Production of the College of Veterinary Medicine and Animal Science of University of São Paulo (Pirassununga City, Sao Paulo State, Brazil). Regarding ethic aspects, this paper did not use living animals.

For ensiling, the hybrid corn Ag 1051 with high grain productivity was used (Agroceres). Corn plants were harvested with 96 days of growth, 50% milk line, in farinaceous state. After manual harvesting, corn plants were chopped (Chopper Nogueira, model EM-9F3B) to obtain average fragments of 1.43 cm. The evaluation of mean theoretical particle size was performed according to the sieves methodology *Penn State Particle Size Separator* proposed by Lammers *et al.* (1996). Chopped material was separated on a plastic packing-sheet and the treatments were applied on it using a manual spray. Each treatment had its own plastic packing-sheet and spray. Inoculants were diluted with distilled water (with no chloride), dosed according to the recommendations of each manufacturer and manually mixed to material to be ensiled.

The treatments were:

a) **Control:** Corn forage without additive.

b) **BAP (By-product from Amino acid Production):** Corn forage with addition of 1% of by-product from monosodium glutamate amino acid production. This product contains 55.2% of dry matter and 80.7% of crude protein at DM basis. The natural basis product was used at dose of 10.0 mL/kg of forage (1%).

c) **Pionner:** Corn forage with addition of bacterial inoculant Pioneer 1174®. Each gram of this product has $1.0x10^{11}$ colony-forming units (CFU) of *Streptococcus faecium* and *Lactobacillus plantarum*. The commercial product was used at dose of 1.0 mg/kg of forage.

d) **Mercosil:** Corn forage with addition of bacterial inoculant Mercosil Mais 11C33®. Each gram of this product has $1.0x10^{11}$ CFU of *Lactobacillus buchneri*, $8.0x10^{9}$ CFU of *Lactobacillus plantarum* and $2.0x10^{9}$ CFU of *Enterococcus faecium*. The commercial product was used at dose of 1.0 mg/kg of forage.

A completely randomized experimental design was adopted with four repetitions per treatment. Sixteen plastic buckets (12 liters of capacity each) were used as experimental silos. The respective forage masses were placed inside each silo and compacted to the density of 500 kg of corn plant/m^3. Silos were sealed with lids, weighed and vertically stored in a covered area at room temperature and only opened after 61 days of storage.

Laboratory analysis

before ensiling

After applying microbial inoculants or by-product from amino acids production to the fresh chopped

material, one sample of each treatment was obtained and stored at -20 ºC for later determination of dry matter (DM), crude protein (CP), acid detergent insoluble nitrogen (ADIN), acid detergent fiber (ADF), neutral detergent fiber (NDF), water soluble carbohydrates (WSC), *in vitro* dry matter digestibility (IVDMD), and buffer capacity (BC) according to methodologies described below. After thawing, this fresh material was analyzed at the same time with the silages in order to avoid analytical source of variation, especially for dry matter losses data.

After ensiling

Prior to opening, silos were weighed to later determination of dry matter (DM) losses during fermentation. After opening silos, the mass was homogenized and a fraction was separated for determination of dry matter (DM) and crude protein (CP), according to AOAC (1995); neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin, according to Van Soest *et al.* (1991). In the determination of DM, the final dry weight of the sample after 48-72 h at 55 ºC and 4 h at 105 ºC in oven with forced-air circulation is divided by the initial wet weight and multiplied by 100. Water soluble carbohydrates (WSC) were determined according to methodology proposed by Johnson *et al.* (1966) and the acid detergent insoluble nitrogen (ADIN), according to Van Soest and Robertson (1985). One fraction was immediately frozen for future counterproof and another was placed in hydraulic press for extraction of silage juice. Buffer capacity is measured by the quantity in meq of H^+ that is spent to decrease pH values of forage from 6.0 to 4.0, indicating the resistance of organic and inorganic components to acidification, during preservation (Playne and McDonald, 1966; McDonald *et al.*, 1991).

Immediately after material pressing during the opening, 50 mL of silage juice were used for pH determination with a portable digital pH meter (Procyon, model 310), calibrated with pH buffer solutions of 4.0 and 7.0. Moreover, 2 mL of silage juice was collected and added to 0.4 mL of formic acid and frozen at -20 ºC for further determination of organic acids and ethanol concentration. Determination of organic acids and ethanol concentration was done by gas chromatography, according to the methodology proposed by Erwin *et al.* (1961), using a gas chromatographer (Finnigan, model 9001), equipped with silica glass column MEGABOR (Ohio Valley, model OV-351) of 30 m x 0.53 mm and stationary phase of 1.0 micron. The determinations were performed injecting 1.0 µL of the sample in the chromatographer, which was integrated to a computer that processed the quantification calculations through the software Borwin (version 1.21) for chromatography, using a standard solution as basis for organic acids concentrations in the sample. The number of repetitions done per sample was the one needed to achieve a difference between readings below 5 %. The standard solution was injected every ten successive injections aiming to avoid possible deviations of readings due to column contamination. Organic acids concentration calculations were performed in a computer by the comparison of samples with the standard solution.

Another silage juice subsample of 2 mL was added to 1 mL of sulfuric acid 1 N and frozen at -20 ºC until analysis of ammonia nitrogen (NH_3-N) concentration by colorimetric assay, according to methodology proposed by Kulasek (1972) and adapted by Foldager (1977). Absorbance measurements were performed in spectrophotometer (Beijing Rayleigh AIC model VIS-7220) set in 630 nm. Values of absorbance were used to calculate NH_3-N concentrations in mg of NH_3-N/100 mL, by linear regression equation obtained by the calibration of the equipment with standard solution in different concentrations. Ammoniacal nitrogen-free protein (NH_3–FP) was also calculated discounting CP content from ammonia nitrogen.

Dry matter (DM) losses during fermentation were calculated as the difference between the weights of masses obtained at the moments of filling up and opening silos, multiplied by the respective dry matter contents. Losses were transformed in percentage of initial mass.

In vitro dry matter digestibility (IVDMD) was determined according to Tilley and Terry (1963). Duplicate samples from oven-dried forage (0.5 g) were weighed in test tubes, which were previously dried and calibrated. In the test tubes, 40 mL of McDougall solution (artificial saliva) was added to 10 mL of rumen inoculum of animals kept grazing *Brachiaria decumbens* pasture, supplemented with 3.0 kg of DM of sugarcane with *ad libitum* mineral salt. Tubes were sealed with rubber corks containing a Bunsen valve (immediately after flushing out with CO_2) and incubated in oven for 48 h in controlled temperature (39 ºC), where they were agitated at least 3 to 4 times during fermentation. The second phase occurred after centrifugation and discard of supernatant. Pepsin solution (1:10.000) at 0.2 % (50 mL) was added to each tube, followed by agitation at 39 ºC for another 48 hours. After washing, drying and weighing the tubes, calculations were performed as the formula below:

$$\text{IVDMD} = \frac{100 \times \text{g of DM in sample} - (\text{g of residual DM} - \text{g of DM of inoculum without sample})}{\text{g of DM in sample}}$$

For silage aerobic stability determination, subsamples of roughly 3.0 kg of fresh silage were taken from each bucket, placed in Styrofoam boxes (12 liter capacity) and stored in controlled temperature room (25 °C). Silage temperatures were monitored every 30 min, during seven days, using the *Monitoring and Acquiring Data System* (SIMAD), with 16 temperature sensors, 2 data acquiring modules, 1 net converter and 1 software for monitoring, acquiring and controlling environmental variables (MACVA version 1.2 from AUTSENS – Industry and Trade Electronic Devices). Maximum temperatures (°C), time to reach the maximum temperature (h) and time to raise the temperature in 2 °C (h) were recorded. Aerobic stability was calculated as the

Table I. Nutritive composition and buffer capacity of ingredients used for ensiling (Composição nutritiva e capacidade tampão de ingredientes utilizados para ensilagem).

Item	DM	CP	ADIN	ADF	NDF	WSC	IVDMD	BC
Corn plant	22.9	7.2	20.8	36.8	57.8	16.5	59.0	16.8
BAP	55.2	80.7	0.0	0.0	0.0	0.0	—	83.9

BAP= by-product from amino acids production; DM= total dry matter (%); CP= crude protein (% DM); ADIN= acid detergent insoluble nitrogen (% of total N); ADF= acid detergent fiber (% DM); NDF= neutral detergent fiber (% DM); WSC= water soluble carbohydrates (% DM); IVDMD= *in vitro* dry matter digestibility (% DM); BC= buffer capacity (meq/100 g DM of forage).

temperature rise rate (ºC/h), dividing the maximum temperature by the time to reach it (Ruppel *et al.*, 1995).

Statistical analysis

Results were analyzed by Statistical Analysis System software (SAS, 2002), after verifying normality of residues by Shapiro-Wilk test (PROC UNIVARIATE). Data (dependent variable), which did not attend to this premise, were subjected to logarithmic [Log (X+1)] or by square root [RQ (X+1/2)] transformation. Original or transformed data, when this procedure was necessary, were submitted to variance analysis by GLM procedure. Differences between treatments were separated by Tukey test. Significance level adopted was 5%.

RESULTS AND DISCUSSION

Nutritive composition and buffer capacity of corn plant and by-product from amino acids production used for ensiling are presented at **table I**. Nutrient content of corn plant was compatible with the expected, except from dry matter content that was below the expected. The low dry matter content observed in corn silage could be attributed to strong rain that anticipated crop harvesting at the moment of ensiling. The by-product from amino acid production used in this experiment had high dry matter (55.2% DM) and crude protein (80.7% DM) content. Macitelli *et al.* (2003) evaluated monosodium glutamate, as a feed additive for corn silage, and observed higher values of DM (62.5% DM) and lower of CP (42.0% DM), compared with product used in the present study.

Data of silage fermentative pattern treated with different additives are shown at **table II**. All additives (BAP, Pioner and Mercosil) slightly increased silage pH in 1.7%, 0.8% and 1.4%, respectively, in relation to control group. The BAP group showed higher pH than the other treated silages and control group, but Mercosil treatment did not differ from the other groups. The higher pH value of silage treated with BAP could be related to higher crude protein content, especially non-protein nitrogen.

Acidity is a critical point while evaluating the process of silage quality. Butyrate-producing bacteria are very susceptible to acid environment. So the faster the pH drops down to values close to 4.0, the more they will be inhibit, resulting in greater conservation of ensiled material and lower losses by protein fraction transformations. According to Muck and Shinners (2001), silages that underwent appropriate fermentation had pH values between 3.8 and 4.2. No silage had pH higher than 4.2, what would classify them as good fermentative quality. Similar results were found by Rodrigues *et al.* (2004) who evaluated corn silages treated with microbial inoculants.

By-product from amino acids production increased ($p<0.05$) in 88.30% the ammonia nitrogen concentration of silage when compared to control group. This effect is explained by the fact that the product is rich in nitrogen compounds (protein, amino acids and ammonium). Rodrigues *et al.* (2004) studied corn silages treated with different microbial additives and observed values of 6.50% of NH_3-N/TN, which were lower than the values observed in the present study. It is important to highlight that NH_3-N concentration found in Mercosil group is below 12% that allows classifying it as good quality silage (Fahey, 1994). The quick drop in pH and its maintenance at low values limit the occurrence of high proteolysis rates, resulting in better protein conservation of ensiled forage.

No effect of treatment ($p>0.05$) was observed on ethanol, propionic, butyric or lactic acid concentrations. These results show the limit capacity of BAP and

Table II. Fermentative patterns of corn silage treated with additives (Padrões fermentativos de silagens de milho tratadas com aditivos).

Variable	Treatment				SEM	Prob.
	Control	BAP	Pioneer	Mercosil		
pH	3.48^{c}	3.54^{a}	3.51^{b}	3.53^{ab}	0.007	0.0001
NH_3-N (% of total N)	12.51^{b}	23.56^{a}	13.03^{b}	11.07^{b}	1.317	0.0001
Ethanol (% DM)	0.44	0.52	0.52	0.43	0.020	0.2583
Acetic acid (% DM)	1.91^{b}	1.90^{b}	2.10^{b}	2.86^{a}	0.113	0.0001
Propionic acid (% DM)	0.02	0.02	0.02	0.03	0.002	0.2922
Butyric acid (% DM)	0.02	0.03	0.02	0.03	0.002	0.3610
Lactic acid (% DM)	7.97	7.76	8.01	7.89	0.081	0.7570
Lac: Ace ratio	4.17^{a}	4.11^{a}	3.83^{a}	2.78^{b}	0.155	0.0001
DM loss (% DM)	-1.42^{b}	-1.70^{b}	-2.09^{b}	7.72^{a}	1.297	0.0034

BAP= by-product from amino acid production; Ethanol (% DM); Acetic acid (% DM); Propionic acid (% DM); Butyric acid (% DM); Lactic acid (% DM); Lac: Ace ratio= lactate:acetate ratio; NH_3-N= ammonia nitrogen (% of total N); DM Loss= dry matter loss (% DM); SEM= standard error of mean; Prob.= probability of treatment effect. ^{abc}Rows with superscript letters differ by Tukey test ($p<0.05$).

Table III. Nutrient composition of corn silage treated with additives (Composição nutricional da silagem de milho tratada com aditivos).

Variable	Treatment				SEM	Prob.
	Control	BAP	Pioneer	Mercosil		
DM	23.38	23.88	23.23	22.95	0.164	0.2533
CP	7.56^{bc}	9.30^{a}	7.46^{c}	7.84^{b}	0.198	0.0092
NH_3-FP	6.62^{ab}	7.11^{a}	6.49^{b}	6.97^{ab}	0.091	0.0272
ADIN	21.89^{a}	13.95^{b}	17.33^{ab}	16.35^{b}	0.892	0.0002
ADF	38.50^{a}	35.68^{c}	37.40^{ab}	36.87^{bc}	0.297	0.0010
NDF	57.71^{a}	54.56^{b}	57.95^{a}	56.57^{a}	0.406	0.0001
WSC	7.96	8.63	8.00	6.48	0.345	0.1476
BC	49.42^{a}	35.14^{b}	37.10^{ab}	46.15^{ab}	2.097	0.0206
IVDMD	50.32	50.27	47.37	49.87	0.955	0.6998

BAP= by-product from amino acids production; DM= total dry matter (%); CP= crude protein (% DM); NH_3-FP= ammoniacal nitrogen-free protein (% DM); ADIN= acid detergent insoluble nitrogen (% of total N); ADF= acid detergent fiber (% DM); NDF= neutral detergent fiber (% DM); WSC= water soluble carbohydrates (% DM); BC= buffer capacity (meq/100 g DM of forage), IVDMD= in vitro dry matter digestibility (% DM); SEM= standard error of mean; Prob.= probability of treatment effect. ^{abc}Rows with superscript letters differ by Tukey test ($p<0.05$).

Pionner in substantially improve corn silage quality, especially when it is produced in good conditions, as observed in the present experiment. Mercosil additive increased acetic acid concentration of silage ($p<0.05$), as it was expected, once this product has the heterofermentative bacteria *Lactobacillus buchneri* in its composition. This microorganism is known to produce this organic acid, which was considered the responsible to improve aerobic stability of silages. Control silage presented acetate concentration below 2.0%, butyrate below 0.2%, lactate:acetate ratio above 3.0 and pH below 4.0. Regarding the treatments effect, Mercosil increased in 49.7% acetate concentration ($p<0.05$) when compared to control silage. This effect resulted in a decrease of 33.3% of lactate:acetate ratio by Mercosil ($p<0,05$), although Pioneer and BAP did not show significant effect on any of these parameters.

Mercosil treatment showed the highest dry matter losses when compared to control, BAP and Pionner treatments ($p<0.05$). It is important to point out that Mercosil altered dry matter losses, in spite of not showing any alteration on ethanol concentration. According to McDonald *et al.* (1991), significant increase in dry matter loss occurs when ethanol is produced due to the action of yeasts and bacteria like heterofermentative, enterobacteria and *Clostridium* spp. Therefore, this effect might be associated to organic acids concentrations, once Mercosil increased acetic acid concentration and, consequently, decreased lactate:acetate ratio. The treatments control, BAP and Pionner showed dry matter losses slightly negative. It means that more dry matter was observed at the silos opening. However, when these data were submitted to T test for mean equal to zero, they presented p value higher than 0.05, which means that these negative averages did not differ from zero. This suggests that this negative value is due to small variation in the technique of dry matter determination and average losses were very close to zero.

At evaluating the nutritive composition of silages **(table III)**, differences for dry matter, water soluble carbohydrates and *in vitro* dry matter digestibility of corn silages treated with different additives were not observed when compared with control group. A higher DM content in BAP treatment was expected as this additive already had high DM content before ensilage. *In vitro* dry matter digestibility varied from 50.3% in control silage to 50.3; 47.4 and 49.9% in silages treated with BAP, Pioneer and Mercosil, respectively.

The BAP treatment increased ($p<0.05$) CP content of corn silage in 23.0%, resulting in drop of ($p<0.05$) 36.3% in ADIN content. The raise of CP content caused by BAP is due to high concentrations of nitrogen sources in this product, although higher protein content was also observed even when CP was discounted from ammonia nitrogen content. ADIN decrease is compatible to dilution effect, observed when this variable is expressed in relation to total nitrogen. Higher CP contents in Mercosil silage were also observed when compared to Pioneer treatment. This fact suggests lower proteolysis in silage treated with this additive, therefore, resulting in better preservation of protein content of this silage. Ammoniacal nitrogen-free protein content in these silages also supports this observation. However, these results differed from those observed by Gimenes *et al.* (2006), who did not find effect of bacterial inoculants on CP content of corn silage. Average crude protein content in the experiment was 7.6% for control silage, which is close to values cited by Erdman (1993) and Rodrigues *et al.* (2002), who observed average crude protein content of 8.0 and 9.4%, respectively.

The additive BAP decreased ($p<0.05$) ADF and NDF concentrations in 7.3% and 5.3%, respectively, possibly due to dilution effect (inclusion of additive practically free of fiber) or due to a direct effect on fiber degradation, because of its chemical composition or due to both. The inoculant Mercosil also decreased ($p<0.05$) ADF concentrations in 4.2%, when compared to control silage, but the inoculant Pioneer did not alter any of these variables. Diverging from the present study, Ruiz *et al.* (2009) found increments of fiber content of silage inoculated with lactic acid bacteria when some corn hybrids were used, but no response was observed when the same inoculants were used with others corn hybrids.

The additive BAP decreased ($p<0.05$) silage buffer capacity in 28.9% compared with control silage. La-

Table IV. Aerobic stability of corn silage treated with additives (Estabilidade aeróbia de silagem de milho tratada com aditivos).

Variable	Treatment				SEM	Prob.
	Control	BAP	Pioneer	Mercosil		
Max (ºC)	29.57	30.14	29.85	31.75	0.524	0.2689
Time (h)	85.88	81.50	91.38	123.00	8.674	0.3415
Rate (ºC/h)	0.118	0.143	0.108	0.093	0.010	0.3960
Time 2ºC (h)	9.50	18.88	19.25	11.25	1.719	0.0685

BAP= by-product from amino acids production; Max= maximum temperature reached (ºC); Time= time spent to reach maximum temperature (hours); Rate= temperature rise rate (ºC/hour); Time 2ºC= time to raise the temperature in 2ºC; SEM= standard error of mean; Prob.= probability of treatment effect. ^{abc}Rows with superscript letters differ by Tukey test ($p<0.05$).

vezzo *et al.* (1997) observed low buffer capacity (15.88 eq.mg HCl/100 g DM) for corn silage (28.65 % of DM).

Data of aerobic stability of corn silage treated with different additives are presented at **table IV**. No effect of treatment was observed on maximum temperature reached, time spent to reach maximum temperature, temperature raise rate and time spent to raise temperature in 2 °C. It is well known the fact that the acetate has an inhibitory effect on yeast population of silages, especially when the material is exposed to atmospheric oxygen at the moment the silo is opened. Currently, it is accepted that this is the effect responsible for aerobic stability of silages with high concentrations of this acid (Matos *et al.*, 2006). Although the inoculant Mercosil had increased acetate concentration in silage, this increase was not sufficient to alter aerobic stability parameters, even though this inoculant has numerically increased time spent to reach maximum temperature in 43.2 % (equivalent to 37 hours) when compared to control **(figure 1)**. Although this desirable effect was not statistically significant, probably due to high variability of this kind of data (SEM= 8.67 h), is also compatible with heterofermentative bacteria *Lactobacillus buchneri* in its composition. These results contrast with those found by Rodrigues *et al.* (2004) who worked with three commercial inoculants and did not observe effect of these additives on maximum temperature and time to reach the maximum temperature. In their work, Martínez-Fernández *et al.* (2010) clearly discus the desirable effects of heterofermentative *Lactobacillus* in improving aerobic stability of silages (Kung and Rajit, 2001). This effect is due the presence of acetic acid (Kung et al, 2003), as this acid passively penetrate cells responsible for heating after opening (as yeast), decreasing cellular pH of these undesirable microorganisms, and resulting in their death (Ruser and Kleinmans, 2005). However, according to Guim *et al.* (2002), the use of microbial additives should be followed by appropriate silage management, from ensiling up to feeding the animals. So, the present experiment is more compatible with this last idea.

The results obtained in the present study suggest that more research are necessary for new products formulation, such as BAP, aiming to increase aerobic stability and maintain fermentative characteristics of ensiled forage for the success of its application.

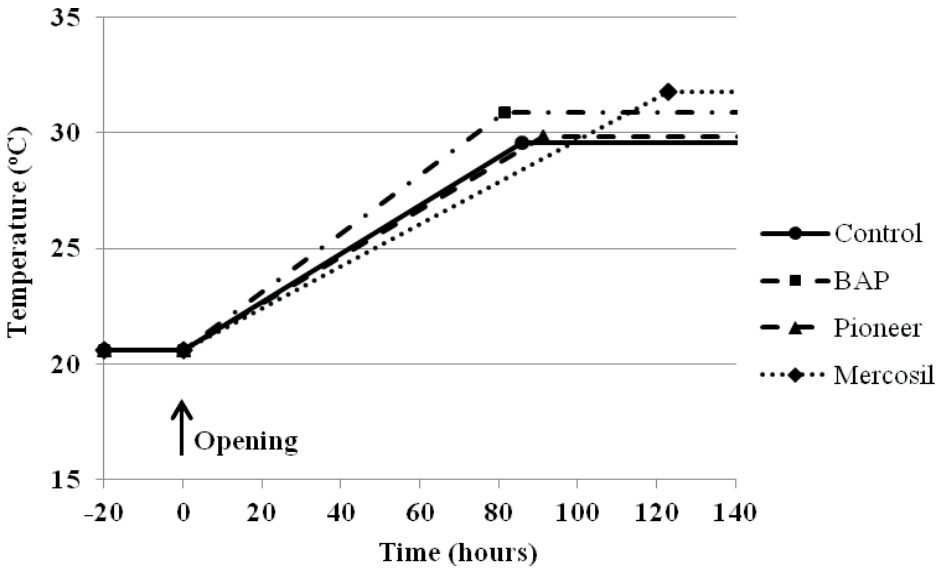

Figure 1. Silage temperature along time after opening (Temperatura da silagem ao longo do tempo após a abertura).

CONCLUSIONS

The additive BAP increased crude protein content of corn silage and can be used to improve its nutrient composition but not its fermentative pattern or aerobic stability. The microbial additives did not promote consistent alterations on nutrient composition, fermentation profile and aerobic stability of corn silage. Although *Lactobacillus buchneri* containing additives increase acetic acid concentration, questionable improvement was observed at aerobic stability of corn silage.

REFERENCES

AOAC [Association of Official Analytical Chemists]. 1995. Official methods of analysis. 15th ed. Association of Official Analytical Chemists. Washington, DC.

Erdman, R. 1993. Silage fermentation characteristics affecting feed intake. In: Silage prodution from seed to animal. Proceedings ... National Silage Production Conference. New York. pp. 210-219.

Erwin, E.S.; Marco, G.J.; Emery, E.M. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J Dairy Sci,* 44: 1768-1771.

Fahey, G.C. 1994. Forage quality, evaluation and utilization. American Society of Agronomy. Crop Science of America. Soil Science Society of America. Madison. Wiscosin. USA. 998 pp.

Foldager, J. 1977. Protein requirement and non protein nitrogen for high producing cow in early lactation. Ph.D. thesis. Michigan State University. East Lasing.

Gimenes, A.L.G.; Mizubuti, I.Y.; Moreira, F.B.; Pereira, E.S.; Ribeiro, E.L. e Mori, R.M. 2006. Composição química e estabilidade aeróbia em silagens de milho preparadas com inoculantes bacteriano e/ou enzimático. *Acta Scient,* 28: 153-158.

Guim, A.; Andrade, P.; Iturrino-Schocken, R.P.; Franco, G.L.; Ruggieri, A.C. e Malheiros, E.B. 2002. Estabilidade aeróbica de silagens de capim-elefante (*Pennisetum purpureum*, Schum). *Rev Bras Zootecn,* 31: 2176-2185.

Henderson, N. 1993. Silage additives. *Ani Feed Sci Technol,* 45: 35-56.

Johnson, R.R.; Balwani, T.L.; Johnson, L.J.; Mcclure, K.E. and Dehority, B.A. 1966. Corn plant maturity effect on *in vitro* cellulose digestibility and soluble carbohydrate content. *J Anim Sci,* 25: 617-623.

Kulasek, G.A. 1972. A micromethod for determination of urea in plasma, whole blood and blood cells using urease and phenol reagent. *Pol Arch Weter,* 15: 801-810.

Kung Jr., L.; Taylor, C.C.; Lynch, M.P. and Neylon, J.M. 2003. The effect of treating alfafa with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. *J Dairy Sci,* 86: 336-343.

Kung Jr., L. and Ranjit, N.K. 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. *J Dairy Sci,* 84: 1149-1155.

Lammers, B.P.; Buckmasster, D.R. and Heinrichs, E.J. 1996. A simple method for the analysis of particle sizes of forages and total mixed rations. *J Dairy Sci,* 79: 922-928.

Lavezzo W.; Lavezzo, E.N.M. e Campos Neto, O. 1997. Estádio de desenvolvimento do milho. Efeito sobre a produção, composição da planta e qualidade da silagem. *Rev Bras Zootecn,* 26: 675-682.

Macitelli, F. ; Berchielli, T.T. e Andrade, P. 2003. Influência do resíduo da produção do glutamato monossódico sobre os parâmetros ruminais e o consumo de bovinos alimentados com silagem de milho. *Arch Lat Prod Anim,* 11: 111-118.

Martínez-Fernández, A.; Soldado, A. and Vicente, F. 2010.Wilting and inoculation of *Lactobacillus buchneri* on intercropped triticale-fava silage: effects on nutritive, fermentative and aerobic stability characteristics. *Agric Food Sci,* 19: 302-312.

Matos, D.L.; Guim, A.; Batista, A.M.V.; Pereira, O.G.; Souza, E.J. e Sumba, E.R. 2006. Estabilidade aeróbica e degradabilidade da silagem de maniçoba (*Manihot* sp.) emurchecida. *Rev Bras Cienc Agr,* 1: 109-114.

McDonald, P.; Henderson, A.R. and Heront, S.J.E. 1991. The biochemistry of silage. 2 ed. Chalcome. Marlow. 340 pp.

Muck, R.E. and Shinners, K.J. 2001. Conserved forage (silage and hay): progress and priorities. In: International Grassland Congress, 19. São Pedro. Proceedings Brazilian Society of Animal Husbandry. Piracicaba. pp. 753-762.

Pereira, R.C.; Evangelista, A.R.; Abreu, J.G.; Amaral, P.N.C.; Salvador, F.M. and Maciel, G.A. 2004. Efeitos da inclusão de forragem de leucena (*Leucena leucocephala* (Lam.) de Wit) na qualidade da silagem de milho (*Zea mays* L.). *Ciênc Agrotec,* 28: 924-930.

Playne, M.J. and McDonald, P. 1966. The buffering constituents of herbage and of silage. *J Sci Food Agric,*17: 264-68.

Pinto, A.P.; Mizubuti, I.Y.; Ribeiro, E.L.A; Fey, R.; Palumbo, G.R. e Alves, T.C. 2007. Avaliação da silagem de bagaço de laranja e silagem de milho em diferentes períodos de armazenamento. *Acta Scient,* 29: 371-377.

Ramos, B.M.O.; Pinto, A.P.; Katsuki, P.A.; Gomes, G.P.; Podleskis, M.R.; Filho, T.J.M.; Soares, L.L.M. e Mizubuti, I. 2002. Composição química de silagens de milho cultivado em dois tipos de solos da região norte do Paraná. *Semina,* 23: 239-248.

Rodrigues, P.H.M.; Andrade, S.J.T. e Ruzante, J.M. 2002. Valor nutritivo da silagem de milho sob o efeito da inoculação de bactérias ácido-láticas. *Rev Bras Zootecn,* 31: 2380-85.

Rodrigues, P.H.M; Ruzante, J.M.; Senatore, A.L.; Lima, F.R.; Melotti, L. e Meyer, P.M. 2004. Avaliação do uso de inoculantes microbianos sobre a qualidade fermentativa e nutricional da silagem de milho. *Rev Bras Zootec*n, 33: 538-545.

Ruiz, B.O.; Castillo,Y.; Anchondo, A.; Rodríguez, C.; Beltrán, R.; La O, O. y Payán, J. 2009. Efectos de enzimas e inoculantes sobre la composición del ensilaje de maíz. *Arch Zootec,* 58: 163-172.

Ruppel, K.A.; Pitt, R.E. and Chase, L.E. 1995. Bunker silo management and its relationship to forage preservation on dairy farms. *J Dairy Sci,* 78:141-153.

SAS Institute Inc. 2002. SAS user's guide: Statistics. ver. 5 ed. SAS Inst. Cary, NC.

Tilley, J.M.A. and Terry, R.A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J Brit Grass Soc,* 18: 104-111.

Van Soest, P.J. and Robertson, J.B. 1985. Analysis of forages and fibrous foods. Cornell University. 202 pp.

Van Soest, P.J.; Robertson, J.B. and Lewis, B.A. 1991. Symposium: Carbohydrate methodology, metabolism, and nutritional implications in dairy cattle methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci,* 74: 3583-3597.

Zopollatto, M.; Nussio, L.G.; Mari, L.J.; Schmidt, P.; Duarte, A.P. e Mourão, G.B. 2009. Alterações na composição morfológica em função do estádio de maturação em cultivares de milho para produção de silagem. *Rev Bras Zootecn,* 38: 452-461.