Research Article

Ruminal kinetics of carbohydrates, gas production and bromatology of sugarcane silage with high levels of sodium benzoate

Celso HEINZEN JUNIOR[1] , Odimári Pricila Prado CALIXTO[2] , Egon Henrique HORST[2],* , Leonardo TROLEGI[2] ,
Fernando Luiz MASSARO JUNIOR[2] , Ivone Yurika MIZUBUTI[2] , Mikael NEUMANN[3] , Valter Harry BUMBIERIS JUNIOR[2]

[1]Departament of Animal Sciences, University of Florida, Gainesville, FL, USA
[2]Departament of Animal Science, State University of Londrina, Londrina, PR, Brazil
[3]Departament of Veterinary Medicine, Midwestern Parana State University, Cascavel, PR, Brazil

Abstract: The objective of this study was to evaluate the chemical composition, ruminal kinetics, gas production and aerobic stability of sugarcane silage added with increasing levels of sodium benzoate up to 1% of the natural matter. After harvesting, the sugarcane was chopped and placed in experimental polyethylene silos for storage for 30 days. An increasing concentration of sodium benzoate in the silage promoted a linear decrease in the crude protein content, varying from 2.79% to 2.51%. The addition of sodium benzoate reduced the fiber concentration of the silage (P = 0.0015) and increased gas production of the fibrous and nonfibrous carbohydrates (R^2 = 0.83 and 0.65, respectively). The volume and rate of degradation of nonfibrous carbohydrates (Vcnf and Kdcnf, respectively) were significantly affected (P < 0.05) by the inclusion of sodium benzoate, with the largest volume of gas produced by fermentation of nonfibrous carbohydrates with a dose 0.25% of sodium benzoate and, not coincidentally, it was the treatment that presented the lowest Kd (5.52 g DM^{-1}). The aerobic stability was higher in the silage with the highest additive content than in the other silages. The high levels of sodium benzoate used in this work, above what is commonly studied, have been shown to improve sugarcane silage with dry matter contents that are normally difficult to control.

Key words: Aerobic stability, benzoic acid, carbohydrate fractionation, chemical additive, dry matter loss

1. Introduction

Yeast control is essential for obtaining high-quality sugarcane silage [1]. Soluble sugars correspond to more than 40% of the organic matter present in the mature plant [2], and, under normal conditions, where the fermentation process of this forage is commonly dominated by yeasts, there is intense metabolization of these sugars into ethanol, resulting in considerable dry matter losses, impoverishing the nutritional quality of the food.

Once added to the ensiled forage, sodium benzoate is converted to benzoic acid, which assist to reduce the pH of the silage more quickly in the initial stage of fermentation. This rapid reduction in pH during the making of silage can both improve fermentation and assist reduce losses, as well as improve aerobic stability [3], especially in grasses [4]. Several studies prove this efficiency in the preservation of dry matter [3,5] but without demonstrating possible changes imposed on the ruminal fermentation of the food obtained. Pedroso et al. [6] demonstrated an increase in the digestibility of the dry matter of sugarcane silage with the addition of sodium benzoate but without a reduction in the production of ethanol, suggesting an intense degradation of nonfibrous carbohydrates by yeasts during silage production.

Related studies demonstrated that the appropriate level of this additive is still debatable. While Pedroso et al. [7] emphasized that 0.05% in natural matter would be sufficient for obtaining an efficient fermentation process, Bernandes et al. [8] described better results after adding a level four times higher. Studies with levels higher than these are scarce, as well as data on interventions in the ruminal kinetics of carbohydrates and gas production in sugarcane silages produced with sodium benzoate.

In vitro analysis techniques are fundamental for the study of these fermentation processes under controlled conditions. Substrates are incubated in cultures of mixed ruminal microorganisms, and the final fermentation products accumulated during the process are measured [9], allowing forthegeneration of information and for obtaining accurate predictions about the quality of forage quickly and on a large scale.

* Correspondence: egonhh@yahoo.com.br

Our objective was to evaluate the chemical composition, ruminal kinetics of carbohydrate degradation, gas production and aerobic stability of sugarcane silage produced with increasing levels of sodium benzoate up to 1% of the total mass.

2. Materials and methods

The sugarcane (*Saccharum officinarum*) used for silage production was tillage in Mandaguari, Paraná, Brazil, located at the coordinates of 23°32'S and 51°40'W at an altitude of 670 m. Immediately after harvest, the sugarcane was choppedto a size of approximately 20 mm, and then manual homogenization with sodium benzoate was performed at proportions of 0.00% (control), 0.25%, 0.50%, 0.75%, and 1.00% in relation to the total mass.

Twenty polyethylene buckets with a capacity of 5 L were used as experimental silos, closed with plastic lids, sealed with adhesive tape and stored indoors. The chopped sugarcane was added to the buckets in quantity to result in 600 kg of DM m^{-3}. After 30 days, the silos were opened, and 5 cm of the upper and lower portions were discarded. Quarterly samples were collected immediately for analysis at the Laboratory of Food and Animal Analysis Nutrition of the State University of Londrina (LANA-UEL). First, the sampleswere predried in a forced ventilation oven at 55 °C for 72 h and then milled at 1 mm in a Wiley mill.

The predried samples were used todetermine the total dry matter (DM), organic matter (OM), ash, crude protein (CP), ether extract (EE), lignin (LIG), hemicellulose (HEM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents according to methods described by the AOAC [10]. The determination of ammoniacal nitrogen (N-NH$_3$) and buffer capacity (BC) was performed using the technique described by Playne and McDonald [11]. The fractionation of carbohydrates was performed according to estimates suggested by Sniffen et al. [12].

For ruminal fluid collection, two sheep with permanent cannulas were used. They were castrated males, 36 months old, weighing approximately 75 kg. The animals received a diet consisting of maize silage and concentrated feed based on corn and soybean meal at a roughage: concentrate ratio of 80:20.

The kinetic parameters of ruminal degradation were estimated using the semi-automatic technique of cumulative in vitro gas production described by Schofield et al. [13]. For this purpose, 300 mg of predried sample ground at 1 mm was weighed and placed in 50 mL bottles. All bottles received 24 mL of McDougal's buffer solution [14], previously reduced with CO_2, until reaching a pH value of 6.9. Subsequently, 6 mL of inoculum from the cannulated sheep was added to each bottle under CO_2. For variation adjustments, bottles without substrate, which were considered blanks, were incubated to discount the

volume of gases from the rumen liquid and the buffer solution.

The bottles were hermetically sealed with a rubber stopper and immediately placed in a bio-oxygen demand (BOD) incubator at a temperature of 39 °C. To ensure that the pressure in the bottles was under the same conditions as the initial pressure, the pressure was depressurized with the aid of needles before the incubation time began. From this moment on, the pressure of the gases that were produced by the fermentation of the substrate and accumulated in the bottles was measured by a MPD-79 model manometer (Instrutherm) at 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 30, 36, 48, 60, 72, 84, 96, and 144 h of incubation. Depressurization was performed after each measurement.

The measured pressure was converted into volume according to the following preestablished equation for local conditions:

$$V = 0{,}5702 + 3{,}2399 \times Psi + 0{,}1074 \times Psi^2$$

where

V = gas volume, in mL;

Psi = gas pressure, in psi.

To estimate the parameters of ruminal kinetics of carbohydrates and gas production, the data were adjusted using the two-compartment statistical model by Schofield et al. (1994), which is described as the following equation:

$$V = \frac{Vcnf}{1 + exp\,2 - 4 \times Kdcnf \times (t - L)} + \frac{Vcf}{1 + exp\,2 - 4 \times Kdcf \times (t - L)}$$

where V = gas volume accumulated at time *t*, in mL; Vcnf = maximum volume of gas formed by the fraction of nonfibrous carbohydrates, in mL; *Kd*cnf = rate of degradation of nonfibrous carbohydrates, in mL hour^{-1}; *L*= lag time, in hours; *t* = incubation time, in hours Vcf = maximum volume of gas by the fraction of fibrous carbohydrates, in mL; *Kd*cf = rate of degradation of fibrous carbohydrates, in mL hour^{-1}.

The experimental design used was completely randomized, with five replications. The F-test was applied at a 5% confidence probability through analysis of variance (ANOVA), and then the Tukey test was implemented for comparing multiple means at the 5% level of significance using the SAS (1993) software. The data were also submitted to polynomial regression analysis, considering the variable content of the additive, through the procedure regression by the same software.

As the distributions for all parameters of ruminal kinetics of carbohydrates and gas production were determined with the Gauss–Newton algorithm through the R software, regression analyses were performed at the 5% significance level.

3. Results and discussion

The silages presented dry matter contents commonly described for sugarcane, with no difference between silages with different levels of added sodium benzoate and an average of 26.14% (Table 1). We opted for early harvest of forage supported by the findings of Knický and Spörndly [3], who concluded that the addition of chemical additives, including sodium benzoate, in silages with a dry matter content similar to that reported here was better for controlling of *clostridium* and deamination.

The increase in the concentration of sodium benzoate in the silage promoted a linear decrease in the crude protein content, which varied from 2.79% to 2.51% between the extremes. The reduction obtained in this study was 0.43 percentage points for each 0.25% increase in the dose of sodium benzoate. Despite the reduction, it is worth noting that the addition of this additive seemed to optimize protein flow to the duodenum [15], improving the productive performance of dairy cows [16].

There was also a significant reduction for all parts of the fiber. No defined behavior was observed among the sodium benzoate levels used for NDF, being that with 0.50% inclusion we obtained the lowest content (51.60%), and the content obtained with 0.75% inclusion

was closest to that of the control silage (55.88 and 58.09%, respectively).

A similar trend was observed for hemicellulose at the same levels, since regardless of the content, the levels of ADF did not differ between the levels of inclusion of sodium benzoate; however, for the ADF content, as for cellulose, there was a reduction in comparison to that of the control silage. Pedroso et al. [17] also described a reduction in the ADF content of sugarcane silage with a much lower level than that of the sugarcane silage we tested (0.5 g per kg); however, this trend was not observed for NDF, which may suggest an advantage for the additions of higher sodium benzoate levels, such as those studied in this work. Despite this, studies with sodium benzoate show great divergence in relation to the chemical characterization of silage, and further studies on the subject are suggested.

The volume and rate of degradation of nonfibrous carbohydrates (Vcnf and Kdcnf, respectively) were significantly affected ($P < 0.05$; Table 2) by the inclusion of sodium benzoate, with the largest volume of gas produced by fermentation of nonfibrous carbohydrates was observed with 0.25%, and, not coincidentally, it was the content that presented the lowest Kd (5.52 g DM^{-1}).

Table 1. Chemical composition and fractionation of carbohydrates from sugarcane silages added with increasing levels of sodium benzoate.

	Control	0.25%	0.50%	0.75%	1.00%	P-value	CV
DM, % NM	24.88	27.62	26.67	26.37	25.18	ns	4.56
OM, % DM	96.50	96.19	97.31	97.91	96.54	ns	1.07
Ash, % DM	3.50	3.81	2.69	2.09	3.46	ns	3.64
CP, % DM†	2.79 a	2.75 a	2.60 ab	2.51 ab	2.36 b	0.0071	8.00
EE, % DM	0.46	0.51	0.22	0.60	0.53	ns	33.66
NDF, % DM	58.09 a	52.55 c	51.60 c	55.88 ab	53.05 bc	0.0015	4.87
ADF, % DM	34.98 a	31.46 b	29.72 b	31.67 b	29.57 b	0.0334	6.89
Hemicellulose, % DM	23.11 abc	21.09 c	21.88 c	24.21 a	23.48 ab	0.0060	6.79
Cellulose, % DM	31.55 a	28.40 ab	26.42 b	28.39 ab	26.40 b	0.0394	9.49
Lignin, % DM	3.43	3.05	3.30	3.28	3.17	ns	20.33
NFC, % DM	38.39	44.10	45.02	42.39	43.51	ns	7.22
CHO, % DM	93.29	92.90	94.50	94.81	93.66	ns	1.10
A+B1, % CHO	45.10	51.20	50.52	47.58	49.86	ns	4.45
B2, % CHO	46.68	41.47	41.57	44.55	42.53	ns	5.67
C, % CHO	8.22	7.33	7.91	7.87	7.61	ns	12.33

†$\hat{Y} = 2.82 - 0.4287x$ (P: <0.0001; R^2: 0.3390; CV: 8.34).

R^2: determination coefficient; CV: coefficient of variation; DM: dry matter; OM: organic matter; CP: crude protein; EE: ethereal extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: nonfibrous carbohydrate; CHO: total carbohydrate.

Averages, followed by different letters in the same row, differ by the Tukey test at 5%.

The highest rate of passage of this fraction occurred with the highest content of addition (1.00%; 8.42 g DM^{-1}).

The lag time was not affected by the addition of sodium benzoate, indicating that this additive did not hinder the access and affinity of the microorganisms for the substrate and probably did not alter the action site of cellulases. Oliveira Filho et al. [18] published lag times longer than ours, which may be related to the lignin content also being higher as described by the authors. Despite the high levels, the use of sodium benzoate as a silage additive did not present a risk to animal and human health (Zwiers and Weissbach, 1989) cited by [8].

The gas production by fermentation of fibrous carbohydrates increased with increasing sodium benzoate levels, which may be more associated with the action of sodium benzoate on the fiber composition (Table 1) than with the action itself on microorganisms. The reduction in ADF caused by the additive facilitated the access of ruminal microorganisms to the most digestible part of the food since it presents itself as a barrier to the substrate.

Despite the differences in Vcf, the final volume (V) of gas produced by the fermentation of carbohydrates was not affected by the addition of sodium benzoate, with an average of 294.96 mL g of DM^{-1}, and was more affected

by the fraction with greater capacity fermentation (Vcnf).

Queiroz et al. [5] demonstrated that in humid and hot conditions, low levels of sodium benzoate in maize silage did not generate improvements in aerobic stability. In a similar environment, we observed that only at the doses of 0.75% and 1.00% sodium benzoate, the aerobic stability was in fact superior (P < 0.05) to the control silage, while the level of 0.25% sodium benzoate reduced the stability after opening the silo. Da Silva et al. [19] described an aerobic stability time of 161 h for silage with added sodium benzoate versus 41 h for the control silage. Many authors expose the high cost of sodium benzoate as an obstacle to its use, but with evidence of improvements such as this, this point is relativized.

This greater postopening stability is based on the control of yeast development in the aerobic phase of fermentation, which is mediated by benzoic acid formed from sodium benzoate. The nondissociated form of this acid crosses microbial cell membranes and releases H$^+$, acidifying the cytoplasm [15]. The presence of oxygen during the initial phase of the fermentation process is inevitable; however, measures to reduce this period, as well as to mitigate the development of yeasts, are essential for obtaining silage with high stability after opening the silo [1].

Table 2. Ruminal kinetics of in vitro carbohydrate degradation of sugarcane silages with increasing levels of sodium benzoate.

	Control	0.25%	0.50%	0.75%	1.00%	Regressione quation	R^2
Vcnf, mL	112.32	150.44	132.64	87.01	93.65	$\hat{Y} = 131.71 + 39.33x - 88.09x^2$	0.6491
Kdcnf, % h^{-1}	6.47	5.52	5.73	8.34	8.42	$\hat{Y} = 0.0619 - 0.0262x + 0.0525x^2$	0.7126
L, hours	6.06	5.90	5.69	4.75	5.75	$\hat{Y} = 5.63$	-
Vcf, mL	152.18	142.85	164.74	205.98	211.65	$\hat{Y} = 138.54 + 72.87x$	0.8297
Kdcf, % h^{-1}	1.74	1.59	1.68	1.80	1.85	$\hat{Y} = 1.73$	-
V, mL	275.34	294.31	297.31	296.48	311.37	$\hat{Y} = 294.96$	-

Vcnf: volume of gas formed by the fraction of nonfibrous carbohydrates; Kdcnf: rate of degradation of nonfibrous carbohydrates; L: lag time; Vcf: maximum volume of gas by the fraction of fibrous carbohydrates; Kdcf: rate of degradation of fibrous carbohydrates; V: total accumulated gas volume.

Table 3. Aerobic stability, ammonium nitrogen (N-NH$_3$) and buffer capacity (BC) of sugarcane silages added with increasing levels of sodium benzoate.

	Control	0.25%	0.50%	0.75%	1.00%	P-value	CV
N-NH$_3$, % N total	16.07	15.13	15.12	16.99	19.38	ns	9.82
BC, mEq kg of DM^{-1}	25.81	22.40	27.69	28.32	21.65	ns	15.64
Aerobic stability, hours	114 bc	102 c	114 bc	128 b	168 a	<0.0001	11.29
Time to maximum temperature, hours	156	138	138	144	144	ns	12.22
pH after stability test	4.04	3.83	3.62	3.71	3.82	ns	3.39

Averages, followed by different letters in the same row, differ by the Tukey test at 5%.

The release of intracellular H^+ that potentiates the antimicrobial action of sodium benzoate also favors an overallreduction in the pH of the silage [3]; however, despite the highest pH value being observed for the control silage, the differences observed after sodium benzoate addition were not significant (4.04; Table 3). Likewise, $N-NH_3$ and BC did not differ among the sugarcane silages with added benzoate, with averages of 16.54% and 25.17 mEq kg of MS^{-1}, respectively. The content of $N-NH_3$ that we observed was below those described by Knicky and Sporndly [3] for sugarcane silage added with a mixture of potassium sorbate, sodium benzoate and sodium nitrite and by Knicky

and Sporndly [20] with a mixture of just the first two additives.

4. Conclusion
The increase in the level of sodium benzoate added increased the gas production of fibrous carbohydrates and theaerobic stability of the sugarcane silage. However, the lower levels reduced the fiber content and stimulated the fermentation of nonfibrous carbohydrates.

The high levels of sodium benzoate used in this work, above what is commonly studied, have been shown to improve the quality of sugarcane silage with a dry matter content that is normally difficult to control.

References

1. Knicky M, Spörndly R. Use of a mixture of sodium nitrite, sodium benzoate, and potassium sorbate in aerobically challenged silages. Journal of Dairy Science 2015; 98: 5729-5734. doi: 10.3168/jds.2015-9332

2. Daniel JLP, Weiss K, Custódio L, Neto AS, Santos MC et al. Occurrence of volatile organic compounds in sugarcane silages. Animal Feed Science and Technology 2013; 185: 101-105. doi: 10.1016/j.anifeedsci.2013.06.011

3. Knický M, Spörndly R. The ensiling capability of a mixture of sodium benzoate, potassium sorbate, and sodium nitrite. Journal of Dairy Science 2011; 94: 824-831. doi: 10.3168/jds.2010-3364

4. Conaghan P, O'Kiely P, O'Mara FP. Conservation characteristics of wilted perennial ryegrass silage made using biological or chemical additives. Journal of Dairy Science 2010; 93: 628-643. doi: 10.3168/jds.2008-1815

5. Querioz OCM, Arriola KG, Daniel JLP, Adesogan AT. Effects of 8 chemical and bacterial additives on the quality of corn silage. Journal of Dairy Science 2013; 96: 5836-5843. doi: 10.3168/jds.2013-6691

6. Pedroso ADF, Nussio LG, Barioni Júnior W, Rodrigues ADA, Loures DRS et al. Performance of Holstein heifers fed sugarcane silages treated with urea, sodium benzoate or *Lactobacillus buchneri.* Pesquisa Agropecuária Brasileira 2007; 41: 649-654. doi: 10.1590/S0100-204X2006000400015

7. Pedroso ADF, Nussio LG, Rodrigues ADA, Santos FAP, Mourão GB et al.Performance of dairy cows fed rations produced with sugarcane silages treated with additives or fresh sugarcane. Revista Brasileira de Zootecnia 2010; 39: 1889-1893. doi: 10.1590/S1516-35982010000900005

8. BernardesTF, Oliveira IL, Lara MAS, Casagrande DR, Ávila CLS et al. Effects of potassium sorbate and sodium benzoate at two application rates on fermentation and aerobic stability of maize silage. Grass and Forage Science 2015; 70 (3): 491-498. doi: 10.1111/gfs.12133

9. Rahman MM, Salleh MAM, Sultana N, Kim MJ, Ra CS. Estimation of total volatile fatty acid (VFA) from total organic carbons (TOCs) assessment through in vitro fermentation of livestock feeds. African Journal of Microbiology Research 2013; 7: 1378-1384. doi: 10.5897/AJMR12.1694

10. AOAC. Animal feed. In: Cunniff PA (editor). Official Methods of Analysis of AOAC International. 16th ed. Washington, DC, USA: AOAC; 1995.

11. Playne MJ, McDonald P. The buffering constituents of herbage and of silage. Journal of the Science of Food and Agriculture 1966; 17: 264-268.

12. Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG, Russell JB. A net carbohydrate and protein system for evaluating cattle diets; II. Carbohydrate and protein availability. Journal of Animal Science 1992; 70: 3562-3577.

13. Schofield P, Pitt RE, Pell AN. Kinetics of fiber digestion from in vitro gas production. Journal of Animal Science 1994; 72: 2980-2991.

14. McDougal EI. Studies on ruminal saliva. 1. The composition and output of sheep's saliva. Biochemical Journal 1949; 43: 99-109.

15. Muck RE, Nadeau EMG, McAllister TA, Contreras-Govea FE, Santos MC et al. Silage review: recent advances and future uses of silage additives. Journal of Dairy Science 2018; 101: 3980-4000. doi: 10.3168/jds.2017-13839

16. Nadeau E, Johansson B, Richardt W, Murphy M. Protein quality of grass silage as affected by silage additives and its effects on dairy cow performance. Journal of Dairy Science 2015; 98: 206-215.

17. Pedroso ADF, RodriguesADA, Barioni Júnior W, Souza GBD. Fermentation parameters, quality and losses in sugarcane silages treated with chemical additives and a bacterial inoculant. Revista Brasileira de Zootecnia 2011; 40: 2318-2322. doi: 10.1590/S1516-35982011001100006

18. Oliveira Filho CAA, Azevêdo JAG, CarvalhoGGP, Silva CFPG, Santos CÍ et al. Crude glycerin combined with sugar cane silage in lamb diets. Tropical Animal Health and Production 2016; 48: 289-295. doi: 10.1007/s11250-015-0948-7

19. Da Silva NC, Santos JP, Ávila CL, Evangelista AR, Casagrande DR et al. Evaluation of the effects of two *Lactobacillus buchneri* strains and sodium benzoate on the characteristics of corn silage in a hot-climate environment. Grassland Science 2014; 60: 169-177. doi: 10.1111/grs.12053

20. Knický M, Spörndly R. Sodium benzoate, potassium sorbate and sodium nitrite as silage additives. Journal of the Science of Food and Agriculture 2009; 89: 2659-2667. doi: 10.1002/jsfa.3771