Effect of different doses of *Lactobacillus buchneri* on the fermentation and *in vitro* dry matter digestibility of sugarcane silage: a meta-analysis

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Introduction In Brazil, sugarcane silage is commonly used to feed beef cattle and dairy cows. However, sugarcane silage may display dry matter (DM) loss of approximately 25% on average (Rabelo et al., 2016) due to the production of high amounts of ethanol caused by yeasts overgrowth during fermentation (Kung and Stanley, 1982). In the last years, *Lactobacillus buchneri* hence has been largely studied as a silage additive to reduce DM loss of sugarcane silage through the increased acetic acid production. However, there is still no consensus regarding which dose is needed applying to alter fermentation, as well as its effectiveness. Thus, our objective was undertaken a meta-analysis to investigate the effects of different doses of *L. buchneri* on the fermentation and *in vitro* DM digestibility (IVDMD) of sugarcane silage.

Materials and Methods A database comprised of 28 articles published in scientific journals (89 treatments means evaluated) spanishing 2007–2016 was used in the meta-analysis. Experiments were excluded if silages were ensiled for less than 56 d. Treatments were classified into three categories: 1) silage with no inoculant (untreated), 2) silage treated with *L. buchneri* at a rate $\leq 1 \times 10^5$ ufc/g of fresh forage (low LB), and 3) silage treated with *L. buchneri* at a rate $> 1 \times 10^5$ ufc/g of fresh forage (high LB). Treatments comprising untreated, low LB and high LB silages accounted for 43.8%, 39.3% and 16.9% of the database, respectively. The meta-analysis was performed through the MIXED procedure of SAS using the study as a random effect (St-Pierre, 2001). Studentized residuals with absolute values exceeding 3 were considered outliers and deleted. Data were weighted by the inverse of the standard error of the mean or the n of observations of the mean (when squared standard error was lacking) using the WEIGHT statement. The covariates (length of fermentation and type of silo) were removed from the model if the random covariance was not significant ($P>0.05$). Differences between means were determined using the P-DIFF option of the LSMEANS statement at $P\leq0.05$.

Results and Discussion In comparison with the untreated silage, the high LB silage had lower ($P<0.001$) gas and DM losses (–22.4% and –21%, respectively; Table 1). Inoculation of sugarcane silage with high LB increased residual water-soluble carbohydrates – WSC (P = 0.011; +96.3%) and lactic acid (P = 0.025; +31.8%), and decreased yeasts count (P = 0.010; –0.55 $\log_{10}$ cfu/g). Surprisingly, the low LB silage had higher concentration of acetic acid (P < 0.001; +71.5% as compared with untreated silage) and lower ethanol (P < 0.001; –68.7% as compared with untreated silage). In contrast, silage pH, ammonia-N and butyric acid were unaffected (P > 0.07) by adding *L. buchneri*. *Lactobacillus buchneri* is a heterofermentative lactic-acid bacterium largely used to enhance the aerobic stability of silages by producing high concentrations of acetic acid under anaerobic conditions (Driehuis et al., 1999). Specifically for sugarcane silage, the high production of acetic acid by *L. buchneri* may decrease alcoholic fermentation by avoiding yeasts overgrowth (acetic acid contains antifungal properties), and hence reduce fermentative loss. However, even though lower yeasts count and fermentative loss were found in the high LB silage, the higher acetic acid concentration and lower ethanol concentration were observed in the low LB silage, for unknown reasons. Additionally, in comparison with the untreated silage, the high LB silage had lower contents of DM (P < 0.001) and lignin (P = 0.004), but increased (P < 0.001) contents of crude protein, neutral detergent

fiber, and acid detergent fiber (ADF). Conversely, the low LB silage had increased IVDMD ($P < 0.001$; +6.2% as compared with the untreated silage). Because the high LB silage had decreased fermentative loss and higher residual WSC, we expected to find a higher IVDMD in the high LB silage. However, the higher IVDMD found in the low LB silage is most likely due to the reduced ADF content, which is negatively associated with digestibility.

Table 1 Fermentation profile, chemical composition, and *in vitro* digestibility of sugarcane silages treated with different doses of *L. buchneri* (data are given in % DM, unless otherwise stated).

Item	Untreated	*Lactobacillus buchneri*		SEM	*P*-value
		Low	High		
Gas loss	21.9[a]	21.3[ab]	17.0[b]	2.38	<0.001
DM loss[1]	27.1[a]	24.1[ab]	21.4[b]	2.59	<0.001
pH	3.51	3.48	3.49	0.035	0.078
Ammonia-N, % TN	5.18	4.16	5.44	1.19	0.081
WSC	1.35[c]	1.88[b]	2.65[a]	0.206	0.011
Lactic acid	2.45[b]	2.50[b]	3.23[a]	0.520	0.025
Acetic acid	3.40[b]	5.83[a]	3.39[b]	0.168	<0.001
Butyric acid	0.238	0.173	0.349	0.164	0.071
Ethanol	5.31[a]	1.66[b]	6.59[a]	0.950	<0.001
Yeasts, $\log_{10}$ cfu/g	4.98[b]	5.29[a]	4.43[c]	0.464	0.010
DM, % as fed	25.2[b]	25.6[a]	24.7[c]	0.810	<0.001
Ash	5.67	4.58	5.57	0.660	0.289
CP	3.13[b]	3.21[b]	3.37[a]	0.192	<0.001
NDF	65.7[b]	65.4[b]	67.0[a]	1.14	<0.001
ADF	45.8[b]	41.0[c]	46.6[a]	1.64	<0.001
Lignin	9.07[a]	8.17[b]	8.22[b]	1.06	0.004
IVDMD	46.7[b]	49.6[a]	44.9[b]	2.06	<0.001

[a-c]*Means in the same row with different superscripts differed (P≤0.05).*
[1]DM = dry matter; TN = total nitrogen; WSC = water-soluble carbohydrates; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; IVDMD = *in vitro* DM digestibility.

Conclusion Applying high doses of *L. buchneri* (i.e. application rate > 1×10^5 ufc/g of fresh forage) at sugarcane ensiling is recommended to reduce fermentative loss, but with no additional gain on *in vitro* DM digestibility.

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