

## Research Paper

# Whole corn grain addition in sugarcane silage avoids fermentative losses and improves in situ degradation of silage

*La adición de granos enteros de maíz a ensilaje de caña de azúcar reduce pérdidas por fermentación y mejora la degradación in situ del ensilaje*

TIAGO A. DEL VALLE<sup>1,2</sup>, GABRIELA DO NASCIMENTO<sup>1</sup>, LUANA S. FERNANDEZ<sup>1</sup>, MARISA M. FRANÇA<sup>3</sup>, JESUS A.C. OSÓRIO<sup>4</sup>, JEFFERSON R. GANDRA<sup>5</sup>, JÚLIA A. MARQUES<sup>3</sup>, JOHNNY M. DE SOUZA<sup>3</sup>, MARIANA CAMPANA<sup>1</sup> AND JOZIVALDO P.G. MORAIS<sup>1</sup>

<sup>1</sup>Departamento de Biotecnologia e Produção Vegetal e Animal, Centro de Ciências Agrárias, Universidade Federal de São Carlos, Araras, SP, Brazil. [cca.ufscar.br](http://cca.ufscar.br)

<sup>2</sup>Universidade Federal do Pampa, Campus Itaqui, Itaqui, RS, Brazil. [unipampa.edu.br/itaqui](http://unipampa.edu.br/itaqui)

<sup>3</sup>Departamento de Nutrição e Produção Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, SP, Brazil. [vnp.fmvz.usp.br](http://vnp.fmvz.usp.br)

<sup>4</sup>Departamento de Ciência Animal, Universidade Estadual de Maringá, Maringá, PR, Brazil. [www.uem.br](http://www.uem.br)

<sup>5</sup>Departamento de Ciência Animal, Universidade Federal da Grande Dourados, Dourados, MS, Brazil. [www.ufgd.edu.br](http://www.ufgd.edu.br)

## Abstract

Sugarcane silage (SS) is generally susceptible to yeast action, resulting in dry matter losses due to high soluble carbohydrate concentration. We evaluated the effects of adding corn grain and microbial inoculant at ensiling on fermentative profile, losses, chemical composition and degradation of silages. Forty experimental silos (PVC tubing) were assigned at random to a  $5 \times 2$  factorial arrangement with: (1) 5 corn additions at ensiling: CONT - straight sugarcane silage; GC2 - sugarcane with ground corn (processed through a 2 mm sieve) added at ensiling; GC8 - sugarcane with ground corn (processed through an 8 mm sieve) added at ensiling; WC - sugarcane with whole corn grain added at ensiling; and RCS - rehydrated corn ensiled without sugarcane; and (2) 2 microbial inoculant additions at ensiling: 0 and 8 mg of commercial inoculant per kg of feed. Corn grain was added at the rate of 100 g per kg of fresh sugarcane. Adding corn grain to sugarcane at ensiling improved SS fermentation and silage chemical composition. There was no benefit from grinding the grain before adding it to sugarcane. Microbial inoculant had little effect on SS fermentation. Studies comparing corn grain with other energy sources, e.g. molasses or cassava, for addition at ensiling sugarcane seem warranted along with feeding studies with livestock to assess intake and subsequent performance. The overall benefits of adding the energy sources at ensiling versus feeding them directly to animals with untreated sugarcane silage should be determined.

**Keywords:** Corn processing, digestibility, grain kernels, microbial inoculant, water activity.

## Resumen

El ensilaje de caña de azúcar es generalmente susceptible a la acción de levaduras resultando en pérdidas de materia seca (MS) debido a la alta concentración de carbohidratos solubles. En un estudio realizado en la Universidade Federal de São Carlos, Araras, Brazil, se evaluó el efecto de la adición, al momento de ensilar, de maíz en grano e inoculante microbiano en el perfil fermentativo, la pérdida de MS, la composición química y la degradación del ensilaje. Cuarenta silos experimentales (tubos de PVC) fueron distribuidos aleatoriamente en un diseño factorial  $5 \times 2$ . Se evaluaron: (1) cinco tratamientos de adición de maíz: CONT - ensilaje de caña de azúcar sin maíz; GC2 - ensilaje con maíz procesado por un tamiz de 2 mm; GC8 - ensilaje con maíz procesado por un tamiz de 8 mm; WC - ensilaje con granos enteros de

Correspondence: Tiago Antonio del Valle, Universidade Federal do Pampa, Rua Luiz Joaquim de Sá Brito, Itaqui, CEP 97.650-000, RS, Brazil. Email: [tiagodelvalle@unipampa.edu.br](mailto:tiagodelvalle@unipampa.edu.br)

maíz; y RCS - ensilaje de maíz rehidratado sin caña de azúcar; y (2) dos tratamientos de adición de inoculante microbiano: 0 y 8 mg de inoculante comercial por kg de material a ensilar. Se utilizaron 100 g de maíz por kg de caña de azúcar fresca. Los resultados mostraron que la adición de maíz a la caña de azúcar al momento de ensilar mejoró la fermentación y composición química del ensilaje. La molienda del grano antes de adicionarlo a la caña de azúcar no mostró beneficios en la calidad del producto final. El inoculante microbiano tuvo poco efecto sobre la fermentación. Estudios para comparar el maíz en grano con otras fuentes de energía, p.ej. adicionando melaza y yuca al momento de ensilar, parecen justificados, igual que estudios de alimentación del ganado para evaluar el consumo y la producción animal subsiguiente. También se debe determinar si el suministro de las fuentes de energía en forma de aditivos al ensilaje es más favorable que el suministro directo a los animales como complemento de ensilaje no tratado de caña de azúcar.

**Palabras clave:** Actividad de agua, digestibilidad, inoculante microbiano, procesamiento de maíz.

## Introduction

In subtropical conditions, sugarcane generally produces higher dry matter (DM) yields per unit area and energy value at maturity than other tropical forages (Daniel et al. 2013) and fresh sugarcane is traditionally fed to cattle during periods of low pasture availability (Santos et al. 2010). Conserving sugarcane as silage would allow greater flexibility in feeding strategies. However, sugarcane silage (SS) is generally susceptible to the action of yeast fungi owing to high soluble carbohydrate concentration, producing a typical alcoholic fermentation of soluble carbohydrates into ethanol, CO<sub>2</sub> and water (Sá Neto et al. 2013) and, consequently, increased DM losses (Pedroso et al. 2008). Soluble carbohydrate concentration in the final product is lower and the level of fibrous components is higher than in the raw material, while ruminal degradation of the ensiled material is lower than that of fresh sugarcane.

One alternative to counteract the undesirable outcomes from natural fermentation in SS is the inclusion of corn grain and other additives at ensiling. Incorporating corn grain with fresh sugarcane when making SS could reduce ethanol production and DM losses (Gómez-Vázquez et al. 2011), while the use of other additives, e.g. inoculants, should help to inhibit epiphytic yeast populations and mitigate nutrient losses (Ávila et al. 2014).

Maize cultivars produced in Brazil have a vitreous endosperm, which limits starch digestibility in the gastrointestinal tract of animals. Rehydrated corn grain silage (RCS) has been used to improve starch digestibility of corn kernels in Brazilian production systems (Silva et al. 2018). Ethanol produced in SS can also solubilize proteins of the grain's endosperm (Zhang et al. 2011), improving starch availability. In addition, grinding of corn grain could affect starch digestibility by animals and starch solubilization in the silos. As suggested by Junges et al. (2017), bacterial activity is the most critical determinant of protein degradation and could be enhanced by increasing the soluble carbohydrate concentration in SS.

Among silage additives, microbial inoculants have been used to reduce the undesirable effects of SS fermentation (Carvalho et al. 2014; Santos et al. 2015; Jacovaci et al. 2017). Inoculants containing homolactic bacteria improve lactic acid production and reduce silage pH, without positively affecting alcohol fermentation (Pedroso et al. 2008; Santos et al. 2015). *Pediococcus acidilactici* establishes a low silage pH (Fitzsimons et al. 1992) and *Propionibacterium* spp. produce propionic acid from lactic acid (McDonald et al. 1991) with potential negative effects on yeast growth.

The present study aimed to evaluate any benefits from the inclusion of corn grain, processed at different particle sizes, and bacterial inoculants at ensiling on fermentation, chemical composition and in situ degradation of DM and neutral detergent fiber (NDF) of SS. We hypothesized that the addition of corn grain when ensiling sugarcane, regardless of the microbial inoculant supply, would reduce fermentative losses in SS and improve chemical composition and DM degradation relative to straight SS without corn or rehydrated corn grain silage (RCS).

## Materials and Methods

The experiment was carried out at the Federal University of São Carlos, Araras, São Paulo, Brazil. Sugarcane (variety RB83-5054), at 8 months of growth (first cut) and 17.5% Brix, was used. Sugarcane from 5 locations/plots was manually harvested and chopped in a stationary cutter (Dedini®, Piracicaba, Brazil) to an ideal cut length of 10 mm.

Forty experimental silos (PVC tubes - 28 cm diameter and 25 cm long) were randomly assigned to a 5 × 2 factorial arrangement to evaluate: (A) 5 levels of corn addition: 1) control (CONT) - sugarcane ensiled without corn addition; 2) GC2 - SS with 100 g ground corn (2 mm sieve-processed)/kg fresh sugarcane added at ensiling; 3) GC8 - SS with 100 g 8 mm sieve-processed corn/kg fresh sugarcane; 4) WC - SS with 100 g whole corn grain/kg fresh sugarcane; and 5) RCS - rehydrated corn silage

(whole grain ensiled without sugarcane); and (B) 2 levels of microbial inoculant addition at ensiling: 0 and 8 mg commercial inoculant/kg total ensiled material. Each kg of inoculant contained  $3.9 \times 10^{10}$  colony-forming units (CFU)/g of *Pediococcus acidilactici* and  $3.75 \times 10^{10}$  CFU/g of *Propionibacterium acidipropionici*. Samples of fresh sugarcane and corn grain were collected for chemical analyses (Table 1).

**Table 1.** Chemical composition of sugarcane and corn grain before ensiling.

Item	Sugarcane <sup>1</sup>	Corn grain
Dry matter (DM) (g/kg)	257	870
Organic matter (OM) (g/kg DM)	961	986
Neutral detergent fiber (NDF) (g/kg DM)	527	139
Acid detergent fiber (ADF) (g/kg DM)	229	17.8
Non-fiber carbohydrates <sup>2</sup> (NFC) (g/kg DM)	396	753
Crude protein (CP) (g/kg DM)	27.0	74.3
Ether extract (EE) (g/kg DM)	10.8	17.4

<sup>1</sup>Sugarcane cultivar RB83-5054: 8 months of growth and Brix 17.5%.

<sup>2</sup>Calculated as:  $\text{NFC (g/kg)} = 1,000 - (\text{ash} + \text{CP} + \text{NDF} + \text{EE})$ .

Ensiling was performed using PVC tubes equipped with Bunsen valves. Sand (2 kg) was placed in the bottom of the tubes and separated from the ensiled material by a nylon mesh screen to drain effluent. Inoculant and corn were added individually to the sugarcane and the total thoroughly mixed manually before being assigned to a tube. Microbial inoculant was diluted in water and sprayed onto the fresh sugarcane. Ensiled material was compacted manually ( $650 \text{ kg/m}^3$  for SS and  $1,000 \text{ kg/m}^3$  for RCS) and tubes were sealed, weighed and stored at room temperature (about  $25^\circ\text{C}$ ) in a shed for 60 days. Immediately before opening, the silos were reweighed to determine DM and gas losses, expressed as a proportion of the DM ensiled.

Samples (500 g) from the center of the mass of those silos treated with whole corn grain (WC) were used for whole corn grain selection and recuperation calculation. Subsamples of corn grain from the WC treatment were called 'recovered' and used for chemical analysis and in situ degradation assay.

Fresh forage and silages were analyzed for DM concentration in a forced-air oven at  $60^\circ\text{C}$  for 72 h, then ground through a 2 mm screen (SL-31, Solab Científica, Piracicaba, Brazil) and analyzed for ash, crude protein

(CP) and ether extract (EE) according to AOAC (2000). Neutral detergent fiber (NDF, with heat-stable amylase, without sodium, and expressed inclusive of residual ash) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991). Two cannulated dairy cows previously adapted to a diet with 60:40 forage:concentrate ratio were used for in situ degradation assays. Ruminal incubation was performed for 96 hours, using  $5 \times 5 \text{ cm}$  non-woven tissue bags (Casali et al. 2008). After removal, bags were washed in running water and evaluated for NDF concentration.

To identify and quantify yeasts and molds, 10 g silage was diluted with 90 mL sterilized peptone water (1%, w/v). Serial dilutions were pour-plated on Dichloran Rose Bengal Chloramphenicol Agar. Agar plates were incubated aerobically at  $28^\circ\text{C}$  for 7 days. Colony-forming units were transformed into  $\log_{10}/\text{g}$  values (Downes and Ito 2001). Water activity (WA) was assessed using a benchtop water activity meter (Aqualab 4T, Decagon Devices Inc., Pullman, USA).

Another sample (500 g) of SS was used for silage juice extraction with a hydraulic press. The extract was filtered through cheesecloth and pH was determined immediately. The silage juice sample was centrifuged ( $500 \times g$  for 15 min) and the supernatant was used for  $\text{NH}_3\text{-N}$  and organic acid evaluation. Ammonia nitrogen was determined by the colorimetric phenol-hypochlorite method (Broderick and Kang 1980). Concentrations of ethanol plus acetic, propionic and butyric acids were determined by gas chromatography (GC-2010 Plus chromatograph, Shimadzu, Barueri, Brazil), fitted with a flame-ionization detector and automatic sample injection. Lactic acid concentration in silage samples was assessed using the spectrophotometric method (Pryce 1969).

Gas and effluent losses were calculated according to the following 3 equations:

$$\text{GL (g/kg)} = \frac{\text{SWE (g)} - \text{SWO (g)}}{\text{EDM (kg)}}$$

where: GL is gaseous loss; SWE and SWO are the silo weights at ensiling and opening, respectively; and EDM is the ensiled dry matter.

$$\text{EL (g/kg)} = \frac{\text{ESWO (g)} - \text{ESWE (g)}}{\text{EDM (kg)}}$$

where: EL is effluent loss; ESWO and ESWE are the empty (but including the sand plus effluent) silo weights at opening and ensiling, respectively.

$$\text{DMR (g/kg)} = \frac{\text{DMO (g)}}{\text{DME (kg)}}$$

where: DMR is dry matter recovery; DMO is dry matter at silo opening; and DME is dry matter at ensiling.

### Statistical analysis

For fermentative profile, losses and chemical composition, data from RCS were removed for statistical analysis. The PROC MIXED of SAS 9.3. (SAS Institute Inc., Cary, USA) was used, according to the following statistical model:

$$Y_{ijk} = \mu + C_i + I_j + C \times I_{ij} + e_{ijk}$$

with  $e_{ijk} \sim N(0, \sigma_{ij}^2)$ ;

where:  $Y_{ijk}$  is the value of the dependent variable;  $\mu$  is the overall mean;  $C_i$  is the fixed effect of corn ( $i = 1$  to 4);  $I_j$  is the fixed effect of microbial inoculant ( $j = 1, 2$ );  $C \times I_{ij}$  is an interaction term;  $e_{ijk}$  is the residual error; and  $N$  stands for Gaussian distribution.

The corn effect was separated into 3 orthogonal contrasts: C1: corn addition effect (CONT vs. GC2 + GC8 + WC); C2: corn grinding effect (GC2 + GC8 vs. WC); and C3: sieve size effect (GC2 vs. GC8). For recovered

corn analysis, we used a similar model, changing corn by processing effect ( $P_i$ ,  $i = 1$  and 2).

### Results

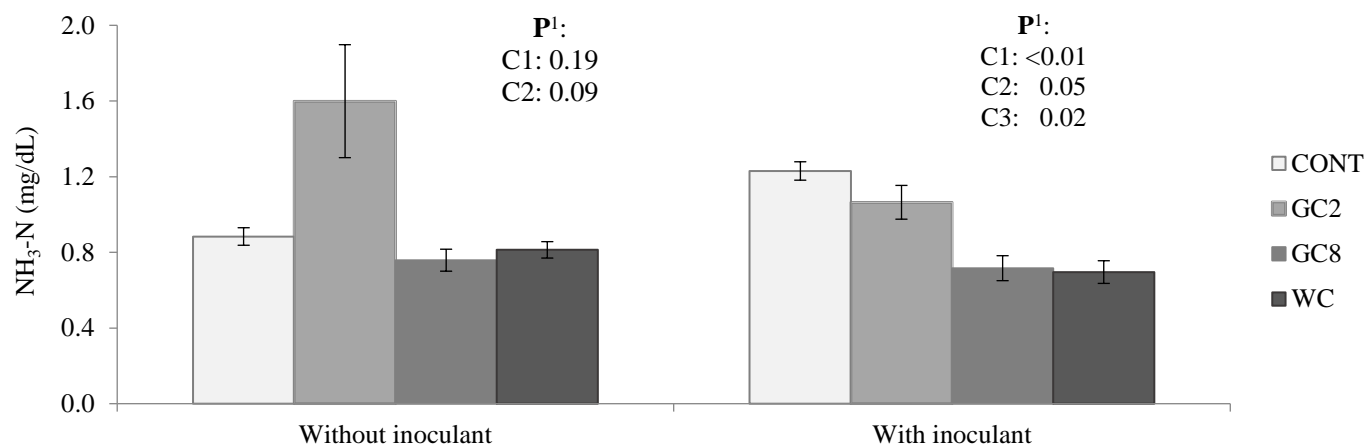
Corn addition decreased ( $P < 0.01$ ) acetic, propionic and butyric acid concentrations in SS and tended to decrease ( $P = 0.07$ ) ethanol concentration, while decreasing ( $P \leq 0.01$ ) losses and increasing ( $P < 0.01$ ) DM recovery (Table 2). Microbial inoculant addition increased ( $P \leq 0.04$ ) silage pH plus ethanol and butyric acid concentrations, but decreased ( $P \leq 0.04$ ) acetic acid concentration, mold and yeast counts and DM recovery, and tended ( $P \leq 0.07$ ) to increase lactic acid concentration and effluent losses. Compared with ground corn, WC tended ( $P \leq 0.07$ ) to decrease mold and yeast counts and to increase DM recovery. The corn  $\times$  microbial inoculant interaction was significant ( $P \leq 0.02$ ) for  $\text{NH}_3\text{-N}$  and water activity (WA) plus gaseous and total losses. Corn addition decreased ( $P < 0.01$ )  $\text{NH}_3\text{-N}$  only in silages treated with microbial inoculant (Figure 1). Ground corn tended to increase ( $P \leq 0.09$ )  $\text{NH}_3\text{-N}$  in silages relative to whole corn, regardless of microbial inoculant application (Figure 1) and the effect was stronger with finer grinding ( $P \leq 0.06$ ).

Regardless of inoculation, corn addition decreased ( $P < 0.01$ ) gaseous and total fermentative losses of silage (Table 2; Figure 2). Whole corn decreased ( $P = 0.02$ )

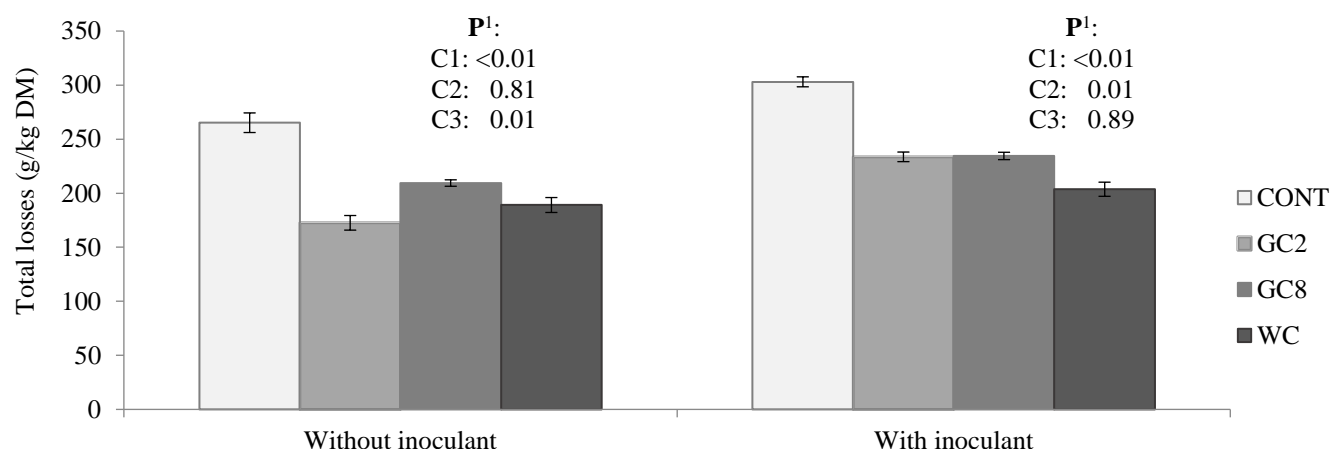
**Table 2.** Effects of addition of ground or whole corn grain and microbial inoculant at ensiling on fermentation, mold counts and fermentative losses in sugarcane silage.

Item	Corn addition <sup>1</sup>				INO <sup>2</sup>		s.e.m.	P <sup>3</sup>				
	CONT	GC2	GC8	WC	–	+		INO	CORN $\times$ INO	C1	C2	C3
pH	3.91	3.88	4.00	3.88	3.83	4.00	0.014	<0.01	0.38	0.78	0.15	0.15
$\text{NH}_3\text{-N}$ (mg/dL)	1.06	1.33	0.74	0.75	1.01	0.93	0.034	0.35	0.01	0.11	0.02	0.77
Ethanol and organic acids (g/kg DM)												
Ethanol	24.9	20.5	14.4	14.0	12.6	24.3	1.37	0.02	0.51	0.07	0.32	0.38
Lactic acid	45.6	39.4	41.2	42.7	38.2	46.2	1.94	0.07	0.21	0.45	0.56	0.75
Acetic acid	15.3	10.5	11.2	10.5	14.6	9.17	0.32	<0.01	0.45	<0.01	0.51	0.46
Propionic acid	0.48	0.18	0.23	0.25	0.27	0.31	0.018	0.31	0.97	<0.01	0.40	0.43
Butyric acid	0.13	0.08	0.08	0.07	0.08	0.09	0.002	0.04	0.42	<0.01	0.36	0.83
Microbial evaluation												
Yeast and mold (log <sub>10</sub> /g as fed)	15.7	12.2	14.0	10.6	16.0	10.3	0.70	<0.01	0.19	0.20	0.07	0.10
Water activity	0.99	0.99	0.98	0.93	0.99	0.95	0.003	<0.01	<0.01	0.01	<0.01	0.04
Fermentative losses (g/kg DM)												
Gaseous	152	102	109	100	103	128	0.8	<0.01	<0.01	<0.01	0.05	0.01
Effluent	133	101	113	96.3	106	115	2.0	0.06	0.24	0.01	0.03	0.01
Total	284	203	222	196	209	244	2.0	<0.01	0.02	<0.01	0.01	<0.01
DM recovery	657	768	760	825	777	728	10.9	0.04	0.40	<0.01	0.06	0.82

<sup>1</sup>CONT = Sugarcane silage (SS) without corn; GC2 = SS with 2 mm sieve-processed corn; GC8 = SS with 8 mm sieve-processed corn; WC = SS with whole corn grain. <sup>2</sup>Microbial inoculant. <sup>3</sup>Probabilities: INO: microbial inoculant effect; CORN  $\times$  INO: interaction between corn and inoculant effects; C1 = Control vs. corn addition; C2 = whole corn vs. ground corn; and C3 = 2 mm grind vs. 8 mm grind.



**Figure 1.** NH<sub>3</sub>-N concentration in sugarcane silage with additions of corn grain and microbial inoculant. CONT = Sugarcane silage (SS) without corn; GC2 = SS with 2 mm sieve-processed corn; GC8 = SS with 8 mm sieve-processed corn; WC = SS with whole corn grain. <sup>1</sup>Probabilities (P): C1 = Control vs. corn addition; C2 = whole corn vs. ground corn; and C3 = 2 mm grind vs. 8 mm grind.



**Figure 2.** Total fermentative losses of sugarcane silage treated with corn grain and microbial inoculant. CONT = sugarcane silage (SS) without corn; GC2 = SS with 2 mm sieve-processed corn; GC8 = SS with 8 mm sieve-processed corn; WC = SS with whole corn grain. <sup>1</sup>Probabilities (P): C1 = Control vs. corn addition; C2 = whole corn vs. ground corn; and C3 = 2 mm grind vs. 8 mm grind.

gaseous and total losses relative to ground corn only in silage treated with inoculant. Finer grinding reduced gaseous and total fermentative losses relative to coarser grinding ( $P \leq 0.01$ ) only without addition of microbial inoculant (Table 2; Figure 2).

Adding corn decreased water activity (WA) in inoculated silages ( $P = 0.01$ ). In addition, silage with WC showed lower WA if treated with microbial inoculant ( $P < 0.01$ ; Table 2) and a tendency for lower WA in non-inoculated silage ( $P = 0.09$ ). There was no corn addition  $\times$  INO interaction effect on chemical composition and DM degradation of silage ( $P \geq 0.22$ ; Table 3). Corn addition increased ( $P \leq 0.01$ ) silage DM, OM, NFC, CP and EE concentrations and DM degradation and decreased ( $P < 0.01$ ) NDF and ADF concentrations.

Microbial inoculant addition decreased ( $P = 0.03$ ) silage OM concentration and increased ( $P = 0.04$ ) CP concentration (Table 3). Ground corn tended to decrease NFC and to increase NDF concentrations, in comparison with whole corn ( $P \leq 0.09$ ). Particle size of ground corn had no effect ( $P \geq 0.13$ ) on SS composition and DM degradation (Table 3).

Recovered corn from WC and RCS showed similar OM, NDF, ADF and NFC concentrations ( $P \geq 0.12$ ; Table 4). However, recovered corn had lower ( $P \leq 0.01$ ) DM and CP concentrations and higher ( $P < 0.01$ ) EE concentration than RCS. Additionally, recovered corn tended to have higher ( $P = 0.09$ ) DM degradation than RCS. Microbial inoculant had no effect on any aspects of corn grain chemical composition and DM degradation ( $P \geq 0.15$ ).



**Table 3.** Effects of addition of microbial inoculant and corn grain at ensiling on chemical composition and in situ ruminal degradation of sugarcane silage.

Item	Corn addition <sup>1</sup>				INO <sup>2</sup>		s.e.m.	P <sup>3</sup>			
	CONT	GC2	GC8	WC	–	+		INO	C1	C2	C3
DM (g/kg)	182	261	260	280	252	240	3.6	0.10	<0.01	0.28	0.15
OM (g/kg DM)	959	970	970	972	969	966	0.5	0.03	<0.01	0.45	0.24
NDF	758	593	618	544	635	619	12.8	0.59	<0.01	0.09	0.61
ADF	438	261	264	243	307	296	4.8	0.35	<0.01	0.14	0.84
NFC	154	301	265	336	252	276	9.1	0.55	<0.01	0.06	0.27
CP	34.7	54.9	56.0	59.8	49.4	53.3	0.87	0.04	<0.01	0.57	0.13
EE	11.3	20.8	42.6	32.6	36.2	17.5	3.25	0.07	0.01	0.34	0.29
DM degradation	477	658	630	657	603	608	6.1	0.71	<0.01	0.19	0.97

<sup>1</sup>CONT = Sugarcane silage (SS) without corn; GC2 = SS with 2 mm sieve-processed corn; GC8 = SS with 8 mm sieve-processed corn; WC = SS with whole corn grain. <sup>2</sup>Microbial inoculant. <sup>3</sup>Probabilities: INO: microbial inoculant effect; CORN × INO: interaction between corn and inoculant effects  $P \geq 0.22$ ; C1 = Control vs. corn addition; C2 = whole corn vs. ground corn; and C3 = 2 mm grind vs. 8 mm grind.

**Table 4.** Chemical composition and in situ ruminal degradation of rehydrated corn silage and recovered whole corn grain from sugarcane silage.

Item	–INO <sup>1</sup>		+INO		s.e.m.	P <sup>4</sup>	
	RCS <sup>2</sup>	RWCG <sup>3</sup>	RCS <sup>2</sup>	RWCG <sup>3</sup>		PROC	INO
DM (g/kg)	616	574	625	561	1.7	<0.01	0.50
OM (g/kg DM)	987	990	985	988	0.8	0.12	0.23
NDF	95.6	89	97.9	93	2.61	0.31	0.56
ADF	26.2	24.4	26.1	24.8	0.65	0.27	0.92
NFC	757	778	752	764	4.8	0.25	0.47
CP	95	74	95.7	71.2	1.66	<0.01	0.80
EE	39.3	49	39.1	61.5	2.15	<0.01	0.15
DM degradation	856	864	861	873	2.6	0.09	0.23

<sup>1</sup>Microbial inoculant. <sup>2</sup>Rehydrated corn silage. <sup>3</sup>Recovered whole corn grain from sugarcane silage with grain added at ensiling.

<sup>4</sup>Probabilities: PROC - processing effect; INO - microbial inoculant effect; PROC × INO - interaction between processing and inoculant effects  $P \geq 0.14$ .

## Discussion

Fresh sugarcane used in the present study averaged 17.5% Brix and 257 g DM/kg, which is lower than the recommended level for ensiling (300 g/kg; [McDonald et al. 1991](#)). However, these values are similar to those reported by other authors, e.g. Sá Neto et al. (2013) for fresh sugarcane. We chose this material to provide a higher challenge for evaluation of the treatments, as ensiling of sugarcane with low DM concentration could benefit more from corn addition.

As expected, corn addition to sugarcane at ensiling increased DM concentration of ensiled material and consequently improved fermentation conditions, decreasing the production of acids and WA, especially in inoculant-treated silos. According to Greenhill (1964), after the breakdown of the cell walls, WA depends mainly on the moisture content of the ensiled material. Material with high DM concentration shows decreased WA, lower

bacterial counts and delayed growth of lactic acid bacteria (LAB) ([Castro et al. 2006](#)). Bernardes et al. (2007) showed that WA is directly associated with counts of mold and yeast. While corn addition decreased acid concentration and ethanol production in the present study, ground corn increased WA relative to whole corn.

Although concentrations of acids in corn-treated silages were lower than in straight sugarcane silage, in general, corn addition had no effect on pH of the silage. This result supports the findings of Andrade et al. (2001), who added ground corn ears to sugarcane with urea at ensiling and found no differences in silage pH. In the present study, the production of lactic acid, the primary acidogenic acid evaluated in the current trial, was not affected by corn addition at ensiling. Similarly, Bernardes et al. (2007) evaluated the addition of dehydrated corn grain with cob and straw to sugarcane at ensiling and also found no effects on silage pH, but chemical composition was improved.

Water may be produced during bacterial fermentation of sugars, mainly during the conversion of substrate to ethanol by yeasts ([Pedroso et al. 2008](#)). This water accumulation results in increased effluent losses and decreased silage DM concentration and DM recovery in silos without corn treatment. Additionally, WC decreased total losses and improved DM recovery, relative to GC, especially in inoculant-treated silos. Besides higher WA, GC2 addition resulted in lower effluent and gaseous losses, without affecting DM recovery, relative to GC8. Finely ground corn with smaller particle size can make compaction of material and expulsion of air from the ensiled material easier than whole or coarsely ground corn.

Furthermore, corn processing increases microbial adhesion to the endosperm and consequently increases  $\text{NH}_3\text{-N}$  concentration ([Lee et al. 2002](#)). The critical interaction between corn particle size and action of microbial inoculant was highlighted by the more evident corn effect in inoculated silos. As  $\text{NH}_3\text{-N}$  is produced from proteolysis ([Albrecht and Muck 1991](#)), decreased microbial action could negatively affect the  $\text{NH}_3\text{-N}$  level, as observed in corn-treated silos. Although Oliveira et al. (2017) found decreased  $\text{NH}_3\text{-N}$  concentration in silages treated with homofermentative LAB regardless of forage type, inoculant had no effect in the present study. Despite the presence of propionic acid bacteria in the evaluated inoculant, the addition of inoculants showed no effect on propionic acid concentration but increased ethanol concentration of the silage. Borreani et al. (2018) observed that yeast activity converted glucose to ethanol, resulting in high fermentative losses in silage. Both homofermentative and heterofermentative lactic acid bacteria potentially improve silage fermentation, but heterofermentative bacteria are more effective in inhibiting fungal growth ([Filya 2003](#)). Santos et al. (2015) observed that adding homofermentative LAB (*Propionibacterium acidipropionici*, *Lactobacillus plantarum* and *Enterococcus faecium*) to sugarcane at ensiling increased ethanol production and DM losses, even when heterofermentative LAB were included, whereas silage inoculated exclusively with heterofermentative LAB (*L. buchneri*) had reduced DM losses and alcoholic fermentation. In the present study, microbial inoculant decreased DM recovery and concentrations of DM and OM in silage.

In general, corn addition to SS reduced fermentative losses and, consequently, improved chemical composition of silage. Traditionally, in SS, high yeast populations ([Ávila et al. 2010](#)) convert most of the water-soluble carbohydrate, which is in the NFC component, into fermentation end-products, such as volatile organic compounds and mainly ethanol ([Daniel et al. 2013](#)). In keeping with these

outcomes, corn addition reduced fermentative losses in SS and increased the concentration of NFC in our study, resulting in decreased fiber concentration via a dilution effect and improved DM degradation. The different responses in NDF and NFC as a result of the addition of ground and whole corn seem related to lower fermentative losses in silos containing WC grain.

In evaluating recovered whole corn grain (RWCG) relative to rehydrated corn silage (RCS), we found similar OM, NDF, ADF and NFC concentrations for both forms of corn, while RWCG had lower DM and CP concentrations and higher EE concentration than RCS. Although Junges et al. (2017) attributed 60% of protein degradation in RCS to bacterial activity, solubilization in fermentation end-products could increase protein degradation ([Lawton 2002](#)) and improve silage DM degradation. Lower CP concentration and increased DM degradation observed in RWCG relative to RCS are reflected in increased acid levels and bacterial activity in the silos. Higher ethanol concentration in SS could solubilize kernel protein ([Zhang et al. 2011](#)), which was not recovered in the silage samples.

At ensiling rehydrated corn contained 660 g DM/kg and DM recovery after ensiling was 926 g DM/kg silage, which is in accordance with average recovery levels reported by Kung Jr et al. (2004). On the other hand, RWCG showed a recovery rate of 966 g DM/kg ( $P>0.05$ ). However, owing to the importance of this variable in silage making, more studies are necessary to evaluate the role of adding corn in improving DM recovery in sugarcane silage. Our results suggest that there is little merit in grinding the grain before adding it to the sugarcane at ensiling. In some situations, other energy sources like molasses or cassava may be a cheaper source than corn grain and studies are needed to test their efficacy relative to corn. In addition, feeding studies to evaluate feed intakes by livestock and subsequent performance using whole corn grain and other energy additives in SS at ensiling or fed directly with sugarcane silage are warranted.

## Conclusions

Microbial inoculant containing mainly homolactic bacteria had little effect on fermentation of sugarcane silage and there appears little merit in adding it to fresh sugarcane at ensiling. The addition of corn grain at ensiling improved SS fermentation and silage composition but there seems little value in grinding the grain before adding it to the sugarcane. Further studies comparing other energy sources with corn as additives at the ensiling of sugarcane seem warranted as well as feeding studies to compare intakes of

the various products by livestock and subsequent performance. The alternative of feeding the energy sources with straight sugarcane silage as opposed to adding them at ensiling should be assessed.

## References

(Note of the editors: All hyperlinks were verified 7 November 2019.)

- Albrecht KA; Muck RE. 1991. Proteolysis in ensiled forage legumes that vary in tannin concentration. *Crop Science* 31:464–469. doi: [10.2135/cropsci1991.0011183X003100020048x](https://doi.org/10.2135/cropsci1991.0011183X003100020048x)
- Andrade JB de; Ferrari Júnior E; Braun G. 2001. Nutritive value of sugarcane silage treated with urea and added ground corn ears. *Pesquisa Agropecuária Brasileira* 36:1167–1174. (In Portuguese). doi: [10.1590/S0100-204X2001000900010](https://doi.org/10.1590/S0100-204X2001000900010)
- AOAC (Association of Official Analytical Chemists). 2000. Official methods of analysis. 17th Edn. AOAC Inc., Arlington, VA, USA.
- Ávila CLS; Valeriano AR; Pinto JC; Figueiredo HCP; Rezende AV de; Schwan RF. 2010. Chemical and microbiological characteristics of sugarcane silages treated with microbial inoculants. *Revista Brasileira de Zootecnia* 39:25–32. doi: [10.1590/S1516-35982010000100004](https://doi.org/10.1590/S1516-35982010000100004)
- Ávila CLS; Carvalho BF; Pinto JC; Duarte WF; Schwan RF. 2014. The use of *Lactobacillus* species as starter cultures for enhancing the quality of sugarcane silage. *Journal of Dairy Science* 97:940–951. doi: [10.3168/jds.2013-6987](https://doi.org/10.3168/jds.2013-6987)
- Bernardes TF; Reis RA; Siqueira GR; Berchielli TT; Coan RM. 2007. Effect of burning and addition of dehydrated corn cob and straw on sugar cane silage. *Revista Brasileira de Zootecnia* 36:269–275. (In Portuguese). doi: [10.1590/S1516-35982007000200001](https://doi.org/10.1590/S1516-35982007000200001)
- Borreani G; Tabacco E; Schmidt RJ; Holmes BJ; Muck RE. 2018. *Silage review*: Factors affecting dry matter and quality losses in silage. *Journal of Dairy Science* 101:3952–3979. doi: [10.3168/jds.2017-13837](https://doi.org/10.3168/jds.2017-13837)
- Broderick GA; Kang JH. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. *Journal of Dairy Science* 63:64–75. doi: [10.3168/jds.S0022-0302\(80\)82888-8](https://doi.org/10.3168/jds.S0022-0302(80)82888-8)
- Carvalho BF; Ávila CLS; Pinto JC; Neri J; Schwan RF. 2014. Microbiological and chemical profile of sugarcane silage fermentation inoculated with wild strains of lactic acid bacteria. *Animal Feed Science and Technology* 195:1–13. doi: [10.1016/j.anifeedsci.2014.04.003](https://doi.org/10.1016/j.anifeedsci.2014.04.003)
- Casali AO; Detmann E; Valadares Filho SC; Pereira JC; Henriques LT; Freitas SG de; Paulino MF. 2008. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos *in situ*. *Revista Brasileira de Zootecnia* 37:335–342. doi: [10.1590/S1516-35982008000200021](https://doi.org/10.1590/S1516-35982008000200021)
- Castro FGF; Nussio LG; Haddad CM; Campos FP de; Coelho RM; Mari LJ; Toledo PA. 2006. Effects of additive application on the microbial profile, physical parameters and aerobic stability of Tifton 85 (*Cynodon* sp.) silages with different dry matter contents. *Revista Brasileira de Zootecnia* 35:358–371. (In Portuguese). doi: [10.1590/S1516-35982006000200005](https://doi.org/10.1590/S1516-35982006000200005)
- Daniel JLP; Amaral RC; Sá Neto A; Cabezas-Garcia EH; Bispo AW; Zopollatto M; Cardoso TL; Spoto MHF; Santos FAP; Nussio LG. 2013. Performance of dairy cows fed high levels of acetic acid or ethanol. *Journal of Dairy Science* 96:398–406. doi: [10.3168/jds.2012-5451](https://doi.org/10.3168/jds.2012-5451)
- Downes FP; Ito K. 2001. Compendium of methods for the microbiological examination of foods. 4th Edn. APHA Press, Washington, DC, USA.
- Filya L. 2003. The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn sorghum silages. *Journal of Dairy Science* 86:3575–3581. doi: [10.3168/jds.S0022-0302\(03\)73963-0](https://doi.org/10.3168/jds.S0022-0302(03)73963-0)
- Fitzsimons A; Duffner F; Curtin D; Brophy G; O'Kiely P; O'Connell M. 1992. Assessment of *Pediococcus acidilactici* as a potential silage inoculant. *Applied Environmental Microbiology* 58:3047–3052. [bit.ly/2qumUGS](https://doi.org/10.1128/aem.58.12.3047-3052.1992)
- Gómez-Vásquez A; Pinos-Rodríguez JM; García-López JC; Cruz-Lázaro E; Luna-Palomera C; Sánchez-Hernández R. 2011. Nutritional value of sugarcane silage enriched with corn grain, urea, and minerals as feed supplement on growth performance of beef steers grazing stargrass. *Tropical Animal Health and Production* 43:215–220. doi: [10.1007/s11250-010-9678-z](https://doi.org/10.1007/s11250-010-9678-z)
- Greenhill WL. 1964. Plant juices in relation to silage fermentation. III. Effect of water activity of juice. *Grass and Forage Science* 19:336–339. doi: [10.1111/j.1365-2494.1964.tb01182.x](https://doi.org/10.1111/j.1365-2494.1964.tb01182.x)
- Jacovaci FA; Jobim CC; Schmidt P; Nussio LG; Daniel JLP. 2017. A data-analysis on the conservation and nutritive value of sugarcane silage treated with calcium oxide. *Animal Feed Science and Technology* 225:1–7. doi: [10.1016/j.anifeedsci.2017.01.005](https://doi.org/10.1016/j.anifeedsci.2017.01.005)
- Junges D; Morais G; Spoto MHF; Santos PS; Adesogan AT; Nussio LG; Daniel JLP. 2017. Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. *Journal of Dairy Science* 100:9048–9051. doi: [10.3168/jds.2017-12943](https://doi.org/10.3168/jds.2017-12943)
- Kung Jr L; Myers CL; Neylon JM; Taylor CC; Lazartie J; Mills JA; Whiter AG. 2004. The effects of buffered propionic acid-based additives alone or combined with microbial inoculation on the fermentation of high moisture corn and whole-crop barley. *Journal of Dairy Science* 87:1310–1316. doi: [10.3168/jds.S0022-0302\(04\)73280-4](https://doi.org/10.3168/jds.S0022-0302(04)73280-4)
- Lawton JW. 2002. Zein: A history of processing and use. *Cereal Chemistry* 79:1–18. doi: [10.1094/CCHEM.2002.79.1.1](https://doi.org/10.1094/CCHEM.2002.79.1.1)
- Lee SY; Kim WY; Ko JY; Ha JK. 2002. Effects of corn processing on *in vitro* and *in situ* digestion of corn grain in Holstein steers. *Asian-Australasian Journal of Animal Sciences* 15:851–858. doi: [10.5713/ajas.2002.851](https://doi.org/10.5713/ajas.2002.851)
- McDonald P; Henderson AR; Heron SJ. 1991. The biochemistry of silage. 2nd Edn. Chalcombe Publications, Marlow, UK.



- Oliveira AS; Weinberg ZG; Ogunade IM; Cervantes AAP; Arriola KG; Jiang Y; Kim D; Li X; Gonçalves MCM; Vyvas D; Adesogan AT. 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. *Journal of Dairy Science* 100:4587–4603. doi: [10.3168/jds.2016-11815](https://doi.org/10.3168/jds.2016-11815)
- Pedroso AF; Nussio LG; Loures DRS; Paziani SF; Ribeiro JL; Mari LJ; Zoopellatto M; Schmidt P; Mattos WRS; Horii J. 2008. Fermentation losses and aerobic stability of sugarcane silages treated with chemical or bacterial additives. *Scientia Agricola* 65:589–594. doi: [10.1590/S0103-90162008000600004](https://doi.org/10.1590/S0103-90162008000600004)
- Pryce JD. 1969. A modification of Barker-Summerson method for determination of lactic acid. *Analyst* 94:1151–1152. doi: [10.1039/AN9699401151](https://doi.org/10.1039/AN9699401151)
- Sá Neto A de; Nussio LG; Zopollatto M; Junges D; Bispo AW. 2013. Corn and sugarcane silages with *Lactobacillus buchneri* alone or associated with *L. plantarum*. (In Portuguese). *Pesquisa Agropecuária Brasileira* 48:528–535. doi: [10.1590/S0100-204X2013000500009](https://doi.org/10.1590/S0100-204X2013000500009)
- Santos WCC dos; Nascimento WG do; Magalhães ALR; Silva DKA; Silva WJCS; Santana AVS; Soares GSC. 2015. Nutritive value, total losses of dry matter and aerobic stability of the silage from three varieties of sugarcane treated with commercial microbial additives. *Animal Feed Science and Technology* 204:1–8. doi: [10.1016/j.anifeedsci.2015.03.004](https://doi.org/10.1016/j.anifeedsci.2015.03.004)
- Santos WP; Carvalho BF; Ávila CLS; Dias Júnior GS; Pereira MN; Schwan RF. 2010. Glycerin as an additive for sugarcane silage. *Annals of Microbiology* 65:1547–1556. doi: [10.1007/s13213-014-0993-x](https://doi.org/10.1007/s13213-014-0993-x)
- Silva NC de; Nascimento CF; Nascimento FA; Resende FD de; Daniel JLP; Siqueira GR. 2018. Fermentation and aerobic stability of rehydrated corn grain silage treated with different doses of *Lactobacillus buchneri* or a combination of *Lactobacillus plantarum* and *Pediococcus acidilactici*. *Journal of Dairy Science* 101:4158–4167. doi: [10.3168/jds.2017-13797](https://doi.org/10.3168/jds.2017-13797)
- Van Soest PJ; Robertson JB; Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74:3583–3597. doi: [10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Zhang B; Luo Y; Wang Q. 2011. Effect of acid and base treatments on structural, rheological, and antioxidant properties of  $\alpha$ -zein. *Food Chemistry* 124:210–220. doi: [10.1016/j.foodchem.2010.06.019](https://doi.org/10.1016/j.foodchem.2010.06.019)

(Received for publication 28 November 2018; accepted 5 September 2019; published 30 November 2019)

© 2019



*Tropical Grasslands-Forrajes Tropicales* is an open-access journal published by *International Center for Tropical Agriculture (CIAT)*, in association with *Chinese Academy of Tropical Agricultural Sciences (CATAS)*. This work is licensed under the Creative Commons Attribution 4.0 International ([CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)) license.