



## Does compaction degree and particle size influence bromatological composition and mycotoxin levels in corn silage?

A densidade da compactação e o tamanho da partícula influenciam na composição bromatológica e nos níveis de micotoxinas em silagens de milho?

E. A. de Toledo<sup>1</sup>; S. F. N. Pertile<sup>1\*</sup>; J. Sifuentes dos Santos<sup>2</sup>; J. V. P. Barreto<sup>1</sup>; G. Banchemo<sup>3</sup>; M. D. Dos Santos<sup>4</sup>; R. L. Venancio<sup>2</sup>; E. L. A. Ribeiro<sup>5</sup>; M. Zamboti<sup>1</sup>; M. Zundt<sup>6</sup>; F. C. de A. Rego<sup>1</sup>

<sup>1</sup> Programa de Mestrado em Saúde e Produção de Animal, Universidade Pitágoras Unopar, 86702-670, Arapongas - PR, Brasil.

<sup>2</sup> Programa de Mestrado em Ciência e Tecnologia de Leite e Derivados, Universidade Pitágoras Unopar, 86040-141, Londrina - PR, Brasil.

<sup>3</sup> Beef and Wool Program, Instituto Nacional de Investigación Agropecuaria (INIA), Colonia, Uruguay.

<sup>4</sup> Programa de Mestrado e Doutorado em Biociência Animal, Universidade de Cuiabá (UNIC), 78015-480, Cuiabá - MT, Brasil.

<sup>5</sup> Departamento de Zootecnia, Universidade Estadual de Londrina (UEL), 86057970, Londrina - PR, Brasil.

<sup>6</sup> Programa de Pós-graduação em Ciência Animal, Universidade do Oeste Paulista, 19050-920, Presidente Prudente - SP, Brasil.

\* s.pertile@zootecnista.com.br

(Recebido em 08 de maio de 2020; aceito em 13 de julho de 2020)

The objective of this study was to evaluate the effects of compaction degree and particle size on the presence of mycotoxins and the bromatological composition of corn silages. Samples from 47 silos with forage corn silage were collected from the northern region of Paraná State, Brazil. The bromatological composition and concentration of mycotoxin in silages were analyzed according to both the compaction degree (600 kg.m<sup>-3</sup> and 700 kg.m<sup>-3</sup>) and particle size. Mycotoxins were detected using enzyme-linked immunosorbent assay, and test results were considered positive when the mycotoxin concentration was above the detection limit of 100 µg.kg<sup>-1</sup> for deoxynivalenol, 2.5 µg.kg<sup>-1</sup> for aflatoxin, 5 µg.kg<sup>-1</sup> for zearalenone, and 200 µg.kg<sup>-1</sup> for fumonisin. Silages with 600 kg.m<sup>-3</sup> compaction degree had higher initial and final pH than silages with a higher compaction degree. Silage with a mean particle size less than or equal to 14.56 mm had 12% higher dry matter content than silage with a mean particle size more than 14.56 mm. There was no effect of the compaction degree or particle size on the concentration of mycotoxin, and 83% of the analyzed samples had contamination above the detection limits according to at least one of the studied mycotoxins. Silages with smaller average particle sizes had higher dry matter content. Thus, the degree of compaction and particle size did not affect the presence of mycotoxins in corn silage.

Keywords: silage, secondary fungi metabolite, forage quality.

O objetivo do presente estudo foi avaliar os efeitos da densidade de compactação e tamanho de partícula na presença de micotoxinas e composição bromatológica de silagens de milho. Amostras de 47 silos contendo silagem de milho foram coletadas da região Norte do Estado do Paraná, Brasil. A composição bromatológica e os níveis de micotoxinas foram analisados de acordo com a densidade de compactação (600 kg.m<sup>-3</sup> and 700 kg.m<sup>-3</sup>) e o tamanho de partícula. Micotoxinas foram avaliadas utilizando imunoensaio imunoenzimático, e os resultados foram avaliados considerando os seguintes limites de detecção: 100 µg.kg<sup>-1</sup> para desoxinivalenol, 2,5 µg.kg<sup>-1</sup> para aflatoxina, 5 µg.kg<sup>-1</sup> para zearalenona e 200 µg.kg<sup>-1</sup> para fumonisina. Silagens com densidade de compactação de 600 kg.m<sup>-3</sup> apresentaram maior pH inicial e final que as silagens com maior densidade de compactação. Silagens com tamanho de partícula inferior ou igual a 14,56 mm apresentaram 12% maior conteúdo de massa seca que as silagens com tamanho de partícula maior que 14,56 mm. Não houve efeito da densidade de compactação ou tamanho de partícula nos níveis de micotoxinas, e 83% das amostras analisadas apresentaram contaminação acima dos limites de detecção para ao menos uma das micotoxinas estudadas. Silagens com menor tamanho de partícula apresentaram maior conteúdo de massa

seca. Assim, a densidade de compactação e o tamanho de partícula não influenciaram a presença de micotoxinas em silagem de milho.

Palavras-chave: silagem, metabólitos secundários fúngicos, qualidade de silagem.

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## 1. INTRODUCTION

Corn (*Zea mays*) is one of the most planted forage crops both in Brazil and globally for silage production. Silage is obtained by fermentation under anaerobic conditions and, consequently, from spontaneous lactic acid production, leading to material preservation as the final pH is unfavorable for the growth of deteriorating microorganisms [1]. The final quality of corn silage is dependent on several factors, such as the nutritional value of the forage, how the silage is processed, opening of the silo, and mechanism by which it is supplied to animals.

The degree of compaction of the silo and the average particle size of the silage can determine the final quality of the silage. Compaction is carried out to eliminate the amount of air, and consequently oxygen, as much as possible to allow anaerobic fermentation. The size of the silage particles influences the removal of air and, consequently, the compaction of the silage [2]. In addition, toxicogenic fungi can contaminate corn during the planting, harvest, and storage periods. The three main fungal genera, which are mycotoxin producers and found in animal and human feeds, are *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. Numerous fungal species belonging to these genera produce mycotoxin secondary metabolites such as aflatoxins, ochratoxins, trichothecenes (deoxynivalenol, nivalenol, T-2 toxin, among others), zearalenone (ZEA), fumonisins, and several other mycotoxins that are considered minor or emerging mycotoxins [3].

Mycotoxins can cause metabolic disorders and diseases in animals [4] and humans [5]. In animals, the consumption of mycotoxins can cause acute effects, such as reduced feed intake, reduced weight gain, and decreased reproductive performance as well as chronic effects such as liver damage and depression of the immune system [6]. In addition, metabolites of mycotoxins may be present in products of animal origin, especially in cattle, which metabolize aflatoxin B1 and excrete aflatoxin M in milk [7]. In humans, ingestion of mycotoxin products is associated with the development of several types of cancer, and both aflatoxin B1 and the metabolite aflatoxin M1 are considered to be substances with potential hepatocarcinogenicity [5].

Knowledge regarding the presence and identification of fungi and mycotoxins in silages is extremely important, especially because this raw material makes up a large part of the diet of ruminants, as silages are favorable for fungal growth and consequent contamination. Thus, the objective of this study was to evaluate the effect of compaction degree and particle size on the presence of mycotoxins and the bromatological composition of corn silages.

## 2. MATERIAL AND METHODS

Corn silage (whole plant) samples were collected from 47 silos of rural properties located at the northern region (23°33'03"S, 51°27'39"W) of Paraná State, Brazil. The Köppen climate classification for the region is Cfb [8], with an average temperature of 18.8°C and average annual rainfall of 1507 mm.

The dimensions of the evaluated silos were between 25 and 50 m for length, 6 and 12 m for width, and 2 to 3.5 m for height. Silage confection was performed during January 2016, and the silos were kept closed until May 2016, when they were opened. The sample collection period was May to August 2016. The samples were collected at several points throughout each silo and at different depths. The edges and the material in contact with the floor or silage with mold or dirt were excluded. The amount of silage withdrawn per silo accounted for approximately 2 kg of fresh matter, which was packed in a plastic bag. Air from the bag was removed, and the bag was properly labeled and then transported to the bromatology laboratory in a Styrofoam box with ice.

Fresh samples from the same silage were homogenized in plastic containers, and 10-g aliquots were taken for pH measurement, according to the methods described by Cherney and Cherney (2003) [9], in which the samples were placed in a container with 100 mL of distilled water for 30

s, followed by homogenization of the mixture using a glass stick. The pH was measured immediately after the samples arrived at the laboratory (initial pH) and again after 1 h (final pH).

Silages were classified according to the amount of material ensiled and the size of the silo. They were grouped into two degrees of compaction: 600 kg.m<sup>-3</sup> (22 corn silage samples) and 700 kg.m<sup>-3</sup> (25 corn silage samples).

Particle size was assessed using the Penn State Particle Size Separator technique [10] adapted by Mari and Nussio (2002) [11]. For this purpose, 250 g of fresh silage from each sample was placed on the upper sieve (38 mm). Stirring was manually performed on a flat and smooth surface by vigorously shaking the sieves for five times in eight sets (after every five agitations, sieve set was rotated by 90°), totaling 40 movements. After shaking, the weight of the sample retained in each sieve was recorded, and the average size of 20 particles retained in the upper sieve was measured using a ruler. The samples were divided into two groups based on the mean particle size: one particle group with size smaller than the median (14.56 mm; 21 corn silage samples) and the other with size larger than the median (26 corn silage samples).

The concentrations of aflatoxin (AFLA B1, B2, G1, and G2), zearalenone (ZEA), deoxynivalenol (DON), and fumonisins (FB, fumonisin B1 and B2) were determined using the methods developed by Neogen (2019) [12]. For this, 10 g of each sample was diluted in 200 mL of distilled water. The samples were shaken and sequentially filtered, and mycotoxin detection was performed using an enzyme-linked immunosorbent assay with the Neogen kit (Veratox). A sample was considered positive when the contamination level was above the detection limit (DON, 100 µg.kg<sup>-1</sup>; AFLA, 2.5 µg.kg<sup>-1</sup>; ZEA, 5 µg.kg<sup>-1</sup>; FB, 200 µg.kg<sup>-1</sup>).

Dry matter (DM) and mineral matter contents of the silages were determined according to the methodology described by AOAC (2000) [13]. Neutral detergent fiber, acid detergent fiber, ethereal extract, and crude protein fractions were obtained according to the methodologies described by Mizubuti et al. (2009) [14]. The estimation of the total digestible nutrient and digestible DM values were conducted using the equations described by Capelle et al. (2001) [15]. Ammoniacal nitrogen was determined using methods described by Mizubuti et al. (2009) [14].

Statistical analysis was carried out using the software STATA 16.0. The studied variables were analyzed according to the degree of compaction and particle size. A Shapiro-Wilk test was used to evaluate the normality of the residuals, and since they did not present a normal distribution, the variables were analyzed using a non-parametric Mann-Whitney U test (p<0.05) to compare the means between the groups.

### 3. RESULTS

The degree of compaction only affected the initial and final pH of the forage corn silage (Table 1). Silages with a medium compaction degree had higher initial and final pH values than silages with a high compaction degree (p<0.05). Particle size only affected the DM content of the silage. Silage with a mean particle size less than or equal to 14.56 mm had 12% higher (p<0.05) DM content than silage with a mean particle size over 14.56 mm.

Table 1. Bromatological composition and fermentative parameters of forage corn silage according to compaction degree and mean particle size.

Variable*	Compaction degree		Particle size	
	Medium (600 kg.m <sup>-3</sup> )	High (700 kg.m <sup>-3</sup> )	≤ 14.56 mm	> 14.56 mm
DM (g.kg <sup>-1</sup> )	331.0	324.0	348.0 <sup>a</sup>	311.0 <sup>b</sup>
MC (g.kg <sup>-1</sup> DM)	34.9	37.5	31.6	36.6
CP (g.kg <sup>-1</sup> DM)	65.1	62.0	60.9	66.1
EE (g.kg <sup>-1</sup> DM)	15.4	15.7	16.0	14.8
NDF (g.kg <sup>-1</sup> DM)	473.0	463.4	463.0	481.0
ADF (g.kg <sup>-1</sup> DM)	277.0	261.3	262.0	268.0
DDM (g.kg <sup>-1</sup> DM)	668.0	671.0	671.0	665.0
TDN (g.kg <sup>-1</sup> DM)	702.0	706.0	706.0	699.0
Initial pH	3.94 <sup>a</sup>	3.76 <sup>b</sup>	3.87	3.80
Final pH	3.92 <sup>a</sup>	3.75 <sup>b</sup>	3.91	3.85
AN (g.kg <sup>-1</sup> TN)	29.5	33.5	30.9	31.3

Data are presented as median. \*DM: Dry matter; MC: Mineral content; CP: Crude protein; EE: Etheral extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; DDM: Digestible dry matter; TDN: Total digestible nutrient; AN: Amoniacal Nitrogen, TN: Total Nitrogen. Different letters denotes difference among compaction degree or particle size.

The evaluation of particle size proportion across all silages indicated a spread of sizes as follows: 14% of particles were larger than 19 mm, 50.7% of particles had sizes between 7.8 and 19 mm, 33.4% of particles were between 4 and 7.8 mm, and 1.9% of particles were smaller than 4 mm.

There was no effect ( $p>0.05$ ) of compaction degree or mean particle size on the concentration of mycotoxin in silage samples (Table 2). All silo samples analyzed had no contamination or had levels below the detection limit for fumonisin mycotoxin. Eighty-three percent of the silo samples had contamination above the detection limits with at least one of the other evaluated mycotoxins.

Table 2. Mycotoxins concentrations in forage corn silage according to compaction degree and mean particle size.

Mycotoxin	Compaction degree				Particle size			
	Medium (600 kg.m <sup>-3</sup> )		High (700 kg.m <sup>-3</sup> )		≤ 14.56 mm		> 14.56 mm	
	N	Me	N	Me	N	Me	N	Me
Deoxynivalenol (µg.Kg <sup>-1</sup> )	07/22	306.8	10/25	328.6	10/21	328.6	07/26	293.2
Aflatoxin (µg.Kg <sup>-1</sup> )	09/22	4.9	18/25	8.6	11/21	5.9	16/26	8.7
Zearalenone (µg.Kg <sup>-1</sup> )	10/22	252.1	23/25	348.5	13/21	193.5	20/26	323.0

N= Number of positive samples/total; Me=Median.

#### 4. DISCUSSION

Despite most samples being contaminated with mycotoxins, the nutritional value was similar among silages, which were within the observed standard values for forage corn silages in Brazil [16]. In this study, the compaction degree did not affect the nutrient composition of silages. Velho et al. (2007) [17] evaluated corn silages made in 30 L mini-silos with a compaction degree of 500 and 600 kg.m<sup>-3</sup>. Similar to our study, their study demonstrated no significant differences in DM content ( $p>0.05$ ). However, the DM values determined in our study were higher (331 and 324 g.kg<sup>-1</sup> for compaction degrees of 600 kg.m<sup>-3</sup> and 700 kg.m<sup>-3</sup>, respectively) than the 28.7% value obtained by Velho et al. (2007) [17] for DM with similar compaction (600 kg.m<sup>-3</sup>). This difference was possibly due to differences in the ensiling process and the nutritional quality of the corn used, as Velho et al. (2007) [17] harvested corn and assessed silages in May, which was earlier than those in our study.

Samples with a lower mean particle size had higher DM content than samples with larger average particle size, as particle size influences air expulsion and consequently silage compaction, which

may cause changes in the final DM content of the silage. According to Heinrichs and Jones (2016) [18], the ideal composition of particle size in forage corn silage should be as follows: 3%-8% of particles larger than 19 mm, 45%-65% of particles between 7.8 and 19 mm, 10%-30% of particles between 4 and 7.8 mm, and less than 10% of particles smaller than 4 mm. According to this classification, in the present study, 14% of particles were larger than 19 mm, whereas the proportions of other particles were within or near the ideal proportion.

The median values obtained for the initial and final pH values were approximately 5% higher for silages with compaction degree of 600 kg.m<sup>3</sup> than those for silages with higher compaction degrees. pH can be influenced by silage compaction, with the highest pH values being obtained in low-compacted materials [19], similar to the results obtained in the present study. Although the pH of silages with different compaction degrees significantly differed, it is important to note that all observed pH values were lower than 6.0, which is related to a reduction in the development of fungi [1].

The median values obtained for the ammoniacal nitrogen variable in relation to the total nitrogen varied from 29.5 to 33.5 g.kg<sup>-1</sup> total nitrogen (TN), which are lower than the average values reported by Pinto et al. (2007) [20] and by Velho et al. (2007) [17] of 58 and 41 g.kg<sup>-1</sup> TN, respectively, for corn silage and similar to those reported by Santos et al. (2010) [21], with a mean of 29 g.kg<sup>-1</sup> TN. The ammoniacal nitrogen quantity varies in relation to the position in the feed-out face (core, lateral, and apical parts) of the silage and the ensiling procedure [22]. This variable is indicative of the degradation of protein in the fermentation phase of silage formation, which is one of the most important parameters for determining the quality of fermentation [21] and should be below 10% in quality silage [23]. Therefore, the results obtained for the ammoniacal nitrogen variable in the present study are indicative of the quality of the forage corn silage samples analyzed.

The compaction degree and particle size used in this study did not influence the presence of mycotoxins in the silages, with mycotoxins identified in all samples. However, the quantities of mycotoxins found were within the limits allowed by the European Union for maize-based foods for animal consumption [24, 25, 26]. Most of the samples evaluated presented the mycotoxins aflatoxin, zearalenone, and/or deoxynivalenol in the forage corn silages studied, demonstrating the importance of measures that control the development of mycotoxigenic fungi in forage corn silages.

The concentrations of DON and ZEA obtained in the present study were similar to those obtained by Schimidt et al. (2015) [27], 259 and 181 µg.kg<sup>-1</sup>, respectively, when evaluating the mycotoxin concentration in corn silage from five Brazilian dairy-production regions. The same authors stated that the critical value for ZEA concentration for corn silage is 285 µg.kg<sup>-1</sup>, which varies with several factors including the region, climate, and planting season [27]. The ZEA concentrations obtained in the present study are above this value, suggesting that environmental conditions of the places these silos were from are favorable for ZEA production.

## 5. CONCLUSIONS

Compaction degree did not influence the bromatological composition of corn silage. Additionally, the degree of compaction and particle size did not affect the presence of mycotoxins in corn silage. Corn silage produced in both compaction degree and particle size presented good mycotoxicological quality, did not presenting a toxicological risk to animals and human health.

## 6. ACKNOWLEDGMENT

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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