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RESEARCH ARTICLE



## Cottonseed cake as nutritional additive for sorghum silages

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### ABSTRACT

This study aimed to determine the optimal proportion of cottonseed cake (CSC) as an additive in sorghum silage to improve its chemical composition, fermentative profile, microbial populations, losses, dry matter recovery, and aerobic stability. Five treatments were evaluated, varying the inclusion of CSC at ensiling (0, 50, 100, 200, and 200 g kg<sup>-1</sup> on fresh matter basis). The results showed that the inclusion of CSC significantly increased the content of dry matter, crude protein, and ether extract in the silages. Water-soluble carbohydrates, buffering capacity, and ammoniacal nitrogen exhibited a linear decrease with increasing CSC inclusion. The populations of lactic acid bacteria and moulds showed a quadratic response to CSC inclusion. The yeast population was completely inhibited with a minimum inclusion of 200 g kg<sup>-1</sup> CSC. Aerobic stability and dry matter recovery showed a linear increase with CSC inclusion. These findings indicate that adding CSC to sorghum silage enhances its nutritive value, fermentative profile, dry matter recovery, and effectively inhibits yeast growth, which is recommended to include 200 g kg<sup>-1</sup> of CSC for good cost-effectiveness.

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By-product; dry matter recovery; *Gossypium*; lipids; *Sorghum bicolor*

## Introduction

The use of silage in animal feeding is a widespread practice that contributes to the intensification of production systems (Daniel et al. 2019). Sorghum (*Sorghum bicolor* L. Moench) is a commonly used crop for silage production due to its good nutritive value and high yields (Perazzo et al. 2017). Some reports have shown that sorghum may have a high moisture content at harvest, especially during periods of high-water availability, such as irrigation or intense rainy seasons (Perazzo et al. 2013, 2017; Pinho et al. 2015). It is important to consider that high moisture content can result in effluent losses that leach nutrients from the forage to the bottom of the silo, thereby reducing the nutritive value of the produced silage (Borreani et al. 2018).

Furthermore, sorghum contains a high content of water-soluble carbohydrates (160–300 g kg<sup>-1</sup> of dry matter) (Behling Neto et al. 2017; Santos et al. 2018), which may be prone to alcoholic fermentation by acid-tolerant yeasts, leading to increased dry matter losses (Pahlow et al.

2003). These microorganisms compete with lactic acid bacteria for carbohydrates and other nutrients during the fermentation process and pose as the main initiators of aerobic deterioration due to lactate-assimilating species acting on both the fermentative and feed-out phases (Pahlow et al. 2003; Rooke and Hatfield 2003). Additionally, as a warm-season grass, sorghum has a low protein content (approximately  $80 \text{ g kg}^{-1}$  of dry matter), thus limiting its use as sole roughage for ruminants (Santos et al. 2018; Gois et al. 2022).

In this context, nutritional additives have been proposed to improve the fermentative process, aerobic stability, and nutritive value of crops with high water-soluble carbohydrate content, such as sugarcane, pearl millet, and sorghum (Carvalho et al. 2016; Trevisoli et al. 2017; Santana et al. 2022; Zanine et al. 2022). Therefore, unsuitable forages and concentrates for ensiling are often mixed with these crops to reduce the proportion of fermentable carbohydrates, promoting proper fermentation of both feedstuffs. An ideal additive should have high availability and low cost due to the large quantities required for silage production.

Cottonseed cake (CSC) can be considered an important nutritional additive as it is traditionally used in livestock nutrition as a protein source (Silva et al. 2016) and is a by-product of oil extraction from cottonseeds (*Gossypium hirsutum* L.), which has high dry matter ( $>900 \text{ g kg}^{-1}$  of dry matter), crude protein ( $>220 \text{ g kg}^{-1}$  of dry matter), and lipid content ( $>70 \text{ g kg}^{-1}$  of dry matter) (Santana et al. 2022). The high dry matter content of CSC can decrease effluent losses and have negative effects on yeasts, as they require higher water activity than lactic acid bacteria (Pahlow et al. 2003). The protein content of CSC can enhance the nutritive value of mixed sorghum silage, eliminating the need for additional protein sources for ruminants (Silva et al. 2016; Dias et al. 2019).

Another advantage of this technique is the improvement in digestibility and reduction of energy losses compared to diets containing concentrates offered in their natural form, without ensiling (Cao et al. 2010; Bueno et al. 2020). Additionally, the residual oil content present in cottonseed cake can contribute to enhancing the fermentative process by inhibiting yeasts. Unsaturated fatty acids in the residual oil cause membrane instability and cytoplasmic disorganisation, ultimately leading to the inhibition of yeast growth (Pohl et al. 2011; Clitherow et al. 2020).

Currently, there is no available literature regarding the ideal proportion of cottonseed cake to be mixed with sorghum to achieve the aforementioned improvements in silage quality. Therefore, the objective of this study was to determine the optimal inclusion level of cottonseed cake with sorghum to produce mixed silages, focusing on chemical composition, fermentative profile, microbial populations, losses, dry matter recovery, and aerobic stability of the silages.

## Materials and methods

### *Experimental site and sorghum cultivation*

The experiment was conducted at the Forage Crop Laboratory in the Animal Science Department, Agricultural Science Center of the Federal University of Paraíba, located in Areia, Brazil ( $06^{\circ}57'48'' \text{ S}$ ,  $35^{\circ}41'30'' \text{ W}$ , 618 m altitude). Forage sorghum (*Sorghum bicolor* L. Moench) cv. “BRS Ponta Negra” was cultivated on a private property in Barra de Santana, Paraíba, Brazil ( $07^{\circ}31'13'' \text{ S}$ ,  $35^{\circ}59'59'' \text{ W}$ , 350 m altitude).

According to Alvares et al. (2013), the region has a BSh climate classification, which indicates a semiarid climate with a hot rainy summer. The average annual temperature is 27.6°C, and even the coldest months have temperatures above 18°C. Irrigation was applied to the sorghum crop based on the evapotranspiration rate, with a total of 440 mm of water applied throughout the crop cycle. Additionally, organic fertilisation was performed using cattle manure at a rate of 15 tons per hectare.

### ***Experimental treatments and design***

A completely randomised design with 5 treatments and 4 replications was used, resulting in a total of 20 experimental units (mini-silos). The treatments consisted of the inclusion of cottonseed cake (CSC) in sorghum at ensiling, with the following proportions on a fresh matter basis (expressed in g kg<sup>-1</sup>): (1) solely sorghum (control), (2) 950 sorghum + 50 CSC, (3) 900 sorghum + 100 CSC, (4) 800 sorghum + 200 CSC, and (5) 600 sorghum + 400 CSC. The silos were opened after 55 days of fermentation.

### ***Ensiling***

Sorghum was manually harvested at the milky/dough stage (approximately 100 days) and transported from Barra de Santana, PB to the Forage Crop Laboratory in Areia, PB. The sorghum was then chopped into particles of approximately 2 cm in length using a traditional forage chopper (Laboremus<sup>®</sup>, model LC 6000, Campina Grande - PB, Brazil).

The chopped material was mixed according to each treatment and manually ensiled with the assistance of wooden sockets until reaching a density of approximately 500 kg m<sup>-3</sup>. The weights of the silos were recorded at the time of ensiling and at the time of opening for subsequent evaluations. Experimental silos were constructed using polyvinyl chloride tubes (PVC) measuring 20 cm in width and 40 cm in height. A layer of 1 kg of sand was placed at the bottom of each silo to allow effluent drainage, and the silos were fitted with adapted PVC lids equipped with Bunsen valves to release the gas produced during the fermentation period.

### ***Sampling and fermentative profile***

Prior to ensiling, the material was sampled and characterised for chemical composition, pH, buffering capacity and microbial populations, as shown in Table 1. After the fermentation period, the opened silos had the surface material discarded, and the remaining silage was homogenised. A sample of approximately 300 g of each silo was taken for further analysis of the fermentative profile, microbial populations, and chemical composition.

The pH evaluation was performed in duplicate for each experimental unit following the methodology described by Jobim et al. (2007). Ammoniacal nitrogen (NH<sub>3</sub>-N) was determined using a colorimetric method based on the procedure outlined by Chaney and Marbach (1962). Buffering capacity (BC) was assessed according to the methods described by Playne and McDonald (1966). Water-soluble carbohydrates (WSC) were quantified using a colorimetric method based on the procedure outlined by Dubois

**Table 1.** Chemical composition, pH, water-soluble carbohydrates, buffering capacity and microbial population of sorghum added with cottonseed cake (CSC) prior to ensiling.

Item <sup>a</sup>	CSC inclusion (g kg <sup>-1</sup> FM)					CSC only
	0	50	100	200	400	
Chemical composition						
Dry matter (g kg <sup>-1</sup> FM)	295.6	313.5	344.8	445.3	516.0	945.0
Organic matter (g kg <sup>-1</sup> DM)	933.6	944.0	949.6	958.5	972.6	960.6
Crude protein (g kg <sup>-1</sup> DM)	67.8	105.6	114.5	173.8	212.4	228.1
Ether extract (g kg <sup>-1</sup> DM)	20.2	35.0	41.2	59.3	81.2	88.7
NDF (g kg <sup>-1</sup> DM)	635.8	619.3	607.1	573.4	556.0	487.0
ADF (g kg <sup>-1</sup> DM)	346.2	379.9	366.6	372.4	374.7	364.7
pH	53.1	54.2	55.1	56.3	57.3	6.24
WSC (g kg <sup>-1</sup> DM)	202.0	188.3	168.7	120.2	101.7	79.3
BC (eq mg HCl 100 g <sup>-1</sup> DM)	0.074	0.085	0.082	0.077	0.079	–
Microbial population						
LAB (log CFU g <sup>-1</sup> )	6.77	6.47	6.44	6.38	6.39	–
Moulds (log CFU g <sup>-1</sup> )	4.69	4.58	4.57	4.49	4.61	–
Yeasts (log CFU g <sup>-1</sup> )	5.14	4.89	4.90	4.81	4.70	–

<sup>a</sup>NDF: neutral detergent fibre, ADF: acid detergent fibre, pH: hydrogen potential, WSC: water-soluble carbohydrates, BC: buffering capacity, LAB: lactic acid bacteria.

et al. (1956) and the extraction method described by Corsato et al. (2008). Detailed information on these variables can be found in Table 1 (prior to ensiling).

### Microbial populations

Microbial populations were assessed both before ensiling and immediately after the silos were opened. Selective culture media were used to isolate different microbial groups. For the isolation of lactic acid bacteria (LAB) population, De Man, Rogosa, and Sharpe agar medium (MRS, KASVI<sup>®</sup>, São José dos Pinhais, Paraná, Brazil) supplemented with 0.1% acetic acid was utilised. Potato-dextrose-agar (PDA, KASVI<sup>®</sup>, São José dos Pinhais, Paraná, Brazil) supplemented with 1% tartaric acid was employed for quantifying moulds (M) and yeasts (Y). Due to the rapid acidification of sorghum silages, enterobacteria were not quantified in this study. Fresh silage samples weighing 10 g each were subjected to serial dilutions ( $10^{-2}$ – $10^{-6}$ ) by manual shaking. The plating was performed in duplicate for each experimental unit. The petri dishes containing 30–300 colony forming units (CFU) were counted and converted to a logarithmic scale (log<sub>10</sub> basis). The microbial populations of the mixtures prior to ensiling and of the silages are presented in Table 1.

### Chemical analysis

Samples collected at ensiling and after the fermentation period were dried in a forced air oven at 55°C for 72 h and then ground using a 1 mm Wiley Mill screen (Marconi, Piracicaba, SP, Brazil). The chemical composition of the silages was evaluated following the methods described by Detmann et al. (2012). Dry matter (DM) was determined using method G-003/1, ash content was determined using method M-001/1, total nitrogen (N) was determined using method N-001/1, ether extract (EE) was determined using the ANKOM<sup>®</sup> model XT10, neutral detergent fibre (NDF) was determined using method F-002/1, acid detergent fibre (ADF) was determined using method F-004/1, and acid

detergent lignin (ADL) was determined using method F-005/1. Crude protein was calculated by multiplying the total nitrogen by 6.25.

### **Losses and dry matter recovery**

Gases losses (GL), effluent losses (EL), and dry matter recovery (DMR) were determined by calculating the weight difference of the experimental silos, following the equations described by Jobim et al. (2007).

### **Aerobic stability**

After sampling, approximately 2 kg of silage were transferred to clean silos without compaction for assessing the aerobic stability (AS). Silage temperature was manually measured every 30 min for 96 h using digital thermometers (Incoterm<sup>®</sup>, model 9197, Porto Alegre, Rio Grande do Sul, Brazil; temperature range:  $-50$ – $300^{\circ}\text{C}$  with a resolution of  $1 \pm 0.1^{\circ}\text{C}$ ). The room temperature was controlled to remain constant at  $25^{\circ}\text{C}$ . The aerobic stability was determined as the duration for which the silages did not exceed  $2^{\circ}\text{C}$  above the room temperature, following the method described by Taylor and Kung (2002).

### **Statistical analysis**

The data were analysed using the Statistical Analysis System version 9.0 (SAS Institute Inc., Cary, NC, 2004) (SAS 2004). An analysis of variance and regression analysis were conducted using the GLM procedure. The mathematical model used is described as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where  $Y_{ij}$  represents the observed value of the studied variable,  $\mu$  is the overall mean,  $T_i$  represents the treatment effect (cottonseed cake inclusion), and  $e_{ij}$  represents the residual error. Regression models were selected based on the significance of estimated parameters (linear effect,  $L$ , and quadratic effect,  $Q$ ) as well as the coefficient of determination ( $R^2$ ). All tests performed considered a significance level of  $P < 0.05$ .

## **Results**

The chemical composition of the silages showed a positive linear effect on DM, OM, CP, and EE with the inclusion of CSC. Additionally, NDF exhibited a significant linear decrease ( $P < 0.0001$ ), while no effects were observed for ADF content ( $P = 0.9187$ ) (Table 2).

The fermentative profile of the silages demonstrated linear effects on the evaluated variables ( $P < 0.0001$ ) (Table 3). Higher inclusion of CSC resulted in a linear decrease in WSC,  $\text{NH}_3\text{-N}$ , and BC, while pH exhibited a linear increase (Table 3).

The microbial populations in the silages exhibited both linear and quadratic effects (Table 4). The LAB population showed a negative quadratic effect with the inclusion of CSC ( $P < 0.0001$ ), reaching a maximum point of  $\pm 7.26 \log \text{CFU g}^{-1}$  silage at 200 g

**Table 2.** Chemical composition of sorghum silages added with cottonseed cake (CSC) after 55 days of fermentation.

Item <sup>a</sup>	CSC inclusion (g kg <sup>-1</sup> FM)					SEM <sup>b</sup>	P-value	
	0	50	100	200	400		L	Q
DM (g kg <sup>-1</sup> DM) <sup>c</sup>	260.1	289.2	313.9	373.2	513.0	20.7	<0.0001	<0.0001
OM (g kg <sup>-1</sup> DM) <sup>d</sup>	936.3	936.9	942.9	946.9	960.4	8.6	0.0002	0.8920
CP (g kg <sup>-1</sup> DM) <sup>e</sup>	65.2	103.9	124.1	203.8	216.6	10.7	<0.0001	<0.0001
EE (g kg <sup>-1</sup> DM) <sup>f</sup>	62.3	59.3	66.6	76.9	94.9	04.3	<0.0001	0.3334
NDF (g kg <sup>-1</sup> DM) <sup>g</sup>	647.5	636.1	626.0	594.3	573.8	20.0	<0.0001	0.2588
ADF (g kg <sup>-1</sup> DM)	398.9	385.9	400.4	386.0	395.1	22.2	0.9187	0.6094

<sup>a</sup>DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fibre, ADF: acid detergent fibre.

<sup>b</sup>Standard error of mean.

<sup>c</sup> $\hat{Y} = 30.0935 - 5.0745 \times X$  ( $r^2 = 0.97$ ); <sup>d</sup> $\hat{Y} = 95.54 + 0.062 \times X$  ( $r^2 = 0.54$ ); <sup>e</sup> $\hat{Y} = 8.08 + 0.36 \times X$  ( $r^2 = 0.96$ ); <sup>f</sup> $\hat{Y} = 5.86 + 0.09 \times X$  ( $r^2 = 0.91$ ); <sup>g</sup> $\hat{Y} = 64.39 - 0.19 \times X$  ( $r^2 = 0.67$ ).

**Table 3.** Mean values of pH, water-soluble carbohydrates (WSC), buffering capacity (BC) and ammoniacal nitrogen (NH<sub>3</sub>-N) of sorghum silages added with cottonseed cake (CSC) after 55 days of fermentation.

Item <sup>a</sup>	CSC inclusion (g kg <sup>-1</sup> FM)					SEM <sup>b</sup>	P-value	
	0	50	100	200	400		L	Q
pH <sup>c</sup>	3.64	3.64	3.71	3.86	4.09	0.04	0.0006	<0.0001
WSC (g kg <sup>-1</sup> DM) <sup>d</sup>	29.5	19.0	5.2	8.6	7.4	2.3	<0.0001	0.0021
BC (eq mg HCl 100 g <sup>-1</sup> DM) <sup>e</sup>	0.0749	0.0745	0.0731	0.0664	0.0629	0.0028	<0.0001	0.3840
NH <sub>3</sub> -N (g kg <sup>-1</sup> TN) <sup>f</sup>	0.93	0.86	0.86	0.72	0.52	0.03	<0.0001	0.0824

<sup>a</sup>pH: hydrogen potential, WSC: water-soluble carbohydrate, BC: buffering capacity, NH<sub>3</sub>-N: ammoniacal nitrogen.

<sup>b</sup>Standard error of mean.

<sup>c</sup> $\hat{Y} = 3.7270 - 0.1179 \times X$  ( $r^2 = 0.96$ ); <sup>d</sup> $\hat{Y} = 4.8215 - 2.0779 \times X$  ( $r^2 = 0.76$ ); <sup>e</sup> $\hat{Y} = 0.075 - 0.00033 \times X$  ( $r^2 = 0.74$ ); <sup>f</sup> $\hat{Y} = 1.04 - 0.09 \times X$  ( $r^2 = 0.64$ ).

kg<sup>-1</sup> of CSC inclusion (Table 4). The moulds population also displayed a negative quadratic effect ( $P = 0.0042$ ), reaching a maximum point of  $\pm 3.91$  log CFU g<sup>-1</sup> silage at 50 g kg<sup>-1</sup> of CSC inclusion (Table 4). The yeasts population exhibited a linear decrease ( $P < 0.0001$ ), and CSC inclusion above 200 g kg<sup>-1</sup> strongly affected this microbial group, completely suppressing it (Table 4).

Dry matter recovery of the silages demonstrated a linear increase with the inclusion of CSC ( $P < 0.0001$ ). Conversely, gases and effluent losses exhibited a linear decrease with CSC inclusion ( $P < 0.0001$ ) (Table 5).

**Table 4.** Microbial population of sorghum silages added with cottonseed cake (CSC) after 55 days of fermentation.

Item <sup>a</sup>	CSC inclusion (g kg <sup>-1</sup> FM)					SEM <sup>b</sup>	P-value	
	0	50	100	200	400		L	Q
LAB (log CFU g <sup>-1</sup> ) <sup>c</sup>	5.21	6.17	6.98	7.69	5.24	0.232	0.1097	<0.0001
Moulds (log CFU g <sup>-1</sup> ) <sup>d</sup>	2.92	3.91	3.90	3.37	3.21	0.124	0.9513	0.0042
Yeasts (log CFU g <sup>-1</sup> ) <sup>e</sup>	5.21	3.64	2.54	0.00	0.00	0.496	<0.0001	0.3242

<sup>a</sup>LAB: lactic acid bacteria.

<sup>b</sup>Standard error of mean.

<sup>c</sup> $\hat{Y} = 2.3325 + 3.1210 \times X - 0.4939 \times X^2$  ( $r^2 = 0.71$ ); <sup>d</sup> $\hat{Y} = 2.0370 + 1.2195 \times X - 0.2025 \times X^2$  ( $r^2 = 0.39$ ); <sup>e</sup> $\hat{Y} = 7.3555 - 2.1374 \times X$  ( $r^2 = 0.85$ ).



**Table 5.** Gases losses, effluent losses, and dry matter recovery of sorghum silages added with cottonseed cake (CSC) after 55 days of fermentation.

Item <sup>a</sup>	CSC inclusion (g kg <sup>-1</sup> FM)					SEM <sup>b</sup>	P-value	
	0	50	100	200	400		L	Q
GL (g kg <sup>-1</sup> DM) <sup>c</sup>	45.7	45.1	40.6	33.8	22.7	0.216	<0.0001	0.0095
EL (kg FM t <sup>-1</sup> ) <sup>d</sup>	100.39	90.69	67.02	13.69	3.65	9.264	<0.0001	0.2458
DMR (g kg <sup>-1</sup> DM) <sup>e</sup>	886.1	892.3	917.2	967.7	984.6	0.937	<0.0001	0.0804

<sup>a</sup>GL: gases losses, EL: effluent losses, DMR: dry matter recovery.

<sup>b</sup>Standard error of mean.

<sup>c</sup> $\hat{Y} = 4.3245 + 0.4207 \times X$  ( $r^2 = 0.90$ ); <sup>d</sup> $\hat{Y} = 121.0735 - 14.05 \times X$  ( $r^2 = 0.82$ ); <sup>e</sup> $\hat{Y} = 87.14 + 0.70 \times X$  ( $r^2 = 0.90$ ).

**Table 6.** Aerobic stability and maximum temperature of sorghum silages added with cottonseed cake (CSC) after 55 days of fermentation.

Item	CSC inclusion (g kg <sup>-1</sup> FM)					SEM <sup>a</sup>	P-value	
	0	50	100	200	400		L	Q
Aerobic stability (hours) <sup>b</sup>	35	37	53	78	88.87	5.06	<0.0001	0.0575
Maximum temperature (°C) <sup>c</sup>	30.12	29.00	28.63	28.62	28.12	2.89	<0.0001	0.1237

<sup>a</sup>Standard error of mean.

<sup>b</sup> $\hat{Y} = 27.9250 + 2.7821 \times X$  ( $r^2 = 0.90$ ); <sup>c</sup> $\hat{Y} = 31.0250 - 1.1339 \times X$  ( $r^2 = 0.63$ ).

Regarding aerobic stability, a significant increase was observed with the inclusion of CSC ( $P < 0.0001$ ). The mixed silage with 400 g kg<sup>-1</sup> of CSC inclusion showed approximately twice the aerobic stability compared to the other silages (Table 6). The maximum temperature followed a similar trend, decreasing linearly with CSC inclusion (Table 6). It is worth noting that within the range of 100–400 g kg<sup>-1</sup> CSC inclusion, the mixed silages did not exceed 29°C in temperature (Table 6).

## Discussion

Overall, the inclusion of CSC in sorghum silages has demonstrated potential as an additive, improving the nutritive value of mixed silages by increasing the digestibility of DM, OM, CP, and EE and decreasing the NDF content (Table 2). The CP content showed a significant increase of almost 2 and 3-fold in inclusions of 100 and 200 g kg<sup>-1</sup>, respectively, making these silages suitable for animal feeding without requiring significant amounts of additional protein concentrates, such as soybean meal (Table 1). The chemical composition of the silages remained stable during the fermentation period, indicating good preservation (Table 2).

Moreover, the silages had a good fermentative process, as demonstrated by low pH, WSC, BC, and NH<sub>3</sub>-N even with high CSC inclusion levels (Table 3). The reduction in WSC content during ensiling did not affect fermentation, as it was still within the optimal range (Table 1). Intermediate treatments (50–200 g kg<sup>-1</sup> of inclusion) showed a similar pattern, indicating that the mixture did not impact fermentation and that there was enough substrate to be fermented. The decrease in NDF content is particularly noteworthy, as this fraction is known to negatively impact intake and digestibility (Ma et al. 2015; Castro-Montoya and Dickhoefer 2018). Therefore, mixed silages containing CSC within the range of 100–400 g kg<sup>-1</sup> are appropriate for animal feeding without requiring significant amounts of additional protein concentrates, but energy concentrates



and other ingredients remain essential in this context to ensure proper diet balance. However, higher inclusions of CSC may have low cost-effectiveness and should be carefully considered within the economic context.

The silages exhibited a favourable fermentative process, characterised by low pH values, WSC, BC, and  $\text{NH}_3\text{-N}$  levels, even with the inclusion of high proportions of CSC (Table 3). Despite a 50% reduction in WSC content during ensiling due to the maximum inclusion of CSC (Table 1), fermentation proceeded effectively, as the content remained within the recommended range of 60–120 g  $\text{kg}^{-1}$  DM (Kung et al. 2018). Similar results were observed for intermediate treatments (50–200 g  $\text{kg}^{-1}$  of inclusion), indicating that the mixture did not negatively impact fermentation, and sufficient substrate was available for fermentation to occur properly.

According to McDonald et al. (1991), the recommended pH range for silages is 3.8–4.2, which was achieved in silages with CSC inclusions above 200 g  $\text{kg}^{-1}$  (Table 3). The low  $\text{NH}_3\text{-N}$  content, below 100 g  $\text{kg}^{-1}$  of total nitrogen, indicates minimal proteolysis, which can be attributed to the rapid acidification commonly observed in these silages (Kung et al. 2018). Despite its high CP content, the inclusion of CSC did not affect the buffering capacity (BC) of the silages (Table 3), indicating that it did not interfere with the acidification process. This demonstrates the potential of CSC as an additive for sorghum and other sugar-rich crops during ensiling.

Regarding microbial populations, the LAB successfully established in the silages of this study, even with the reduction in WSC, which serves as the primary substrate for the anaerobic conversion of sugars to lactic acid (Pahlow et al. 2003). The microbial populations in the silages remained highly active throughout the fermentation period ( $>5.2$  log CFU  $\text{g}^{-1}$  silage), with the highest values observed in the treatment containing 200 g  $\text{kg}^{-1}$  of CSC inclusion (Table 4). The decrease in LAB population at the highest CSC inclusion level may be attributed to the limited availability of WSC in the late stages of fermentation (Table 3).

The results regarding yeast inhibition are noteworthy, especially considering the high initial counts of epiphytic yeast populations observed in the sorghum samples (Table 1). Sorghum silage is known to be susceptible to yeast growth throughout the fermentation process, leading to ethanol production, low aerobic stability, and high gases losses (Filya and Sucu 2007; Abrão et al. 2017; Santos et al. 2018). The complete suppression of yeast populations in the mixed silages with CSC inclusion (200–400 g  $\text{kg}^{-1}$ ) (Table 4) is a remarkable finding. This can be attributed to the higher levels of CSC inclusion, which have a high lipid content (Table 2). The presence of lipids in these silages likely disrupted yeast development by affecting membrane permeability and causing cytoplasmic disorganisation (Pohl et al. 2011; Clitherow et al. 2020).

The inhibition of yeasts is significant because it leads to an improvement in aerobic stability as the CSC inclusion increases (Table 6). This is particularly important considering that lactate-assimilating yeast species are commonly found in sorghum silages (Abrão et al. 2017), and these yeasts are known as major contributors to aerobic deterioration in silages (Pahlow et al. 2003; Wilkinson and Davies 2013). Therefore, the use of CSC as an additive demonstrates its potential in limiting yeast growth and improving various aspects of silage quality, including nutritive value, fermentation profile, and aerobic stability.

Furthermore, the presence of non-fermented water-soluble carbohydrates (WSC) at the time of silo opening is crucial, as these carbohydrates can be oxidised in the presence of air and promote the growth of spoilage microorganisms (Wilkinson and Davies 2013; Borreani et al. 2018). Therefore, the observed low levels of WSC in the silages at opening in our study are linked to improved aerobic stability. These levels restrict the growth of undesirable microorganisms by constraining substrate availability.

## Conclusion

The addition of cottonseed cake (CSC) as an additive to sorghum silage leads to improvements in its nutritive value, fermentative profile, dry matter recovery, aerobic stability, and inhibition of yeast population. Based on our findings, we recommend a CSC inclusion level of 200 g kg<sup>-1</sup> for producing high-quality silages and good cost-effectiveness.

## Disclosure statement

No potential conflict of interest was reported by the authors.











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## Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article, but they are available from the corresponding author (Cavalcanti, H. S.) upon reasonable request.

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