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Untreated or ammoniated cotton gin trash in the ensiling of elephant grass reduces silage quality

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ABSTRACT. This study examined the inclusion of untreated or urea-ammoniated CGT on the chemical composition, pH, gas, and effluent losses, dry matter recovery (DMR), and aerobic stability of elephantgrass silage. The experiment was laid out in a randomized design with a 3 × 3 factorial arrangement represented by three levels of inclusion of CGT during ensiling (0, 5, or 10%) and three levels of ammoniation of CGT with urea before ensiling (0, 4 or 8% for 28 days), using four replications. Data were subjected to analysis of variance and Tukey's test, considering a 5% significance level. The pH and DM, NDIN, ADIN, NDF and ADF contents of the silages increased ($p < 0.05$) with the inclusion of CGT, regardless of ammoniation. The lowest ($p < 0.05$) effluent loss was achieved using 10% of CGT ammoniated with 8% urea. In contrast, the elephantgrass silage exhibited the lowest ($p < 0.05$) gas loss and the greatest ($p < 0.05$) DMR. The inclusion of CGT compromised the aerobic stability of the silage. Therefore, the inclusion of CGT – either untreated or ammoniated – in the ensiling of elephantgrass is not recommended.

Keywords: aerobic stability; ammoniation; pH; post-opening; urea.

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Introduction

Elephantgrass (*Pennisetum purpureum* Schum.) has high herbage mass during the rainy season (Silva et al., 2020). Its vigorous growth culminates in rapid drop in nutritional value. Ensiling is the most recommended way of preserving its nutritional value and increasing the supply of forage during the dry season of the year (Andrade et al., 2012; Santos et al., 2013).

Nonetheless, elephantgrass exhibits some characteristics that limit the fermentation process (e.g., high moisture, low soluble carbohydrate, and high buffering capacity) (Oliveira et al., 2017; Barcelos et al., 2018). Therefore, ensiling elephantgrass alone results in high effluent losses; decrease in the concentrations of digestible nutrients, lactic acid, minerals, and soluble nitrogen compounds; and increase in cell wall components, which are responsible for reducing the nutritional value of silage (Ferreira et al., 2017; Lira Junior et al., 2018).

To overcome the intrinsic limitations of elephant grass for silage production, several studies have tested the addition of by-products and agricultural wastes (e.g., straw, husks) in its ensiling with promising results (Viana et al., 2013; Guerra et al., 2016; Silva et al., 2020). In addition to favoring the fermentation process, this technique allows the use of products with a more restricted potential for animal feed that usually ends up being discarded. In this scenario, cotton gin trash (CGT) stands out as a potential additive for elephantgrass silage. The CGT is a low-quality roughage that can have its nutritive value improved through urea-ammoniation (Andrade et al., 2020).

Thus, this study objected to evaluate the inclusion of CGT, untreated or urea-ammoniated, on the chemical composition, pH, gas and effluent losses, dry matter recovery (DMR), and aerobic stability of elephantgrass silage. We hypothesized that CGT may lower the pH and increase DMR, whereas ammoniating CGT before ensiling might improve the chemical composition and aerobic stability of silage.

Material and methods

This study was carried out at the Bahia State University, Barreiras campus, Bahia, Brazil (12°9' S, 45°0' W, 439 m above sea level).

The experiment was laid out in a completely randomized design with a 3×3 factorial arrangement represented by three levels of CGT inclusion during ensiling (0, 5, or 10%) and three levels of ammoniation of the waste before ensiling (0, 4, or 8% urea), using four replications.

The elephantgrass had 60-d regrowth and had not received any type of fertilizer. The CGT was obtained from a cotton mill in Guanambi, Bahia, Brazil.

For the treatments that involved ammoniation, before ensiling the CGT was treated with urea ($\text{NH}_2\text{COONH}_2$) at the rates of 4 and 8% (dry matter, DM, basis), which had been diluted in enough water to raise the moisture to 30%. The solution was applied to the CGT using a watering can. Ground soybeans were added as a source of urease, at the rate of 1.5% (DM basis) (Andrade et al., 2020). After homogenization, the CGT was packed in plastic bags, sealed, and treated for 28 days. Then, the material was subjected to aeration for 72 h to release the excess of ammonia.

Elephantgrass was chopped in a stationary forage chopper to particles of 2 to 3 cm. The chopped forage was ensiled according to the following treatments: elephantgrass only (EG), and elephantgrass with the inclusion of 5% (CGT5) or 10% (CGT10) of CGT untreated or ammoniated with 4% (CGT5U4, CGT10U4) or 8% (CGT5U8, CGT10U8) of urea (DM basis). Before ensiling, three 250-g samples of the ingredients were dried in a forced-air oven at 60°C for 72 h, ground in a Wiley mill with a 1-mm sieve, and finally analyzed for the chemical composition according to the methodologies described by Detmann et al. (2012) (Table 1).

The experimental silos were made of plastic buckets with a capacity of 20 L. Before filling them, 4 kg of dry sand were placed at the bottom of each bucket and a thin and a thick shade net were placed between the sand and the material to retain and quantify effluent losses.

After the material was mixed according to the treatments, the silos were filled and compacted. The lab silos were closed hermetically with the respective lids (adapted with a Bunsen valve), sealed with adhesive tape, labeled, weighed, and stored for 60 days. Then, the silos were weighed again to quantify gas loss and opened. Samples were collected for chemical composition, pH, and aerobic stability analyses.

Table 1. Chemical composition of elephantgrass and cotton gin trash (CGT) untreated and ammoniated with 4 and 8% of urea.

Composition ¹	Elephantgrass	CGT	CGT 4% urea	CGT 8% urea
DM (% AF)	16.2	88.2	72.1	71.6
CP (%DM)	7.63	8.76	16.8	22.8
NDIN (%DM)	0.28	0.73	1.20	1.27
ADIN (%DM)	0.17	0.68	1.10	1.23
Fat (%DM)	1.38	3.01	2.84	2.96
NDF (%DM)	70.7	70.3	68.9	65.7
ADF (%DM)	46.9	64.9	59.2	56.7
Ash (%DM)	7.51	12.8	13.6	13.9

¹DM= dry matter; AF = as-fed; CP=crude protein; NDIN= neutral detergent insoluble nitrogen; ADIN= acid detergent insoluble detergent; NDF=neutral detergent fiber; ADF= acid detergent fiber.

The DM, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose, lignin, and ash were measured following the methodologies described by Detmann et al. (2012). The nitrogenous fractions were separated into neutral (NDIN) and acid (ADIN) detergent insoluble nitrogen relative to total nitrogen, according to Detmann et al. (2012).

Immediately after opening the silos, a 9-g sample was immersed in 60 mL of distilled water for 30 min. After this period, the pH was measured using a digital pH meter.

A sample of each silage, weighing approximately 500 g, was transferred to 5-L capacity extruded polystyrene foam containers, in which were kept at ambient temperature to assess aerobic stability. The silage temperatures were monitored at 0, 3, 6, 12, 18, 24, 48, 72, 96, and 120 h after silo opening, using a mercury thermometer inserted 10 cm into the mass. The ambient temperature was measured with a digital thermometer placed close to the containers, and the following values were recorded at the different post-opening times (in °C): 1 h = 26.8; 6 h = 24.4; 12 h = 23.6; 24 h = 27.9; 48 h = 27.5; 72 h = 26.4; 96 h = 26.9; and 120 h = 27.1. At each time, the silage was sampled to measure the pH, as previously discussed. The break of aerobic stability was determined as the time, in hours, after silo opening for the silages to reach a temperature of 2 °C above the ambient temperature (Andrade et al., 2012).

Effluent losses were estimated as the increase in the weight of the set (bucket, sand, screen, and shade nets), as described in the equation below (Santos et al., 2013):

$$E = \left(\frac{WOP - WEN}{EFFW} \right) * 1000,$$

where E = effluent production (kg t⁻¹ of fresh mass); WOP = weight of the set (bucket + sand + screens) at opening (kg); WEN = weight of the set (bucket + sand + screens) at ensiling (kg); EFFW = ensiled forage fresh weight (kg).

Gas losses were calculated as the difference in bucket weight after ensiling and opening (Santos et al., 2013):

$$G = \frac{(BW_i - BW_f)}{(FW_i * DM_i)} * 100,$$

where G = gas losses (% DM); BW_i = full-bucket weight at closing (kg); BW_f = full-bucket weight at opening (kg); FW_i = forage weight at closing (kg); DM_i = forage dry matter content at closing.

The DMR of the silages was estimated by using the equation described by Santos et al. (2013):

$$DMR = \frac{(FW_f * DM_f)}{FW_i * DM_i} * 100,$$

where DMR = dry matter recovery rate (%); FW_f = forage weight at opening (kg); DM_f = forage dry matter content at opening (%). FW_i = forage weight at closing (kg); DM_i = forage dry matter content at closing (%).

To estimate the density of the silages, the silos were weighed before ensiling and the weight of the ensiled mass was found gravimetrically. Next, the following equation, described by Santos et al. (2013), was applied:

$$\text{Density} \left(\frac{\text{kg}}{\text{m}^3} \right) = \frac{Em}{V},$$

where Density (kg m⁻³); Em = ensiled mass (kg); V = silo volume (m³).

The obtained data were subjected to analysis of variance. Means were compared by Tukey's test at the 5% significance level, using Assistat (version 7.6 beta) statistical software.

The mathematical model used is represented by the following equation:

$$Y_{ijk} = \mu + G_i + E_{ik},$$

where Y_{ijk} = observed values for treatment i; μ = overall mean; G_i = treatment effect; E_{ik} = effect of uncontrolled factors (experimental or residual error).

The effects of time on temperature and pH in the evaluation of aerobic stability were analyzed by orthogonal polynomials. Results are presented with the regression equations and their respective coefficient of determination (R²).

Results and discussion

The CGT increased DM of silages (*p* < 0.05, Table 2) when compared to the EG silage due to the higher DM content of CGT (Table 1). Moisture-absorbing additives are an important tool to minimize the effects of low DM of grasses at the time of ensiling (Dias et al., 2019; Silva et al., 2020). However, the observed values were still below 20% reported by McDonald et al., (1991) as the minimum acceptable for the preservation of grasses in the form of silage, except in the CGT10U8 treatment. High moisture in silages can lead to DM losses, the occurrence of unwanted fermentation, and increased storage costs (Wilkinson & Rinne, 2018; Borreani et al., 2018).

There was no significant change in CP content of silages, which the average of 7.2% (Table 2). It was expected the inclusion of urea-ammoniated CGT could have induced an increase in CP of the silages. Possibly, the proportions of ammoniated CGT used in this trial (maximum of 10%) were not sufficient to alter the results significantly.

The NDIN and ADIN contents were greater (*p* < 0.05) in the silages containing ammoniated CGT than EG silage (Table 2). On average, the CGT5U8, CGT10U4, and CGT10U8 treatments had 180 and 517% higher (*p* < 0.05) NDIN and ADIN, respectively, than EG. The ammoniation of CGT contributed to these changes due to the higher levels of nitrogen linked to the cell wall (Table 1). The NDIN can show considerable digestibility when soluble in acid detergent, but this nitrogen has a slower digestion rate, while ADIN is unavailable to rumen microorganisms (Andrade et al., 2020).

The NDF, ADF, cellulose, and lignin increased (*p* < 0.05) in the silages with CGT, relative to the EG silage (Table 2). The addition of CGT influenced the fiber content of the silage because of its high NDF and ADF contents (around 68 and 59%, respectively; Table 1). Increases or decreases in fiber of grass silages depend on the type and inclusion rate of a byproduct added to them (Quadros et al., 2003; Andrade et al., 2010).

Table 2. Chemical composition of elephantgrass silage with untreated or urea-ammoniated cotton gin trash (CGT).

Treatments ¹	DM ² (% AF)	CP ² (% DM)	NDIN ² (%N)	ADIN ² (%N)	NDF ² (% DM)	ADF ² (% DM)	CEL ² (% DM)	HEM ² (% DM)	LIG ² (% DM)	Ash (% DM)
EG	13.3 ^{c3}	6.89 ^a	19.0 ^b	6.47 ^d	65.4 ^{cd}	39.7 ^b	35.5 ^b	25.6 ^a	2.47 ^d	9.82 ^{bc}
CGT5	15.7 ^b	7.13 ^a	22.5 ^b	16.8 ^{cd}	61.5 ^{de}	41.0 ^b	34.2 ^{bc}	20.4 ^b	5.15 ^c	10.1 ^{bc}
CGT10	19.8 ^a	7.26 ^a	28.5 ^b	25.5 ^{bc}	59.3 ^e	40.8 ^b	31.9 ^c	18.6 ^b	7.25 ^{bc}	9.02 ^c
CGT5U4	16.7 ^b	6.61 ^a	54.3 ^a	35.5 ^{ab}	72.2 ^{ab}	52.5 ^a	39.5 ^a	19.7 ^b	9.74 ^{ab}	10.3 ^{bc}
CGT10U4	16.6 ^b	7.81 ^a	49.2 ^a	38.0 ^a	72.8 ^a	53.1 ^a	38.4 ^a	19.7 ^b	11.3 ^a	11.8 ^a
CGT5U8	19.5 ^a	7.43 ^a	56.8 ^a	44.4 ^a	68.8 ^{bc}	51.4 ^a	38.4 ^a	17.4 ^b	11.5 ^a	10.8 ^{ab}
CGT10U8	21.2 ^a	7.34 ^a	52.6 ^a	41.7 ^a	70.7 ^{ab}	50.7 ^a	38.0 ^a	20.0 ^b	11.5 ^a	9.66 ^{bc}
C.V. ⁴ (%)	3.59	8.05	14.0	13.1	2.09	2.68	2.35	5.82	11.2	5.11

¹Elephantgrass (EG), EG with the inclusion of 5% (CGT5) and 10% (CGT10) of CGT untreated or ammoniated with 4% (CGT5U4, CGT10U4) and 8% (CGT10U8, CGT10U8) of urea. ²DM= dry matter; CP=crude protein; NDIN= neutral detergent insoluble nitrogen; ADIN= acid detergent insoluble detergent; NDF=neutral detergent fiber; ADF= acid detergent fiber; CEL=cellulose; HEM=hemicellulose; LIG=lignin. ³Means followed by the same letter do not differ by Tukey's test ($p < 0.05$). ⁴CV= coefficient of variation.

The expected benefits of ammoniation in reducing the fibrous fraction were not reflected in the composition of the silages containing ammoniated CGT, as also was described by Kung Junior et al. (1989). However, hemicellulose decreased ($p < 0.05$) with the inclusion of both untreated and ammoniated CGT when compared with EG (Table 2). The CGT has low hemicellulose content and, unlike what is observed for other low-quality roughages, ammoniation does not always promote its solubilization (Andrade et al., 2020).

The pH of the silage increased ($p < 0.05$) with the addition of 10% untreated CGT and with the inclusion of ammoniated CGT, as compared with the EG silage (Table 3). Only EG and the silages that received untreated CGT (CGT5 and CGT10) were within the ideal pH range of 3.8 to 4.2 (McDonald et al., 1991). The increased pH with ammoniated CGT can be attributed to residual ammonia from urea treatment, which has high alkalinizing power, corroborating with Kung Junior et al. (1989). Silage pH greater than 4.5, as those with ammoniated CGT (Table 3), enable the action of proteolytic bacteria and high production of butyric acid, indicating poor silage quality (McDonald et al., 1991).

Table 3. Losses, pH, density, and dry matter recovery (DMR) of elephantgrass silage with untreated or urea-ammoniated cotton gin trash (CGT).

Treatments ¹	pH	As-fed density (Kg m ⁻³)	DM density (Kg m ⁻³)	Effluent losses (Kg Mg ⁻¹)	Gaseous losses (% DM)	DMR (%)
EG	3.70 ^{e2}	626 ^a	90.0 ^c	58.4 ^a	1.29 ^e	87.0 ^a
CGT5	3.77 ^{de}	571 ^{abc}	95.9 ^{bc}	54.7 ^a	2.96 ^{abc}	70.4 ^{cde}
CGT10	4.02 ^d	528 ^{cd}	111.9 ^{ab}	55.8 ^a	3.31 ^a	66.8 ^e
CGT5U4	5.10 ^c	599 ^{ab}	106.1 ^{ab}	54.2 ^a	3.06 ^{ab}	69.4 ^{de}
CGT10U4	5.48 ^b	593 ^{ab}	115.5 ^a	53.8 ^a	2.16 ^d	78.4 ^b
CGT5U8	5.24 ^{bc}	557 ^{bcd}	103.8 ^{abc}	54.7 ^a	2.30 ^{bcd}	76.9 ^{bcd}
CGT10U8	7.36 ^a	504 ^d	114.8 ^a	9.97 ^b	2.26 ^{cd}	77.3 ^{bc}
C.V. ³ (%)	1.97	3.81	6.12	8.28	11.21	3.70

¹Elephantgrass (EG), EG with the inclusion of 5% (CGT5) and 10% (CGT10) of CGT untreated or ammoniated with 4% (CGT5U4, CGT10U4) and 8% (CGT10U8, CGT10U8) of urea. ²Means followed by the same letter do not differ by Tukey's test ($p < 0.05$). ³CV= coefficient of variation.

The silage as-fed (AF) and DM densities changed ($p < 0.05$) with the addition of CGT in different ways. The AF density of the EG silage (626 kg m⁻³) differed ($p < 0.05$) by more than 100 kg m⁻³ in relation to CGT10U8 (504 kg m⁻³) (Table 3). However, DM density increased ($p < 0.05$) with the inclusion of 10% CGT, either untreated or ammoniated, in comparison with EG.

The inclusion of CGT did not promote a reduction in effluent losses, except in the treatment CGT10U8 ($p < 0.05$), which produced approximately five times less effluent than the other treatments (Table 2). A partial reduction in effluent losses was expected with increased DM promoted by the inclusion of CGT (Table 2) (Andrade et al., 2010; Andrade et al., 2012; Negrao et al., 2016). This only occurred, however, in CGT10U8, which was the only treatment to exceed 20% DM. Silages with DM greater than 20% have little effluent loss in comparison to the silages with higher moisture levels (Razak et al., 2012). It is important to reduce effluent losses in high-moisture silages, since effluents drain highly digestible nutrients, reduce the amount of material stored and cause environmental pollution (Gebrehanna et al., 2014; Borreani et al., 2018). In this regard, dry additives can be a good alternative to reduce effluent losses (Andrade et al., 2010). However, depending on the moisture content of the forage, the rate of inclusion of the additive must be sufficient to absorb excess fluids.

The EG silage had lower ($p < 0.05$) gaseous losses (1.29%) than the others (from 2.16 to 3.31%, Table 3). Conversely, Viana et al. (2013) reported decreased gaseous losses after adding increasing rates of cottonseed cake to elephantgrass silage originally containing 18% of DM. Gaseous losses may be related to fermentation performed by heterofermentative bacteria, enterobacteria, and yeasts inside the silo (McDonald et al., 1991; Wilkinson & Rinne, 2018). According to Ferreira et al. (2013), increased lactic acid production can lead to lower DM losses in grass silages, considering lactic fermentation minimize losses while acetic and butyric fermentations are associated with secondary fermentation and gaseous losses. Other factors related to gaseous losses are high pH and increased ammoniacal N, especially in silages with ammoniated CGT, which may have favored butyric fermentation (Borreani et al., 2018).

The DMR was greater ($p < 0.05$) in the EG silage than in the silages that contained CGT (Table 3). Thus, regardless of whether it was ammoniated or untreated, CGT was not as efficient for increasing DMR as other agricultural byproducts and wastes (i.e., cassava peel, coffee husks, cacao bran, and cottonseed cake, Andrade et al., 2010; Viana et al., 2013).

The aerobic stability of all silages was broken 12 h after silo opening (Table 4). According to Wilkinson and Davies (2012), the goal would be to maintain the aerobic stability of the silage for seven days, including the time in the trough. Air exposure can provide a favorable environment for the proliferation of fungi and yeasts, which use fermentation products as a substrate, causing heat production and resulting in increased losses of DM and nutrients (Andrade et al., 2012).

Table 4. Aerobic stability of elephantgrass silage with untreated or urea-ammoniated cotton gin trash (CGT).

Treatments ¹	Aerobic exposure (h)								Regression equation	R ²
	0	6	12	24	48	72	96	120		
Temperature (°C)										
EG	26.5	27.1	27.5	28.2	28.7	28.8	29.1	28.8	Y= 26.496 + 0.103*x - 0.001*x ²	0.84
CGT5	26.4	26.7	27.4	28.9	31.3	31.8	31.9	31.5	Y= 26.432 + 0.017*x + 0.006*x ²	0.94
CGT10	26.7	26.8	27.5	28.2	30.8	33.5	36.3	34.8	Y= 26.670 - 0.010*x + 0.005*x ²	0.95
CGT5U4	26.8	27.0	27.4	27.7	28.2	28.5	28.7	28.9	Y= 26.768 + 0.059*x	0.92
CGT10U4	26.7	27.1	27.1	27.8	27.9	28.0	28.8	31.5	Y= 26.752 + 0.073*x	0.81
CGT5U8	26.6	27.1	27.3	27.6	27.8	28.4	29.4	33.4	Y= 26.564 + 0.119*x	0.80
CGT10U8	27.0	27.2	27.4	27.7	28.2	31.4	36.7	35.2	Y= 26.910 + 0.048*x - 0.0003*x ²	0.90
pH										
EG	3.79	3.98	4.09	4.14	4.13	4.04	3.79	3.98	Y= 3.796 + 0.009*x - 0.00006*x ²	0.99
CGT5	3.74	4.04	5.37	7.31	8.02	8.27	3.74	4.04	Y= 3.739 - 0.040*x	0.94
CGT10	4.02	4.08	4.34	6.73	7.83	9.03	4.02	4.08	Y= 4.02 + 0.046*x	0.91
CGT5U4	5.09	5.18	5.21	5.25	5.35	5.56	5.09	5.18	Y= 5.096 + 0.004*x	0.89
CGT10U4	5.44	5.57	5.45	5.64	5.88	7.11	5.44	5.57	Y= 5.440 - 0.015*x + 0.0005*x ²	0.91
CGT5U8	5.29	5.36	5.43	5.50	5.57	5.63	5.29	5.36	Y= 5.289 + 0.003*x	0.89
CGT10U8	7.36	6.99	6.91	7.14	7.68	8.59	7.36	6.99	Y= 7.36 - 0.019*x + 0.00014*x ²	0.99

¹Elephantgrass (EG), EG with the inclusion of 5% (CGT5) and 10% (CGT10) of CGT untreated or ammoniated with 4% (CGT5U4, CGT10U4) and 8% (CGT10U8, CGT10U8) of urea.

Acetic acid inhibits the development of undesirable microorganisms during the period of exposure of the silage to air and may be an explanation for the superior performance of the EG silage over those that received CGT (Quadros et al., 2003; Gerlach, Daniel, Jobi, & Nussio, 2021; Randby and Bakken, 2021). Unlike in the EG silage, the inclusion of untreated CGT was responsible for raising the temperature above 30 °C after 48 h, whereas the CGT10U8 exceeded this temperature after 72 h. In the EG, CGT5, CGT10, and CGT10U8 silages, the temperature rose quadratically ($R^2 > 0.84$), whereas, in CGT5U4, CGT10U4, and CGT5U8, it increased linearly ($R^2 > 0.80$). Silage density and porosity are key physical factors that determine the rate of oxygen entering the silage mass during the unloading period (Wilkinson & Davies, 2012; Borreani et al., 2018; Wilkinson & Rinne, 2018), and these factors may also be related to the results obtained in this experiment.

All treatments increased pH as a function of post-opening time (Table 4). After silo opening, the pH rises when organic acids that preserve the silage are consumed by microorganisms (Wilkinson & Davies, 2012; Wilkinson & Rinne, 2018). The EG, CGT10U4 and CGT10U8 silages showed a quadratic increase in pH ($R^2 > 0.91$), whereas in the other treatments the pH rose linearly ($R^2 > 0.89$).

However, only the EG silage remained within the ideal pH range of 3.8 to 4.2 (McDonald et al., 1991), even up to 120 h post-opening. To be considered stable, the silage must show a pH increase of < 0.5 units in five days of exposure to air (Wilkinson & Davies, 2012). With untreated CGT, the pH exceeded 4.2 after 48 h, while

with ammoniated CGT the pH was always higher than 4.2 due to the alkalizing power of the ammonia released by the action of urease on urea (Andrade et al., 2020).

Conclusion

The use of untreated or ammoniated cotton gin trash resulted in silages with signs of low fermentation quality. Even with increased dry matter content, the inclusion of cotton gin trash worsened the chemical composition, dry matter recovery, and aerobic stability. Therefore, its inclusion in elephantgrass ensiling is not recommended.

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