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USE OF VINASSE AS AN ADDITIVE IN SILAGES OF *TITHONIA DIVERSIFOLIA* (HEMSL) L. VC. ICACUBA OCC-10, ALONE AND IN MIXTURE WITH *CENCHRUS PURPUREUS* CV. CT-608

UTILIZACIÓN DE LA VINAZA COMO ADITIVO EN ENSILAJES DE *TITHONIA DIVERSIFOLIA* (HEMSL) L. VC. ICACUBA OCC-10, SOLA Y EN MEZCLA CON *CENCHRUS PURPUREUS* VC. CT-608

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To evaluate the quality of silages of *Tithonia diversifolia* vc. IcaCuba Occ-10, alone or mixed with forage of *Cenchrus purpureus* cv. CT-608, preserved with or without vinasse, a completely random design was used, with a factorial arrangement (2x2) and 10 microsilos per treatment. All treatments showed similar dry matter content (P>0.05), lower than the recommended values (30-35 %). The inclusion of vinasse reduced the pH compared to the treatment without it (P<0.01). Similarly, the dry matter:pH ratio improved when vinasse was included as an additive. The treatments of Tithonia alone showed a dark green color, the mixture of Tithonia and Cenchrus forages resulted in olive green with dark tones that disappeared when vinasse was added. It was the latter that had a coloration most in line with an excellent quality silage. In the silages with vinasse, a pleasant smell was obtained, similar to that suggested for silages of very good characteristics. The silages obtained from *T. diversifolia,* alone or mixed with *C. purpureus* cv. CT-608 forage, showed sufficient quality indicators to recommend their evaluation on a larger scale, even starting from materials with low dry matter contents. The organoleptic and chemical evaluation supports its stability and acceptability. The inclusion of vinasse in the preservation process of these silages is confirmed as an economical and effective strategy for the conservation of tropical forages, even forages with high protein content and high buffering capacity such as tithonia. Its use as an additive reduces the acid detergent fiber content, pH, and improves dry matter yield, the dry matter:pH ratio, and its organoleptic characteristics, such as color and odor.

Key words: *food preservation, livestock supplementation, microsilos, pH, tropical forages*

Para evaluar la calidad de ensilados de *Tithonia diversifolia* vc. IcaCuba Occ-10, sola o en mezcla con forraje de *Cenchrus purpureus* vc. Cuba 608, conservados con vinaza o sin ella, se empleó un diseño completamente aleatorizado, con arreglo factorial (2x2) y 10 microsilos por tratamiento. Todos los tratamientos mostraron similar contenido de materia seca (P>0.05), inferior a los valores recomendados (30-35 %). La inclusión de vinaza redujo el pH con respecto al tratamiento sin ella (P<0.01). De igual forma, la relación materia seca: pH mejoró al incluir vinaza como aditivo. Los tratamientos de tithonia sola mostraron un color verde oscuro, la mezcla de forrajes de tithonia y cenchrus resultó en verde aceituna con tonos oscuros que desaparecieron al añadir vinaza. Fue este último el que tuvo una coloración más acorde con un ensilaje de excelente calidad. En los ensilajes con vinaza, se obtuvo un olor agradable, similar al sugerido para ensilajes de muy buenas características. Los ensilados obtenidos a base de *T. diversifolia*, sola o en mezcla con forraje de *C. purpureus* vc. CT- 608, mostraron indicadores de calidad suficientes para recomendar su evaluación a escala mayor, aun partiendo de materiales con bajos contenidos de materia seca. La evaluación organoléptica y química respalda su estabilidad y aceptabilidad. La inclusión de vinaza al proceso de conservación de estos ensilajes se confirma como una estrategia económica y efectiva para la conservación de forrajes tropicales, incluso en forrajes con altos contenidos de proteínas y elevada capacidad amortiguadora como Tithonia. Su uso como aditivo reduce el contenido de fibra detergente ácido, el pH, y mejora el rendimiento en materia seca, la relación materia seca: pH y sus características organolépticas, como el color y el olor.

Palabras clave: *conservación de alimentos, forrajes tropicales, microsilos, pH, suplementación del ganado*

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Introduction

Ruminants have a very important role in human nutrition because they produce practically all the milk and a third of the meat consumed in the world. The grasses and forages constitute the natural food of ruminants and represent the most abundant and least costly food source (Ruiz *et al.* 2021 and Arias *et al.* 2023). However, much of the forage used are grasses of moderate productivity and quality, which require certain levels of supplementation to improve animal productivity.

In Cuba, the cooperative sector has the main weight of animal production, but it lacks products and technologies that allow it to enhance its production through the efficient use of local resources and minimize production costs. Therefore, under the current conditions in the country, this sector must be provided with technologies and products adapted to the production scale, so as to guarantee improved quality and efficiency of nutrient use by ruminants and, consequently, increased productivity.

Silage can be a way to preserve and obtain food with adequate nutritional quality for the dry period. However, grass forages have a variable nutritional composition (Rojas-Cordero *et al.* 2020), high fiber content and low digestibility (Ku Vera *et al.* 2014). Therefore, the production of protein shrub silages or their inclusion in mixtures in grass silages can improve the quality of the product obtained under the practical conditions of the tropics.

Among the protein plants used as forage in the tropics is *Tithonia diversifolia* (Hemsl.) Gray, a species of high nutritional quality that provides high protein content and lower fiber and lignin content, which can complement the deficiencies of grasses, generating positive effects on digestibility and ruminal methane emissions (Arias *et al*. 2023).

However, its protein content can cause unwanted fermentations that affect the preservation of the material, so the use of acidifying products that limit these fermentations should be considered. Therefore, the objective of this study was to evaluate the quality of silages obtained from *T. diversifolia*, alone or mixed with forage from *Cenchrus purpureus* vc. Cuba 608, ensiled with or without vinasse.

Materials and Methods

The experiments were conducted from November to December 2023 at Instituto de Ciencia Animal (ICA), located between 22º 58 N and 82º 02 W and at 80 m o. s. l.

Obtaining silage: For the preparation of silages, forages of *C. purpureus* cv. CT-608 and the shrubby *T. diversifolia* vc. IcaCuba Occ-10 were used, both with a regrowth age between 90 and 100 d. The collection of the two materials was carried out in the experimental areas of Miguel Sistach Experimental Station, established on typical red ferralitic soil, with rapid drying and uniform profile (Hernández *et al.* 2015).

Vinasse was used as an additive, obtained in the final alcoholic fermentation process of molasses at Habana Club S.A. distillery in San José de las Lajas, Mayabeque.

The following treatments were evaluated:

1. Fresh Tithonia forage silage (100 %)
2. Fresh Tithonia forage silage (100 %) + vinasse (4 % on a wet basis)
3. Fresh forage silage from Tithonia and Cenchrus [50:50]
4. Fresh forage silage of Tithonia and Cenchrus [50:50] + vinasse (4 % on a wet basis)

The grass and tree material were obtained by hand cutting. Both were transported fresh and chopped in a forage mill to a particle size of 20-30 mm. The different proportions of forages were mixed with vinasse and compacted in microsilos made of PVC pipes (24 cm x 10 cm) (Gutiérrez *et al.* 2015). Finally, the microsilos were hermetically sealed and placed for 60 days in a protected and dry location. At the end of the ensiling process, the microsilos were opened and a 10 g sample was taken from each one, 90 mL of distilled water was added and it was mixed in an orbital sieve at 250 r.p.m for 15 min at 20 °C.Then, the mixture was filtered through gauze and the pH of the filtrate was measured (Everich pH meter, PHSJ-3F, China). Approximately 200 g were taken from each microsilo and dried to constant weight in a forced air oven with regulated temperature (60 ºC). They were ground in a hammer mill until a particle size of 1 mm was reached. The dried material from each microsilo was stored individually in sealed nylon bags for chemical composition analysis.

Chemical analysis: To determine the chemical composition of forages and silages, dry matter (DM), organic matter (OM) and crude protein (CP) were determined (AOAC 2016). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were quantified by the procedure described by van Soest *et al.* (1991).

At the end of the ensiling process, the organoleptic characteristics of the silage were determined, according to table 1 (Chaverra Gil and Eusse 2000). For this purpose, an evaluation panel made up of nine members was used, all associated with the Ruminant and Grasses and Forage Departments from ICA (five researchers and four research technicians). The panelists described the organoleptic characteristics of the samples made available to them, without previously knowing which treatment they corresponded to.

Statystical analysis: For the analysis of the chemical composition of the evaluated silages, a completely random experimental design with a factorial arrangement (2x2) was applied, where factor A was the type of ensiled forage (Tithonia alone or Tithonia with Cenchrus) and factor B was the level of vinasse used (0 or 4 %). The microsilos were considered as an experimental unit (10).

Table 1. Organoleptic characteristics for the evaluation of silage quality (Chaverra Gil and Eusse 2000)

Indicator	EXCELLENT	GOOD	REGULAR	BAD
COLOR	Olive green or dark yellow	Yellowish-green, Stems with a paler tone than the leaves	Dark green	Dark brown, almost black, or black
ODOR	Molasses or sugared ripe fruit	Pleasant, with a slight vinegar smell	Strong, acidic, vinegar odor (butyric acid)	Unpleasant, like rancid butter
TEXTURE	It retains its continuous contours	Same as previous	The leaves separate easily from the stems, tend to be transparent, and the venous vessels are very yellow	There are no differences between the stems and leaves. It is more amorphous and soapy. It feels moist and shiny to the touch

The variables were analyzed by ANOVA. When differences were found (P<0.05), the treatment means were compared by Duncan (1955) multiple range test The statistical package Infostat was used for these analyses (Di Rienzo *et al.* 2012).

Results and Discussion

The chemical composition of *T. diversifolia* forage and the mixture with *C. purpureus* used for silage is showed in table 2. The chemical composition of Tithonia was similar to that recorded by Ontivero (2021), except CP which was lower. The DM values were similar to those obtained for tithonia forage by Rodríguez-Oliva *et al.* (2022) and in all cases were less than 30 %, a characteristic range of tropical forages with high moisture that can present challenges for optimal fermentation, by favoring the production of effluents and clostridial fermentations (Ávila *et al.* 2022). Tithonia alone showed lower NDF content and higher CP content compared to the mixture, which was expected given the protein nature of this species in relation to the Cenchrus grass.

Figure 1 shows the effect of the interaction between forage type and vinasse level on the DM yield of the evaluated silages (P<0.001). Silage of Tithonia alone showed better yield than mixed silage, and in both cases, the addition of vinasse increased the yield. This increase can be attributed to the contribution of soluble solids and organic compounds from vinasse that are incorporated into the final DM of the silage (Prado *et al*. 2023).

Regarding the effect of the evaluated treatments on the chemical composition, there was no effect of the interaction between the forage type and vinasse level, nor of the individual factors on the DM and OM content (table 3). Regarding the ADF of the evaluated silages, there was interaction between the evaluated factors (P=0.0269) and the results are also shown in table 3.

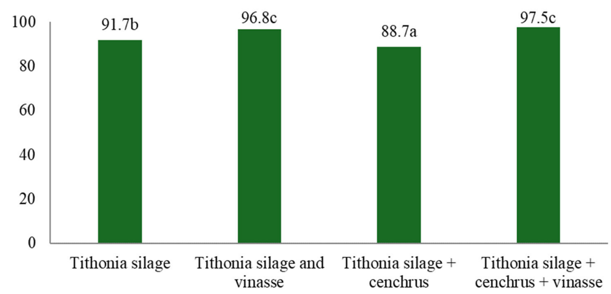

Figure 1. Effect of the interaction between forage type and vinasse level on the DM yield of the evaluated silages (SE = ± 4.23)

Regarding NDF content, there was no effect of the interaction between forage type and vinasse level (P=0.2278), but there was an effect of the individual factors forage type (P<0.0001) (figure 2a) and vinasse level (P<0.0001) (figure 2b).

Regarding the CP content of the evaluated silages, there was no effect of the interaction between the forage type and vinasse level (P=0.9024) nor of the vinasse level factor (P=0.4236), but there was an effect of the forage type factor (P<0.0001), since a decrease in protein content was observed when mixing Tithonia with Cenchrus, as expected when incorporating a tropical grass with low protein content (figure 3).

Regarding the pH values of the evaluated silages, there was interaction between the evaluated factors (P<0.01) and the results are shown in figure 4.

Table 2. Chemical composition of tithonia forage and the tithonia and cenchrus mixture

Treatment	DM, %	OM, %	NDF, %	ADF, %	CP, %
Tithonia	21.8	86.9	49.8	43.3	13.4
Tithonia silage + Cenchrus	23.1	88.7	63.4	47.9	10.9

Table 3. Effect of the interaction between the forage type and vinasse level used on the DM, OM and ADF content of the evaluated silages.

Treatments	DM, %	OM, %	ADF, %
Tithonia silage	20.0	92.6	47.0b
Tithonia silage and vinasse	21.1	92.6	42.7a
Tithonia silage + Cenchrus	20.5	91.3	52.1c
Tithonia silage + Cenchrus + vinasse	23.5	89.3	50.6c
SE ±	1.75	6.22	0.60
P (interaction)	0.056	0.346	0.027
P (forage type)	0.609	0.257	0.0002
P (vinasse level)	0.311	0.348	< 0.0001

Different letters show differences between treatments (P<0.05)

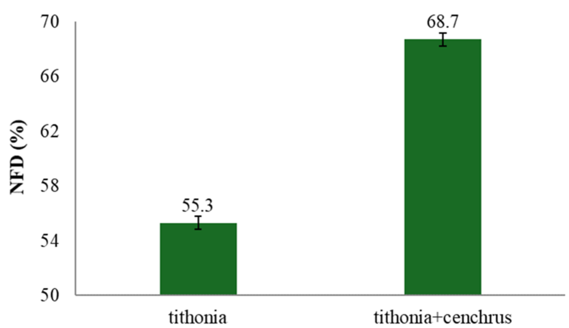

Figure 2a.

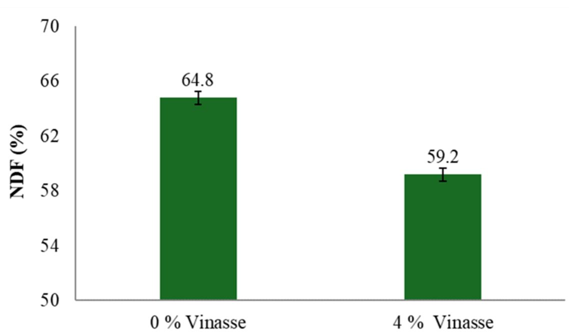

Figure 2b.

Figure 2. Effect of forage type (figure 2a, SE= ± 0.47) and vinasse level (figure 2b, SE= ± 0.47) on the NDF content of the evaluated silages

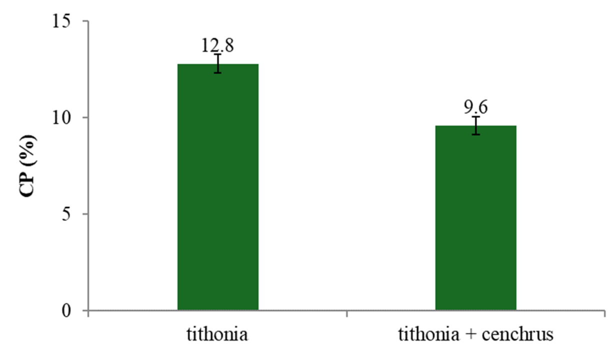

Figure 3. Effect of forage type on CP content (%) of the evaluated silages (SE= ± 0.33)

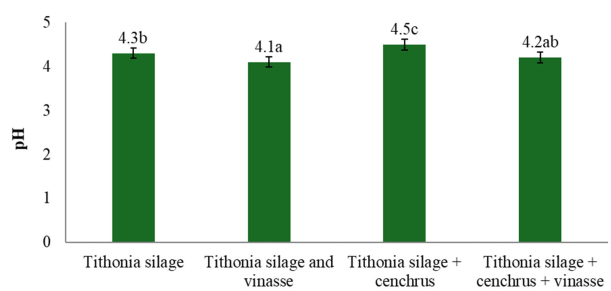

Figure 4. Effect of forage type on the pH of the evaluated silages (SE= ± 0.12)

The highest values were obtained when the forage mixture was ensiled. The inclusion of vinasse reduced the pH compared to the corresponding treatment without vinasse (P<0.01). All pH values were higher than those recommended by Sánchez (2018) for this type of silage (pH=4.0), but were lower than those recorded by Rodríguez-Oliva *et al.* (2022) when ensiling pre-dried Tithonia plants and were within the range observed in silage of the whole plant of *Sorghum bicolor* (L.) Moench (Morales *et al*. 2019) and of *Manihot esculenta* Crantz (Sánchez *et al.* 2021). In the case of silages with vinasse, the pH values were at the level of maximum pH values (3.8 to 4.2) recommended by McDonald *et al.* (1991).

This acidifying effect of vinasse is directly attributed to the high concentration of organic acids (acetic, lactic, propionic) and its low pH (Li *et al.* 2022 and Prado *et al*. 2023). Vinasse proved to be an effective additive to mitigate the high buffering capacity of tithonia, by promoting faster and more efficient acidification, which is essential to suppress undesirable bacteria and preserve the nutritional value of the forage (Ávila *et al*. 2022 and Li *et al*. 2022).

The dry matter:pH ratio is an important indicator related to silage quality. Comparing the values obtained with the criteria proposed by Ojeda *et al*. (1991), it was observed that the inclusion of vinasse as a silage additive improves this ratio. The worst DM:pH ratio was found in the silage of Tithonia and Cenchrus mixture, and an intermediate value in Tithonia alone.

The results of this study show that it is possible to obtain good quality silage from *T. diversifolia*, alone or mixed with *C. purpureus*, despite the low dry matter content (DM < 24 %) of the forages used, which is below the recommended optimum (30-35 %) to avoid losses through effluents and undesirable fermentations (Ávila *et al.* 2022).

The inclusion of vinasse showed a consistent acidifying effect, significantly reducing (P<0.01) the pH of the silages (figure 3). This result was expected, given the high concentration of organic acids (acetic, lactic) and the low pH inherent in vinasse (Prado *et al.* 2023). The acidifying effect of vinasse is relevant to production practice, since this byproduct of the sugar industry, widely available in Cuba, is confirmed as an effective and low-cost additive to improve the fermentation process.

The effect of vinasse was more pronounced in the silage of tithonia alone, a forage with high protein content that usually presents difficulties in reaching a low pH, due to its high buffering capacity (Ávila *et al*. 2022). Vinasse, by providing organic acids and possibly stimulating a more efficient lactic fermentation (Prado *et al.* 2023), mitigated this problem, opening a promising way for the conservation of high-quality protein shrubs. Furthermore, vinasse not only acts as a source of acids, but also as a substrate that can promote efficient lactic fermentation, even in materials with high buffering capacity such as protein legumes and shrubs (Li *et al.* 2022).

Regarding the chemical composition, the reduction in ADF content in Tithonia silage with vinasse (table 3) could show a partial degradation of the fiber during fermentation, possibly mediated by fibrolytic enzymes present in the vinasse or by the activation of native microorganisms. If this finding is confirmed *in vivo*, it could have practical relevance, since improving fiber digestibility is one of the main challenges in ruminant nutrition in the tropics (Arias *et al.* 2023).

It was not expected that vinasse would not influence on the CP content of the silages, since this additive provides non-protein nitrogen (NPN) and nitrogenous compounds awaiting quantification as CP by the Kjeldahl method (AOAC 2016).

Table 4 shows the organoleptic characteristics of the evaluated silages. The higher pH values recorded in the treatments, even without vinasse, did not affect the indicators evaluated in the microsilos. There was not evidence of putrefaction or presence of filamentous fungi, which is an indication of aerobic stability and good preservation of the silage (Kung *et al.* 2018).

There were not differences in the texture and moisture of the silage. The Tithonia alone treatments showed a dark green color that must have been related to the oxidation of proteins and secondary compounds. Meanwhile, the mixture of Tithonia and Cenchrus forages showed an olive green color with dark shades that disappeared when vinasse was added, the latter having a coloration more in line with an excellent quality silage. Regarding the smell, a slight vinegar smell was observed in the silage of tithonia alone and more intense in that of the forage mixture, although in the silages with vinasse a pleasant smell was obtained similar to that suggested for silages of very good quality (Ojeda *et al*. 1991). Morales *et al*. (2022) noted a slight vinegar smell in sorghum silage and, applying the same methodology as this study qualified that silage as "Excellent".

The organoleptic evaluation confirmed the chemical and fermentative results. The pleasant colors and smells, the absence of putrefaction and the preserved texture in all treatments, especially those with vinasse, classify these silages as 'Good' to 'Excellent' according to the criteria of Chaverra Gil and Eusse (2000). This is crucial for adoption by farmers, since sensory characteristics are the first criterion for acceptance of silage.

Table 4. Organoleptic characteristics of the evaluated silages

Treatment	Color	Odor	Texture	Humidity	Evaluation
Tithonia silage	Dark green	Slight vinegar smell. Doesn't leave an unpleasant odor when touched.	The forage retains its shape. Leaves joined to the stems	It doesn't wet hands	Good
Tithonia silage and vinasse	Dark green	Pleasant. It doesn't leave an unpleasant odor when touched	The forage retains its shape. Leaves joined to steams	It doesn't wet hands	Good to Excellent
Tithonia silage + Cenchrus	Olive green with dark shades	Stronger vinegar smell. Doesn't leave an unpleasant odor when touched	The forage retains its shape. Leaves joined to the stems	It doesn't wet hands	Good
Tithonia silage + Cenchrus + vinasse	Olive green	Slight vinegar smell. Doesn't leave an unpleasant odor when touched	The forage retains its shape. Leaves joined to the stems	It doesn't wet hands	Good

Conclusions

The silages obtained from *T. diversifolia*, alone or mixed with *C. purpureus* cv. CT-608 forage, showed sufficient quality indicators to recommend their evaluation on a larger scale, even starting from materials with low DM content. The organoleptic and chemical evaluation supports its stability and acceptability.

The inclusion of vinasse in the conservation process of these silages is confirmed as an economical and effective strategy for the conservation of tropical forages, even in forages with high protein content and high buffering capacity such as Tithonia. Its use as an additive reduces ADF content and pH, and improves DM yield, DM:pH ratio and its organoleptic characteristics such as color and odor.

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