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Effect of two additives on the bromatological quality of Cuba 22 grass and buttercup biomass in different proportions

Efecto de dos aditivos en la calidad bromatológica de biomasa de pasto Cuba 22 y botón de oro en distintas proporciones

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ABSTRACT

The research was carried out at the Zoila Luz Hacienda of the University of the Armed Forces – ESPE, Santo Domingo de los Tsáchilas, with the objective of determining the bromatological quality of the biomass of Cuba 22 grass (*Pennisetum purpureum* × *Pennisetum glaucum*) and buttercup (*Tithonia diversifolia*) in different proportions and two silage additives. This method constitutes an effective alternative for feeding cattle during critical times of forage scarcity. The study included a field phase, where silage was prepared in variable proportions of both forages with two additives (why and biological inoculant), followed by a laboratory phase for chemical (protein, fiber, dry matter, ash and pH) and physical (odor, color, texture and acceptability) analyses. A completely randomized design with a factorial arrangement (3×2) was used, generating six treatments with four repetitions each, evaluated using ADEVA and Fisher's LSD test at 5%. Significant differences were found in proteins (15.03%-17.95%) and fiber (20.13%-24.78%). The best results were obtained using Silamix and whey with 75% Cuba 22 grass and 25% buttercup, thus achieving a silage of excellent bromatological quality and good acceptability for use in animal feed.

Keywords: composition; evaluation; forages; nutrients; silage

RESUMEN

La investigación se realizó en la Hacienda Zoila Luz de la Universidad de las Fuerzas Armadas – ESPE, Santo Domingo de los Tsáchilas, con el objetivo de determinar la calidad bromatológica de la biomasa del pasto Cuba 22 (*Pennisetum purpureum* × *Pennisetum glaucum*) y botón de oro (*Tithonia diversifolia*) en diferentes proporciones y dos aditivos para ensilaje. Este método constituye una alternativa eficaz para alimentar ganado bovino durante épocas críticas de escasez forrajera. El estudio contempló una fase de campo, donde se preparó ensilaje en proporciones variables de ambos forrajes con dos aditivos (suero de leche e inoculante biológico), seguida de una fase de laboratorio para análisis químicos (proteína, fibra, materia seca, cenizas y pH) y físicos (olor, color, textura y aceptabilidad). Se utilizó un diseño completamente al azar con arreglo factorial (3×2), generando seis tratamientos con cuatro repeticiones cada uno, evaluados mediante ADEVA y prueba LSD Fisher al 5%. Se encontraron diferencias significativas en proteína (15,03%-17,95%) y fibra (20,13%-24,78%). Los mejores resultados se obtuvieron usando Silamix y suero de leche con 75% pasto Cuba 22 y 25% botón de oro, logrando así un ensilaje de excelente calidad bromatológica y buena aceptabilidad para uso en alimentación animal.

Keywords: composición; ensilaje; evaluación; forrajes; nutrientes

1. INTRODUCTION

In a global context, food security and the sustainability of agricultural systems are fundamental challenges in achieving efficient production of high-quality biomass to meet supply needs, improve livestock efficiency, and avoid compromising environmental health (Tricarico et al., 2020). In this regard, the adoption of strategies that enhance forage nutritional quality through additives is considered a promising alternative for rational resource use and the development of sustainable systems (Castaño-Jiménez et al., 2023).

According to Sánchez et al. (2019), in Ecuador, and Polanía et al. (2013), 90% of livestock producers engage in daily grazing, as it is considered the most "economical" way to feed cattle. Ecuador's national territory has two well-defined seasons. During the rainy season, food production is abundant, while the dry season usually brings scarcity, leading to economic losses in cattle herds (Polanía et al., 2013). Therefore, strategies must be developed to mitigate the impact of the dry season, such as producing or storing surplus food from the rainy season to use during critical times.

Cuba 22 grass (*Pennisetum purpureum x Pennisetum glaucum*) is considered a highly productive forage, valued globally for its adaptability to tropical and subtropical soils and conditions. It has very rapid growth and high regrowth capacity with multiple harvests (Paredes et al., 2014). It provides consistent biomass, with thick stems and broad leaves that give volume and facilitate handling. Additionally, its ability to withstand adverse climatic conditions like mild droughts makes it a resilient and sustainable option for livestock systems (Cerdas Ramírez et al., 2021).

Mexican sunflower (*Tithonia diversifolia*), known as Botón de Oro, is valued as forage for its wide adaptability to various soil types and climates, rapid growth, and high biomass production. Its nutritional profile—rich in proteins, minerals, and bioactive compounds—is noteworthy, especially considering that most tropical plants are sources of such compounds. Its multifunctional characteristics enable its use both in animal feed and as a green manure to significantly enhance soil fertility with a low production cost-benefit ratio. These features make it a central component in any sustainable agricultural system (Botero Londoño et al., 2019).

Cuba 22 grass and Mexican sunflower are valuable ingredients in animal feed. Cuba 22 stands out for its high biomass production, adaptability, and high nutritional value (Rivas Avellán & Vera Mera, 2023). Meanwhile, Mexican sunflower demonstrates high forage potential with unique nutritional levels, rapid growth, hardiness, and mineral richness, making it a model ingredient for improving silage biomass quality (Cerdas Ramírez et al., 2021).

The simultaneous incorporation of Cuba 22 and Mexican sunflower helps to compensate for each other's individual weaknesses, improving overall nutritional quality (Angulo et al., 2021). Silage production is crucial for biomass conservation and fermentation, particularly in tropical and subtropical areas. Selecting specific additives is essential in preserving and fermenting forage to retain nutrients during storage (I. Gonzalez et al., 2011).

Therefore, this research aimed to determine the bromatological quality of biomass from Cuba 22 grass and Mexican sunflower in different proportions using two silage additives.

2. MATERIALS AND METHODS

2.1. Experiment Location

The research was conducted at Hacienda Zoila Luz, part of the Universidad de las Fuerzas Armadas ESPE in Santo Domingo, Ecuador (688,139.15 m E; 9,954,386.36 m S) [Figure 1]. The area is characterized by a humid tropical climate, with an average altitude of 605 meters above sea level, temperatures between 22°C and 28°C, and average annual rainfall of around 2,500 mm. These conditions are suitable for forage grass growth and silage processes.

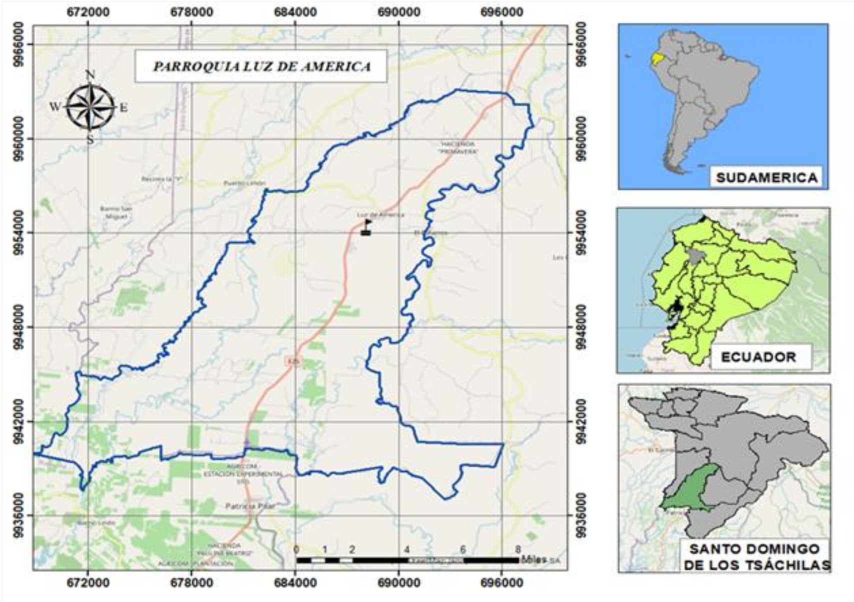

Figure 1. Spatial location of the experiment

2.2. Silage Preparation

Cuba 22 grass was harvested at 65 days at a height of 1.60 meters, and Mexican sunflower at 60 days, then stored at the University's Forest Nursery. Each forage was chopped separately using a forage chopper. Additives (whey and Silamix) were applied using a 20 L sprayer, and the predetermined forage proportions were mixed for each treatment. Molasses was added as an energy source. The mixtures were packed into 25x35 cm polyethylene bags (2 kg per bag), vacuum sealed, and stored in 50 kg silage bags to ensure an optimal internal fermentation environment.

2.3. Protein Determination

Protein content was measured using the Kjeldahl method on pre-dried and ground samples. A 0.3 g sample was digested at 420°C with sulfuric acid and catalysts, followed by ammonia neutralization and distillation. The ammonia was captured in 50 ml of 2% boric acid and titrated. The following formula was applied (ITW Reagents, 2023):

Equation 1. Protein Percentage:

$$\%\ PB=\frac{(VHCL\text{-}Vb)*1.401*NHCL*F}{\text{Peso de la muetsra (gr)}}$$

Where: VHCL: Volume of HCl used in titration; Vb: Blank volume (0.3); 1.401: Atomic weight of nitrogen; N HCL: Normality of HCl (0.1N); F: Conversion factor (6.25).

2.4. Fiber Determination

Fiber was determined using the Weende method on 2 g of pre-dried, ground samples. Samples underwent successive extraction, filtration, and washing with sulfuric acid and sodium hydroxide in the Dosi-Fiber apparatus, then dried at 105°C for 12 hours and ashed at 550°C for 3 hours. The Weende formula was then applied:

Equation 2. Fiber Percentage:

$$\% \text{ FB}=\frac{\text{W1-W2}}{\text{Wo}}*100$$

Where: W1: Weight of crucible + dried sample; W2: Weight of crucible + ashed sample; W0: Sample weight

2.5. Ash Determination

Two grams of each sample were incinerated in pre-weighed crucibles at 600°C for 3 hours. The following formula was used (Prado-Martínez et al., 2012):

Equation 3. Ash Percentage:

$$\% \text{ C}=\frac{\text{W2-W1}}{\text{Wo}}*100$$

Where: W2: Crucible + ashed sample; W1: Empty crucible; W0: Sample weight (g).

2.6. Dry Matter Determination

To determine the dry matter, each crucible to be used was prepared and placed in an oven at 60 °C for 30 minutes to record its weight. Then, the sample was homogenized and 2 grams were weighed, after which it was placed in the oven at 105°C for 12 hours. After this time, the sample was allowed to dry in a desiccator for 30 minutes, and its weight was recorded again. For this method, the following formula was applied (García Martínez & Fernández Segovia, 2019):

Equation 4. Percentage of moisture:

$$\%\text{H}=\frac{\text{W2-W1}}{\text{Wo}}*100 \qquad \%\text{MS}=100-\%\text{H}$$

Where: W2: Weight of the crucible + dry sample; W1: Weight of the crucible + sample before drying; Wo: Weight of the sample (g); %MS: Dry matter; %H: Moisture.

2.7. pH Determination

Samples were taken from various parts of the opened silage bag, homogenized, and 20 g was mixed with 20 ml of distilled water in a mortar. The suspension was measured three times using a pH meter to obtain an average.

2.8. Experimental design

A completely random design (CRD) with a factorial arrangement AB (32) was applied, in which 3 levels for factor A were evaluated: (a0: 80% Cuba 22 and 20% Botón de Oro; a1: 75% Cuba 22 and 25% Botón de Oro; a2: 70% Cuba 22 and 30% Botón de Oro) and two levels for factor B: (b0: Whey; b1: Silamix), as shown in Table 1. This resulted in 6 treatments and 3 replications, yielding 18 experimental units, which are shown in Table 2. To determine the differences between the means of the treatments, an LSD Fisher significance test ($p<0.05$) was applied, using the statistical software Infostat, Statistica, and Statgraphics.

Table 1. Study Factors

Factor A	Factor B
a0: 80% Cuba 22 con 20% Botón de Oro	b0: Whey
a1: 75% Cuba 22 con 25% Botón de Oro	b1: Silamix
a2: 70% Cuba 22 con 30% Botón de Oro	

Table 2. Factorial arrangement of treatments

Treatments	Codes	Factor A + Factor B
1	a0b0	80% Cuba 22 con 20% Botón de Oro + Suero de leche
2	a0b1	80% Cuba 22 con 20% Botón de Oro + Silamix
3	a1b0	75% Cuba 22 con 25% Botón de Oro + Suero de leche
4	a1b1	75% Cuba 22 con 25% Botón de Oro + Silamix
5	a2b0	70% Cuba 22 con 30% Botón de Oro + Suero de leche
6	a2b1	70% Cuba 22 con 30% Botón de Oro + Silamix

3. RESULTS and DISCUSSION

3.1. Bromatological Characteristics of Cuba 22 Biomass and Button of Gold

In this research, the bromatological analysis was conducted on the silage samples, which were obtained from the combination of different types of forage and two specific additives. These analyses were essential to evaluate the nutritional quality and viability of the silage produced under various treatment conditions. At the end of the study, significant differences were observed between the treatments, suggesting the influence of the components used on the bromatological composition of the silage, particularly in terms of dry matter, protein, crude fiber, ash, and pH levels.

3.2. Dry Matter

Regarding dry matter content, Gonzalez et al. (2022) in their study focused on the silages of Pennisetum purpureum and Button of Gold in a 75:25 ratio, recorded a percentage of 24% for this variable. On the other hand, Rodríguez-Badilla et al. (2022) reported achieving a dry matter content of 18.40% with a combination of Cuba Grass (75%) and Button of Gold (25%). This value, in the first case, is similar to that obtained in the present study for treatment T3, where 24.14% dry matter was achieved. According to Pacheco Ramos et al. (2021), dry matter content is a key factor during silage production because it helps reduce water activity, thus delaying the onset of deterioration caused by the anaerobic action on the silage mass. Therefore, maintaining an adequate level of dry matter is essential to prolong the shelf life and nutritional quality of the silage. Demant, 2011 suggests that silage should contain at least 20% dry matter; however, if this percentage exceeds 25%, the production of effluents is reduced, indicating that the optimal range for dry matter content in these cases is between 28% to 35%, as shown in (Table 3).

3.3. Protein

On the other hand, Rodríguez-Oliva et al. (2022), in their study on the inclusion of *Tithonia diversifolia* at 25% in the quality of silages made from Cuba Grass OM22 and Musa sp., determined a crude protein level of 9.90%. However, Gonzalez et al. (2022), using a 75% Elephant Grass + 25% Button of Gold ratio, obtained 7.09% crude protein. These values differ significantly from those obtained in all treatments in this study, where T3 (75:25 + whey) stood out with 17.96% protein. According to Pazla et al. (2024), *Tithonia diversifolia* is a plant that can reach a high crude protein content of up to 22.98%. Moreover, Rodríguez-Oliva et al. (2022), determined that the inclusion of *T. diversifolia* directly increases protein levels in silage. On the other hand, whey has been determined to have protein levels of up to 8.8%, which makes T3 a promising alternative for animal feed, as it favors better digestibility and a higher nutritional contribution for ruminants—key factors for optimizing growth, milk production, and animal health (Tabla 3).

3.4. Crude Fiber

In Table 3, it could be observed that the high level of crude fiber was found in T2 (80:20 + Silamix), which stood out among the other treatments with 24.78%. This percentage is not far from that obtained by Dueñas & Burgos (2021), who silaged Cuba Grass 22 and obtained 22.75% fiber at 60 days of evaluation.

According to Zanin et al. (2022), silage improves the digestibility of feed by breaking down plant fibers through acid hydrolysis during anaerobic fermentation; however, several studies have shown that inoculants can slightly reduce the degradability of crude fiber and non-protein dry matter in silages, thus achieving more efficient material preservation (Hurtado et al., 2020).

3.5. Ash

Regarding ash, as shown in Table 3, it was determined that the highest value was found in T3 (75:25 + Whey) with 7.54%. While, Zanin et al. (2022) in their study with fluid whey, obtained 11.8% ash during the rehydration of corn silage, a value higher than the one obtained in this study, indicating that the ash values obtained in this research are within a favorable range. According to Encalada et al. (2018), ash levels in silage are influenced by several factors such as the mineral composition of the material used, the mineral richness of the soil, fertilization, harvest stage, and climatic conditions. Additionally, the biofermentation process during silage can increase the ash content. Therefore, the interaction of these factors determines the amount of minerals in the final silage, which impacts its nutritional quality.

3.6. pH

In Table 3, it is observed that the lowest values in this study were obtained with T1 (80:20 + Whey) and T3 (75:25 + Whey) with 4.18 and 4.24, respectively. Rodríguez et al., (2022) on the other hand, obtained a pH of 6.5 in silage with whey. Rodríguez-Oliva et al. (2022), when using a ratio of Cuba Grass (75%) + Button of gold (25%) in silage, reached a pH of 3.83. While, Gonzalez et al., (2022), reported a pH of 4.01 when using Elephant grass + Button of gold (75:25). Only in the last case do the values resemble those obtained in the current research. Therefore, whey may have an effect on the pH of the silage. According to a study, the whey content can affect the pH of silage. Tirira Pusdá (2018) y Paredes et al. (2014), state that whey provides acid-lactic microorganisms and 0.46% lactic acid, which actively participate during lactic acid fermentation as they convert sugars in the forage to lactic acid. According to Fernández et al. (2017), a silage should have a pH lower than 4.5, although ideally, it should be around 4.2. Therefore, T5 (70:30 + whey), with a pH of 4.73, is outside the acceptable range; however, this can be attributed to the type of epiphytic flora present in the material at the time of silage, its proportion, and the species, as stated by (Genero et al., 2022).

Table 3. Determination of the bromatological characteristics of Cuba 22 grass and botón de oro biomass with different proportions of additives

Treatments	Dry Matter	Protein	Crude Fiber	Ash	pH
a0b0	20,29^{b}	15,62^{b}	23,65^{e}	6,47^{b}	4,18^{a}
a0b1	21,43^{c}	15,04^{a}	24,78^{f}	6,95^{c}	4,39^{ab}
a1b0	24,14^{f}	17,96^{f}	20,45^{b}	7,54^{f}	4,24^{a}
a1b1	23,61^{e}	17,34^{d}	20,14^{a}	7,16^{d}	4,53^{ab}
a2b0	19,51^{a}	17,53^{e}	22,47^{c}	5,73^{a}	4,73^{b}
a2b1	22,95^{d}	16,64^{c}	23,27^{d}	7,51^{e}	4,48^{ab}
C.V (%)	0,04	0,07	0,05	0,14	4,74

3.7. Correlation of the variables

An analysis of the principal components was conducted, shown in figure 2, to reduce the dimensionality of the data, and 87.7% of the total variability is explained by the first two principal components (PC1: 52.7%, PC2: 35.0%). The first principal component is primarily associated with protein, dry matter, and ash, as it shows a high correlation between them. The second principal component is mainly related to crude fiber; therefore, it is negatively correlated with the variables of PC1. Protein and dry matter were strongly highlighted in observations 3 and 4, while crude fiber was more prominent in observation 5, with low levels of all other variables. This clearly segregates the samples based on their bromatological properties and indicates that these variables may be determinant in the data variability. Based on these results, it is recommended to prioritize treatments with higher protein and dry matter content (as observed in treatments 3 and 4), as these contribute to a better nutritional quality of the silage and greater efficiency in animal feeding.

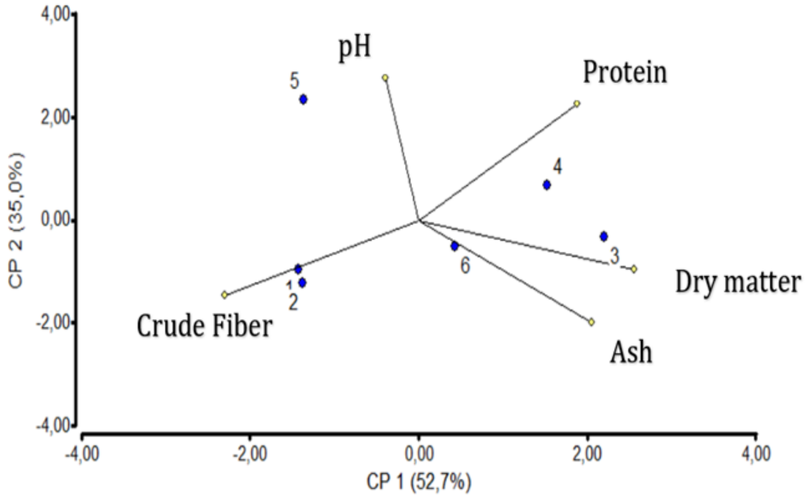

Figure 2. Principal Components

3.8. Organoleptic Analysis

Odor

Figure 3 provides a visual evaluation of the organoleptic characteristic of odor in the silage. Treatments T3 and T4 stand out with the highest ratings, achieving an excellent level in terms of the odor of the silage. With scores of 3.83 and 4, respectively, these treatments are classified as "Excellent," indicating that they produce silage with an exceptionally pleasant odor, similar to that of ripe fruit. On the other hand, treatments T1, T2, T5, and T6, while not reaching the excellence of T3 and T4, still show positive evaluations regarding the odor of the silage. T1 and T5 received a score of 3, indicating a good odor, while not exceptional. T2 and T6 scored 3.33 and 3.17, respectively.

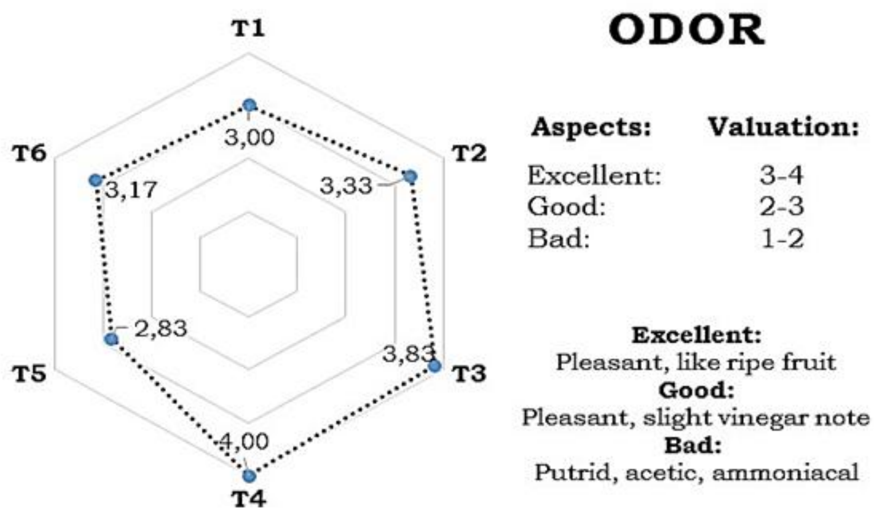

Figure 3. Organoleptic characteristic corresponding to the odor of the silage

Color

Regarding the color of the silage shown in Figure 4, treatments T2, T3, T4, and T5 stand out with high ratings, indicating that they produce silage with an excellent color, an olive-green tone. The high score obtained by these treatments suggests proper fermentation and effective preservation of nutrients. In particular, T3 and T4 received the highest scores, with values of 3.83 and 4, respectively. This indicates that they produce silage with exceptional color, suggesting excellent preservation of the nutritional components and minimal degradation during the silage process. However, although treatments T1 and T6 have slightly lower ratings with values of 2.83 and 2.67, respectively, they still show a yellowish-green color, which is considered good.

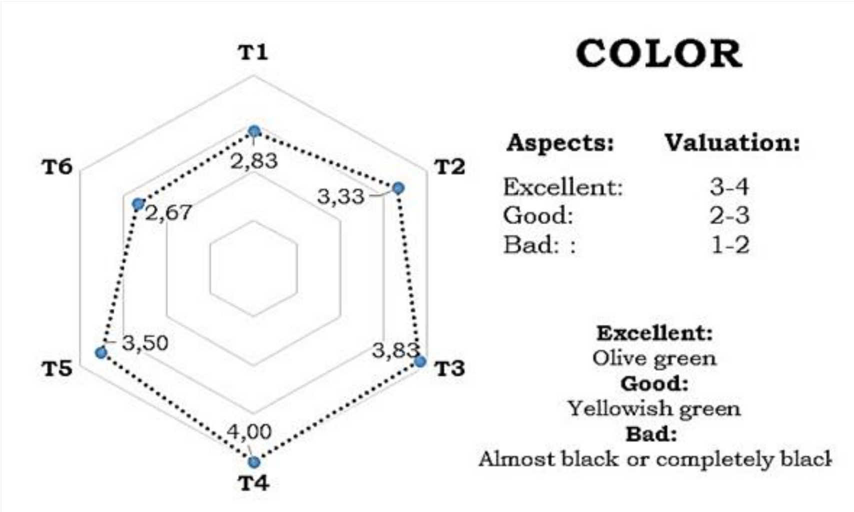

Figure 4. Organoleptic characteristic corresponding to the color of the silage

Texture

In the case of texture, shown in Figure 5, it was observed that treatments T2, T3, T4, and T5 received the highest ratings, with scores of 3.33, 3.83, 4, and 3.50, respectively. These scores reflect excellent texture in the produced silage, characterized by continuous contours and leaves firmly attached to the stem. The consistency in texture indicates good compaction and fermentation. On the other hand, treatments T1 and T6, with scores of 2.83 and 2.67, respectively, showed slightly lower ratings. Although these scores indicate good texture, it was observed that the leaves were more transparent, and the edges were less defined.

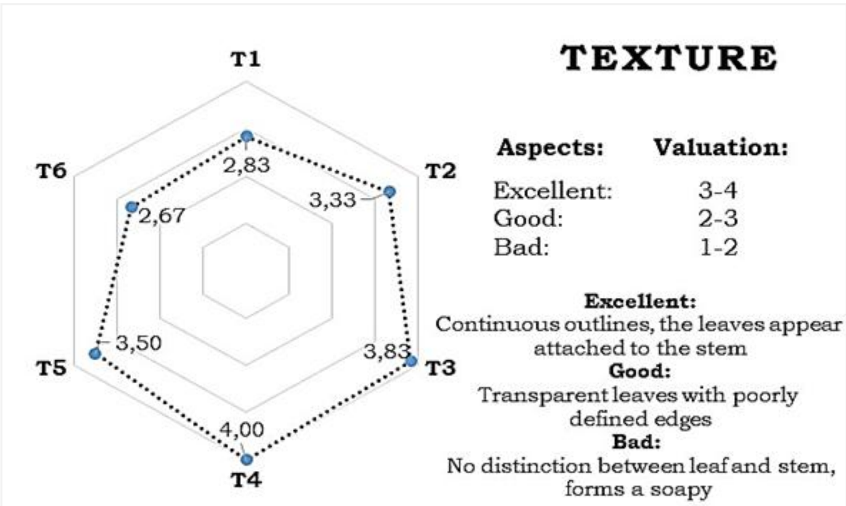

Figure 5. Organoleptic characteristic corresponding to the texture of the silage

Moisture

In the moisture results shown in Figure 6, treatments T2, T3, T4, T5, and T6 received ratings of 3.17, 3.50, 3.83, 3.17, and 3.17, respectively. The high scores indicate that the silage produced by these treatments exhibits excellent moisture management. Treatment T1, although it received a slightly lower rating compared to the other treatments, still falls within the good to excellent category.

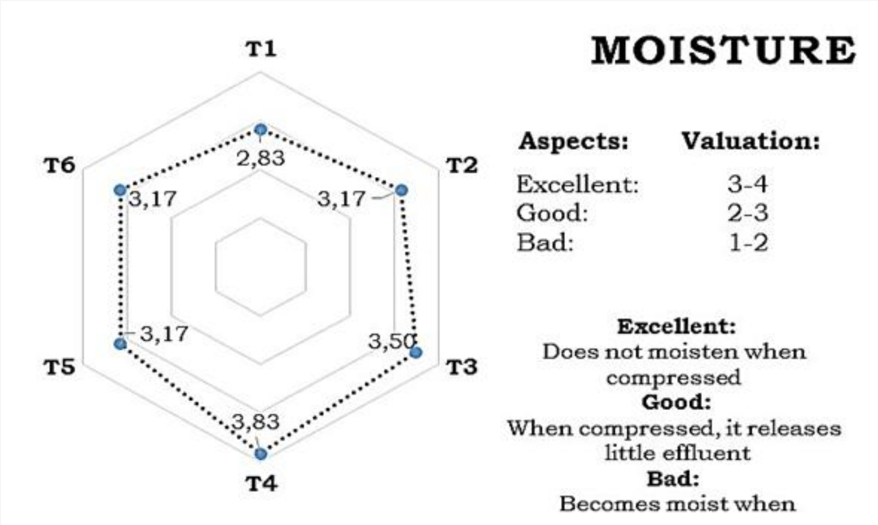

Figure 6. Organoleptic characteristic corresponding to the moisture of the silage

In general, the silage showed a good taste, texture, and appearance at all evaluated stages, demonstrating consistent quality during storage. This indicates that the silage process was effective and that the forage was well-preserved, making it suitable for animal consumption and easy to handle. The uniform quality suggests that there was no significant deterioration. These results are comparable to those obtained by Granados et al., 2014, who also achieved successful fermentation after 60 days of ensiling African star grass with molasses. According to Fernández et al., 2017, well-made silos have distinctive characteristics. The color can range from yellow, brown, or green, depending on the forage and fermentation. The odor should be pleasant and slightly sour, indicating correct fermentation. The texture should be firm and not viscous, as a viscous consistency can promote the growth of fungi.

CONCLUSIONS

The research characterized the bromatological properties of silage made from Cuba 22 grass biomass and Botón de oro in different proportions, using two additives. The results showed that the evaluated combinations caused significant variations in dry matter, crude protein, crude fiber, ash, and pH levels. Among the treatments, T3 (75% Cuba 22 + 25% Botón de oro with the addition of whey) exhibited the highest protein content and an optimal pH for preservation. Through principal component analysis, it was identified that protein, dry matter, and ash are key variables to differentiate the treatments. Complementarily, organoleptic characteristics — odor, color, texture, and moisture management — were evaluated favorably, highlighting the fermentative and nutritional quality of the silage. These findings support the strategic use of additives such as whey and Botón de oro to improve the preservation and nutritional value of the produced silage.

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CONFLICT OF INTEREST

There is no conflict of interest related to the material in the article.

AUTHORSHIP CONTRIBUTION

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