

Proximate analysis and aerobic stability of forage mixtures composed of *Cenchrus purpureus* cv. Taiwan A-146 and *Paspalum virgatum* L. ensiled under laboratory conditions

Análise aproximada e estabilidade aeróbica de misturas de forragem compostas por *Cenchrus purpureus* cv. Taiwan A-146 e *Paspalum virgatum* L. ensiladas em condições de laboratório

Análisis proximal y estabilidad aeróbica de mezclas forrajeras compuestas por *Cenchrus purpureus* cv. Taiwan A-146 y *Paspalum virgatum* L. ensiladas en condiciones de laboratório

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Juan Pablo Uzcátegui-Varela

Research Group on Animal Science and Tropical Plants. Universidad Nacional Experimental Sur del Lago "Jesús María Semprum" (UNESUR) Núcleo La Victoria

Agricultural Research Institute, Faculty of Forestry and Environmental Science. Universidad de Los Andes (ULA)

Mérida. Venezuela

E-mail: uzcateguij@unesur.edu.ve

Osmar Araque

Agricultural Research Institute, Faculty of Forestry and Environmental Science. Universidad de Los Andes (ULA)

Mérida. Venezuela

E-mail: osmarydeleon@gmail.com

Adriana Morgado-Osorio

Agricultural Research Institute, Faculty of Forestry and Environmental Science. Universidad de Los Andes (ULA)

Mérida. Venezuela

E-mail: adelcmo@gmail.com

Héctor Nava-Trujillo

Division of Animal Sciences, University of Missouri

Columbia. USA

E-mail: hectornava00@gmail.com

ABSTRACT

To evaluate the quality of silage composed of *Cenchrus purpureus* cv. Taiwan A-146 and *Paspalum virgatum* L. under laboratory conditions, both forage species were harvested south of Lake Maracaibo-Venezuela. The plant material was reduced to 2.5 cm and distributed according to the treatments (T): T₀: 100% *C. purpureus* (CPT); T₁: 100% *P. virgatum* (PV); T₂: 50% CPT + 50% PV; T₃: 75% CPT + 25% PV and T₄: 25% CPT + 75% PV; then, polyethylene bags were used to fill the silo, in which each mixture was placed, incorporating molasses at 6% of its weight as an additive; then, oxygen was extracted to seal the bags and they were kept in darkness for 30 days.

All mixtures were subjected to proximate analysis before and after storage. A completely randomized design was chosen, using Tukey's test for multiple comparisons of means. Statistical analysis revealed that CPT and fresh PV were different ($p < 0.05$) for the variables moisture, dry matter, crude protein and ash, but similar ($p > 0.05$) in etheral extract, crude fiber, neutral detergent fiber, acid detergent fiber and nitrogen free extract. After fermentation, crude protein (CP) content increased ($p < 0.05$) in CPT, while the other parameters increased without changes ($p > 0.05$), except for nitrogen free extract, which reduced its post-silage concentration, although energy increased ($p > 0.05$), since the calculated theoretical value (kcal/g) was higher in the silage but similar ($p > 0.05$) with respect to the initial fresh forage; Aerobic stability was influenced by the proportion of CPT, because the high moisture content reduced its suitability time to be consumed. It is concluded that, theoretically, under laboratory analysis conditions, the forage species evaluated represent a feasible alternative for supplementing large ruminants, since they are medium quality pastures with a better nutritional profile when ensiled.

Keywords: dry matter, fermentation, forage preservation, pH, temperature.

RESUMO

Para avaliar a qualidade da silagem composta por *Cenchrus purpureus* cv. Taiwan A-146 e *Paspalum virgatum* L. em condições de laboratório, ambas as espécies forrageiras foram colhidas ao sul do Lago Maracaibo, na Venezuela. O material vegetal foi reduzido a 2,5 cm e distribuído de acordo com os tratamentos (T): T₀: 100% *C. purpureus* (CPT); T₁: 100% *P. virgatum* (PV); T₂: 50% CPT + 50% PV; T₃: 75% CPT + 25% PV e T₄: 25% CPT + 75% PV; de uma vez, foram utilizados sacos de polietileno para enchimento do silo, no qual foi depositada cada mistura, incorporando melaço a 6% do seu peso como aditivo; o oxigênio foi então removido para selar os sacos e eles foram armazenados no escuro por 30 dias. Todas as misturas foram submetidas à análise de proximidade antes e depois do armazenamento. Foi escolhido um projeto completamente aleatório, usando o teste de Tukey para comparações múltiplas de médias. A análise estatística revelou que o CPT e o PV fresco são diferentes ($p < 0,05$) para as variáveis umidade, matéria seca, proteína bruta e cinzas, mas semelhantes ($p > 0,05$) para extrato etéreo, fibra bruta, fibra detergente neutra, fibra detergente ácida e extrato livre de nitrogênio. Após a fermentação, o teor de proteína bruta (PB) aumentou ($p < 0,05$) na CPT, enquanto os outros parâmetros aumentaram sem alterações ($p > 0,05$), exceto o extrato livre de nitrogênio, que reduziu sua concentração após a ensilagem, embora a energia tenha aumentado ($p > 0,05$), pois o valor teórico calculado (kcal/g) foi maior na silagem, mas semelhante ($p > 0,05$) em relação à forragem fresca inicial; A estabilidade aeróbica foi influenciada pela proporção de CPT, pois o alto teor de umidade reduziu seu tempo de adequação para consumo. Conclui-se que, teoricamente, em condições de análise laboratorial, as espécies forrageiras avaliadas representam uma alternativa viável para a suplementação de grandes ruminantes, pois são pastagens de média qualidade com melhor perfil nutricional quando ensiladas.

Palavras-chave: matéria seca, fermentação, preservação da forragem, pH, temperatura.

RESUMEN

Para evaluar la calidad del ensilaje compuesto por *Cenchrus purpureus* cv. Taiwán A-146 y *Paspalum virgatum* L. bajo condiciones de laboratorio, ambas especies forrajeras se cosecharon al sur del Lago de Maracaibo-Venezuela. El material vegetal se redujo a 2,5 cm y fue distribuido según los tratamientos (T): T₀: 100% *C. purpureus* (CPT); T₁: 100% *P. virgatum* (PV); T₂: 50% CPT + 50% PV; T₃: 75% CPT + 25% PV y T₄: 25% CPT + 75% PV; a continuación, se emplearon bolsas de polietileno para el llenado del silo, en las cuales, se depositó cada mezcla, incorporando melaza en 6% de su peso como aditivo; seguidamente, se extrajo el oxígeno para sellar las bolsas y fueron resguardadas en oscuridad durante 30 días. Todas las mezclas fueron sometidas a análisis proximal antes y después del almacenamiento. Se optó por un diseño completamente aleatorizado,

empleándose la prueba de Tukey para realizar comparaciones múltiples de media. El análisis estadístico reveló que CPT y PV en fresco son diferentes ($p < 0,05$) para las variables humedad, materia seca, proteína cruda y cenizas, pero semejantes ($p > 0,05$) en extracto etéreo, fibra cruda, fibra neutro detergente, fibra ácido detergente y extracto libre de nitrógeno. Superada la fermentación, el contenido de proteína cruda (PC) se incrementó ($p < 0,05$) en CPT, mientras que los otros parámetros aumentaron sin cambios ($p > 0,05$), excepto el extracto libre de nitrógeno que redujo su concentración post-ensilaje, aunque la energía aumento ($p > 0,05$), pues el valor teórico calculado (kcal/g) fue mayor en el ensilaje pero semejante ($p > 0,05$) con respecto al forraje fresco inicial; por su parte, la estabilidad aeróbica estuvo influenciada por la proporción de CPT, debido a que el alto contenido de humedad redujo su tiempo de idoneidad para consumo. Se concluye que, teóricamente, bajo condiciones de análisis en laboratorio, las especies forrajeras evaluadas representan una alternativa factible para suplementar grandes rumiantes, ya que son pastos de mediana calidad con mejor perfil nutricional al ser ensilado.

Palabras clave: materia seca, fermentación, conservación de forrajes, pH, temperatura.

1 INTRODUCTION

Animal production systems with grazing large ruminants are recognized as an indispensable bio-economic dynamic model for agrifood activities in the Latin American tropics; in this regard, in recent years, cattle and buffalo farming in these rural areas have gradually incorporated animal husbandry strategies to reduce costs and increase the sustainability of the production process, since the need to offer healthy food with minimal negative impact on the natural environment is the key to guarantee food and nutritional security (Oosting *et al.*, 2022).

In tropical livestock farming, any nutritional plan to improve and maintain the performance of cattle must start from pasture consumption as a crucial element of productivity, since they are biologically efficient animals that feed essentially on highly fibrous grasses that offer green matter in quantity and quality, but that varies significantly due to seasonal differentiation. In this sense, the practice of controlled grazing to obtain meat and milk in a profitable way, has been clearly promoted for decades to guarantee in the future, economy and environmental sustainability in agrosystems; however, costly balanced diets based on concentrate mixtures continue to be employed among livestock farmers in economically more favored countries, where the profitability of the system basically depends on feed efficiency, leaving aside the enteric emissions generated by high protein feeds (Uzcátegui-Varela *et al.*, 2022).

Currently, livestock experts comment that, although ruminants can consume low quality forage species and convert them into animal protein of high biological value, a diet based exclusively on grazing considerably restricts animal performance, but by diversifying pastures and including functional supplements based on minerals and conserved pasture, productivity indicators increase their value, which represents a feasible practical scenario for the producer to contribute to

the reduction of the environmental impact naturally caused by rumen methanogenesis (Hubbart *et al.*, 2023).

Globally, forages offered to cattle as silage remain popular among dairy farms because of minimized nutrient loss through trampling during grazing, suggesting greater utilization of the pasture resource at critical times of the year when forage supply is severely compromised; hence, silage is known as an effective management tool to conserve enriched fresh forage, ensuring a continuous supply of non-synthetic feed for animals (Piltz *et al.*, 2022). Silage is a preservation technique achieved by spontaneous lactic fermentation under anaerobic conditions, whose nutritional composition varies according to the chemical quality of the forage species selected for preservation, i.e., in the absence of air, the transformation of soluble carbohydrates produces organic acids and alcohols that create an acidic environment (pH= 4.0) that delimits microbial growth, these components being determining indicators of fermentative efficiency at the silo level; in addition, they are recognized for their rapid metabolic utilization that makes efficient use of energy, protein and fiber from grass to improve rumen function; in this sense, a combination of silage forage species generates a positive associative effect to maximize feed utilization (Kung *et al.*, 2018).

For Hahn *et al.* (2021) silage sustainably reduces the seed load generated by weeds, therefore, by minimizing genetic material through silage, it is possible to exclude weeds from the field, thus reducing their density and delaying regrowth before it can interfere with the growth of traditional pastures; in this sense, the mixture of forage species, including those with low palatability in the diet for ruminants has been proposed as a possibility for animals to access nutrients from fibrous feeds of reduced nutritional quality, in this way, they could be used more efficiently while including highly palatable forages, hence there is interest in combining plants morphologically recognized as forages (mixed silages) to reduce fermentation losses by improving the nutritional quality of the silage. On the other hand, pasture productivity depends on the conversion efficiency of atmospheric CO₂, available nutrients, solar energy and rainfall, which, under tropical rainforest conditions, are sufficient to satisfy the water demand of forage plants during most of the year, indicating adaptation and high productive potential of the agroecosystem based on tropical pastures. However, since tropical pastures are composed of grasses and legumes selected for their high forage yield, the selective consumption of plant biomass through grazing has an impact on their structural features, floristic composition and nutritional quality; therefore, grazing pressure represents the practical factor that conditions the richness of consumable or non-consumable forage species (Fraser *et al.*, 2022).

The diversity of native and introduced grasses in the humid tropics includes species that are not very palatable to animals during grazing, which favors their dominance and limits the richness

of the pasture; however, many of these plants, when harvested at a specific age and treated as conserved forages, can be an alternative for nutritional maintenance in large ruminants. Jaipolsaen *et al.* (2022) comment that sustainable livestock production is highly dependent on forage yield, an indicator well represented by *Cenchrus purpureus* cv. Taiwan A-146 formerly, *Pennisetum purpureum*, a fast-growing grass with high biomass production, acceptable nutritional quality, undemanding and used as fresh forage in tropical livestock; it is a perennial, invasive, rhizomatous, robust grass, commonly used for cutting because of its outstanding palatability between barns, confinement systems and as a reserve for livestock supplementation. Although *C. purpureus* has a high moisture content at the time of cutting, few soluble carbohydrates and great buffering capacity can inhibit the fermentative process; therefore, Piltz *et al.* (2022) consider that appropriate forage conservation practices are necessary to guarantee the required volume, reduce loss and minimize silage deterioration in favor of rumen health.

The cespitose grass *Paspalum virgatum* commonly known as "cabezona" or "razor grass" is considered a non-palatable species for cattle in tropical pastures, although there is evidence in the field that confirms its use prior to flowering, because as the days pass, its leaves become rough with very sharp edges, which generates rejection during grazing; therefore, it is possible to consider that forage conservation practices that include *P. virgatum*, could be a way to partially control this weeds in the pasture (Labegalini *et al.*, 2023). In practice Bureenok *et al.* (2012) recall that, in some tropical forage grasses, dry matter (DM) content and water soluble carbohydrate (WSC) concentration are too low to obtain excellent silage, hence molasses is an energy ingredient widely used as an additive to improve the silage process due to its concentration of easily fermentable carbohydrates. Fahmi *et al.* (2019) and Rambau *et al.* (2022) proved that by including molasses in *C. purpureus* silage, a product of good physical and chemical quality is obtained, highlighting that the respiration and proteolysis process are the biochemical events that determine the real nutritional value of the silage.

In this sense Wróbel *et al.* (2023) documented that, in order to obtain quality silage, it is necessary to take care of aerobic stability, since losses during the fermentation process can reduce DM recovery, since oxygen penetration tends to increase on the silo surface, causing significant oxidation on the organic substrate and, consequently, a reduction in dry content, i.e., aerobic damage represents the main process that reduces silage efficiency. The palatability of *C. purpureus* due to its physical and chemical qualities may allow *P. virgatum* to be used as silage, a management initiative to improve the utilization of forage biomass on the farm; but, since evaluations on silage of grasses little consumed during grazing are scarce, the elaboration of silos in the laboratory represents the first analytical step to ensure that the original nutrients express their bioavailability during the conservation period. The objective of the trial was to evaluate the nutritional composition,

aerobic stability and organoleptic characteristics of microsilos made from experimental mixtures of *C. purpureus* and *P. virgatum* using molasses as fermentative substrate to balance nutrient supply.

2 MATERIALS AND METHODS

2.1 SILAGE PREPARATION

An area cultivated with *Cenchrus purpureus* grass with 32 days of regrowth was identified, as well as a pasture for rotational grazing of dual-purpose cattle composed mainly of *Paspalum virgatum* with 18 days (d) of regrowth after the last cutting, both established the farm San Pedro, an agricultural production unit located on clay loam soils of the entisol and inceptisol order, typical of the tropical rainforest in the southern zone of Lake Maracaibo, Venezuela between coordinates 08° 47' 37.3''N; 71° 37' 6.2'' W, mean temperature 34.2 degrees Celsius (°C), 76% relative humidity (RH) and 46 meters above sea level (m.a.s.l.) according to the report generated by a GPS (GARMIN® Etrex 10 Lcd. 2.2-USA) and a weather station (Celestron®-USA) installed on the farm. Following the methodological recommendations of Fahmi *et al.* (2019) and Kara *et al.* (2021) both grasses were harvested early in the day (7:00 h) making the cut from 10 centimeters (cm) from the ground; the forage biomass was transferred in independent nylon bags per forage species with a capacity of 40 kilograms (kg) and deposited on a concrete surface to be subjected to a change in feed size, this consisted of reducing the plant material to 2.5 cm particles using a DOMOSA-Venezuela 1.5 HP Model 22-GP-100 220W electric grass chopper. Twenty 1 kg transparent polyethylene bags were placed in which 230 grams (g) of each experimental mixture (EM) to be ensiled were introduced according to the treatments (T): T₀: 100% *C. purpureus* (CPT); T₁: 100% *P. virgatum* (PV); T₂: 50% CPT + 50% PV; T₃: 75% CPT + 25% PV and T₄: 25% CPT + 75% PV. The weight of the ensiling bags was recorded before the trial, immediately, taking care not to puncture the bag, the plant material corresponding to each treatment was manually compressed and, with the help of a domestic vacuum cleaner EQP10 1600W Electrolux®-Brazil, the air was extracted to avoid undesirable biochemical reactions, they were also sealed with adhesive tape to guarantee the anaerobic environment and reweighed. To ensure good preservation of the silage, molasses was added to each package with its respective EM at 6% of its weight (13.8 milliliters (mL)) as an energy source necessary for the effective colonization of lactic acid bacteria, as demonstrated by Fahmi *et al.* (2019) and Wahyudi *et al.* (2019). To conclude, the sealed bags were stored for 30 d according to the work on *Cenchrus* sp. silage developed by Rahman *et al.* (2021) inside an extraction hood guaranteeing darkness in the laboratory of crop ecophysiology, Faculty of Forestry and Environmental Sciences, Universidad de Los Andes (ULA), Mérida-Venezuela (08° 37' 23' 23''N; 71° 08' 20''W) with average temperature of 24.4 °C and 59% RH.

2.2 NUTRITIONAL QUALITY OF FORAGE

A sample of 500 g was taken in each EM at the beginning of the trial and post-conservation from the center of the forage mixture to be subjected to chemical analysis and to determine the value of various parameters associated with the nutritional quality of the forage in the *in vitro* crops laboratory of the Faculty of Forestry and Environmental Sciences-ULA (08° 37' 22.7''N; 71° 08' 23.3''W). For the bromatological analysis, the samples were dehydrated at 65°C for 48 hours (h) in a 30 liters (L) capacity Globe®-USA ventilated oven, then the already dehydrated substrate was subjected to a DOMOSA®-Venezuela Willey mill using a 1 millimeter (mm) sieve following the technical recommendations of Soe-Htet *et al.* (2021). Analytical determinations were carried out according to the protocols described by the Association of Official Analytical Chemists (AOAC) and also detailed in the reports of Latimer (2023), which specify the calculation of moisture (% H) and dry matter (% DM) through the difference between fresh and dry weight, crude protein (% CP) following the Kjeldahl method, ether extract (% EE) using ether as solvent for the extraction of free lipids and crude fiber (% CF) as detailed by the AOAC; with respect to neutral detergent fiber (% NDF) and acid detergent fiber (% ADF), these were quantified following the generic protocol of Van Soest *et al.* (1991) and ash (%) by combustion of the samples at 550 °C for 2 h in a Thermo Scientific®-USA muffle furnace. Soluble carbohydrates or nitrogen free extract (% NFE) was calculated using the formula: $\%DM - (\%ASH + \%CP + \%EE + \%CF)$ shared by Abd El-Wahab *et al.* (2022); while the gross energy content (GE) in feeds, was found theoretically as documented by Velayudhan *et al.* (2015) taking into account the ratio of 4.16 kilocalories (kcal)/g for carbohydrates, 9.51 kcal/g in lipids and 5.64 kcal/g in protein supply.

2.3 SILAGE pH AND AEROBIC STABILITY

To measure the acidity of fresh and ensiled plant material, an aqueous extract was prepared with 30 g of the forage and 250 mL of distilled water macerating for 4 minutes (min); then, the resulting compound was filtered through Fisherbrand®-USA filter paper obtaining a homogeneous solution to which pH was measured with a Sper Scientific®-USA portable potentiometer (Da Silva *et al.*, 2018). After the fermentation stage (30 d), the bags were opened and a minimal portion of the forage in poor condition was quickly discarded; from this moment on, the ensiled forage came into contact with oxygen and initiated the natural oxidative process of the sugars, which favored the degradation of the lactic acid of the ferment, causing deterioration in the silage and, with it, a negative impact on its nutritional quality. For Ferrero *et al.* (2021) aerobic stability (AS) refers to the number of hours that silage remains stable before its internal temperature increases 2 °C above ambient; To monitor the AS of the silage studied, it was considered to follow the practical principles

of the protocol adopted by Araújo *et al.* (2022) which consisted of measuring the temperature at 1 h intervals for 120 h or until aerobic stability was broken with a standard laboratory digital thermometer Traceable®-France, which was inserted into the ensiled mass; likewise, during the stability test, the pH was recorded every 6 h until 102 h of exposure using a portable pH meter with a Milwaukee®-USA brand insertion probe, considering an ideal reference value of 3.8 when opening the silos and >4.5 as an indicator of deterioration based on the arguments presented by Kung *et al.* (2018).

2.4 ORGANOLEPTIC CHARACTERISTICS OF SILAGE

Since molasses was used as an additive, Xie *et al.* (2021) comment that in the practice of preserving forages this factor does not influence animal performance, but favors the fermentation process, inhibiting the appearance of undesirable microorganisms and increasing its palatability; in this sense, color, odor and texture are considered by Kung *et al.* (2018) the three most important attributes to evaluate by direct observation the quality of the silage immediately after opening the bags. Qualitative assessments were taken into account according to the recording instrument and weighted scale adopted by Fahmi *et al.* (2019) including moisture and presence of fungi (mold) in the assessment, as moldy silage is considered a source of mycotoxins that compromise animal health.

2.5 EXPERIMENTAL DESIGN

The information gathered in each experience was analyzed using a completely randomized design with five treatments and four replications. The normality of the data distribution in all the studied variables was verified using the Shapiro-Wilk test, and the homogeneity of variance was assessed using Levene's test. Significant differences observed between treatments were calculated through multiple mean comparisons using Tukey's test ($p < 0.05$), employing the Statistical Analysis Software (2012).

3 RESULTS AND DISCUSSION

3.1 PROXIMAL COMPOSITION

Analytical experimentation protocols focused on nutrition and animal feeding available in specialized literature begin by recognizing the nutritional demands based on species and physiological state. In this regard, determining the nutrients that make up each diet or its ingredients is essential for planning timely supplementation strategies. Table 1 summarizes values derived from the proximate analysis conducted on mixtures of fresh and silage forage composed of *C. purpureus*

(CPT) and *P. virgatum* (PV). The results obtained for each parameter indicate that the CPT grass harvested at 32 days has a high moisture concentration (84.27%), low protein content (8.88%), and a high percentage of crude fiber (31.02%). This suggests that it is an ideal forage grass for maintaining ruminants, albeit with moderate nutritional value, as reported by Holguín *et al.* (2020), Botero-Londoño *et al.* (2021) and Okukenu *et al.* (2021), who conducted bromatological assessments of *C. purpureus* in their studies on forage yield, *in vitro* ruminal digestibility, and silage potential; however, they emphasize that, in the fermentative dynamics of *Pennisetum purpureum*, moisture content is crucial for its preservation. The acceptable range should be between 66% and 72% at most; therefore, the moisture content determined for CPT is excessive. This can affect the efficiency of the fermentation process, increase losses due to drainage (nutrient loss), and even promote the growth of pathogenic microorganisms such as *Clostridium* spp. and mycotoxins. This implies that *C. purpureus*, when harvested for silage, has a short shelf life, and its ideal preservation time to ensure better nutritional characteristics for animals should be planned for 21 to 30 days, as proposed by Fahmi *et al.* (2019) and Rahman *et al.* (2021).

Table 1. Proximal analysis of forage mixtures composed of *C. purpureus* and *P. virgatum* in their fresh presentation and post-silage at 30 days.

Parameter	Experimental forage mixture									
	Fresh					Silage				
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₀	T ₁	T ₂	T ₃	T ₄
H (%)	84.27 ± 0.24 ^a	75.89 ± 0.16 ^b	79.16 ± 0.12 ^{ab}	80.88 ± 0.08 ^b	73.55 ± 0.13 ^b	82.19 ± 0.19 ^a	75.92 ± 0.19 ^b	78.50 ± 0.15 ^{ab}	79.43 ± 0.18 ^b	78.46 ± 0.11 ^b
DM (%)	15.73 ± 0.21 ^a	24.11 ± 0.19 ^b	20.84 ± 0.10 ^{ab}	19.12 ± 0.10 ^b	26.45 ± 0.11 ^b	17.81 ± 0.17 ^a	24.08 ± 0.17 ^b	21.50 ± 0.12 ^{ab}	20.57 ± 0.20 ^b	21.54 ± 0.10 ^b
CP (%)	8.88 ± 0.11 ^a	12.45 ± 0.05 ^b	10.75 ± 0.15 ^b	10.91 ± 0.17 ^b	11.57 ± 0.12 ^b	11.71 ± 0.85 ^b	12.84 ± 0.84 ^b	12.28 ± 0.83 ^b	12.43 ± 0.13 ^b	12.00 ± 0.04 ^b
EE (%)	1.91 ± 0.04 ^a	1.88 ± 0.06 ^a	1.89 ± 0.12 ^a	1.90 ± 0.21 ^a	1.87 ± 0.09 ^a	2.21 ± 0.16 ^a	2.10 ± 0.14 ^a	2.18 ± 0.12 ^a	2.34 ± 0.15 ^a	2.12 ± 0.32 ^a
ASH (%)	10.77 ± 0.75 ^a	7.67 ± 0.44 ^b	9.81 ± 0.17 ^a	10.75 ± 0.16 ^a	8.70 ± 0.20 ^b	10.84 ± 0.18 ^a	7.76 ± 0.19 ^b	10.55 ± 0.09 ^a	10.83 ± 0.14 ^a	9.61 ± 0.11 ^b
CF (%)	31.02 ± 0.29 ^a	35.69 ± 0.09 ^a	3.44 ± 0.11 ^a	32.19 ± 0.19 ^a	34.52 ± 0.10 ^a	31.48 ± 0.18 ^a	35.77 ± 0.17 ^a	33.63 ± 0.36 ^a	32.55 ± 0.88 ^a	34.70 ± 0.98 ^a
NDF (%)	56.41 ± 0.19 ^a	56.26 ± 0.14 ^a	56.35 ± 0.37 ^a	56.38 ± 0.16 ^a	56.30 ± 0.36 ^a	58.94 ± 0.11 ^a	57.32 ± 0.37 ^a	58.17 ± 0.33 ^a	58.34 ± 0.75 ^a	57.73 ± 0.8 ^a
ADF (%)	32.43 ± 0.07 ^a	30.23 ± 0.03 ^a	31.33 ± 0.33 ^a	31.88 ± 0.42 ^a	30.78 ± 0.94 ^a	32.76 ± 0.27 ^a	30.62 ± 0.38 ^a	31.60 ± 0.30 ^a	32.07 ± 0.72 ^a	31.11 ± 0.87 ^a
NFE (%)	47.42 ± 0.27 ^a	42.31 ± 0.32 ^a	44.11 ± 0.19 ^a	44.25 ± 0.10 ^a	43.34 ± 0.24 ^a	43.76 ± 0.26 ^a	41.53 ± 0.12 ^a	42.86 ± 0.16 ^a	41.85 ± 0.76 ^a	41.19 ± 0.32 ^a
Energy (kcal de EB/g)	3.90 ± 0.18 ^a	4.13 ± 0.08 ^a	4.01 ± 0.13 ^a	3.98 ± 0.20 ^a	4.07 ± 0.19 ^a	3.93 ± 0.02 ^a	4.08 ± 0.03 ^a	4.08 ± 0.01 ^a	4.15 ± 0.03 ^a	4.04 ± 0.03 ^a

*The values with different superscripts within the same row differ significantly from each other ($p < 0.05$).

Source: Prepared by the authors

Regarding crude protein (CP) intake, the cut grass *C. purpureus* recorded a percentage value that is considered acceptable, referring to Carrasco & Drewery (2024) who pointed out that a minimum of 7% CP in the diet (pasture) guarantees the biological dynamics of ruminal bacteria without negatively impacting forage intake and digestibility. The proportion of ether extract (EE) was quantified at 1.91% for *C. purpureus*, aligning with the data published by Queiroz *et al.* (2020) and Silveira *et al.* (2020) concerning samples of *Pennisetum* spp. The EE in forage grasses has a heterogeneous composition, primarily consisting of lipids and other non-polar compounds like vitamins and pigments, which vary according to the phenological state of the cultivar (Barbosa *et al.*, 2017). However, the value determined here conforms to the general average described in most practical experiences with *C. purpureus*, which is quite different from the 5% threshold contraindicated for ruminants.

The ash parameter is perhaps the most variable when evaluating grass varieties for nutritional purposes, due to the ability of forage grasses to absorb nutrients from the soil. For CPT, the laboratory value obtained (10.77%) shows little variation compared to findings by Weerathunga *et al.* (2023), who assessed elephant grass (*Pennisetum purpureum*) and its hybrids as an important forage resource in pasture-based livestock systems and they concluded that forage samples collected in the tropics have high ash levels (>10%). Another indicator evaluated was crude fiber (CF), a structural component of the plant cell wall formed by molecular interactions of microfibrils corresponding to cellulose, hemicellulose, and lignin, which influence feed digestibility; in this sense, the determined FC content exceeds 20%, which is in agreement with the findings of Santika *et al.* (2023) in Taiwan grass (*P. purpureum*) harvested at different ages, reporting an average par FC of $22.73 \pm 1.66\%$ FC. This categorizes *C. purpureus* as a highly fibrous tropical forage that, when chopped and ensiled, enhances nutrient consumption and digestibility, making it a viable part of a sustainable feeding plan for cattle due to its biochemical advantages during the fermentation process, all corroborated by Pratama *et al.* (2021).

Carrillo-Díaz *et al.* (2022) note that when determining the crude fiber content, the portion of digestible fiber is not taken into account, despite being a nutritionally valuable fraction for the animal as a source of metabolizable energy. Additionally, it serves as a substrate that ruminal bacteria utilize to produce acetate. Neutral detergent fiber (NDF) is an accurate measure of material that is digested slowly; it is also inversely related to voluntary feed intake. When analyzing the *C. purpureus* sample, the percentage (56.41%) obtained of NDF is very close to the range (60-65%) suggested as a critical limit above which the metabolic efficiency of a tropical forage would be affected, as considered by Okukenu *et al.* (2021). Acid detergent fiber (ADF) corresponds to cellulose and lignin, the first is a structural polysaccharide of the plant cell formed by glucose

subunits and the other represents the determining phenolic polymer to reduce the efficiency indicators during enzymatic hydrolysis during digestion, which conditions the availability of nutrients in feed and accelerates rumen filling, negatively correlating with consumption (Teixeira *et al.*, 2023); meanwhile, the ADF obtained in *C. purpureus* (32.43%) coincided with the analysis reports presented by Feng *et al.* (2023).

Much of the field evaluations with *C. purpureus* focus on studying the effects of age, cutting frequency, and agronomic management of the grass on nutrient bioavailability, reporting values for NDF and ADF that are higher than those presented here, this indicates that tropical grasses grow and mature rapidly, leading to significant changes in their chemical composition and a decrease in nutritional quality as demonstrated by Dios-León *et al.* (2022). Regarding non-structural carbohydrates, Rahman *et al.* (2023) consider them the main component of animal diets, recommending that they should make up between 40% and 70% of the total dry matter, as they represent the primary immediate energy source needed for body development. In the current study, the nitrogen free extract (NFE) was quantified for *C. purpureus* at 47.42%, a figure very close to the 42.96% calculated by Jagadeesh *et al.* (2017) and the 42.20% obtained in the study by Umami *et al.* (2023) while analyzing a local cultivar of *Pennisetum purpureum* in Indonesia under environmental conditions similar to those of the American tropics. For its part, the gross energy (GE) of the grass was theoretically estimated at 3.90 kcal of GE/g, a result similar to the caloric contribution quantified by Srichana *et al.* (2022) in *P. purpureum* when they reported 3.89 and 3.78 kcal of GE in their order, also highlighting the efficiency of this forage at the field level to transform light energy into glucose, while presenting a high biomass production.

The other forage component studied was *Paspalum virgatum*, a perennial subtropical grass that, when harvested, showed a set of tillers at different stages of growth on the plant, that is, various shoots at variable ages of vegetative development that, in particular, rapidly invade the areas where cattle graze, an ecological aspect that can be used to associate its level of infestation-colonization with the degree of degradation in tropical pastures, a valid approach based on the criteria set forth by Marques *et al.* (2019). From a nutritional point of view, its moisture content (75.89%), dry matter (24.11%), crude protein (12.45%) and ash (7.67%) differ significantly ($p < 0.05$) when compared with the analytical determinations made to *C. purpureus*. It is known that the DM content influences the efficiency of the fermentation process when ensiling any forage and, although the optimal proportion ranges between 30% and 40%. Khota *et al.* (2018) concluded that when forage grasses at the time of cutting have a minimum value of 21% DM, it is suitable for ensiling, which gives *P. virgatum* favorable characteristics during an eventual conservation of more than 30 days, a scenario

that is not viable for *C. purpureus* without prior dehydration, since it would increase the proximity of aerobic instability.

Regarding the crude protein levels, the results align with reports by Jiang *et al.* (2022), who indicated that most *Paspalum* spp. hybrids contain between 12% and 15% crude protein. This range corresponds with the findings obtained and significantly differs ($p < 0.05$) when compared to the CP recorded for *C. purpureus*, which clearly meets the basic metabolic requirements for calves and lactating cows. However, recent evaluations have established that *P. virgatum*, grazed every 21 days, ensures its. The ash content was similar to the average (7.87%) established by Salamanca-Carreño *et al.* (2022) for *Paspalum* sp., indicating that it falls within the expected range for grasses commonly used in animal feed; additionally, variations in mineral content between pastures are due to specific biological characteristics of the soil, the age of the plants and the physiological adaptation mechanisms that are constantly active in the plant.

The EE, CF, NDF, ADF, NFE and GE parameters found in *P. virgatum* were statistically similar ($p > 0.05$) to those determined in *C. purpureus*, so both grasses are considered energetic forage grasses with good biological characteristics, including high biomass yield, adequate adaptability and strong regeneration capacity; although the availability of nutritional data on *P. virgatum* is very scarce due to the limited interest in studying the species, the results allow us to consider that from a morphological point of view *C. purpureus* and *P. virgatum* have a structure composed mainly of cellulose, covered by a moderate network of lignin and hemicellulose. In addition, they are C4 plants with the ability to make the most of solar radiation, which has an impact on maintaining an important photosynthetic rate that is reflected in a high capacity to recover sugars, an approach that was presented by Sinche *et al.* (2021). The results presented so far coincide with the literature, indicating that tropical grasses have an accelerated growth and maturation rate, which generates changes in their chemical composition.

The results from the proximal analysis conducted at the beginning of the trial on each experimental mixture identified as T₂, T₃, and T₄, in which *C. purpureus* and *P. virgatum* were distributed in different proportions, revealed a significant difference ($p < 0.05$) concerning some nutritional variables of interest. The moisture and dry matter factors of the forage corresponding to the mixture composed equally of the studied grasses (T₂) were statistically similar ($p > 0.05$) to the values individually obtained for *C. purpureus* and *P. virgatum*. Nevertheless, the mixtures prepared for ensiling in T₃ and T₄ were equal ($p > 0.05$) to each other and in comparison to T₁, but differed ($p < 0.05$) from T₀, indicating that the ensiled mixtures transitioned from a fully partitioned state to one that was ideally mixed.

The additive effect between the DM and humidity components was equal to the average inclusion proportionally distributed by each mixture, in this case, corresponding to each forage species used to be evaluated, a principle conceptually defined as practical mixing by Brandani *et al.* (2013). However, once the conservation stage was over, when determining DM and humidity, the calculation showed higher figures for DM and lower figures for humidity ($p>0.05$) with respect to the beginning in T_0 and T_3 , these treatments where *C. purpureus*, the wettest grass, dominated the silage mixture, a physical-chemical response that suggests a quantifiable loss of effluents that begins with the mechanical transformation of the forage, which is why Wróbel *et al.* (2023) assure that pastures with significant portions of moisture require increasing the DM content by wilting or partial dehydration, since the microbiological risk conditions the quality of the preserved material; on the other hand, T_1 , T_2 and T_4 , although without significant effect ($p>0.05$) when compared between fresh and silage, there was an increase in DM and a reduction in humidity in the mixtures where *P. virgatum* apparently exerted a buffering or regulating effect on these parameters, since containing percentages higher than 20% of dry matter favors the obtaining of stable silage feed, a phenomenon that, even naturally, is observed in tropical pastures with the dynamism that *P. virgatum* physiologically demonstrates with adaptability while recovering from water stress or flooding, as justified by Laurindo-Silva *et al.* (2019).

When the dietary supplementation strategy for ruminants consists of offering preserved forages, it is essential to carry out a critical evaluation of the nutrient content, especially the protein contribution; in this regard, after 30 days of the trial, the percentage of CP ($>11.71\%$) increased in all treatments, only with a statistically significant effect ($p<0.05$) compared to the protein load of T_0 in fresh or pre-silage; in all cases, an adequate level of degradable protein persisted as recommended by Xia *et al.* (2018) with this, the concentration of ammonia (NH_3) in the ruminal broth can be reduced and thus offer a favorable metabolic environment for microbial growth and endogenous protein synthesis. Teles-Rêgo *et al.* (2010) concluded that, in principle, the addition of molasses contributes to maintaining the protein level of the ensiled material because the endogenous lactic acid bacteria (LAB) present in plant tissues convert fermentable carbohydrates into organic acids ($\text{C}_3\text{H}_6\text{O}_3$) that make the silage reach early stability ($\text{pH}\leq 4.5$), consequently, the proteolytic activity generating NH_3 is inhibited and the bacteria present in the substrate, mostly of a protein nature, do not have the opportunity to deteriorate the ensiled mixture, with microorganisms becoming part of the medium; likewise, Rambau *et al.* (2022) assure that when the pH of the silage decreases to 4.3 or less, proteolytic activity is reduced, which indicates adequate fermentation thanks to the LAB that use as an energy source, the carbohydrate with the greatest metabolic use (molasses), creating an acidic medium that limits microbiological activity.

The post-fermentation forage mixtures showed a slight increase in the lipid and mineral portion without significant effect ($p>0.05$) with respect to their pre-silage composition. The data obtained coincide with those discussed by Rahman *et al.* (2021) who obtained an acceptable response by reporting an EE range between 2.1% and 5.8%; in addition, Prado *et al.* (2023) report that well-preserved silages have EE levels very similar to the value of the forage material harvested in the field; likewise, the authors agree when they mention an increase to a certain extent during ensiling of the ash without risk of causing a nutritional imbalance. Regarding the fibrous contribution (CF, NDF and ADF), the data collected from the proximate analysis does not show a statistically significant difference ($p>0.05$) for any of these variables in the preserved mixtures, therefore, there is no effect of the fermentation process that alters the fiber in the evaluated pastures; the reason is that plant cells have complex links in their structure that are difficult to degrade by microorganisms that gradually naturally colonize the silage, evidence that was also shared by Khota *et al.* (2018).

The estimated content of simple carbohydrates in the ensiled mixtures was between 41.19-43.76%, being a smaller scale than that considered at the beginning of the trial but compatible with the results tabulated by Jagadeesh *et al.* (2017) and Rahman *et al.* (2023), however, in the experience of Baba *et al.* (2018), although they foresee an increase in NFE and a reduction in the CF component caused by hydrolysis of hemicellulose, in this case a considerable increase in CP was observed in the ensiled mixtures after 30 d, hence, it is possible to notice that the additional increase displaced part of the fraction that corresponds to NFE and therefore there is a reduction in its contribution without representing a significant statistical difference ($p>0.05$) on the NFE in the forages evaluated. Baba *et al.* (2018) also highlight that molasses improves the fermentation process thanks to the available soluble carbohydrates and, therefore, the energy load of the ensiled feed; in this case, the minimal reduction or increase in energy that was recorded in the mixtures did not represent a significant energy change ($p>0.05$) of the preserved forage material. This difference is regularly associated with biological factors inherent to each species, since Khota *et al.* (2016) and Rahman *et al.* (2023) recall that the nutritional values of ensiled plants are significantly altered with the maturity of the plant, additives incorporated into the silo and even agroecological variations specific to the sector where it was harvested; even so, the variables derived from the proximal analysis can hardly accurately show the complex process of biochemical transformations that occurs during ensiling.

3.2 pH AND AEROBIC STABILITY (AS) TEST IN ENSILED MIXTURES

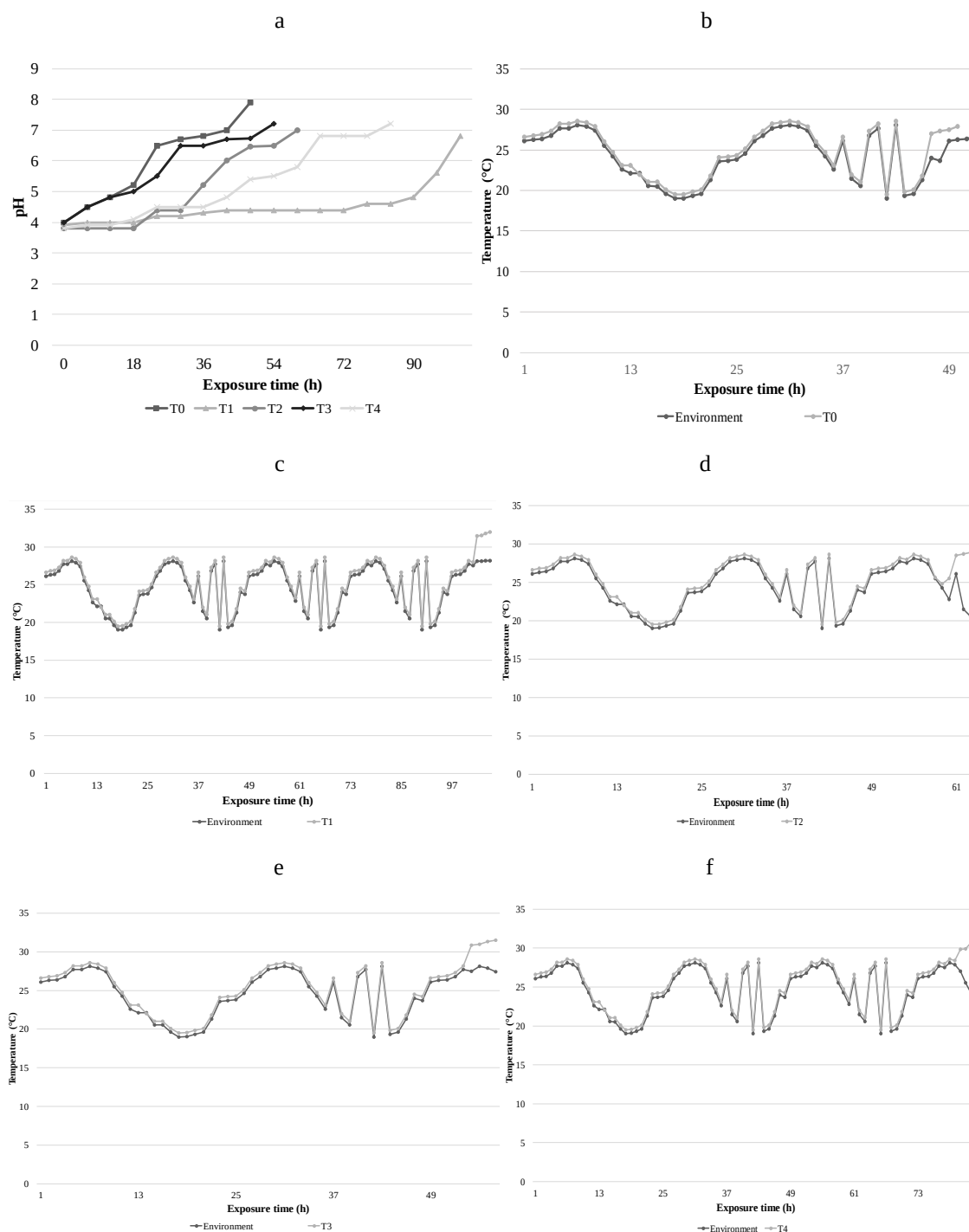
The pH is one of the key variables for estimating silage quality, since it is directly correlated with the regulation of microbial growth associated with spoilage. The average initial fresh pH in *C.*

purpureus and *P. virgatum* was 6.0 ± 0.14 , a value compatible with the range pH=5.5 to 6.5 considered optimal by Ren *et al.* (2022).

Figure 1(a) illustrates the pH behavior in each experimental mixture after opening the microsilos, in this regard, the potentiometer emitted a data ratio that averaged a pH= 3.9 ± 0.1 without statistically significant difference between treatments ($p > 0.05$), resulting in a medium with a high degree of acidity. On the other hand, the addition of molasses during silage decreased the pH value and favored the production of lactic acid, a reaction compatible with the experiences of Msimbira & Smith (2020) who used a referential of pH= 3.8-4.0 as an ideal mean for silages with tropical grasses. The graph shows that pH increases as the time of exposure to the natural environment elapses, regardless of the treatment; in this regard, Wang *et al.* (2017) consider that silages with pH greater than 4.8 are poor quality food substrates; in this case, during the trial, treatments T₀, T₂ and T₃ were equal ($p > 0.05$) when reaching a pH > 4.8 at 12, 24 and 18 h, respectively, while T₁ registered the undesired pH after 84 h, differing statistically ($p < 0.05$) from the other experimental mixtures and, for T₄, the statistical analysis showed that the hours elapsed from the opening of the silage until exceeding pH=4.8 was equal ($p > 0.05$) with respect to T₂, but different ($p < 0.05$) from T₀, T₁ and T₃; therefore, if silage pH is influenced by the lactic acid generated by the bacteria that proliferate during the anaerobic stage, an increase in pH is for Kung *et al.* (2018) the reflection of clostridial metabolism, which is based on transforming lactic acid to a weaker organic acid such as butyric acid (C₄H₈O₂), which generates the accelerated deterioration of silage, a phenomenon observable in mixed silos made with C4 grasses and DM less than 30%, so that the instability of silage exposed to air is determined by the colonization of microorganisms capable of metabolizing previously formed organic acids and residual sugars, which causes an increase in pH and temperature that conditions its aerobic stability (AS).

Figure 1(b-f) shows graphically the influence of time (h) from the exposure of the ensiled mixtures to air, until the preserved forage exceeded its own temperature with respect to the environment by 2°C. During the first evaluation phase, all experimental units presented a good fermentative profile with constant aerobic stability, observing that the substrate temperature decreased during the first 24 h, a common dynamic among the investigations cited by Nurjanah *et al.* (2023). However, at 46 h after opening the microsilos, the temperature at T₀ was higher than ambient temperature and, at 48 h, the remaining treatments recorded internal temperatures close to ambient, as occurred to Figueredo *et al.* (2020).

Figure 1. Behavior of the pH variables of silage, ambient temperature, and internal temperature of the ensiled forage mixture when opening the bags until aerobic stability is broken. a. Acidity of the ensiled mixtures and exposure time to the environment. b-f. Internal temperature of the evaluated silages until aerobic stability is lost.



Source: Prepared by the authors themselves.

The statistical test revealed that there was a significant effect ($p < 0.05$) of the time factor on the treatments to reach aerobic deterioration, possibly due to the low buffering capacity of the grasses to buffer the pH increase, which promotes the establishment of pathogenic colonies demanding oxygen, generating metabolic heat that increases the internal temperature of the

substrate, increases protein hydrolysis and thus reduces the sustainable quality of the fermentative process; in this case, the silage composed entirely of *P. virgatum* (T₁) required about 120 h to break the AS, while T₀, T₂, T₃ and T₄ were equal ($p>0.05$) to each other because they required between 46 and 80 h to show signs of aerobic detriment; a similar situation was commented by Andrade *et al.* (2023) in assuring that silages made with tropical grasses, especially employing *P. purpureum*, the high moisture content is a biological characteristic in this forage species that accelerates aerobic damage immediately upon opening the bags, this probably due to the rapid metabolism that in very humid environments, yeasts carry out during the first 36-45 h exposed to oxygen; therefore, based on the elements of the present study, it is inferred that when ensiling *C. purpureus* without previous dehydration for 30 d, the forage should be consumed by the animals within 46 h after feeding, while the utilization time of *P. virgatum* is longer when it is ensiled individually or its proportion is greater in the mixture, but it is fundamental to take into account that the biochemical characteristics of *C. purpureus* are determinant to evaluate the quality of the silage.

3.3 ORGANOLEPTIC EVALUATION OF SILAGE FORAGE MIXTURES

Table 2 groups the parameters considered by Fahmi *et al.* (2019) to evaluate the quality of the silage according to the organoleptic characteristics identified when the microsilos were opened. During fermentation (anaerobic time), no unpleasant aromas were detected; on the contrary, the persistent odor was slightly vinegary, typical of canned vegetables, due to the presence of lactic acid and the very volatile acetic acid, whose intensity increased as the days passed without being perceived as sweet or fruity, a premise that according to Kung *et al.* (2018) and Kara *et al.* (2021) indicates minimal concentrations of ethanol, which when generated in high quantities, is the main sign of pathogenic microbial activity in alcoholic fermentations; in addition, when molasses was used as an additive, the conversion of soluble carbohydrates into organic acids originated the acid odor until 30 d, adhering to the basic criteria of good quality considered by Umami *et al.* (2023).

Table 2. Organoleptic characteristics of various forage mixtures ensiled for 30 d composed of *C. purpureus* and *P. virgatum* under experimental laboratory conditions.

Treatment	Odor	Color	Texture	Moisture	Mold
T ₀	Strong smell	Yellowish green	Soft	Hydrates when compressed	With mold
T ₁	Sour smell	Brownish yellow	Firm and consistent	Does not hydrate when compressed	Without mold
T ₂	Strong smell	Yellowish green	Firm and consistent	Does not hydrate when compressed	Without mold
T ₃	Strong smell	Yellowish green	Firm and consistent	Hydrates when compressed	With mold
T ₄	Sour smell	Brownish yellow	Firm and consistent	Does not hydrate when compressed	Without mold

Source: Prepared by the authors

When the bags were opened, the strong but pleasant aroma of alcohol, without ammonia odor and yellowish green color caused by the enzymatic activity of the plant tissue, was characteristic of the T₀, T₂ and T₃ treatments, unlike the other mixtures (T₁ and T₄) in which sour odor and brownish yellow color prevailed; about that, Vu *et al.* (2019) argue that the presence of excessive moisture (>80%) in silages without biological additives with high buffering capacity, can cause undesirable contamination by molds and yeasts as occurred with T₀ and T₃, both treatments with the highest proportion of *C. purpureus*; although the species and fungal load varies not only by the practical conditions of filling and conservation, but also by the origin of the ensiled plant material, an important remark that was exposed by Penagos-Tabares *et al.* (2022). Among other sensory variables, all silages had a firm texture, although some were slightly moist to the touch, in this sense, according to the considerations of Fahmi *et al.* (2019) the sensation to the touch of the T₀ silage was influenced by the water content of the forage material at the beginning of the process, since it was viscous, soft and moldy, while the silages whose moisture content was <80% (T₁, T₂, T₃ and T₄) the texture was firm and consistent, but no post-fermentation mixture possessed the ideal moisture content <30% that would give it dry, solid, whole and slightly lumpy texture suggested by the specialized literature.

4 CONCLUSION

The proximal analysis, pH, aerobic stability and organoleptic characteristics obtained when ensiling *Cenchrus purpureus* cv. Taiwan A-146 and *Paspalum virgatum* L. under laboratory conditions, revealed that both forage species represent a feasible alternative for supplementing large ruminants, since they are medium quality pastures that improve their nutritional profile when ensiled and can cover the maintenance requirements in view of the ecological difficulties faced by the pasture agroecosystem, as well as the high economic cost of using commercial feedstuffs. However, for the fermentation process to be optimal and successful, it is essential to harvest the fresh forage at an early age, subject the pastures to wilting or partial dehydration to control the high moisture content, reduce the leaves to a size of 1.5 to 2.5 cm, add molasses at 6% of the total volume of the forage mixture to stimulate fermentation in an acid medium (pH<4.0), 30 d maximum of conservation, and make sure to extract all the oxygen when sealing the bags, only in this way, it is possible to efficiently take advantage of the prolonged conservation of both grasses, especially *P. virgatum*, a tropical grass that dominates degraded pastures south of Lake Maracaibo, Venezuela, and is not consumed by grazing. Finally, it is important to comment that most researchers working in applied sciences, especially those derived from biology, consider that scientific approaches have intrinsic limitations due to the nature of knowledge; In this case, the operational and budgetary

difficulties to access cattle herds for experimental purposes, conditioned the verification of acceptance and metabolic utilization of the ensiled material, which was only evaluated based on analytical variables of the dynamics for laboratory evaluations; in this regard, it would be interesting to include *in vitro* digestibility tests, and to ensure access to animals that have the necessary conditions to carry out an optimal feeding protocol.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

CONFLICT OF INTEREST

There is no conflict of interest among the researchers involved in this scientific work.

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