LITERATURE REVIEW

Tropical grasses used in haylage production: An integrative review

Pastos tropicales utilizados en la producción de henolage: Una revisión integradora

Gramíneas tropicais utilizadas na produção de pre-secado: Uma revisão integrativa

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Abstract

Background: Haylage is a way of preserving forage with good nutritional value, consisting of the partial removal of water from the plant through the wilting technique. **Objective:** To identify the main tropical grasses used in haylage production, we developed an integrative review. **Methods:** Through the PVO mnemonic strategy, which consists of population (P): tropical forage grasses, variables (V): haylage production, and outcome (O): qualitative parameters, we raised the following question: "What are the main tropical forage grasses used in haylage production?" Papers were selected from three different databases: SCOPUS (Elsevier), Web of Science, and Science Direct. **Results:** The search identified 1,049 articles, but only 10 were considered suitable and included for data extraction. Among the grasses used, 50% were of *Cynodon* spp., 30% of *Panicum maximum*, 10% of *Festuca rubr*, 10% of Dactylis glomerata, and 10% of *Trisetum flavescens*. **Conclusion:** We conclude that the main forage grasses used in pre-dried silage production belong to the genera *Cynodon* spp. and *Panicum* spp., highlighting Tifton-85 grass and Tanzania grass, respectively.

Keywords: *Conservation; forage; grass; haylage; nutritional characteristics; pre-dried silage; review; tropical grasses; wilting.*

Resumen

Antecedentes: henolaje es una forma de preservación de forraje con buen valor nutritivo, que consiste en una retirada parcial de agua de la planta a través de la técnica de emurchecimento. **Objetivo:** Para identificar las principales gramíneas tropicales utilizadas en la producción presecada, desarrollamos una revisión integradora. **Métodos:** A través de la estrategia mnemotécnica PVO, que consta de población (P): pastos forrajeros tropicales; variables (V): producción de presecado, resultados (O): parámetros cualitativos, planteando la siguiente interrogante: "Cuáles son las principales gramíneas forrajeras tropicales utilizadas en la producción de henolaje?". Los artículos se seleccionaron de tres bases de datos diferentes, SCOPUS (Elsevier), Web of Science y Science Direct. **Resultados:** La búsqueda identificó 1.049 artículos, pero sólo 10 se consideraron adecuados e incluidos para la extracción de datos. Entre las gramíneas utilizadas, el 50% fueron del género *Cynodon* spp., el 30% cultivares de *Panicum máximo*, el 10% cultivares de *Festuca rubr*, el 10% cultivares del género *Dactylis glomerata* y el 10% cultivar *Trisetum flavescens*. **Conclusão:** Se concluye que las principales gramíneas forrajeras utilizadas en la producción presecada pertenecen al género *Cynodon* spp. y *Panicum* spp., destacando el pasto Tifton 85 y el pasto Tanzania, respectivamente.

Palabras clave: *Características nutricionales; conservación; forraje; henolage; marchitez; pasto; pastos tropicales; revisión.*

Resumo

Antecedentes: O pré-secado é uma forma de preservação de forragem com bom valor nutritivo, que consiste na retirada parcial de água da planta através da técnica de emurchecimento. **Objetivo:** Identificar as principais gramíneas tropicais utilizadas na produção de pré-secado, desenvolvemos uma revisão integrativa. **Métodos:** Através da estratégia mnemônica PVO, em que consiste em população (P): gramíneas forrageiras tropicais; variáveis (V): produção de pré-secado, resultados (O): parâmetros qualitativos, levantando a seguinte questão: "Quais são as principais gramíneas forrageiras tropicais utilizadas na produção dos pré-secados?". Foram selecionados artigos em três bases de dados diferentes, SCOPUS (Elsevier), Web of Science e Science Direct. **Resultados:** A busca identificou 1.049 artigos, mas apenas 10 foram considerados aptos e incluídos para extração de dados. Entre as gramíneas utilizadas 50% eram do gênero *Cynodon* spp., 30% cultivares de *Panicum maximum*, 10% cultivares de *Festuca rubr*, e 10% gênero *Dactylis glomerata*, 10% cultivar *Trisetum flavescens*. **Conclusão:** Concluímos que as principais gramíneas forrageiras utilizadas na produção de pré-secado pertencem aos gêneros *Cynodon* spp. e *Panicum* spp., destacando-se o capim-tifton 85 e o capim-tanzânia, respectivamente.

Palavras-chave: *Características nutricionais; capim; conservação; forragem; gramíneas tropicais; haylage; murchando; pré-secado; revisão.*

Introduction

Feeding is one of the most important points within the animal production system, and it is essential to search for low-cost alternatives applicable to the field, which maximize animal performance. With seasonality throughout the year, the forage supply to ruminants is limited. This scenario leads to a drop in animal productivity and one of the alternatives found to overcome productive losses during this dry period is the conservation of forage (Fluck *et al*., 2018).

Haylage (pre-dried silage) is a way of preserving forage with good nutritional value, consisting of the partial removal of water from the plant through the wilting technique, with levels between 400 and 600 g/kg DM (Edvan *et al*., 2023). The technique aims to control the fermentation

process during conservation and reduce undesirable secondary fermentations (Horst *et al*., 2016).

The choice of forage species for making haylage depends on the region's climatic conditions, soil fertility, applied technology, nutritional requirements of the animals, and the economics of the process. Pre-dried grasses or legumes from temperate climates have lower contents and better quality of the fibrous fraction in their composition when compared to pre-dried tropical forages (Jimenez Filho *et al*., 2013).

The microbiome present in forage is favorable for the growth of bacteria that produce desirable products in anaerobic fermentation, contributing to a balanced pH, and maintaining the nutritional quality and stability of the product (Costa *et al*., 2019). In European countries, pre-dried feed is widely used in horse feeding (Muller, 2011). In Brazil, haylage use is more evident in feeding ruminants, as it is possible to store surplus forage and later use it to feed these animals during periods of scarcity (Pereira, 2007).

Research comparing two forage preservation techniques, haying, and pre-drying, showed positive results regarding the effectiveness of pre-drying as a means of preserving forage quality even in colder environments (Borreani *et al.*, 2007). However, to bring a broader view of the results available through primary studies extracted from the database and to provide information and suggestions for future research, the objective was, through an integrative review, to identify the main tropical forage grasses used in haylage production.

Methods

The present study consists of an integrative review that aims to generate a broader view of the main grasses used in the pre-drying conservation technique. Currently, this is the broadest method of review and research to define current knowledge on a specific topic.

Preparation of the protocol and guiding question

The integrative review protocol was developed so that the research carried out has a high-standard scientific basis, extracting correct data using a search strategy that meets the objective presented. To ensure that the terms used for the search were non-random, an analysis was made of each scientific article published on the topic under study. To elaborate on the guiding question of this review, the PVO mnemonic strategy was adopted, which consists of population (P) tropical forage grasses, variables (V) haylage production, and outcome (O) were qualitative

parameters. Raising the following question: "*What are the main tropical forage grasses used in haylage production?*"

Database search

The selected scientific papers were searched up to September 8, 2023, in three databases that presented studies that answered the guiding question: SCOPUS (Elsevier), Web of Science, and Science Direct. The CAPES journal portal was used to access the database through the Federal University of Piauí (UFPI, Brazil). Table 1 shows the terms used in the search and their synonyms.

The articles were available in electronic databases, so articles that answered the guiding question were selected. Studies were considered important when (1) they contained primary research published in the format of a scientific article, (2) they included the use of forage grasses in the production of pre-dried silage, (3) and the chemical and fermentative characteristics of the pre-dried grass. Duplicate articles in the same database and other databases were considered only once. Articles in letter forms to the editor, abstracts, theses and dissertations, books or book chapters, lectures, other reviews, and correspondence were discarded. Articles that contained silage and other forms of conservation that were not pre-dried, as well as fresh forage that were not tropical grasses were excluded.

Table 1. Terms used in a unicross and high sensitivity search to study the main tropical forage grasses used in the production of haylage crops.

	Keywords
Population (P)	"**Tropical forage grasses**" OR "Forage crops" OR "Crops forage" OR "Fodder" OR "Grass" OR "Grasses" OR "Forage grasses" OR "Grasses forage" OR "Pasture" OR "Tropical pasture" OR "Pasture tropical" OR "Tropical" OR "Forages" OR "Forage" OR "Lawn" OR "*Brachiaria*" OR "*Urochloa*" OR "*Panicum*" OR "*Megathyrsus*" OR "*Cynodon*" OR "*Pennisetum*" OR "Foragers" OR "Tropical forages" OR "Forages tropical" OR "Grass tropical" OR "Tropical grass" OR "Guinea grass" OR "*Megathyrsus maximus*" OR "*Andrapogon gayanus*"
Variables (V)	"**Haylage**" OR "Production haylage" OR "Pre dry forages" OR "confection haylage" OR "fodder pre dry"
Outcome (O)	**Qualitative parameters**" OR "pH" OR "N-NH_3" OR "ammonial nitrogen" OR "aerobic stability" OR "ethanol content and organic acids" OR "microbiological determination" OR "chemical composition" OR "determination of dry matter" OR "crude protein"

	OR “ether extract” OR “dry matter mineral” OR “detergent fiber neutral” OR “acid detergent Fiber OR “total soluble carbohydrates” OR “*In vitro* ruminal degradability” OR “gas production” OR “fermentation parameters”

Data selection and collection

In the first data collection, articles were evaluated by individually reading the title and summary, then the exclusion criteria were used together with the research objective. In the second stage, the chosen articles were read in full, undergoing a new screening and selecting those corresponding to the research. A form was designed to extract data for study purposes, including information about the publication (article title, indexed databases, authors, country, language, and year of publication), name of the scientific journal, methodological aspects of the study (description of the experiment including treatments and experimental period, analyzed variables, and results) the most used grasses in pre-drying, study limitations and conclusions.

Assessment of included studies

The studies were classified according to their level of evidence, prepared (JBI level of evidence) and those that presented level 2 support were added only to guarantee studies with greater evidence. The JBI classifies research according to its level of evidence into: 1) experimental studies; 2) quasi-experimental studies; 3) observational and analytical studies; 4) observational and descriptive studies; and 5) expert opinion. To estimate the methodological quality used in the studies, the CASP instrument was used for the type of methodological structure (available at: https://casp-uk.net/casp-tools-checklists/) this includes only those that met the criteria according to the research method presented in each study.

Results

Overall, 1,049 studies were identified, including 1,000 scientific articles. Of this total, 50 articles were identified as data of possible interest. After complete reading, and searching for articles that answered the guiding question, ten publications were considered suitable and included due to the solidity of the methodology for data extraction (Figure 1). All results presented level 1 evidence. The selected articles involve publications dated from 2006 to 2023, 40% of which were published in the last 5 years. The studies used were carried out in Brazil (8), Italy (1), and the United States of America (1), published in English and Portuguese.

Among the selected studies, there was a variation in the duration of the experiments, ranging from 3 months to 1 year.

Among the forage grasses most used in haylage production, we can highlight the genera *Cynodon* spp. (60%) and *Panicum* spp. (30%) (Table 2). The pre-dried chemical composition parameters analyzed most frequently in studies were dry matter (DM, 100%), crude protein (CP, 100%), neutral detergent fiber (NDF, 100%), acid detergent fiber (FDA, 90%), mineral matter (MM, 60%), lignin (LIG, 50%), hemicellulose (HEM, 60%), organic matter (MO, 60%), ether extract (EE, 50%), total carbohydrates (CHOT, 30%) and cellulose (CEL, 10%) (Table 3). Fermentative parameters such as pH, soluble carbohydrates (SC), ammonia nitrogen (N-NH_3), lactic acid, acetic acid, butyric acid, and aerobic stability were present in 80, 40, 50, 40, 50, 40, 30% of the studies, respectively (Table 2 and 4).

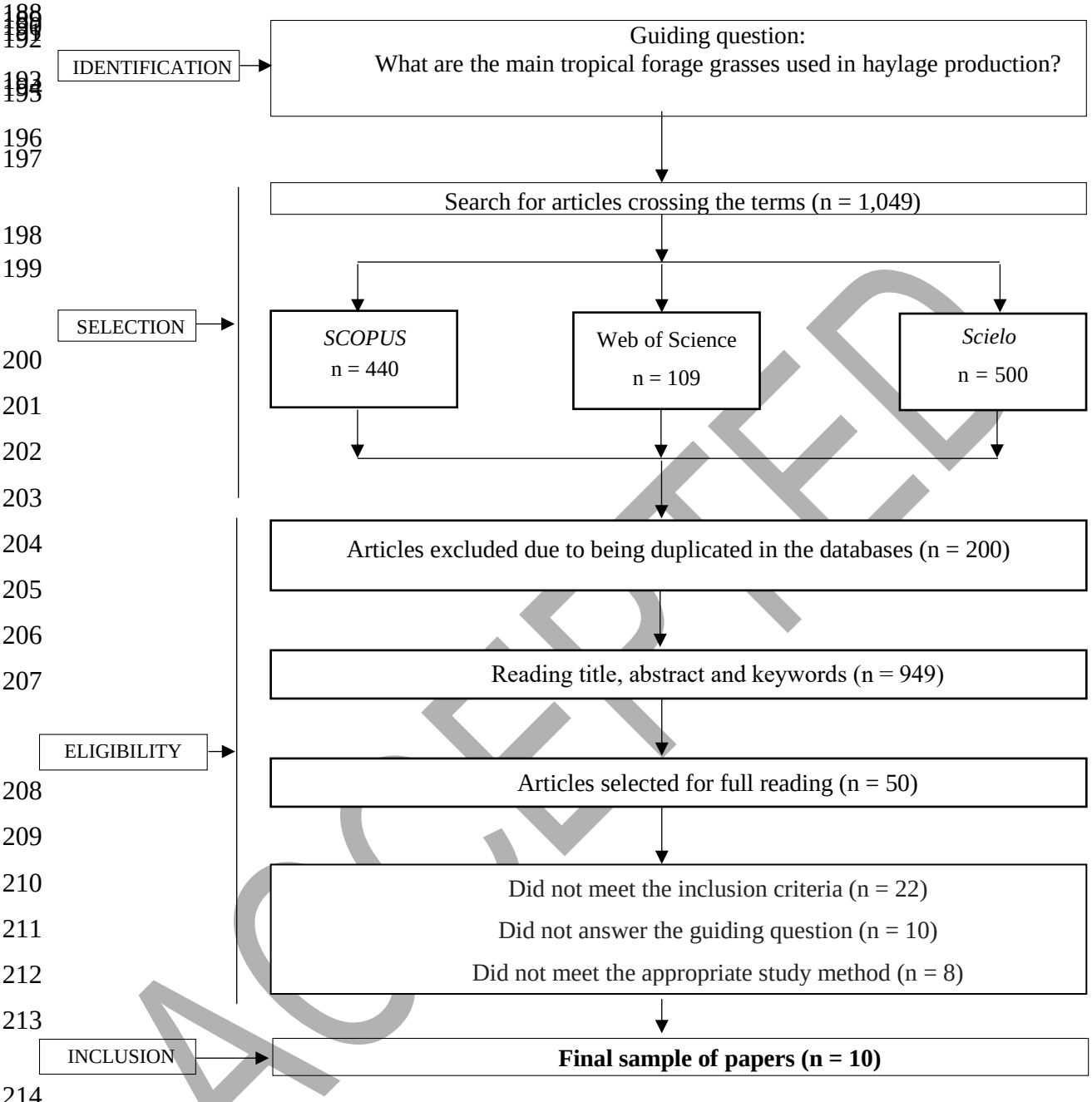

Figure 1. Flowchart of the final sampling selection method for the integrative review

219 **Table 2.** Main tropical forage grasses used in haylage production.

Author/year	Country	Duration	Common name	Scientific name	Treatments	Level of evidence
Souza *et al.* (2006)	Brazil	90 days	Tifton-85 grass	*Cynodon* spp.	The isonitrogenous diets were formulated to contain approximately 12% CP and a roughage: concentrate ratio of 60:40. As a source of roughage, pre-dried Tifton-85 grass and sorghum silage from the forage hybrid AG 2002 (Agroceres) were used in proportions 100:0, 68:32, 34:66 and 0:100, respectively, based on the MS.	1
Gomes *et al.* (2006)	Brazil	65 days	Tifton-85 grass	*Cynodon* spp.	1 - chopped Elephant grass (Pennisetum purpureum Schum. cv. Cameron), of medium maturity, 2 - pre-dried Tifton-85 grass (*Cynodon* spp.), 3 - Signal grass hay (*Brachiaria decumbens* Stapf), 4 - signal grass hay and concentrate 1 supplied at 0.5% BW, and 5 - signal grass hay and concentrate 2 supplied at 1% BW.	1
Borreani *et al.* (2007)	Italy	1 year	Orchardgrass Red fescue Yellow oats	*Dactylis glomerata* *Festuca rubra* *Trisetum flavescens*	Natural meadow hay harvested late with the addition of 5.1 kg of DM per cow and, pre-dried from natural meadow with early cut 3.5 kg of DM of concentrate/cow.	1
Pereira *et al.* (2007)	Brazil	90 days	Tifton-85 grass	*Cynodon* spp.	Pre-dried Tifton 85 grass (Cynodon spp.) + Concentrate (corn meal and ground soybeans) in a 60:40 ratio.	1

	Arriola *et al.* (2015)	Florida (USA)	112 days	Bermuda grass	*Cynodon* spp.	Use of four inoculants in pre-dried Tifton 85 bermudagrass (Buchneri 500 inoculant, Biotal Plus II inoculant, Silage II inoculant, Silo King inoculant).	1

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221 **Table 2 cont.** Main tropical forage grasses used in haylage production.

Guimarães *et al.* (2016)	Brazil	86 days	Tifton-85 grass	*Cynodon* spp.	The Tifton-85 grass (Cynodon spp.) was cut after 30 days of growth to produce haylage and remained in the field until reaching 70% dry matter (DM), Silobac® biological additive was added (2g of product in 2L of water to inoculate each ton of forage destined for silage), totaling 56 days of storage.	1
Costa *et al.* (2019)	Brazil	56 dyas	Tifton-85 grass	*Cynodon* spp.	The evaluated treatments consisted of storage times of 1, 3, 7, 14, 28 and 56 days of pre-dried Tifton-85 grass (with sprinkled Silobac® biological additive).	1
Galeano *et al*. (2022)	Brazil	90 days	Tamani grass	*Panicum maximum*	Monoculture of Tamani grass, Tamani grass + Crotalaria ochroleuca, Tamani grass + cowpea and Tamani grass + soybeans and Tamani grass + pigeon peas.	1
Silva *et al*. (2023)	Brazil	60 days	Tanzania grass	*Panicum maximum*	Plastic films of different thicknesses were tested on the Tanzania grass wrap, 27 µm polyethylene plastic film and three PVC films, which were 10 µm, 11 µm and 13 µm.	1

	Edvan *et al.* (2023)	Brazil	90 days	Tanzania grass	*Panicum maximum*	Four groups of pre-dried Tanzania grass that varied in DM content as follows: fresh plant (not dehydrated), 400, 500 and 600 g kg-1 DM (dehydrated in the field until reaching DM content of treatment).	1

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224 **Table 3**. Chemical characteristics (g/kg DM) of grasses used for haylage production.

Author/year	Species	DM (g/Kg de Fresh matter	CP	SC	EE	Ash	NDF	ADF	CEL	HEM	LIG	OM
Souza *et al*. (2006)	*Cynodon* spp.	536.5	59.9	85.71	18.5	-	783.7	446.7	-	337.0	75.1	935.6
Gomes *et a*l. (2006)	*Cynodon* spp. *Dactylis glomerata*	467.1	169.7	71.41	28.9	87.3	642.2	319.8	-	-	58.0	912.7
Borreani *et al*. (2007)	*Festuca rubr Trisetum flavescens*	522.0	107.0	-	21.0	110	570.0	386.0	-	-	59.0	636
Pereira *et al*. (2007)	*Cynodon* spp.	542.5	57.7	85.23	13.5	-	792.8	444.4	-	348.4	77.8	923.5
Arriola *et al*. (2015)	*Cynodon* spp.	536.5	188.8	-	-	-	691.0	321.0	-	352	-	-
Guimarães *et al.* (2016)	*Cynodon* spp.	947.1	127.1	-	-	91.5	547.7	224.8	-	3229	-	-
Costa *et al.* (2019)	*Cynodon* spp.	622.8	173.3	-	28.7	-	723.3	320.4	-	402.9	30.2	-
Galeano *et al*. (2022)	*Panicum maximum*	669.6	57.4	-	-	86.8	700.8	310.5	239.2	394.8	77.6	-
Silva *et al.* (2023)	*Panicum maximum*	655.0	140.9	-	-	64.8	857.4	493.3	-	-	-	935.1
Edvan *et al*. (2023)	*Panicum maximum*	581.6	99.9	45.4	-	71.0	653.9	-	-	-	-	943.6

225 DM – dry matter; CP – crude protein; EE – ether extract; Ash – mineral matter; NDF –neutral detergent fiber; ADF – acid detergent fiber; CEL – cellulose;
226 HEM – hemicellulose; LIG – lignin; OM - organic matter; SC - soluble carbohydrates.

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229 **Table 4**. Fermentative parameters of the main grasses used for haylage production, organic acids (g/kg DM), pH, aerobic stability

Author/year	Species	pH	N-NH_3	Lactic acid	Acetic Acid	Butyric acid	Propionic acid	Microbiology	Break in aerobic stability
Souza *et al*. (2006)	*Cynodon* spp.	4.37	-	-	-	-	-	-	-
Gomes *et al*. (2006)	*Cynodon* spp.	-	-	-	-	-	-	-	-
Borreani *et al.* (2007)	*Dactylis glomerata* *Festuca rubr* *Trisetum flavescens*	5.13	50.6	11.8	6.4	0.8	-	-	-
Pereira *et al.* (2007)	*Cynodon* spp.	4.37	-	-	-	-	-	-	-
Arriola *et al*. (2015)	*Cynodon* spp.	5.37	-	10.4	2.8	-	-	-	yes
Guimarães *et al*. (2016)	*Cynodon* spp.	-	-	-	-	-	-	-	-
Costa *et al*. (2019)	*Cynodon* spp.	4.70	22.4	-	-	-	-	-	-
Galeano *et al.* (2022)	*Panicum maximum*	4.53	103.6	22.4	28.9	1.3	2.7	-	-
Silva *et al.* (2023)	*Panicum maximum*	5.83	8.5	26.30	1.40	1.0	1.50	5.40 UFC g^{-1}	no
Edvan *et al.* (2023)	*Panicum maximum*	6.37	3.5	-	48.9	3.2	4.1	5.95 UFC g^{-1}	no

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Discussion

The studies verified in this review present a high degree of confidence due to the planning and scientific search criteria. In addition, all stages undergo analysis by two reviewers, thus ensuring the reliability of the data presented and certifying the inclusion of studies that do not lead to review. After reading the full texts, most of the studies that were excluded by the reviewers were due to the use of conservation techniques with grasses other than pre-drying. Studies used pre-dried Tifton grass 85 associated with sorghum silage supplied together in cattle feed, however, research involving the use of these feeds as a source of roughage is limited, including their evaluation and the animal's physiological response to consume this feed (Pereira *et al*., 2007).

Other studies were excluded because they presented legumes as a source of forage for haylage production. The first studies with pre-drying in Brazil date back to 2006, as a way of conserving forage instead of traditional silage to feed beef cattle (Souza *et al*., 2006). There was a variation in the duration of published experiments, however, 80% of the studies were carried out within three months and 20% within a year. Studies using the pre-drying conservation technique tend to last 90 to 120 days, as most pastures are already pre-established and at the cutting point for making bales.

The main forage grasses used in haylage production were Tifton-85 (*Cynodon* spp.) and Tanzania (*Panicum maximum*). These grasses showed great production potential and are widely used in direct grazing, with high adaptive capacity to tropical regions around the world and with potential for hay and haylage production (Edvan *et al*., 2023). Cultivars of the *Cynodon* genus gained prominence in this study probably because they have good productivity and high nutritional value, high DM production, and fast growth rate, in addition to thin culms (Souza *et al*., 2006).

Forage grasses of the genus Brachiaria are the most used in Brazil, occupying approximately 85% of cultivated pasture areas. Of this total, marandu grass occupies 50% and is considered an excellent option for forming pastures (Medica *et al.*, 2016). However, cultivars such as Marandu grass (*Brachiaria brizantha*) did not appear in the list of tropical grasses used in pre-dried preparation, probably because it is a grass with medium-low size characteristics not commonly used for conservation but rather for direct grazing (Macedo, 2006).

Of the treatments applied in the research, 70% used additives on pre-dried grasses, which included bacterial inoculants or concentrates. The works that did not contain any additives also

had relatively satisfactory results, when compared to pre-dried Tifton 85 and other roughage, the pre-dried presented a higher apparent digestibility (Gomes *et al.*, 2006). Silva *et al.* (2023) observed that pre-dried Tanzania grass wrapped in 13-micron thick polyethylene film had the lowest population of enterobacteria, making it safer for use in animal feed.

Most of the experiments took place in Brazil (80%), as the country has regions characterized by irregular rainfall, which is concentrated in a short period of the year (Schmidt *et al.*, 2018), causing seasonality in forage production requiring the use of conservation techniques.

Pre-drying (Haylage) constitutes an alternative for the conservation of forage plants used to feed ruminants, the effects linked to chemical composition, consumption, and animal performance are variables that can be influenced by the production system, forage species, and animals, requiring some care when recommending this practice (Edvan *et al.*, 2023). The lack of standardization in experimental methodologies continues to be a challenge for the conservation and availability of production of good nutritional and microbiological quality. Methodological adaptations or full use of methodology for evaluating silages were commonly used in the studies analyzed, especially concerning fermentative parameters.

Limitations and proposals for future research

One of the limitations of this integrative review is that although the pre-drying technique has been used for a long time, the articles found were limited, thus showing that more studies are needed, especially on how this conservation method affects ruminant nutrition. It is also notable that most of the selected studies did not provide all the data on fermentative parameters, nor was there a standardization of the experimental units, thus suggesting a standardization of the data presented for future studies.

In this sense, to improve the quality of tropical grasses preserved in pre-dried form, research involving the use of additives has been expanding. Studies using fibrolytic enzymes have been highlighted in ruminant nutrition. The use of enzymes in animal feed is biotechnology that became part of the supplementation of these animals in terms of weight gain, and this was confirmed by Burroughs in 1960. These enzymes are extracted from fungi or bacteria, which act in conjunction with enzymes produced by rumen microorganisms (Martins *et al.*, 2007).

Fibrolytic enzymes are used in ensilage to increase the efficiency of the fermentation process, thus collaborating with the action of desirable microorganisms, as in the case of bacteria that produce lactic acid (Muck and Kung Jr., 1997; Kung Jr., 2000). Consequently, Loures *et al.*

(2005), highlighted the main fibrolytic enzymes used, such as hemicellulases, cellulases, pectinases, and xylanases, act to make simple sugars available as a source of nutrients for fermenting bacteria. The use of fibrolytic enzymes that are produced by cultures of filamentous fungi in ruminant feed has shown positive results, with an increase in the digestibility of dry matter and neutral detergent fiber, in milk production and also in the fat content of milk (Schingoethe *et al.*, 1999).

The addition of fibrolytic enzymes to corn silage and Tifton hay favored an increase in the activity of β-1,4-endoglucanase in the rumen fluid in the early stages of incubation, and the average enzymatic activity of β-1,4- Endoglucanase in the ruminal fluid was higher in diets containing corn silage (Martins *et al.*, 2006). The addition of xylanase increased cellulose degradation and tended to increase ADF degradation. The use of multiple enzymes (cellulase, xylanase, and glucose) increased the ruminal degradation of NDF and DM, without affecting the other fractions of the feed (Antonio *et al.*, 2018).

Because of the above, there is a need for studies on the use of fibrolytic enzymes in pre-dried tropical grasses, aiming to enhance the activity of enzymes produced by microorganisms in the rumen, stimulating fiber degradation, increasing DM digestibility, and better animal performance.

Conclusions

The main forage grasses used in haylage production belong to the genera *Cynodon* spp. and *Panicum* spp., highlighting Tifton 85 grass and Tanzania grass, respectively. Around the world, the preservation technique in the form of pre-drying has gradually been adopted with the use of additives, seeking to improve the fermentative parameters and quality of the material.

Declarations

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Conflicts of interest

The authors declare that they have no competing interests regarding the work presented in this report.

Author contributions

ABAD, TPDS ALCG, MJA, LRB, RRN, CATM, RLE, LCVI and AJCC conducted experiment, collected the samples, and wrote the manuscript; ABAD and WCCV conceived and designed the study and wrote the manuscript; ABAD, TPDS ALCG, MJA, LRB, RRN, CATM, RLE, LCVI and AJCC wrote and reviewed the manuscript; All authors read and approved the manuscript.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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