

Research

Standardization of different ensiling method for bamboo leaves (*Melocanna baccifera*) silage preparation and its nutritive, storage quality evaluation

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Abstract

The study was carried out with the main objective to prepare silage using leaves of *Melocanna baccifera* a dominantly available bamboo species in Mizoram. The chemical composition and fermentation coefficient (FC) of the fresh bamboo leaves were analyzed before silage preparation. The DM content of the fresh bamboo leaves (*Melocanna baccifera*) was 40.38 g per 100 g of fresh bamboo leaves. The buffering capacity of the bamboo leaves sample was estimated 30.95 mE per 100 g DM. A lower water soluble content (WSC) of the fresh bamboo (4.8%) leaves makes it difficult for ensiling without additives. A uniform spray of molasses solution (4%) was applied and compacted until filled to the bamboo basket and silo bag capacity. Lastly, the basket and silo bags were made airtight and left unopened for 45, 60, 75 and 90 days. By the end of treatment period, portion of the silage materials were taken to evaluate the quality of ensiled bamboo leaves before offering to the lactating dairy cattle. The present research revealed that the duration of storage of bamboo leaves silage, beyond 45 days, resulted in a decline in its nutrient composition, organic acid content, and fermentation characteristics. In comparison to bamboo basket silage (BBS), it was found that silo bag silage (SBS) had the highest quality at 45 days of ensiling for animal feeding.

Article highlights

- Fresh bamboo leaves (*Melocanna baccifera*) can be utilized as a substrate for silage making.
- A homogeneous application of 4% molasses solution is necessary for better fermentation of fresh bamboo leaves.
- Best quality bamboo leaves silage can be prepared by 45 days of ensiling period.

Keywords Bamboo leaves · Bamboo basket · Silage bag

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1 Introduction

Poaceae, the real grass family, includes a species of perennial evergreens known as bamboo. As a sustainable source of fodder for dairy cattle, bamboo is the best choice due to its high biomass, wide range of adaptation, quick growth, year-round availability of green leaves, low need for heavy inputs (manures, irrigation, labor, insecticides, etc.), ability to maintain soil moisture, and environmental protection. Bamboo leaves might offer cattle farmers an alternative during the winter and rainy season, when there are few species of forage available. According to reports, bamboo leaves have been reported to have the potential to be a reliable feed choice, particularly in scenarios where conventional forage resources are limited. It is also thought to have benefits of possible drought resistance [26, 51]. It is important to note that animals offered bamboo leaves are likely to experience a slightly delayed fermentation process due to the higher concentration of crude fiber in the leaves, which is an essential but less digestible part of the diet [26]. However, the comparable levels of cell wall composition in bamboo leaves can function as a regular feed source for ruminants, providing important sources of dietary fiber that can aid in bulk fill. According to Hummel et al. [24], this bulk fill is necessary to initiate the re-chewing reflex in ruminants, which results in copious amounts of saliva and promotes the health of the rumen. According to Grant et al. [23], improving the starch or neutral detergent fiber (NDF) digestibility of forages may also improve lactation efficiency in dairy cows or lower the cost of purchased feed for dairy farmers. Silage feeding is still a preferred method of feeding forages to dairy farms since it improves both digestibility and palatability. A high-quality feed may be collected for silage making and a unique thing in itself is being able to maintain that quality, which is another benefit. For managing the resources on the land, it gives the farmer more flexibility particularly when there is a surplus of feed, farmers can store it for later use as a feed source for their livestock. Bamboo leaves treated with urea molasses performed better for animals than leaves left untreated, according to studies by Tirusew et al. [51] and Adugna et al. [3]. Although the high nutritional value of bamboo leaves has been shown in numerous studies, the use of bamboo leaves as silage has never been explored. The goal is to generate revenue to support the livelihoods of the local people while simultaneously providing enough food and fodder for cattle. Therefore, bamboo silage could be utilized as an alternative to provide dairy animals with succulent fodder round the year. The purpose of this study was to assess the nutritional characteristics of the widely accessible bamboo species (*Melocanna baccifera*) of Mizoram and standardization of different ensiling method for bamboo leaves silage preparation and its nutritive, storage quality evaluation.

2 Materials and methods

The present study was conducted over a period of six months from April to September 2023, at the Instructional Livestock Farm Complex, CVSc & AH., Mizoram, India, in order to standardize the process of making silage from bamboo leaves and assess the nutritional value of this alternative feed source for lactating dairy cattle.

2.1 Collection and chemical composition of fresh bamboo leaves

Bamboo leaves (*Melocanna baccifera*) were collected from the forest area of Aizawl district based on bamboo availability and accessibility. Water extract of fresh bamboo leaves was prepared by adding 90 ml of distilled water to 10 gm of fresh samples in a beaker and homogenized by mechanical homogenizer. Buffering capacity of the fresh bamboo leaves was estimated as per the method of Playne and McDonald [42]. Total soluble carbohydrate was determined with the method of Yemm and Wills [61]. Proximate analysis of fresh bamboo leaves was done as per AOAC, 2005 [7]. The fraction of cell wall constituents such as neutral detergent fiber, acid detergent fiber, acid detergent lignin were estimated [54]. Oxalate was determined by using the method of Sanchez-Alonso and Lachica [44].

2.2 Preparation of silage in bamboo basket and silo bag for nutritive, storage quality evaluation

The collected bamboo leaves were chopped into partial lengths of 2–4 cm using electrical chaff cutter and treated with molasses based on the soluble sugar content of fresh bamboo leaves. The average DM content of the bamboo leaves was 40 percent at the time of ensiling. For silage preparation and storage, a 500 kg plastic silage bag (BAG SIZE -- 90 × 90 × 70 CM, polypropylene material) and a 5 ft (h) × 2.4 ft (r) bamboo basket (cement and mud plaster) for 100 kg silage



Fig. 1 Preparation of silage in bamboo made basket {mud plaster (a, b) and cement plaster c and silo bag (d)}

making were utilized (Fig. 1). The bamboo basket and silo bag were filled to capacity after a homogenous spray of 4% molasses solution was applied and crushed. After that, the silo bags and basket were sealed airtight and kept sealed for 45, 60, 75, and 90 days. After the treatment period, certain silage components were removed in order to assess the ensiled bamboo leaves' quality. Dry matter in fresh silage samples was determined by hot air oven. Dry matter loss was determined by using the formula of Addah et al. [1]. Short chain fatty acids were measured using a gas chromatograph (GC–MS, Clarus 690, Perkin-Elmer, USA), and the amount of lactic acid in silage samples was estimated using the Barker and Summerson [9] and Barnett [10] modified technique. After the fermentation period, Flieg points from the pH value and DM of the silage were determined using the equation provided by Moselhy et al. [36], in order to determine the quality of the silage.

2.3 Statistical analysis

Statistical analysis was performed with SPSS Version 11.0 statistic software package. Data were analyzed using one way ANOVA test and expressed as means $\pm$ standard deviation (SD). A value of $P < 0.05$ was considered statistically significant.

3 Results and discussion

3.1 Chemical composition of bamboo leaves

Evaluating the nutritional suitability of fodder for animals requires a thorough examination of its chemical composition. The present investigation aimed to provide an understanding of the changes in the chemical composition of fresh bamboo leaves and their potential impact on the quality of feed. The leaves of bamboo have higher quantities of vital nutrients than the branches and stems [58] that looked into the nutritional components of the plant. This shows ruminants can benefit greatly from eating bamboo leaves as a rich source of essential nutrients. In the present study, the DM content of the introduced fresh bamboo leaves (*Melocanna baccifera*) was 40.38 g per 100 g of fresh bamboo leaves (Table 1). The DM content of the fresh bamboo leaves were lower compared with DM values of other studies done for similar bamboo species reported by Datt et al. [19], Bhandari et al. [12] and Bhardwaj et al. [13]. This difference in DM values may have resulted from differences in the soil, climate, and leaf harvesting stage. The fresh bamboo leaf species in another study by Kitaw et al. [26] had a dry matter content that ranged from 40.4 to 46.7 percent, which is comparable with the current finding. The genetic diversity of bamboo species along with regional elements including soil composition, precipitation patterns, and seasonal variations may account for the variations [43]. According to Bhandari et al. [12], species of fodder that have a dry matter content of more than 300.0 g/kg are considered to be of high quality. In this study, the leaves of evaluated bamboo species had 12.35 gm CP per 100 gm DM. The CP values of the bamboo leaves from this investigation

Table 1 Chemical composition (g/100 g DM), pH, buffering capacity and microbial count (Mean $\pm$ SD) of fresh bamboo leaves

Parameter	Mean $\pm$ SD (n = 6)
DM (%)	40.38 $\pm$ 1.26
Organic matter	91.68 $\pm$ 0.12
Crude protein	12.35 $\pm$ 0.97
Ether Extract	5.15 $\pm$ 0.10
Total ash	8.32 $\pm$ 0.12
NDF	68.0 $\pm$ 1.11
ADF	38.81 $\pm$ 0.39
ADL	13.38 $\pm$ 0.74
Oxalate	0.84 $\pm$ 0.16
TDN	51.7 $\pm$ 0.45
pH	6.8 $\pm$ 0.16
Buffering capacity (mE.100 g-1DM)	30.95 $\pm$ 1.62
WSC (g/100 g DM)	4.8 $\pm$ 1.21
LAB ($\log_{10}$ cfu/gm)	4.26 $\pm$ 0.06
Fermentation coefficient	41.62 $\pm$ 2.80

DM dry matter, FM fresh matter, NDF neutral detergent fiber, ADF acid detergent fiber, ADL acid detergent lignin, TDN total digestible nutrient calculated by Martin [33] and Chandler (1990), LAB Lactic acid bacteria; Fermentation coefficient DM(%) + 8 WSC/BC calculated from Weissbach and Honing [56] formula

were within range for a variety of species reported by Sasu et al. [47] in Ghana, and consistent with Bhandari et al. [12] for similar species discovered in India. The range (13.3 to 15.9%) and mean CP, 14.33% and 16.6% for different bamboo species reported by Kitaw et al. [26] and Tirusew et al. [48] in Ethiopia and Altamino-Gutierrez et al. [4] in Peru, respectively, were in contrast with it. The CP content of the bamboo fodder species from the current study was higher than the 7 g per 100 g DM CP content of pasture grasses required for growth and voluntary intake by ruminants [21]. Moreover, compared to crop wastes like rice straw (5 g/100 g DM CP on the average), which is widely fed to ruminants during the dry season [8], the bamboo leaves examined in this study exhibited a better nutritional value. Furthermore, *Brachiaria ruziziensis*, a common Congo signal forage in Mizoram, was observed to have a crude protein content of 10.11% of DM [15], which was comparable to bamboo leaves that had been collected. Thus, the mean CP content of bamboo fodder species from present study would meet the threshold CP needed to maximize nitrogen demand and it is therefore possible to minimize the amount of concentrate feed that ruminants consume, which would ultimately lower the cost of producing milk [12]. The mean EE percentage of bamboo leaves from present study (5.15%) (Table 1) were well above than that reported by Sahoo et al. [45] (1.7%) for similar species and comparable with Bharadwaj et al. [13], which varied from 4.7 to 7.6 including *Melocanna baccifera* species but much lower than reported by Sasu et al. [47] for various other species, which varied from 7.31 to 12.26% and averaging 10.18%. Bamboo leaves would be resistant to decay-causing microbes because of their small amounts of crude fat and low levels of moisture, which would extend their storage lives [25].

Bamboo fodder sample from the present study had a mean total ash content of 8.32 percent which was lower (11.7%) as reported by Sahoo et al. [45] for similar species found in India and it was comparable with other bamboo species reported by Andriarimalala et al. [5], which varied from 6.68 to 18.50 percent of DM. However, it was in contrast with the mean ash percentage reported by Kitaw et al. [26] as 11.1 to 13.2 percent on dry matter basis and Tirusew et al. [48] as 13.83 to 16.37 percent (avg. 10.32 percent) ash in various bamboo species in Ethiopia, Sasu et al. [47] as 13.83 to 16.37 percent ash in various bamboo species in Ghana. According to Bhandari et al. [12] species containing less than 10% ash in dry matter basis is generally consider as good fodder.

The fiber composition of fresh bamboo leaves showed that the mean measurement range of NDF (68%) from the present study (Table 1) was comparable as reported by Sahoo et al. [45] (65.4%) and Bhandari et al. [12] (66.59%) for similar species found in India and various species reported by Altamino-Gutierrez et al. [4] (67.5%) in Peru. However, the NDF percentage of fresh bamboo leaves from the current study was lower than studies from Kitaw et al. [26] (73.4%) and Tirusew et al. [51] (73.44% in Ethiopia. Van Saun [53], states that forage grass with more than 60% NDF are considered poor quality; however, *Melocanna baccifera*, one of the best varieties of bamboo found in India, is rated highly and is expected to have significant nutritional value [45]. Additionally, compared to the bamboo leaves used in the present investigation, *Brachiaria ruziziensis*, a common Congo signal forage in Mizoram, was shown to have a lower NDF

concentration of 56.11% of DM [15]. Furthermore, the comparable levels of cell wall composition in bamboo leaves can function as a regular feed source for ruminants, providing important sources of dietary fiber that can aid in bulk fill. According to Hummel et al. [24], this bulk fill is necessary to initiate the re-chewing reflex in ruminants, which results in copious amounts of saliva and promotes the health of the rumen. ADF content (Table 1) of the bamboo species in the present study (38.31%) were lesser than that reported by Sahoo et al. [45] (41.6%) and Bhandari et al. [12] (46.58%) for the similar bamboo species in India and various other species reported by Kitaw et al. [26] (44.1 to 50.3%), Tirusew et al. [51] (47.9%) in Ethiopia but were considerably higher than that reported by Sasu et al. [47] (25.82%) in Ghana. According to McDonald et al. [35], there is a negative association between feed digestibility and ADF, which means that diets with a high proportion of ADF (more than 45%) have limited nutritional availability. The mean ADF value (38.31%) of the bamboo fodder species examined in this study was lower than what McDonald et al. [35] suggested. This finding further implied that the bamboo leaves (*Melocanna baccifera*) might be utilized as ruminant fodder in Mizoram. The ADL value obtained in the present study (13.38%) are comparable with the studies of Bhandari et al. [12] (12.25%) and higher than that reported by Sahoo et al. [45] (4.9%) for similar species found in India. In other study, Bakshi et al. [11] (9.52%) and Tirusew et al. [51] (10.71) reported lesser ADL but Andriarimalala et al. [5] reported higher ADL (15.20%) in various other bamboo species. ADL content of 10–20% is generally regarded as moderate quality fodder, according to Bhandari et al. [12]; however, polyphenolic compounds, such as tannins, are also significant factors influencing feed digestibility [2], with bamboo leaves having the lowest amount (1.66%) when compared to other tree leaves for feeding dairy cattle [11].

The antinutritional factors such as oxalate content measured in bamboo leaves (Table 1) of *Melocanna baccifera* species (0.84%) from the present study was very narrow as compared to Bakshi et al. [11], who found that the oxalate level for *Bambusa anindinacea* was 0.96%. The soluble oxalate content of studied bamboo leaves was significantly lower than the 4% hazardous threshold [29].

The WSC content of *Melocanna baccifera* species from present study was estimated 4.8 percent which was higher than that reported by Bakshi et al. [11] (2.25%) for *Bambusa anindinacea* species on DM basis. WSC of bamboo shoot shell was quantified as 3.9 (*Bambusa arundinacea*) and 3.6 g/100 g DM reported by Liu et al. [31] and Zhao et al. [63] respectively. An adequate level of WSC should be more than 6–8 g/100 g of dry matter [22] as it is crucial for the efficient fermentation of silage crops because they are easily accessible nutrients for the lactic acid bacteria and other organisms during ensiling to enhance the production of lactic acid, resulting in a sharp pH drop [34]. Thus, to enhance soluble sugar and moisture percentage for ideal fermentation, 4% molasses was applied to fresh bamboo leaves in the current study.

The calculated TDN value of fresh bamboo leaves (*Melocanna baccifera*) from present study was 51.7% as shown in Table 1, which was lower than 68.78% reported by Sasu et al. [47] for various other bamboo species in Ghana and within the range of 44–65% in vitro DM digestibility (IVDMD), according to Teklu et al. [49] and Yeshambel et al. [62]. When comparing bamboo leaves to other leafy vegetable parts, Antwi- Boasiako et al. [6] found that bamboo leaves are carbohydrate-rich, which are typically used as feed or as pasture palatability enhancers. The equivalent carbohydrate values for *Bambusa ventricosa*, *Melocanna baccifera* and *Bambusa vulgaris* (i.e., 30, 31, and 32%) compare favorably to those discovered by Okaraonye & Ikewuchi [39] for the extensively grazed elephant grass (i.e., 30.91%). Because of this, these bamboo leaves are an excellent source of carbohydrates or energy, similar to the plant (*P. purpureum*) that is frequently used as a source of food by cattle and wildlife. It was also reported that, especially during the dry season, bamboo species are a great source of additional nutrients for ruminants grazing low-quality plants including mulberry leaves as reported by Temel and Pehlivan [50], the leaves of *Brighia sapida* and *Mangifera indica* as reported by Sasu et al. [47] and cereal crop residue like paddy straw with low protein levels (about 5 g/100 g of protein in crude form kg^{-1} DM), which is widely used during dry season reported by Babayemi et al. [8]. In contrast, study conducted in Mizoram revealed that *Brachiaria ruziziensis*, a common Congo signal forage, had a greater TDN concentration of 62.18% of DM compared to the bamboo leaves employed in the present study [15].

The mean pH of the fresh bamboo leaves from present study (Table 1) was found to be 6.8. The buffering capacity of the bamboo leaves sample were estimated 30.95 mE per 100 g DM, which could have resulted from a lower ash concentration of leaves. Buffering capacity of forage indicate presence of chemical substances that stop pH drop during ensiling. The pH (5.78) and buffering capacity 58.8 and 54.4 mE. per 100 g DM of bamboo shoot shell was reported by Liu et al. [31] and Zhao et al. [63]. The ideal material for preservation as silage are that it should contain an adequate level WSC 6–7 g/100 g DM, a relatively low buffering capacity (20–30 mE. per 100 g DM) [34]. The buffering capacity of fresh bamboo leaves from the present study was very narrow as compared to the ideal range for ensiling material reported by McDonald et al. [34] but lower WSC (4.8 g/100 g DM) makes it difficult to ensile without additives.

The mean LAB count of the fresh bamboo leaves of *Melocanna baccifera* was 4.26 $\log_{10}$ cfu/g (Table 1) of fresh leaves which was less than 6 $\log_{10}$ cfu/g for efficient fermentation [34]. According to Xin et al. [59], natural fermentation relies

on epiphytic microbial communities, which are more efficient than exogenous origins and play a crucial role throughout the entire process. The *Lactobacilli*, *Weissella*, and *Pediococcus* species are the predominant *Lactobacilli* in silages made from corn, alfalfa, grass, and tropical crops [22]. *Lactobacillus plantarum*, a type of LAB that is frequently found in fermented bamboo shoots, requires one to three months to ferment properly [43].

3.2 Chemical composition and dry matter loss of bamboo silages prepared in bamboo basket and silo bag at different days of ensiling

Bamboo leaves are commonly fed to animals in regions where the plant exists all over the world, especially during the dry season [26], Ghana [47], Colombia [16], Peru [4], and India [12, 45]. Despite being a good source of minerals and protein, its roughage qualities demand feed processing methods such as ensiling. According to the investigations carried out by Tirusew et al. [51] and Adugna et al. [3], bamboo leaves mixed with urea and molasses outperformed untreated bamboo leaves in terms of animal performance. However, before adding bamboo leaf silage to dairy cattle's diet during the dry season, it is crucial to assess its nutritional value.

The purpose of silage production was to create a feed that is stable, has a high dry matter recovery, and is highly digestible as compared to fresh crops. When a crop goes through the fermentation process, a number of factors, such as moisture, buffering capacity, soluble sugar content, and microbial population, determine the quality of the silage. According to Kung et al. [28], silage fermentation and its subsequent quality can also be impacted by management parameters such pack density, the kind of additive employed, cut length, and silo management during ensiling. Thus, during the experimental phase of the current study, two distinct types of silos; bamboo basket and silo bag were taken into consideration for the silage preparation. Lastly, the basket and silo bags were made airtight and left unopened for 45, 60, 75 and 90 days (Figs. 2, 3, 4). Since the pH decline in forages with DM percentages higher than forty (40) is slower, it makes sense to open the silo at 45 days because there is less metabolic water available for fermentation. According to Pahlow et al. (2003) [40], the entire fermentation phase of the ensiling process is estimated to take 7 to 45 days.

The bamboo leaves used in this study contained low epiphytic $4.26 \log_{10}$ cfu/g LAB than $6.0 \log_{10}$ cfu/g for effective fermentation recommended by [34] and FC value 41.62, which particularly are considered as middle ensilable according to Weissbach and Honig [56]. Therefore, 40 ml of molasses was applied per kilogram of fresh bamboo leaves in order to increase the amount of soluble sugar in the leaves. Molasses directly provide soluble sugar for lactic acid production during the initial stage of fermentation. The pH and fleig point value were effected ($p < 0.05$) by both ensiling method (BBS and SBS) and ensiling days (Table 4 and 5). As the number of days increases during the ensiling process, the DM loss increases steadily ($p > 0.05$) in both BBS and SBS. The greatest recorded DM loss was 5.60% in BBS at 90 days, while the lowest was 4.69% in SBS at 45 days (Tables 2 and 3). The observed variations in dry matter loss can be explained by the breakdown of nutrients and the change in fermentation from homofermentive to heterofermentive as a result of insufficient substrate [38]. Before ensiling the pH of the fresh bamboo leaves was 6.8, which was reduced to 4.22 in BBS and 4.20 in SBS at 45 days of ensiling. The pH of the silage varied between 4.2 and 4.5 across all treatments (Table 4 and 5). The pH value dropped during the first 45 days of ensiling; after that, the pH decreased gradually over the course of the ensiling period. An extended period of ensiling (day 45 to day 90)

Fig. 2 Fermentation characteristics of bamboo leaves silage (BLS) prepared in bamboo basket

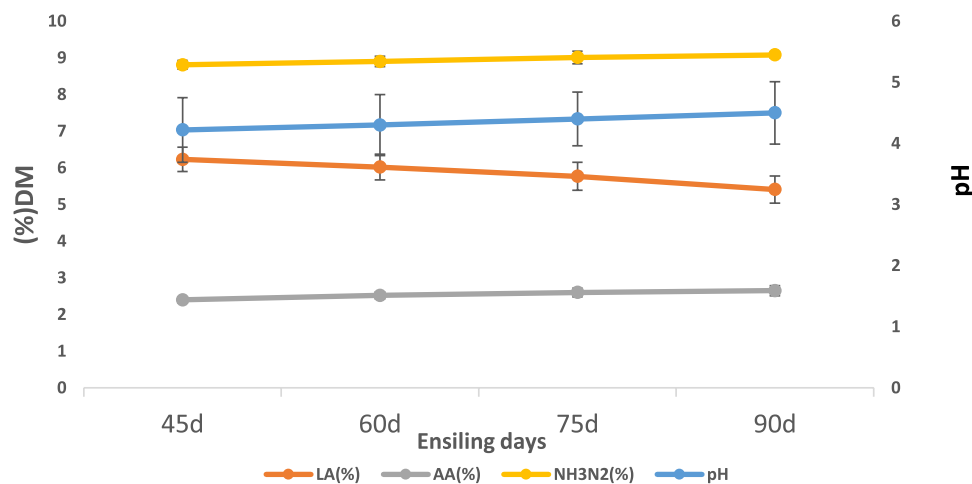


Fig. 3 Fermentation characteristics of bamboo leaves silage (BLS) prepared in plastic silo bag

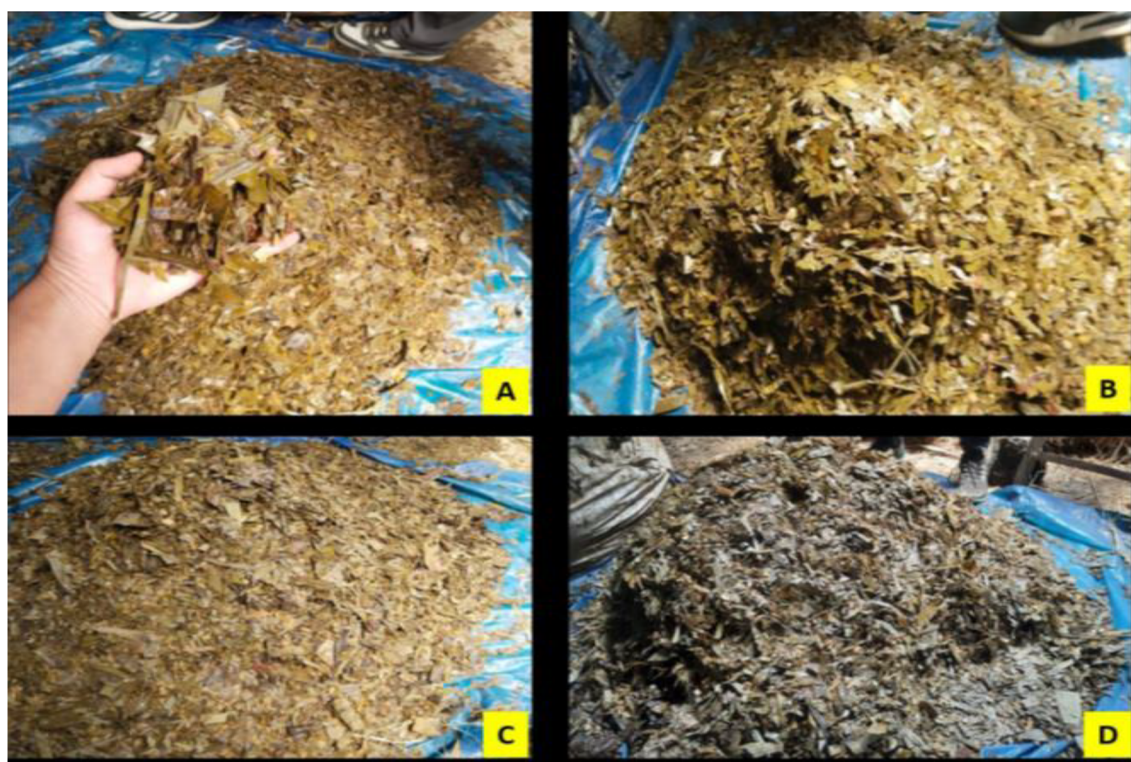
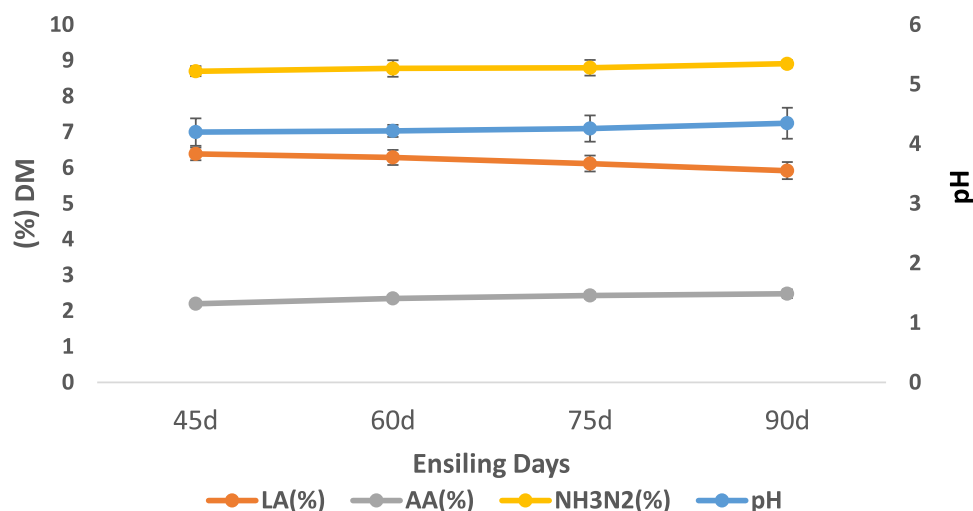


Fig. 4 Bamboo leaves silage (BLS) {45 d (A), 60 d (B), 75 d (C), 90 d (D)}

may be associated with a decrease in pH due to the production of less acidic metabolites (mostly acetic acid; $pK_a = 4.8$) from an acidic product (lactic acid; $pK_a = 3.9$) [20]. Moreover, the final pH of silage is also affected by buffering capacity of forage [34], which was higher in bamboo leaves ($30.95 \text{ mE} \cdot 100 \text{ g}^{-1} \text{ DM}$) and higher DM content of the leaves (40.38) as it reduces the metabolic water availability for growth of LAB [57]. Likewise, low epiphytic bacterial populations prolonged the period during which pH dropped at a very modest rate [41]. Therefore, microbial inoculants and enzyme treatments are both of interest to improve sugarcane tops silage [17] but in the present study only molasses was added to increase the soluble sugar content of the substrate. Based on the reference values, all the silages had a Flieg point above 80, indicating very good quality. According to the results of the current analysis, the highest flieg point was reached on day 45 of ensiling in both SBS (114.0) and BBS (112.2) (Tables 4 and 5). The similar trend was

Table 2 Chemical composition (%DM), WSC (%DM) and dry matter loss (%) of bamboo silages prepared in Bamboo basket (Mean $\pm$ SD) at different days of ensiling

Parameter	BBS (Bamboo basket silage) (n = 6)				p-value
	45 D	60 D	75 D	90 D	
DM (%FM)	38.01 $\pm$ 1.07	37.85 $\pm$ 0.29	37.56 $\pm$ 0.26	37.50 $\pm$ 0.40	0.08
CP	11.03 $\pm$ 0.26	10.96 $\pm$ 0.22	10.78 $\pm$ 0.23	10.73 $\pm$ 0.22	0.52
EE	4.36 $\pm$ 0.08	4.26 $\pm$ 0.09	4.1 $\pm$ 0.13	4.1 $\pm$ 0.15	0.08
ASH	8.78 $\pm$ 0.16	8.80 $\pm$ 0.14	8.79 $\pm$ 0.14	8.83 $\pm$ 0.08	0.14
NDF	65.58 $\pm$ 1.03	65.2 $\pm$ 1.41	64.95 $\pm$ 1.40	64.91 $\pm$ 0.33	0.50
ADF	37.78 $\pm$ 0.93	38.03 $\pm$ 0.55	38.1 $\pm$ 0.76	38.28 $\pm$ 1.16	0.48
ADL	13.25 $\pm$ 0.90	13.19 $\pm$ 0.68	13.09 $\pm$ 0.57	13.02 $\pm$ 0.30	0.2
WSC	3.45 ^a $\pm$ 0.11	3.2 ^b $\pm$ 0.22	2.85 ^c $\pm$ 0.17	2.57 ^d $\pm$ 0.05	0.01
DM loss	5.23 $\pm$ 1.52	5.45 $\pm$ 1.54	5.34 $\pm$ 1.8	5.60 $\pm$ 1.98	0.52

BBS Bamboo basket silage, WSC water soluble carbohydrate, DM loss (%) calculated from Addah et al., [1] formula

Means along a column with different superscripts (^{a,b,c,d}) differed significantly (p<0.05)

Table 3 Chemical composition (%DM), WSC (%DM) and dry matter loss (%) of bamboo silages prepared in silo bag (Mean, SD) at different days of ensiling

Parameter	SBS (Silo bag silage) (n = 6)				p-value
	45 D	60 D	75 D	90 D	
DM (%FM)	38.5 $\pm$ 0.35	37.8 $\pm$ 0.42	37.65 $\pm$ 0.25	37.45 $\pm$ 0.45	0.06
CP	11.2 $\pm$ 0.39	11.06 $\pm$ 0.38	11 $\pm$ 0.41	10.9 $\pm$ 0.34	0.35
EE	4.56 $\pm$ 0.19	4.51 $\pm$ 0.22	4.41 $\pm$ 0.20	4.4 $\pm$ 0.14	0.13
ASH	8.73 $\pm$ 0.20	8.75 $\pm$ 0.20	8.77 $\pm$ 0.20	8.80 $\pm$ 0.11	0.08
NDF	65.05 $\pm$ 0.75	64.21 $\pm$ 0.79	63.98 $\pm$ 0.95	63.23 $\pm$ 0.75	0.08
ADF	37.51 $\pm$ 0.77	37.6 $\pm$ 0.53	37.98 $\pm$ 0.48	37.78 $\pm$ 0.18	0.98
ADL	13.22 $\pm$ 0.62	13.18 $\pm$ 0.35	13.07 $\pm$ 0.27	12.98 $\pm$ 0.30	0.35
WSC	3.12 ^a $\pm$ 0.23	2.98 ^a $\pm$ 0.09	2.70 ^b $\pm$ 0.07	2.68 ^c $\pm$ 0.04	0.01
DM loss	4.69 $\pm$ 1.25	4.98 $\pm$ 1.35	5.10 $\pm$ 1.52	5.45 $\pm$ 1.3	0.4

SBS Silo bag silage, WSC water soluble carbohydrate, DM loss (%) calculated from Addah et al., [1] formula

Means along a column with different superscripts (^{a,b,c}) differed significantly (p<0.05)

Table 4 Fermentation characteristics, microbial population and flieg point of bamboo silages prepared in bamboo basket (Mean $\pm$ SD) at different days of ensiling

Parameter	BBS (Bamboo basket silage) (n = 6)				p-value
	45 D	60 D	75 D	90 D	
pH	4.22 ^a $\pm$ 0.05	4.30 ^b $\pm$ 0.05	4.40 ^{bc} $\pm$ 0.07	4.50 ^c $\pm$ 0.14	0.01
Lactic acid (%DM)	6.23 ^a $\pm$ 0.06	6.02 ^b $\pm$ 0.07	5.77 ^c $\pm$ 0.05	5.41 ^d $\pm$ 0.03	0.01
Acetic acid (%DM)	2.4 ^a $\pm$ 0.01	2.52 ^b $\pm$ 0.02	2.60 ^c $\pm$ 0.01	2.65 ^d $\pm$ 0.03	0.01
Butyric acid (%DM)	ND	ND	ND	ND	
NH ₃ -N ₂ (%/TN)	8.81 $\pm$ 0.23	8.9 $\pm$ 0.26	9.01 $\pm$ 0.26	9.08 $\pm$ 0.22	0.26
Lactic acid bacteria (log ₁₀ cfu/g)	7.07 ^a $\pm$ 0.35	6.28 ^b $\pm$ 0.28	6.12 ^b $\pm$ 0.23	4.5 ^c $\pm$ 0.5	0.03
Yeasts (log ₁₀ cfu/g)	–	–	2.86 $\pm$ 0.45	3.65 $\pm$ 0.6	0.08
Moulds (log ₁₀ cfu/g)	–	–	–	–	
Flieg point	112.2 ^a $\pm$ 2.2	108.7 ^b $\pm$ 2.6	104.1 ^c $\pm$ 2.84	100.0 ^{cd} $\pm$ 6.2	0.04

Means along a column with different superscripts (^{a,b,c,d}) differed significantly (p<0.05)

observed for sugarcane tops silage, highest flieg point at 45 days after that, it decreased as the number of ensiling days extended due to an intensification of pH values and a decrease in the silage's DM content (Chauhan et al., [17].

The proximate analysis showed that, chemical composition of silage decreased compared to the fresh bamboo leaves but were unaffected during the storage period (p > 0.05). Comparing with fresh bamboo leaves the amount of DM, crude protein, ether extract, NDF and ADF decreased, with the exception of ash, which increased with a longer

Table 5 Fermentation characteristics, microbial population and flieg point of bamboo silages prepared in silo bag (Mean $\pm$ SD) at different days of ensiling

Parameter	SBS (n = 6)				p-value
	45 D	60 D	75 D	90 D	
pH	4.20 ^a $\pm$ 0.04	4.22 ^a $\pm$ 0.02	4.26 ^b $\pm$ 0.01	4.35 ^c $\pm$ 0.04	0.01
Lactic acid (%DM)	6.39 ^a $\pm$ 0.01	6.29 ^b $\pm$ 0.01	6.12 ^c $\pm$ 0.02	5.92 ^d $\pm$ 0.03	0.01
Acetic acid(%DM)	2.2 ^a $\pm$ 0.08	2.35 ^b $\pm$ 0.01	2.43 ^c $\pm$ 0.01	2.48 ^d $\pm$ 0.02	0.01
Butyric acid (%DM)	ND	ND	ND	ND	
NH ₃ -N ₂ (%/TN)	8.7 $\pm$ 0.53	8.78 $\pm$ 0.50	8.8 $\pm$ 0.51	8.91 $\pm$ 0.44	0.87
Lactic acid bacteria(log ₁₀ cfu/g)	7.85 ^a $\pm$ 0.22	6.58 ^b $\pm$ 0.19	6.22 ^b $\pm$ 0.18	4.9 ^c $\pm$ 0.35	0.02
Yeasts (log ₁₀ cfu/g)	–	–	1.85 $\pm$ 0.2	2.46 $\pm$ 0.38	0.12
Moulds (log ₁₀ cfu/g)	–	–	–	–	
Fleig point	114.0 ^a $\pm$ 2.43	112.6 ^a $\pm$ 1.16	109.9 ^b $\pm$ 1.03	105.9 ^c $\pm$ 2.50	0.01

Means along a column with different superscripts (^{a,b,c,d}) differed significantly (p<0.05)

storage period in both BBS and SBS (Table 1, 2 and 3). The DM content of bamboo leaves silage ranged from 37.45 to 38.5 percent from 40.38 percent (fresh leaves); this decrease in DM can be explained as a result of breakdown to nutrients by microbes during the stages of anaerobic fermentation [14]. The CP content of bamboo leaves silage decreased and it varies from 10.73 to 11.2% DM during 45 to 90 d of ensiling, which was in accordance with Naeini et al., [37] for sorghum silage and Saricicek et al., [46] for maize silage. The EE percentage of bamboo leaves silage varies from 4.1 to 4.5%, this decrease in EE content of silage could be due to increase in the activity of plant enzymes [46] and lipolysis which was greater during extensive fermentation [52]. Ash percentage increased in bamboo leaves silage and ranged from 8.73 to 8.83% DM, which was in line with Naeini et al. [37] for sorghum silage but in consistence with Sariçiçek et al. [46] for maize silage. The differences among these studies may be related to the plant material and mini silo type used.

NDF content of bamboo leaf silage dropped over storage, ranging from 63.23 to 65.58% FM over 45 to 90 days of ensiling (Tables 2 and 3). According to Naeini et al. [37], for sorghum silage, the NDF content dropped from 54.4 to 49.5 g/100 g FM over the course of 30 to 120 days after ensiling. Similar results was found by Chi et al. [18] in Mulberry Leaves and Pennisetum Hybrid Mix-Silage; these findings, along with our own, may be explained by the bacterial enzymes cellulase and hemicellulase breaking down the cell wall and the fermentation process producing organic acids [60]. However, ADF content of bamboo leaves silage decreased during 45 to 90 days of ensiling and range was 37.51 to 38.28% FM, which was in line with Sariçiçek et al. [46] and Chi et al. [18].

WSC concentration of the bamboo silage decreased markedly (p < 0.05) during first 45 days of ensiling and gradually fall up to 60 days but after 60 days decrease was negligible (Tables 2 and 3). The highest amount of WSC were observed at 45 days as 3.45% DM in BBS and 3.12% DM in SBS. However lowest WSC concentration were observed at 90 days. The duration of bamboo silage beyond 45 days, resulted in a decline in its organic acid concentrations (p < 0.05). The amount of lactic acid varied slightly as the fermentation period was extended and it was at its lowest point, 5.41% DM in BBS, after 90 days and at its highest, 6.39% DM in SBS, after 45 days. The acetic acid concentration increased with the extension of ensiling time and ranged from 2.2 to 2.65% DM. Lowest acetic acid concentration was at 45 days as 2.2% FM in SB and highest at 90 days as 2.65% DM in BBS. Longer fermentation times resulted in a considerable drop in the LAB population, reaching its lowest values in 90 days in both BBS and SBS, at 4.5 and 4.9 log₁₀ cfu/g of FM, respectively. Yeast were only detected at 75 th and 90 th day of storage time in both BBS and SBS (Table 4). Fresh bamboo leaves had an epiphytic LAB count of 4.26 log₁₀ cfu/g, which is more effective than exogenous origin, and this was enough to start lactic acid fermentation in anaerobic circumstances after mixing with 4% molasses. The LAB population in the current study substantially decreased (p < 0.05) with longer fermentation times could be due to a lack of substrate, such as WSC [55], which causes the lactic acid content to drop during ensiling. Additionally, there may be some anaerobic microbes that break down lactic acid to produce acetic acid [45]. Silage made from bamboo leaves has an acetic acid content that suggests the presence of certain heterofermentative LAB [55]. This acetic acid can effectively prevent yeast growth, enhancing stability during feeding, and reducing the amount of nutrients lost from the silage during prolonged storage [30]. According to research by Kleinschmit et al. [27], maize silage possessed in a micro silo for 361 days showed an 80% increase in acetic acid concentration and a 15% decrease in lactic acid concentration. Furthermore, it was intriguing to see that after 60 days of ensiling, a sizable number of yeasts existed in the bamboo leaves silage. Anaerobic yeast competes with LAB for water soluble carbohydrate (WSC), raising pH and significantly reducing silage's DM content [32]. However,

in this investigation, molds were not found after ensiling, which may be because the silage has a low pH or sufficient acidity to prevent the growth of these microorganisms [59].

NH₃-N content often represents the breakdown of proteins [55]. In the study, NH₃-N₂/TN content were unaffected ($p > 0.05$) during ensiling time and ranged between 8.7 to 9.08%TN. The NH₃-N concentration was lowest at 45 days in SBS as 8.7% TN and highest at 90 days in BBS as 9.08% TN (Table 3). The absence of considerable NH₃-N throughout the ensiling period in both BBS and SBS could be explained by the favorable and stable environment that the bamboo leaves silage may have. In general, high moisture silages undergo a stronger fermentation than drier silages, they generally have higher concentrations of NH₃-N [28]. Similarly, Naeini et al. [37] found a similar outcome, demonstrating a steady increase in the NH₃-N content of sweet sorghum throughout a 30- to 120-day storage period.

4 Conclusion

Fresh bamboo leaves (*Melocanna baccifera*) with 4% molasses ensiled for 45 days in both silo bag or bamboo basket could produce good quality silage. However, finding the best time to harvest bamboo leaves is crucial. Furthermore, it is critical to determine the best addition or combination of additives to treat bamboo leaves in order to produce silage with a moderate fiber content and increased digestibility.

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Author contributions All authors contributed to the study's conception and design. The research work was executed by Abhishek Paul (PhD Scholar) ICAR-NDRI with the guidance of Dr.Pawan Singh. The chemical analysis of fresh and bamboo silage was supervised by Dr. Raman Malik, Principal scientist, Animal Nutrition. The organic acid content of silage was evaluated using HPLC under the supervision of Dr. AK Samanta. Dr. Girin kalita helped to analyze the data using suitable software. Dr.Satyabrata Roy supervised the writing of the manuscript. All authors have read and approved the final manuscript.

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Data availability The datasets generated and/or analyzed during the current study are not publicly available as they are part of a PhD dissertation currently under review. However, they are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Plant reproducibility *Melocanna baccifera* was collected in Aizawl, Mizoram. The plant material was identified by Department of Environmental Science, Mizoram University, India, and a voucher specimen was deposited at Herbarium of Mizoram University, Aizawl with ID [ZO16660].

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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