

THE PHYSICAL, NUTRITIONAL, PHYTOCHEMICAL, AND MICROBIOLOGICAL PROPERTIES OF WATER MIMOSA (*Neptunia oleracea* Lour) SILAGE USING DIFFERENT MOISTURE CONTENTS FOR POULTRY FEED

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✉Supporting Information



ABSTRACT: The potential of water mimosa (WM; *Neptunia oleracea* Lour) swamp legumes, as a source of green protein, phytochemical compounds, and probiotics in the form of silage juice for waterfowl such as ducks is quite large, but to date it has not been further investigated. This study aimed to evaluate the physical, nutritional, phytochemical, and microbiological characteristics of WM silage fermented at different moisture levels. This study used a completely randomized design consisting of 5 treatments and 3 replicates. The treatments consisted of control group (fresh WM legumes), WM40, WM50, WM60, and WM70: WM silage with moisture levels of 40%, 50%, 60%, and 70%, respectively. The results of the analysis of variance showed that differences in silage moisture content had a significant effect on color, aroma, and texture scores ($P < 0.05$). Qualitative tests showed that WM silage fermented at moisture levels of 40-70% contained several phytochemical/antibacterial compounds, including flavonoids, steroids, saponins, phenols, and carotenoids. Differences in WM silage moisture content significantly affected dry matter, organic matter, crude protein, crude fiber, carbohydrates, and Fleigh point ($P < 0.05$). Total acid, total bacteria count, and lactic acid bacteria count in WM silage fermented at different moisture contents increased compared to the control group ($P < 0.05$) but showed similar results between moisture levels of 40 and 70%. In conclusion, the making of WM silage with a moisture content of up to 50% provided optimal yields in terms of the physical, microbiological, nutritional, and phytochemical properties of WM silage.

Keywords: Microbiological characteristics, Moisture content, *Neptunia oleracea* Lour, Nutritional content, Phytochemical compounds, Silage.

INTRODUCTION

Feed constitutes the most significant factor in supporting the productivity of poultry. Fishmeal and soybean meal are the primary sources of protein and are extensively utilized in poultry feed formulations (Ravindran, 2013). However, the procurement of these ingredients is often challenging due to their necessity for importation, which introduces variability in availability and sustainability. Consequently, there is a compelling need to identify alternative, readily available, and cost-effective protein sources that maintain adequate nutritional quality.

South Sumatra, Indonesia, is renowned for its extensive swampland potential and a substantial local feed supply for livestock. *Neptunia oleracea* Lour, more commonly referred to as water mimosa (WM), is a species of legume that is native to swampy environments. It has the potential to serve as a source of protein for livestock, with a crude protein content estimated to be approximately 20.5% (Muhakka et al., 2020). However, to date, the utilization of WM legume has been predominantly focused on ruminants, which are provided with fresh WM legume containing a high proportion of crude fiber (Sumantri et al., 2023; Van Chen et al., 2026). In contrast to ruminants, which are capable of digesting complex carbohydrates, poultry are monogastric animals with limited capabilities for digesting crude fiber (Singh and Kim, 2021). Consequently, in order to utilize WM legumes in poultry feed, it is necessary to employ feed processing techniques to reduce the crude fiber content. One such technique involves the production of juice. The objective of juicing is to obtain nutrients from WM legume in a form that is more easily digestible and absorbable by poultry. In recent years, research on the utilization of forage juice for monogastric livestock has witnessed a surge of interest, particularly in the context of swine, as evidenced by the study conducted by Keto et al. (2021). In contrast, the scope of research in poultry remains considerably limited, underscoring the necessity for further investigation to ascertain its full potential, especially for waterfowl species.

To enhance the benefits of utilizing this WM juice, it is essential to process the WM legume into silage. In addition to reducing crude fiber content, increasing nutritional value, and enhancing feed digestibility, the silage process has the capacity to stimulate microbial growth (Kung et al., 2018). This includes lactic acid bacteria and organic acids, such as acetic, propionic, and butyric acids. Notably, the fermentation process leads to the production of lactic acid. In accordance with the findings of our prior in vitro investigations, lactic acid bacteria isolated from swamp grass silage are classified as *Lactobacillus* sp. and possess the capacity to function as probiotics and acidifiers (Sandi et al., 2018; 2020).

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The ensiling process is significantly influenced by the initial moisture content of the raw material, as moisture content plays a pivotal role in determining microbial activity, anaerobic stability, and the nutritional quality of the silage produced (Zheng et al., 2011). Legumes, by their very nature, contain a high moisture content, which must be taken into consideration during the fermentation process to ensure optimal results. An inadequate moisture content in silage production (below 40%) results in dry silage texture, while a moisture content of more than 70% significantly alters the ensilage process and the resulting phases, leading to undesirable clostridial fermentation instead of the beneficial lactic acid fermentation (Kung et al., 2018). Consequently, further research is necessary to assess the physical, nutritional, phytochemical, and microbial characteristics of WM silage fermented at varying moisture levels.

MATERIALS AND METHODS

Experimental design

This study used a completely randomized design with five treatment groups, each repeated three times. The treatment groups were as follows: a control group consisting of fresh WM legume without ensilage; and WM40, WM50, WM60, and WM70, which were groups of WM silage with moisture contents of 40%, 50%, 60%, and 70%, respectively.

Determination of moisture content of WM legume

The moisture content was determined by analyzing the water content of the WM legume to calculate the dry matter content. This data was then used to determine the amount of water to add to the silage according to the treatment. The procedure for determining the moisture content of the WM legume was as follows: First, a porcelain dish was dried in an oven at 105°C for 1 hour. Then, it was placed in a desiccator for 30 min and weighed. Next, 2 g of the sample was weighed and placed in a dish with a known weight. The sample was dried in an oven at 105°C for 24 hours. Afterward, the dish containing the sample was cooled in a desiccator for 30 min and weighed until a constant weight was achieved. Weighing was carried out 3-4 times to ensure consistent results. The moisture content of the WM legume was calculated according to AOAC (2005).

Determination of WM silage moisture content

The determination of silage moisture content is based on Putri (2024). First, the dry matter content of the sample was determined, as well as the amount of dry matter in the sample (g). Then, the moisture content of the sample was calculated using the following formula: Moisture content of WM (%) = 100% - dry matter content of WM (%)

The next step was to calculate the percentage of water added, using the formula:

Percentage of water added (%) = target moisture content in WM silage (%) - moisture content of WM (%)

To determine the amount of water added (mL), the following formula was used:

Amount of water added (mL) = amount of WM dry matter (g) x percentage of water added (%).

The making process of WM silage

The process of making silage referred to our previous research (Sandi et al., 2018; Yosi et al., 2025) with some modifications. Briefly, the WM leaves were harvested fresh, cleaned of debris, separated from the stems, and air-dried for 24 hours. Then, 7.5 kg of the wilted leaves were placed into 15 double-layered one-kilogram plastic bags (each bag containing 500 g) and mixed with predetermined amounts of distilled water according to the treatment group. Each bag received an addition of molasses amounting to 3% of the dry weight. The mixture was homogenized until thoroughly mixed and then compacted to create anaerobic conditions. The bags were vacuum-sealed, labeled, and stored at room temperature for 21 days.

Determination of the physical quality of WM silage

Physical quality was tested by a panel of 10 moderately trained individuals. The silage assessment used an ordinal scale with standard silage assessment criteria referring to Al Biruni et al. (2024). The standard silage assessment criteria for color are as follows: greenish-yellow = 25, brownish-yellow = 10, and blackish, close to compost color = 0. The odor assessment is as follows: fermented sour smell = 25, sour smell not too strong = 20, no smell in silage = 10, and mold growth and compost causing an unpleasant smell = 0. The assessment based on cleanliness was as follows: no mold = 25, mold and slime = 0. The assessment based on texture was as follows: dry, soft to the touch, crumbly, fermented aroma = 25, moist but not wet = 20, and watery with high water content = 0.

Determination of the nutritional quality of WM silage

The moisture content of WM silage was determined using the oven method (AOAC, 2005). The crude protein and crude lipid content of WM silage were analyzed using the Kjeldahl and Soxhlet methods, respectively (AOAC, 2005). Then, the crude fiber content was measured using the Weende method. The ash content was measured by placing the WM

silage sample in a muffle furnace at 550 °C for 6 h. To measure nitrogen-free extract (NFE), the following formula was used: % NFE = % DM - (% EE + % CP + % ash + % CF)

where: NFE = nitrogen free extract, DM = dry matter, EE = ether extract or crude lipid, CP = crude protein, and CF = crude fiber

The fleigh point (Fp) was calculated according to [Ozturk et al. \(2006\)](#) using the following equation:

$Fp = 220 + [2 \times DM\% - 15] - [40 \times pH]$, where % DM denotes the dry matter content of the silage.

Silage quality is characterized as follows: very good (>85), good (60-85), moderate (55-60), satisfactory (20-55), and poor/worthless (<20).

Determination of phytochemical compounds in WM silage

Qualitative testing of phytochemical compounds refers to the research conducted by [Ramli et al. \(2020\)](#). Alkaloid compounds were analyzed using the Dragendroff test. A total of 100 mg of the sample was dissolved in 10 mL of solvent. The sample was filtered, and then 2 mL of the filtrate was added to 1 mL of Dragendroff reagent. The formation of a red precipitate indicated the presence of alkaloids ([Tiwari et al., 2011](#)). Flavonoid compounds were analyzed using the alkaline reagent test. A total of 100 mg of the sample was dissolved in 10 mL of solvent. The sample was filtered, and several drops of NaOH solution were added to the filtrate (2 mL). If a yellow color formed and faded after the addition of acid, the sample tested positive for flavonoids ([Tiwari et al., 2011](#)). For the saponin compound test, 1 g of sample extract was placed in a test tube, then distilled water was added until the entire sample was submerged. The mixture was boiled for 2-3 min, cooled, and shaken vigorously. A positive result is indicated by the formation of stable foam. For the steroid test, 1 mL of the extract is dissolved in 10 mL of chloroform, and an equal volume of sulfuric acid is added. A positive result is indicated by a red upper layer and a yellow sulfuric acid layer with green fluorescence ([Trease and Evans, 1983](#)). The carotenoid test used the Liberman-Burchard reagent. A total of 2 mL of n-hexane extract solution was added to the reagent and H₂SO₄. The sample showed a positive result when a brown ring formed. To test for phenolic compounds, 2 mL of the sample was placed in a test tube, to which 3 drops of 1% FeCl₃ were added. A positive result is indicated by the appearance of a blue or blackish-green color.

Determination of the microbial parameters of WM silage

A total of 5 g of the sample was weighed and dissolved in 50 mL of distilled water. Then, the pH was measured using a calibrated pH meter (pH meter EZ-9908, China). Total acid content was analyzed by titration according to [AOAC \(2005\)](#). The total bacteria and lactic acid bacteria (LAB) were calculated using the total plate count (TPC) method ([AOAC, 2005](#)).

Statistical analysis

The SPSS statistical software package (IBM SPSS, version 26) was used to analyze all experimental data. A one-way analysis of variance (ANOVA) was performed on the nutritional and microbial data of WM silage. Duncan's multiple range test was used to determine the differences between the treatment groups. The physical properties of the WM silage, including color, odor, cleanliness, and texture, were tested using the Kruskal–Wallis test. If a significant effect was found, the analysis continued with Mann–Whitney testing. A statistical difference was defined as $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect on the physical properties of WM silage

According to the ANOVA results, differences in the moisture content of WM silage significantly affected color, aroma, and texture scores ($P < 0.05$, Table 1), but did not affect cleanliness or pH scores. WM silage with 50% and 60% moisture content had higher color scores than WM silage with 60% and 70% moisture content ($P < 0.05$).

Table 1 - Differences in pH, color, odor, cleanliness, and texture score of WM silage with different moisture contents.

Variable	WM40	WM50	WM60	WM70	SEM	P-value
Color	25.0 ^a	25.0 ^a	23.5 ^b	22.4 ^b	0.30	0.004
Odor	24.5 ^a	24.9 ^a	23.4 ^b	21.5 ^c	0.18	<0.001
Texture	24.9 ^a	24.5 ^a	21.4 ^b	19.5 ^c	0.24	<0.001
Cleanliness	25	25	25	25	0	1.0
pH	4.3	4.2	4.3	4.3	0.03	0.16

WM40, WM50, WM60, and WM70: WM silage groups with moisture content levels of 40%, 50%, 60%, and 70%, respectively. ^{a,b,c} Means within a column with different superscripts differ significantly ($P < 0.05$). SEM, standard error of the mean. The standard silage criteria for color are as follows: greenish-yellow = 25, brownish-yellow = 10, and blackish, close to compost color = 0. For odor: fermented sour smell = 25, sour smell not too strong = 20, no smell in silage = 10, and mold growth and compost causing an unpleasant smell = 0. For texture: dry, soft to the touch, crumbly, fermented aroma = 25, moist but not wet = 20, and watery with high water content = 0. For cleanliness: no mold = 25, mold and slime = 0.

A good silage color is a bright light green, yellow, or brownish green. These colors are normal and desirable for well-fermented silage. The color change from green to brownish green is caused by the formation of the pheophytin pigment during anaerobic preservation. This color indicates high dry matter content and optimal fermentation, producing the desired lactic acid (Hidayat, 2014). Similar results were observed in the aroma and texture scores of WM silage at moisture contents between 40 and 50%, but these scores gradually decreased at 60% and 70% moisture content ($P < 0.05$). A good odor in silage is characterized by a distinctive sour smell, pleasant aroma, and slightly sweet taste. This odor indicates successful lactic acid fermentation and minimal activity of spoilage bacteria (Lendrawati et al., 2012). Good silage texture is moist but not dry or slimy. This texture indicates that the raw material was effectively compacted during ensiling, which removes trapped oxygen and creates an anaerobic environment necessary for lactic acid fermentation (Lendrawati et al., 2012).

Effect on the nutritional value of WM silage

The ANOVA showed that differences in moisture content significantly affected the dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), carbohydrates (CH) and Fleigh point (FP) ($P < 0.05$, Table 2). However, it did not affect the crude lipid content or the NFE of WM silage. Further testing indicated a significant increase in the DM content of WM silage fermented with a water content ranging from 40 to 70%, compared to the control ($P < 0.05$). The DM content of WM silage with a moisture content of 40% was higher than that of other moisture contents (50%-70%). The optimal DM content in silage ranges from 35% to 40% (equivalent to a moisture content of 60% to 65%) (Ohmono et al., 2002). This range is ideal because it enables effective compaction, minimizes oxygen entry, and promotes the desired lactic acid fermentation. The OM content of WM silage with various moisture levels (40%-70%) was higher than the unfermented control ($P < 0.05$; Table 2). However, the OM content of WM silage with moisture contents ranging from 40 to 70% yielded similar results. Favorable OM in silage is characterized by high retention of original biomass and low ash content (Kung et al., 2018). The CF content of WM silage with moisture content of 50% or higher was lower than the 40% moisture treatment and the control group ($P < 0.05$; Table 2). Generally, the main anaerobic fermentation process in silos does not significantly alter the total CF concentration. This is because the primary structural carbohydrates (cellulose, hemicellulose, and lignin) are relatively resistant to degradation by the dominant lactic acid bacteria (Ulger and Kaplan, 2017). CH content significantly increased in the control treatment up to WM silage with 40% and 50% moisture content, but stable values were observed above 50% to 70% ($P < 0.05$; Table 2). CP content increased in silage fermented with 40%-70% moisture content compared to the control group ($P < 0.05$). WM silage fermented with a moisture content of 40% or 50% had a higher FP than WM silage fermented with a moisture content of 60% or 70% ($P < 0.05$; Table 2). This indicates that the ensilage process with a moisture content of up to 50% produces the highest quality silage. Higher Fleigh points indicate better silage fermentation quality (Wang et al., 2022).

Table 2 - Differences in nutritional value and Fleigh point of WM silage with different moisture contents

Variable	Control	WM40	WM50	WM60	WM70	SEM	P-value
Dry matter (%)	23.95 ^c	33.54 ^a	29.06 ^b	28.48 ^b	28.05 ^b	1.45	0.001
Organic matter (%)	97.71 ^b	98.21 ^a	98.14 ^a	98.18 ^a	98.18 ^a	0.10	0.002
Crude lipid (%)	1.22	1.31	1.35	1.55	1.40	0.13	0.227
Crude protein (%)	10.38 ^b	11.28 ^{ab}	13.24 ^a	10.31 ^b	11.64 ^{ab}	0.87	0.040
Carbohydrates (%)	7.70 ^c	19.59 ^a	14.58 ^b	14.80 ^b	13.19 ^b	1.80	0.001
Crude fiber (%)	12.20 ^{ab}	13.20 ^a	7.81 ^b	8.14 ^b	7.20 ^b	1.77	0.019
Nitrogen free extract (%)	5.98	6.37	6.76	6.65	5.98	1.44	0.968
Fleigh point	-	101.4 ^a	93.78 ^{ab}	88.63 ^b	87.76 ^b	3.91	0.029
Control group (fresh WM legume), WM40, WM50, WM60, and WM70: WM silage groups with moisture content levels of 40%, 50%, 60%, and 70%, respectively. ^{a,b,c,d} : Means within a column with different superscripts differ significantly ($P < 0.05$). SEM, standard error of the mean							

Effect on the phytochemical compounds in WM silage

A qualitative analysis of WM silage demonstrated that silage fermented at a moisture content ranging from 40 to 70% contained several phytochemical and antibacterial compounds, including flavonoids, steroids, saponins, phenols, and carotenoids. However, it did not contain alkaloid compounds (Table 3). Flavonoids inhibit the growth of unwanted microorganisms, such as fungi (Kiczorowski et al., 2022). Their antioxidant properties help reduce the loss of important nutrients, such as beta-carotene and tocopherol (vitamin E), during storage. Previous studies have shown that the ensilage process affected steroid compounds through microbial degradation and transformation (Bolsen et al., 1996). It was found that the loss of saponins ranged from 12% to 100%, depending on the type of saponin and the ensiling conditions (Kholif, 2023). Phenolic compounds, which are often antinutrients, such as tannins, may decrease in concentration during anaerobic fermentation (Jeyakumar and Lawrence, 2022). Carotenoids in forage are an important source of provitamin A (beta-carotene), a strong antioxidant that boosts the immune system, thereby improving the health and performance of livestock (Miao et al., 2024).

Effect on the microbial count in WM silage

The ANOVA results showed that the total acid content, total bacterial count, and lactic acid bacteria in WM silage fermented with different moisture contents increased compared to the WM group without ensiling ($P < 0.05$; Table 4), but similar results were observed among the groups with 40% to 70% moisture content. The ensiling process increases the total acidity of feed materials by breaking down water-soluble carbohydrates into organic acids through the action of microorganisms, primarily lactic acid bacteria (LAB) (Abedi and Hashemi, 2020). Once anaerobic conditions are achieved in a tightly sealed silo, LAB begins to multiply exponentially. These bacteria ferment carbohydrates into lactic acid, rapidly lowering the pH to 3.8–4.5 (Zhang et al., 2023).

Table 3 - Differences in phytochemical compounds of WM silage with different moisture contents.

Variable	WM40	WM50	WM60	WM70
Alkaloids	Negative (-)	Negative (-)	Negative (-)	Negative (-)
Flavonoids	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Steroids	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Saponins	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Phenols	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Carotenoids	Positive (+)	Positive (+)	Positive (+)	Positive (+)

WM40, WM50, WM60, and WM70: WM silage groups with moisture content levels of 40%, 50%, 60%, and 70%, respectively. ^{a,b,c,d}: Means within a column with different superscripts differ significantly ($P < 0.05$). SEM, standard error of the mean.

Table 4 - Differences in total acids, total bacteria count, and lactic acid bacteria count of WM silage with different moisture contents.

Variable	Control	WM40	WM50	WM60	WM70	SEM	P-value
Total acids (%)	1.29 ^b	2.86 ^a	2.24 ^a	2.03 ^{ab}	2.40 ^a	0.36	0.018
Total bacteria count (log ₁₀ CFU/g)	4.99 ^b	7.86 ^a	7.92 ^a	8.03 ^a	7.97 ^a	0.31	<0.001
Lactic acid bacteria count (log ₁₀ CFU/g)	2.17 ^b	6.98 ^a	6.73 ^a	6.79 ^a	6.61 ^a	0.33	<0.001

control group (fresh WM legume), WM40, WM50, WM60, and WM70: WM silage groups with moisture content levels of 40%, 50%, 60%, and 70%, respectively. ^{a,b,c}: Means within a column with different superscripts differ significantly ($P < 0.05$). SEM, standard error of the mean

CONCLUSION

In conclusion, irrespective of the moisture content level, the ensiling process improved the physical, phytochemical, and nutritional properties of WM compared to the control, fresh WM legumes that were not processed into silage. Furthermore, using 50% moisture content in the ensiling process yielded the optimal results in terms of the physical, microbiological, nutritional, and phytochemical characteristics of the WM silage.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contribution

Conceptualization, F. Yosi; Methodology, F. Yosi and N. Gofar; Investigation, F. Yosi, Muhakka, N. Gofar, N. Hasanah, A. Nababan, I. Lestari; formal analysis, validation, F. Yosi, Muhakka; writing-original draft preparation, F. Yosi; Writing-review and editing, Muhakka, N. Gofar, N. Hasanah, A. Nababan, I. Lestari; Supervision, F. Yosi

Consent to publish

All authors have reviewed and approved the final manuscript for publication.

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Ethical considerations

All related to ethical issues, consisting of plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by the authors.

Competing interests

All authors involved in this research work declare having no competing interests.

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