

Original Research Article

Enrichment of Ensilage Quality and Nutrients of Whole-Plant Water Hyacinth) *Eichhornia crassipes* (Based Silages)

ABSTRACT

Aims:

This study aimed at developing practices for enrichment both ensilaged quality and nutrient contents of whole-plant water hyacinths (WWH) based silage by mixing with various local feed ingredients.

Study design: Twelve recipes with three replications each were assigned in a completely random design.

Place and Duration of Study: Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Lampang Campus, Thailand, between October 2018 and September 2019.

Methodology: Twelve recipes were: T1= 1-d wilted WWH + 1.96 % salt, T2 = 1-d wilted WWH + 4.15 % molasses + 8.27 % rice bran, T3 = 1-d wilted WWH + 6.63 % molasses + 15.86% rice bran, T4 = 1-d wilted WWH +8.91% molasses + 22.83 % rice bran, T5 = 2-d wilted WWH + 1.59% Salt, T6 = 2-d wilted WWH + 11.17 % molasses + 13.33 % rice bran, T7 = 2-d wilted WWH + 10.75 % molasses + 25.67 % rice bran, T8 = 2-d wilted WWH + 10.36 % molasses + 37.11% rice bran, T9 = 4-d wilted WWH + 1.16% salt, T10 = 4-d wilted WWH + 5.10% rice bran, T11 = 4-d wilted WWH + 4.30 % molasses, T12 = 4-d wilted WWH + 4.30% molasses + 5.14 % rice bran. Ingredients were mixed into triplicates of a 30 kg high-density polyethylene plastic bag under anaerobic condition for 30 days.

Results: The results demonstrated that 1-d wilted (T4) and most of the 2-d wilted WWH with rice bran, and molasses supplementations (T6, T7, and T8) had 3.80 - 4.19 pH, 0.44 - 1.21 % lactic acid, 0.48 – 0.81% acetic acid (except T4 = 4.77%), and 0.35 - 0.86 % citric acid that was more superior in chemical properties and physical appearances than those other formulas ($P<0.05$).

Conclusion: WWH should be wilted in sun-dry for approximately one to two days; chopped into 5 to 10 cm length; ensilaged with 13 to 37% rice bran plus about 10 % molasses under anaerobic conditions for 30 days. Based on these practices, WWH based silages would yield appropriate pH, acid contents, and dominants in desired physical appearances except for some 1-d wilt WWH silage that was more dominant in acetic acid content.

Keywords: Water hyacinth, ensiling, silage, feed, ruminant.

1. INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) has been classified as a foreign water weed with severe outbreaks in tropical areas. It can multiply and propagate rapidly, resulting in severe adverse effects by obstruction of water flow and water transport. Water hyacinth obstructs the drainage of sluices in the irrigation system, negatively affecting the water flow rate. Dense water hyacinths also block sunlight, which negatively affects the photosynthesis of underwater plants, retarding their growth

and ruining ecosystems. However, water hyacinths have some advantages in nutritional values that benefit as feed to animals. Suppose large quantities of water hyacinth could be harvested effectively from water. It could be fed directly to some animals or preserved in the ensilaged form. Tham (2015) reported that whole-plant water hyacinth contained high moisture content, approximately 91.3-94.2 % of the dry matter [1]. Besides, fresh leaves of water hyacinth contained 18.1% crude protein; total leaf petiole had 7.6% crude protein; whereas whole-plant water hyacinth comprised approximately 12.8% of crude protein. Several works have reported that freshwater hyacinths, especially the leaves and petioles or the above-water part, can feed pigs [2, 3] or cattle [4, 5]. Water hyacinths may feed cattle in the dried form [6] or the fermented form [7]. Tham and Uden [7] reported that fermented water hyacinths could be used to fatten cattle in the proportion of 30-45% with rice straw. However, the development of water hyacinth in the form of whole plant silage in combination with local additives or feed ingredients to enrich as quality roughage is limited. The objective of this research was to develop the recipe and some techniques as a practical guideline for ensiling of whole-plant water hyacinths combined with readily available local ingredients that might be able to promote the use of whole-plant water hyacinth to become an alternative high-quality roughage; benefit to ruminant production that might help to relieve those problems of dense water hyacinth infestation, especially in some irrigated or public areas.

2. MATERIAL AND METHODS

Whole-plant water hyacinths (WWH) were collected from irrigation canals and public waterways in Mueang District, Lampang province (latitude 18°21'51.4"N 99°35'49.4"E), Thailand. After collection, whole-plant water hyacinths were allowed to wilt under the sun-dried open-air condition to reduce moisture for 1, 2, or 4 days, respectively. Then, it was chopped into small pieces of 5-10 cm length by a multipurpose shredder model MP-003 with a 6.5 horsepower using Hinota® gasoline engine as a supply motor power. Twelve recipes of WWH based silages were developed (Table 1) as the following:

T1= 1-d wilted WWH + 1.96 % salt

T2 = 1-d wilted WWH + 4.15 % molasses + 8.27 % rice bran

T3 = 1-d wilted WWH + 6.63 % molasses + 15.86% rice bran

T4 = 1-d wilted WWH +8.91% molasses + 22.83 % rice bran,

T5 = 2-d wilted WWH + 1.59% salt

T6 = 2-d wilted WWH + 11.17 % molasses + 13.33 % rice bran,

T7 = 2-d wilted WWH + 10.75 % molasses + 25.67 % rice bran,

T8 = 2-d wilted WWH + 10.36 % molasses + 37.11% rice bran

T9 = 4-d wilted WWH + 1.16% salt,

T10 = 4-d wilted WWH + 5.10% rice bran

T11 = 4-d wilted WWH + 4.30 % molasses

T12 = 4-d wilted WWH + 4.30% molasses + 5.14 % rice bran (dry matter basis).

All formulas were thinly sprayed with 5% ~~distilled~~-vinegar to stimulate the acidity during the mixing process. Each recipe was mixed thoroughly into triplicate of 30 kg each in high-density polyethylene plastic bags; then, the anaerobic condition was applied using a handy vacuum cleaner (Mamaru® model MR-2301, China) for 30 seconds before wrapping by ropes. All bags were piled, covered by an opaque tent, and overlaid by wheel tires for 30 days. After finishing ensiling process, approximately 50 g of each replicate was randomly sampled from each bag, cut into small pieces, and crushed within a high-speed blender. About 200 ml of distilled water was added and kept at 4 °C for approximately 24 h [8] filtered with Whatman No. 4 filter paper. Then pH was measured immediately using a pH meter (pHTestr®30, EUTECH, Singapore). Lactic acid, acetic acid, and citric acid were measured by titration [9]. The physical quality characteristics of fermented silages were graded according to the criteria indicated in the handbook of the Department of Livestock Development [10], including 1) odor: fragrant, like pickled fruit or vinegar (12 points); unscented, slightly pungent (8 points); very pungent and slightly foul (4 points); rotten or moldy (0 points); 2) plant texture: firm, with

Comment [pichet1]: For different recipes, how select these, are there references?

leaves and stems intact and free of impurities (4 points); firm, leaves and stems slightly rotting, slimy (2 points); firm, leaves and stems highly

Table 1. Ingredients of WWH based silages in various recipes (Dry matter basis)

Items	Amount (kg)				Total (kg)
	WWH	Rice bran	Molasses	NaCl	
1-d wilted					
T1	98.17	-	-	1.96	100
T2	87.56	8.27	4.15	-	100
T3	77.49	15.86	6.63	-	100
T4	68.25	22.83	8.91	-	100
2-d wilted					
T5	98.39	-	-	1	100
T6	75.48	13.33	11.17	-	100
T7	63.57	25.67	10.75	-	100
T8	52.51	37.11	10.36	-	100
4-d wilted					
T9	98.83	-	-	1.16	100
T10	94.88	5.10	-	-	100
T11	95.68	-	4.30	-	100
T12	90.54	5.14	4.30	-	100

disintegrated, impurity (1 point); mucus and very dirty (0 points); 3) color: greenish-yellow or khaki (3 points); yellowish-green darken (2 points); golden brown (1 point); dark brown or black (0 points); 4) pH 3.5-4.2 (6 points), 4.4-4.7 (4 points), 4.7-5.1 (2 points), > 5.1 (0 points); 5) quality rating: 20-25 (Very Good), 15-19 (Good Quality), 6-14 (Medium Quality), and 0-5 (Low Quality). Only Very Good quality samples were analyzed for dry matter, crude protein, ether extract, ash [9], neutral detergent fiber (NDF), and acid detergent fiber (ADF) [11]. Gross energy (GE) was analyzed using AC500 Isoperibol Calorimeter (Leco, USA). Data were analyzed for variance using Analysis of Variance (ANOVA), mean values were compared by Duncan's new multiple range test using SPSS 15.0 for Windows, and significance was declared when P-value <0.05 [12]. The statistical model was as the following: $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$, where Y_{ij} = observation, μ = Overall mean, τ_i = treatment effect, and ε_{ij} = random error [13]. For physical assessment, data were analyzed and compared means using Kruskal-Wallis of a nonparametric method [12]. This experiment was conducted at the Faculty of Agricultural Science and Technology, Rajamangala University of Technology Lanna, Lampang, Thailand, between October 2018 and August 2019.

3. RESULTS AND DISCUSSION

3.1 Effect of drying on moisture and dry matter of WWH

Before ensiling, moisture and dry matter contents of WWH were observed to determine an appropriate time for a wilting process. The average moisture content of WWH at harvesting from water sources was 63.53% or 36.47% dry matter (Figure 1). Major moisture contents were contained in the root parts. Tropical plants should be wilted to reduce their moisture concentration to obtain at least 30% dry matter to decrease buffering ability that resists changing of pH, enhance the concentration of water-soluble carbohydrate, and prevent loss from silage seepage [14, 15].

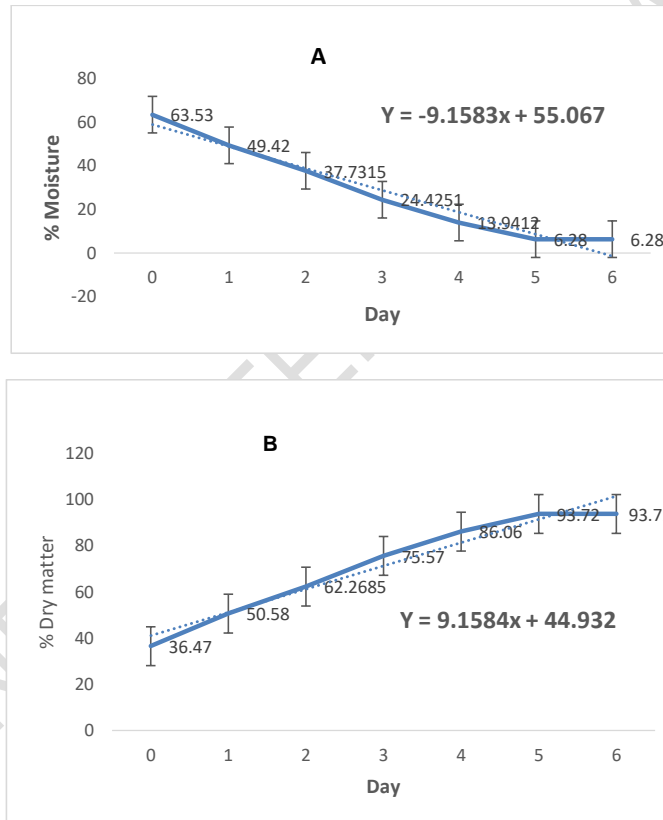


Figure 1. Moisture (A) and dry matter (B) contents of WWH observed in a hot-air oven at 65°C before ensiling

3.2 Effects of ensilage method on Physical qualities of WWH

Comment [pichet2]: If want to show, author should explain more in method and do not confuse with hot air oven drying. When graph showed 6 days of drying, why treatment selected only 1, 2, 4 days, what is criterion. Author studied at any part of plant or whole, it is good if can show data, do not forget that author used whole plant in next study.

Comment [pichet3]: If want to show these data, author should write the clearly method and how much replicate, from the previous method, sun drying period is only 4 days, also not hot air oven drying

Comment [pichet4]: 1-days, trend seemed to be linear, but for day 6 is not, so equation may be change, also r-square is needed

Comment [pichet5]: It is confused that author prepared material by sun drying but showed these data by hot air oven drying, wrong and method indicated only 4 days. How about 6 days?

Values of pH and acidity of whole-plant water hyacinth ensilaged with different methods were shown in Table 2. The results showed that WWH prepared with method T3, 4, 6, 7 and 8 had pH lower 4.2 that were good for inhibiting growth of other microorganisms, enabling the preservation of the quality of fermented whole-plant water hyacinths (reference). These methods were integration of adding high molasses and rice bran to be nutrient sources of useful bacteria. (reference). Growth of bacteria could be reflected by the sums of acid production showed high in T3, 4, 6 and 7. In this study adding salt with different rates (T1, 5 and 9) did not facilitate fermentation since high pH and low acid production of the ensilaged product were shown replied low activity of bacteria. There was feasible that salt might inhibit bacter growth. (then show reference referred o effect of salt on bacteria growth)

Comment [pichet6]: Guidance for writing result and discussion

Adding 1% salt (NaCl) in T1, T5, and T9 was intended to increase the palatability of fermented plants but not relatively improve the fermentation mechanism resulting in low acidity with a pH of about 7.85-8.47.

Comment [pichet7]: 1 or 1.96% or else? Please check treatment

Comment [pichet8]: Author should show and discuss by each criterion i.e. when added salt, it is not working because it does not promote lactic acid bacteria growth, resulted in high Ph, finally not improve nutritional and physical quality

In an efficient ensiling mechanism, pH should be lower than 4.2, which will inhibit the growth of other microorganisms, enabling the preservation of the quality of fermented whole-plant water hyacinths. The pH below 4.2 was found in the 1-d wilted WWH for T3 and T4; the 2-d wilted WWH for T6, T7, and T8, but not for the 4-d wilted (P = 0.001). That low pH relies on the mechanism of natural lactic acid bacteria to convert soluble carbohydrates in each formula into a lactic acid that results in a dropping pH below 4.2 and be able to prevent other microorganisms such as yeast and mold that cause spoilage of fermented plants [16]. This study also showed that WWH wilted for 4 days

Comment [pichet9]: This study clearly showed that integration of molass and rice bran (T3,4,6,7,8) were best for silage, but author showed and discuss unclearly. They should be explained point by point. For example, started from overall results and showed what was the best physical quality, what was the best for nutritional quality, then explain with the result and discuss.

Kung et al. [17] reported that a higher pH than 4.2 may be associated with extremely dry (>42 % of dry matter) before ensiling, overly plant mature, drought-stricken, or low sugar contents in case of corn silage. However, the general pH of grass silage may range between 4.3 to 4.7 [17]. Lactic acid contents were found relatively higher for T4 (0.71%), T6 (0.73%), T7 (0.89%), and T8 (1.21%) compared to other formulas (P = 0.001). These groups of results were similar to those found in high-moisture corn silage in that amount of lactic acid contents ranged 0.5-2.0% due to high moisture contents of WWH, but lower than grass silage that typically ranged 6 to 10% [18]. Surprisingly, that 1-d wilted WWH found high amounts of acetic acids for T2 (2.88%), T3 (3.31%), and T4(4.77%) (P = 0.001). In general, acetic acid concentration in silage usually ranks from 1 to 3 %, but higher than the average concentration of approximately 3 to 4 % is often reported in silages treated with *L. buchneri* because of the conversion of lactic acid to acetic acid [17]. Citric acid concentrations were found higher in T4 (0.51%), T6 (0.52%), T7 (0.57%), and T8 (0.86%) (P = 0.001). Ke et al. [19] reported that citric acid helps improve fermentation quality, limit proteolysis, and improve the fatty acid composition of silage.

Table 2. pH and acids of WWH in different formulas.

Items	pH	%		
		Lactic acid	Acetic acid	Citric acid
1-d wilted				
T1	7.85 ^e	0.07 ^a	0.45 ^{bc}	0.05 ^a
T2	4.59 ^c	0.43 ^c	2.88 ^e	0.22 ^{bc}
T3	4.19 ^b	0.44 ^c	3.31 ^f	0.35 ^d
T4	3.80 ^a	0.71 ^d	4.77 ^g	0.51 ^e
2-d wilted				
T5	8.47 ^f	0.21 ^b	0.14 ^a	0.15 ^{ab}

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T6	4.01 ^b	0.73 ^d	0.48 ^b	0.52 ^e
T7	3.85 ^a	0.89 ^e	0.53 ^c	0.57 ^e
T8	3.90 ^{ab}	1.21 ^f	0.81 ^d	0.86 ^f
4-d wilted				
T9	7.90 ^e	0.07 ^a	0.05 ^a	0.05 ^a
T10	5.38 ^d	0.20 ^b	0.13 ^{ab}	0.14 ^{ab}
T11	4.86 ^c	0.37 ^c	0.29 ^{abc}	0.31 ^{cd}
T12	4.64 ^c	0.46 ^c	0.31 ^{abc}	0.33 ^{cd}
S.E.	0.17	0.04	0.19	0.07
P-value	0.001	0.001	0.001	0.001

^{abcdegh}, different letters in the same column showed a statistically significant difference (P<0.05); T1= 1-d wilted WWH + 1.96 % salt, T2 = 1-d wilted WWH + 4.15 % molasses + 8.27 % rice bran, T3 = 1-d wilted WWH + 6.63 % molasses + 15.86% rice bran, T4 = 1-d wilted WWH +8.91% molasses + 22.83 % rice bran, T5 = 2-d wilted WWH + 1.59% Salt, T6 = 2-d wilted WWH + 11.17 % molasses + 13.33 % rice bran, T7 = 2-d wilted WWH + 10.75 % molasses + 25.67 % rice bran, T8 = 2-d wilted WWH + 10.36 % molasses + 37.11% rice bran, T9 = 4-d wilted WWH + 1.16% salt, T10 = 4-d wilted WWH + 5.10% rice bran, T11 = 4-d wilted WWH + 4.30 % molasses, T12 = 4-d wilted WWH + 4.30% molasses + 5.14 % rice bran (dry matter basis).

Physical quality characteristics of fermented ensilaged with different methods were shown in Table 3. The results showed that WWH prepared with method T3, 4, 6, 7 and 8 obtained scores of odor, Ph, color, total scores highly significant higher than other methods (p<0.01), although odor did not different from WWH prepared by method T12 (p>0.05). This confirmed improvement caused by integration of adding high molasses and rice bran.

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An assessment of the physical characteristics of WWH in different formulas is present in Table 3. The score of odor, color, pH, and the total score was similar for 1-d wilted WWH (T3 and T4), 2-d wilted WWH (T6, T7, and T8), 4-d wilted WWH (T12) (P >0.05); and then had the very good quality. The very good ensiling quality groups characterized odor smelled like pickled fruit or vinegar; color was greenish-yellow; plant texture was firm, with leaves and stems intact and free of impurities; pH 3.5-4.2, and total score ranged between 20-25. The ensilaged effects of WWH by organic acid-producing microorganisms in this experiment were consistent with the fermentation of various forage crops by lactic acid-producing bacteria reported by several works [20, 21, 16],

Table 3. Physical assessment of ensilaged WWH in different formulas.

Items	Odor	Texture	Color	pH	Total score	Quality
1-d wilted						
T1	4.00 ^a	4	2.33 ^{ab}	0.00 ^a	7.33 ^a	Medium
T2	8.00 ^c	4	2.67 ^{bc}	2.67 ^b	18.67 ^e	Good
T3	12.00 ^d	4	3.00 ^c	4.67 ^c	24.33 ^g	Very Good
T4	12.00 ^d	4	3.00 ^c	6.00 ^c	25.00 ^g	Very Good
2-d wilted						
T5	8.00 ^c	4	2.00 ^a	0.00 ^a	14.00 ^{bc}	Medium
T6	12.00 ^d	4	3.00 ^c	6.00 ^c	25.00 ^g	Very Good
T7	12.00 ^d	4	3.00 ^c	6.00 ^c	25.00 ^g	Very Good
T8	12.00 ^d	4	3.00 ^c	6.00 ^c	25.00 ^g	Very Good
4-d wilted						
T9	6.67 ^b	2	2.00 ^a	0.00 ^a	12.67 ^b	Medium
T10	8.00 ^c	4	2.33 ^{ab}	0.00 ^a	14.33 ^c	Good
T11	8.00 ^c	4	2.00 ^a	2.67 ^b	16.67 ^d	Good
T12	12.00 ^d	4	2.00 ^a	2.00 ^b	20.33 ^f	Very Good
S.E.	0.66		0.28	0.82	0.93	
P-value	0.001	na	0.001	0.001	0.001	

^{abcdegh}, different letters in the same column showed a statistically significant difference (P<0.05).

T1= 1-d wilted WWH + 1.96 % salt, T2 = 1-d wilted WWH + 4.15 % molasses + 8.27 % rice bran, T3 = 1-d wilted WWH + 6.63 % molasses + 15.86% rice bran, T4 = 1-d wilted WWH +8.91% molasses + 22.83 % rice bran, T5 = 2-d wilted WWH + 1.59% Salt, T6 = 2-d wilted WWH + 11.17 % molasses + 13.33 % rice bran, T7 = 2-d wilted WWH + 10.75 % molasses + 25.67 % rice bran, T8 = 2-d wilted WWH + 10.36 % molasses + 37.11% rice bran, T9 = 4-d wilted WWH + 1.16% salt, T10 = 4-d wilted WWH + 5.10% rice bran, T11 = 4-d wilted WWH + 4.30 % molasses, T12 = 4-d wilted WWH + 4.30% molasses + 5.14 % rice bran (dry matter basis).

Smell: fragrant, like pickled fruit or vinegar (12 points); unscented, slightly pungent (8 points); very pungent and slightly foul (4 points); rotten or moldy (0 points). Plant texture: firm, with leaves and stems intact and free of impurities (4 points); firm, leaves and stems slightly rotting or slimy (2 points); firm, leaves and stems highly disintegrated, impurity (1 point); mucus and very dirty (0 points). Color: greenish-yellow or khaki (3 points); yellowish-green or dark green (2 points); golden brown (1 point); dark brown or black (0 points). pH 3.5-4.2 (6 points); 4.4-4.7 (4 points); 4.7-5.1 (2 points); > 5.1 (0 points). Quality Rating: 20-25 (Very Good); 15-19 (Good Quality); 6-14 (Medium Quality); and 0-5 (Low Quality).

Overall means of WWH nutritive values in various recipes consisted of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash at: 51.40, 8.62, 2.58, 53.45, 47.97, 20.31% of dry matter, respectively, and gross energy (GE) at 3.31 kcal/kg (Table 4).

Crude protein found in WWH was slightly higher than those reported in Napier grass silage ranged 6.51-7.40% [22, 23]. After the ensiling process, WWH with very good quality had crude protein levels closed to those of corn silage, which had protein levels of about 8.80-9.90% [24], but lower than those legume silages that contained approximately 10.43-11.30 % CP [25]. In terms of fibers, ensiled WWH had fairly levels of NDF and ADF that might allow in greater edible amount and digestible contents for ruminant animals, compared to conventional forage with relatively high levels of NDF and ADF pulp, which are important factors limiting feed intake and digestibility in the rumen. However, only one disadvantage of ensiled WWH found in this study was a relatively high total mineral content (ash) compared to conventional forage due to including the root that can absorb various minerals from the water component of its. The ramifications of feeding high ash diets to ruminant animals are not well understood, but excessive ash contents in forages or dairy cow diets could be a silent antagonist in the performance of dairy nutrition programs [26].

Table 4. Nutrient composition of the selected WWH based silages (% dry matter)

Items	DM	CP	EE	NDF	ADF	Ash	GE
1-d dry in the shade							
T4	42.16	8.59	2.50	54.12	48.84	22.14	2.81
2-d dry in the shade							
T6	48.07	8.26	2.06	51.66	49.20	22.74	3.28
T7	55.29	8.50	2.54	53.37	46.90	18.53	3.54
T8	60.08	9.16	3.25	54.65	46.95	17.83	3.64
Overall mean	51.40	8.62	2.58	53.45	47.97	20.31	3.31

WWH = whole-plant water hyacinth, DM = dry matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, GE = gross energy, kcal/kg. T4 = 1-d wilted WWH +8.91% molasses + 22.83 % rice bran, T6 = 2-d wilted WWH + 11.17 % molasses + 13.33 % rice bran, T7 = 2-d wilted WWH + 10.75 % molasses + 25.67 % rice bran, T8 = 2-d wilted WWH + 10.36 % molasses + 37.11% rice bran (Dry matter basis).

Comment [pichet12]: with different ratio of WWH, rice bran and molasses meant starting from different nutrients it is difficult to compare data, even author will compare % of each nutrient increased after ensilage, see in the example table

Comment [pichet13]: should show all data like table 2, 3 also show WWH without ensilage

Comment [pichet14]: compared with statistics

	TREATMENT BEFORE ENSILAGE						AFTER ENSILAGE						% CHANGE (COULD COMPARE)					
	DM	CP	FAT	ASH	NDF	ADF GE	DM	CP	FAT	ASH	NDF	ADF GE	DM	CP	FAT	ASH	NDF	ADF GE
1																		
2																		
3																		
4																		
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6																		
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8																		
9																		
10																		
11																		
12																		

CONCLUSION

the best method for ensilaging WWH was integration with Whole-plant water hyacinth can be conserved as good-quality silage. To enrich ensilage characteristics and quality, we recommended initiating the wilting process of the whole plants in open air-dry condition for approximately one or two days; chopped the whole-plant into 5 to 10 cm in length; mixed with 13 to 37% rice bran plus approximately 10 % molasses under anaerobic conditions for 30 days. Based on these practices, WWH based silages would yield appropriate pH, acid contents, and dominants in desired physical appearances except the 1-d wilt WWH silage that was quite notable in acetic acid content.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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