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Enrichment of Rice Straw Silage with Sacha Inchi (*Plukentia volubilis* L) Meal and Sambung Nyawa (*Gynura procumbens*) Leaves

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ABSTRACT

Farmers in Indonesia utilize a substantial amount of rice straw to provide feed for their livestock but restricted in terms of its low nutritional value. This study was conducted with objective to evaluate the quality of rice straw silage with addition of sacha inchi (*Plukenetia volubilis* L.) meal and sambung nyawa (*Gynura procumbens*) leaves based on physical, chemical, and microbiological properties. The study employed a completely randomized design with 9 treatments (P0: 100% rice straw, P1: 90% rice straw + 10% sacha inchi meal, P2: 85% rice straw + 15% sacha inchi meal, P3: 80% rice straw + 20% sacha inchi meal, P4: 75% rice straw + 25% sacha inchi meal, P5: P1+10% sambung nyawa leaves, P6: P2+10% sambung nyawa leaves, P7: P3+10% sambung nyawa leaves, P8: P4+10% sambung nyawa leaves) and 5 replications, incorporating the addition of sambung nyawa leaves. The variables examined include the physical quality i.e. colour, flavour, texture, and presence of mold; chemical quality i.e. pH, crude protein (CP), and crude fibre (CF); and microbiological quality i.e. lactic acid bacteria (LAB), yeasts, aerobic bacteria, and pathogenic bacteria. The results of the physical quality test indicated that the addition of sacha inchi meal and sambung nyawa leaves resulted no significant difference. On the other hand, the chemical quality showed that the addition of sacha inchi meal and sambung nyawa leaves have enhanced the rice straw silage nutritional content ($p<0.05$) particularly CP content by about 4 fold. The microbiological tests showed that the addition of sacha inchi meal and sambung nyawa leaves had an impact on the preservation of silage by inhibiting the growth of pathogenic microorganisms. It is concluded that the quality of rice straw silage has improved by the addition of 10-25% sacha inchi meal and 10% sambung nyawa leaves.

Keywords: Gynura procumbens leaves, Plukentia volubilis L. meal, Rice straw, Silage quality

Introduction

Rice straw, as an agricultural by-product, possesses the advantage of being abundantly available, rendering it an affordable option for farmers due to its cost-effectiveness. Indonesia produces 54 million metric tons of rice (BPS, 2022). In terms of quantity, on average every 1 kg of rice produces around 1 to 1.5 kg of straw. Based on the data above, rice straw production in Indonesia can be estimated to reach 54-81 million metric tons per year. However, the extensive employment of rice straw as animal feed faces impediments in the form of its low crude protein (CP) content. Specifically, rice straw entails a CP content of approximately 3.80%, crude fibre (CF) content of approximately 39.81%, lignin content of approximately 6.7%, and a silica content ranging from 12-16% (Maluyu and Suhardi, 2016).

During the harvest season, the copious amounts of rice straw can be conserved via the process of fermentation with the utilization of lactic acid bacteria (LAB), commonly referred to as silage. In the event of ruminant livestock exclusively consuming rice straw, they would be deprived of essential nutrients. The quality of the silage is deemed satisfactory with the additional feed components to enhance the nutritional value. The physical attributes are commendable, the nutritional composition aligns with the demands of the livestock, and it predominantly consists of LAB (Pratiwi *et al.*, 2015). The role of LAB is to convert carbohydrates into lactic acid. The presence of lactic acid will lower the pH of silage, thereby suppressing unnecessary bacterial growth. Lactic acid can be formed during fermentation process with the activity of LAB, mainly of the genus Lactobacillus, which takes place spontaneously and occurs naturally by paying attention to conditions the environment is anaerobic (Perdana *et al.*, 2021).

In the rice straw processing as silage, there would be an opportunity to enhance the quality of silage with agricultural processing by product, such as sacha inchi (*Plukenetia volubilis* L.) meal. Sacha inchi seeds are a potential source of oil and protein which are rich in polyunsaturated fatty acids (PUFA). Sacha inchi seeds contain about 54.7% fat, 29.2% protein, 6.59% carbohydrates, and 6.61% fiber (Bueno *et al.*, 2018). Sacha inchi seeds which are made into oil produce sacha inchi meal which is thought to still contain good nutrients, especially protein. Another potential additive of fresh forage that could be used, was sambung nyawa (*Gynura procumbens)* leaves. Sambung nyawa well known as medicinal plant and contained flavonoid compounds, saponins, steroids or triterpenoids, and tannins, all of which exhibit antibacterial properties against bacteria. In terms of antibacterial efficacy, the extract obtained from sambung nyawa is particularly effective in inhibiting the growth of Gram-negative bacteria (*Escherichia coli*) as opposed to Gram-positive bacteria (LAB) (Mulyani *et al.*, 2018). Therefore, the objective of the study was evaluate the quality of rice straw silage with addition of sacha inchi meal and sambung nyawa leaves to correct the protein deficiency of rice straw based on physical, chemical, and microbiological qualities.

Materials and Methods

Silage materials

Fresh rice straw aromatic variety was collected from the Indonesian Center for Rice Research, Muara, Bogor. Fresh rice straw was wilted overnight and then cut into 3-5 cm long using a chopper. Sacha inchi meal was obtained from the residue of sacha inchi oil production at the experimental garden of the Natural Product Laboratory, SEAMEO BIOTROP, Bogor. Sambung nyawa leaves was from IPB Lecturer Housing. Sacha inchi meal and dried sambung nyawa leaves were ground and sifted to pass 1 mm sieve. Silage additives including inoculum of *Lactiplantibacillus plantarum* 1A-2 (Sarwono *et al.*, 2021), rice bran and molasses. Inoculum of *L. plantarum* with population of $1.65x10^9$ CFU mL^{-1} was diluted ten times using sterile distilled water. Rice bran and molasses were purchased from local market. Rice bran and molasses were used as much as 5% and 1%, respectively.

Silage preparation

Chopped rice straw (1 kg), rice bran (50g), and molasses (10g) were mix thoroughly and sprayed with *L. plantarum* 1A-2 in all treatments. Sacha inchi meal and sambung nyawa leaves were included based on the treatment level (P0: 100% rice straw, P1: 90% rice straw + 10% sacha inchi meal, P2: 85% rice straw + 15% sacha inchi meal, P3: 80% rice straw + 20% sacha inchi meal, P4: 75% rice straw + 25% sacha inchi meal, P5: P1 + 10% sambung nyawa leaves, P6: P2 + 10% sambung nyawa leaves, P7: P3 + 10% sambung nyawa leaves, P8: P4 + 10% sambung nyawa leaves). The mixture was devided into 5 replicates and placed in a vacuum emboss food grade plastic bag (30 x 20 cm) as silo, each weighing approximately of 200 g. The air inside the bags is then removed using a Roudiao vacuum sealer. Subsequently, the fermentation process is carried out anaerobically at room temperature for a duration of 30 days (Cherdthong *et al.*, 2020).

Physical analysis of silage

A quantity of 10 g of samples were segregated for the purpose of conducting physical evaluations on silage based on attributes including aroma, colour, texture, and presence of mold parameters. The evaluation was carried out by four untrained panelists using a table of silage assessment criteria (Table 2).

Microbial analysis of silage

A total of 10 g of silage sample were taken and put in a plastic bag, added with 90 mL of sterile distilled water, serial dilutions were carried out by adding 1 mL of silage liquid to 9 mL of 0.85% sodium chloride. A total of 20 µL of silage liquid from dilutions 10^{-1},10^{-3},10^{-5} were performed. The 20 µL of various solution were then spread on agar plates. The LAB was counted on de Man Rogosa Sharpe agar (MRS, Difco), aerobic microbe were cultivated on nutrient agar (Nissui-Seiyaku), yeast were cultivated on potato dextrose agar (Nissui-Seiyaku) and the presence of coliform was detected on blue light agar (Nissui-Seiyaku).

Chemical analysis of silage

The remaining portion of silage is dried using an oven at 60˚C for a duration of 3 days until the weight reached a constant state. Following this, the sample was ground using a grinder and filtered using a sieve. Dry silage sample are employed for the purpose of examine dry matter (DM), ash, CP, CF, and crude fat analyses followed the AOAC (2005) method. The pH of silage measurement was carried out using solution as prepared for microbial analysis using calibrated pH meter and Fleigh point (FP) followed the Idikut *et al.* (2009) method. $FP = 220 + (2\times \%BK - 15) - (40\times pH)$. Fleigh point indicator : 85 – 100 (very good), 60 – 80 (good), 55 – 60 (fair), 25 – 40 (poor), <25 (bad).

Statistical analysis

To analyze physical and chemical analysis was arranged using thecompletely randomized design with one factor (different levels of sacha inchi meal and sambung nyawa leaves treatment). Data were analyzed by analysis of variance (ANOVA) and if there is signinficant different ($p<0.05$), then tested further by the Duncan;s New Multiple Range Test using SPSS 22 software. The microbial analysis was arranged using descriptive test which was then carried out by the Shapiro-Wilk Test.

Table 1. Silage assessment criteria

Criteria	Assessment			
	Very good (100)	Good (80)	Medium (10)	Bad (0)
Aroma	Very sour (25)	Sour (20)	Less sour (10)	Rotten (0)
Texture	Smooth, dry, soft, dense, not slimy (25)	The water content feels a lot, but doesn't feel wet (20)	A bit soft, a bit slimy (10)	Lots of water content, soft, lumpy, slimy (0)
Color	Yellowish green (25)	Brownish green (20)	Dark brown (10)	Brown (0)
Presence of mold	No mold (25)	A little bit mold (20)	Lots of mold (10)	More of mold (0)

Description: (Direktorat Pakan Ternak, 2011).

Results and Discussion

Overall examination on the quality of rice straw silage produced from this study is satisfactory. As a preliminary study on the use of sacha inchi meal and sambung nyawa leaves, the information obtained from this study is very important for further study. The nutrient content of the feed ingredients used is presented in Table 2.

Physical quality of silage

The assessment of physical attributes can be conducted based on the aroma, colour, texture, and the presence of mold. The physical quality of silage is satisfactory, characterized by a smooth texture, a brownish-green hue, and a pleasant, fermented scent (Patimah *et al.*, 2020). The findings regarding the physical quality of untreated and treated straw silage indicate the same level of excellence. The rice straw silage displays the typical pungent fragrance of fermentation, along with the distinct aroma of sambung nyawa leaves. It possesses a soft texture, a considerable amount of water content without being excessively damp, a brownish colour, and is devoid of any mold (Table 3).

Chemical quality of silage

The addition of sacha inchi meal and sambung nyawa leaves in silage resulted the increasing in DM, CP, and crude fat levels and the decreasing in ash, CF, and pH, with a significance level of p<0.01 (Table 4). This phenomenon could be attributed to the impact of the initial material's quality and nutrient composition on the fermented material's nutrient content (Koni and Foenay, 2020).

The DM value in silage treated with sacha inchi meal increased and was higher with the addition of sambung nyawa leaves. The factor that affects the DM content of silage is the DM content of the forage before ensilaged (Balo *et al.*, 2022).The DM value indicating that there was no loss of DM in the silage. Losing of DM can reduce the quantity of materials (Syahrir *et al.,* 2014). The ash value in the control treatment was the highest due to the presence of 100% rice straw because rice straw has the main component of silica which is found in the ash content of a feed ingredient (Yanuartono *et al.*, 2017). The ash content in rice straw silage with the addition of sacha inchi meal was still high, however the addition of sambung nyawa leaves caused a greater reduction in ash content. The increase in CP levels in rice straw silage was caused by the high CP content of sacha inchi meal used in the rice straw silage treatment (P1-P4) and higher with the addition of sambung nyawa leaves (P5 -P8). The value of CF of treated rice straw silage decreased. One of the factor that reduces the CF content of rice straw silage is the stretchable cell wall fraction caused by LAB during the ensilage process (Sulistyo *et al.*, 2020). In the rice straw silage treatment given sacha inchi meal there was a slight increase in crude fat but it had no effect when given sambung nyawa leaves.

According to Qadarullah *et al.* (2018), high-quality silage pH is less than 4.5. The third treatment's pH started to show good silage results (Table 5). The Fleigh value indicates an increase in fermentation quality and it is very good if it is higher

Table 2. Nutrient content of raw material silage

Raw material	DM	Ash	CP	CF	Crude fat
	%DM				
Rice straw	27.57	26.40	6.79	23.96	1.51
Sacha inchi meal	91.14	5.25	62.86	2.91	5.21
Sambung nyawa leaves	61.11	9.81	28.31	6.48	1.68

Table 3. Physical quality of rice straw silage

Criteria	Aroma	Texture	Colour	Presence of mold	Total
P0	20.00±6.88	22.75±2.06	25.00±0.00	25.00±0.00	92.75
P1	23.75±1.50	21.75±1.26	24.00±2.00	25.00±0.00	94.50
P2	21.25±2.50	21.75±1.50	24.25±1.50	25.00±0.00	92.25
P3	19.25±4.27	21.00±1.15	24.25±1.50	25.00±0.00	89.50
P4	17.75±5.25	22.75±1.89	25.00±0.00	25.00±0.00	90.50
P5	15.00±7.05	24.00±1.50	25.00±2.89	25.00±0.00	89.00
P6	17.25±8.38	21.75±2.36	22.50±2.89	25.00±0.00	86.50
P7	17.50±8.66	21.25±2.50	23.00±2.45	25.00±0.00	86.75
P8	17.50±8.66	22.50±2.89	22.50±2.89	25.00±0.00	87.50
P-value	0.715	0.882	0.451	0.000	

The variance result showed no significant differences (p>0.05) in the physical quality.

P0: 100% rice straw, P1: 90% rice straw + 10% sacha inchi meal, P2: 85% rice straw + 15% sacha inchi meal, P3: 80% rice straw + 20% sacha inchi meal, P4: 75% rice straw + 25% sacha inchi meal, P5: P1 + 10% sambung nyawa leaves, P6: P2 + 10% sambung nyawa leaves, P7: P3 + 10% sambung nyawa leaves, P8: P4 + 10% sambung nyawa leaves.

Table 4. Nutrient content of rice straw silage

Treatments	DM (%)	Ash	CP	CF	Crude fat
		%DM			
P0	51.23±2.13^{cd}	24.98±0.58^{a}	4.30±0.13^{f}	29.92±0.24^{a}	1.63±0.01^{c}
P1	57.89±4.19^{bc}	19.40±0.05^{b}	17.45±1.42^{e}	23.28±0.50^{b}	2.04±0.05^{b}
P2	46.46±3.27^{d}	17.27±0.09^{d}	23.49±0.39^{c}	19.60±0.31^{d}	2.34±0.07^{ab}
P3	57.71±0.77^{bc}	15.57±0.39^{e}	26.57±2.40^{ab}	17.30±0.36^{e}	2.42±0.28^{ab}
P4	61.84±5.19^{b}	13.05±0.13^{g}	28.53±1.10^{b}	19.02±0.53^{d}	2.77±0.09^{a}
P5	53.35±2.58^{c}	17.92±0.29^{c}	19.13±0.95^{e}	21.35±0.14^{c}	2.08±0.14^{b}
P6	61.92±1.82^{b}	14.30±0.04^{f}	27.34±3.22^{c}	19.59±0.60^{d}	2.36±0.03^{ab}
P7	61.00±1.36^{b}	14.34±0.28^{f}	28.37±2.39^{c}	15.47±0.94^{f}	2.62±0.18^{a}
P8	73.38±6.07^{a}	14.54±0.31^{f}	32.12±1.33^{a}	13.40±0.67^{g}	2.45±0.32^{ab}
P-value	<0.01	<0.01	<0.01	<0.01	<0.01

^{a,b,c} Different superscripts in the same column indicate statistical differences among treatments (p<0.01).

P0: 100% rice straw, P1: 90% rice straw + 10% sacha inchi meal, P2: 85% rice straw + 15% sacha inchi meal, P3: 80% rice straw + 20% sacha inchi meal, P4: 75% rice straw + 25% sacha inchi meal, P5: P1+10% sambung nyawa leaves, P6: P2+10% sambung nyawa leaves, P7: P3+10% sambung nyawa leaves, P8: P4+10% sambung nyawa leaves.

than 80. The high Fleigh value is related to the pH and DM values of the silage. The process of decreasing pH gradually and maintaining the DM of silage will increase the Fleigh value (Komalasari *et al.*, 2015).

Table 5. pH and *Fleigh Point* of rice straw silage

Treatments	pH	*Fleigh point*
P0	5.23±0.14^{a}	98.18
P1	4.61±0.19^{b}	136.22
P2	4.52±0.19^{bc}	117.20
P3	4.39±0.07^{cd}	144.74
P4	4.41±0.10^{bcd}	152.12
P5	4.29±0.04^{d}	140.26
P6	4.34±0.02^{cd}	155.40
P7	4.28±0.05^{d}	155.64
P8	4.33±0.06^{cd}	178.40
P-value	<0.01	

^{a,b,c} Different superscripts in the same column indicate statistical differences among treatments (p<0.01).

P0: 100% rice straw, P1: 90% rice straw + 10% sacha inchi meal, P2: 85% rice straw + 15% sacha inchi meal, P3: 80% rice straw + 20% sacha inchi meal, P4: 75% rice straw + 25% sacha inchi meal, P5: P1 + 10% sambung nyawa leaves, P6: P2 + 10% sambung nyawa leaves, P7: P3 + 10% sambung nyawa leaves, P8: P4 + 10% sambung nyawa leaves.

Microbiological characteristics of silage

High-quality silage will be obtained when the process of silage is predominantly controlled by LAB. Lactic acid bacteria, during the fermentation of carbohydrates, have the capability to generate lactic acid, thereby inducing a decline in pH. Low pH possesses the ability to impede the proliferation of various other microorganisms, particularly those that are pathogenic in nature.

The inoculum consisted of *L.plantarum* at a concentration of 1.65x10^{9} CFU g^{-1}. Lactic acid bacteria play a crucial role in supporting the fermentation process. As stated by Harahap *et al.* (2019), a minimum population of LAB exceeding 10^{5} CFU kg^{-1} of fresh material is necessary for successful fermentation. Over time, the population of LAB decreases during the harvest (Table 6). However, despite this decrease, the overall population at harvest is still capable of producing sufficient acid to lower the pH within the range of 4.29-4.34.

Various media have been developed to grow specific microorganism. In the NA media, there exist various bacteria apart from LAB. The introduction of sambung nyawa leaves in rice straw silage showed in an overall reduction in the number of bacteria that proliferated. Nevertheless, the population of bacteria that thrives on NA media remains akin to the population of LAB that flourish on MRS media. PDA media is used for the cultivation of molds, yeasts, and yeasts commonly encountered in the production of silage. The addition of sambung nyawa leaves in rice straw silage exhibited comparable populations of molds, yeasts, and yeasts to the control. The blue light broth agar media serves the purpose of determining the quantity of pathogenic bacteria. In the case of rice straw silage with treatment, the growth of pathogenic bacteria can be hindered. This can be attributed to the presence of alkaloids, saponins, and essential oils in sambung nyawa leaves, which possess antibacterial properties (Sidauruk *et al.,* 2021).

Table 6. Microbial population of rice straw silage growing on various agar media

Treatments	Microbial Population (CFU g^{-1})			
	LAB	Aerobic bacteria	Yeasts	Coliform bacteria
P0	6.00x10^{7}	1.30x10^{8}	1.40x10^{8}	3.00x10^{5}
P1	1.30x10^{7}	7.30x10^{7}	1.20x10^{8}	0.30x10^{5}
P2	7.50x10^{7}	1.80x10^{7}	0.60x10^{8}	0.00
P3	0.40x10^{7}	0.26x10^{7}	1.02x10^{8}	0.00
P4	2.20x10^{8}	0.35x10^{7}	0.60x10^{8}	0.00
P5	2.80x10^{7}	3.50x10^{7}	0.70x10^{8}	0.00
P6	3.50x10^{7}	4.30x10^{7}	0.30x10^{8}	0.00
P7	0.60x10^{7}	8.80x10^{7}	1.10x10^{8}	0.00
P8	0.30x10^{7}	1.30x10^{7}	1.50x10^{8}	0.00
Shapiro-Wilk P-value	0.149	0.216	0.721	<0.05

P-value (>0.05) indicated the data is normally distributed.

P0: 100% rice straw, P1: 90% rice straw + 10% sacha inchi meal, P2: 85% rice straw + 15% sacha inchi meal, P3: 80% rice straw + 20% sacha inchi meal, P4: 75% rice straw + 25% sacha inchi meal, P5: P1 + 10% sambung nyawa leaves, P6: P2 + 10% sambung nyawa leaves, P7: P3 + 10% sambung nyawa leaves, P8: P4 + 10% sambung nyawa leaves.

Conclusion

The quality of rice straw silage has improved by the addition of sacha inchi meal and sambung nyawa leaves. The addition of sacha inchi meal to rice straw silage from 10-25%, increased quality silage. Addition of sacha inchi meal and sambung nyawa leaves in rice straw silage does not have an impact on the physical characteristics. It does lead to an elevation in the levels of CP, while reducing the levels of ash, CF, and pH. With the result, addition of sacha inchi meal and sambung nyawa leaves corrects the CP content deficiency from rice straw and enhanced by about 4 fold. The microbiological quality of rice straw silage is determined by the presence of a suitable quantity of LAB that meet the criteria for high-quality silage, a reduction in the number of other bacteria, and yeasts that are comparable to the control group, and the absence of any growth of pathogenic bacteria.

Conflict of interest

The authors have no conflict of interest to declare. All authors have seen and agree with the contents of the manuscript.

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Author's contribution

The authors confirm contribution to the paper as follows: study conception and design: N. R. K. and Y. W.; data collection: F. F. A.; analysis and interpretation of results: F. F. A.; draft manuscript preparation: F. F. A., Y. W., and N. R. K. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval

There are no human subjects in this article and informed consent is not applicable.

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