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The Quality of Red Napier Grass (*Pennisetum purpureum* cv. Red) Silage at Different Harvest Ages and Addition Level of *Lactobacillus plantarum*

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ABSTRACT: This study aimed at evaluating the pH, nutrient content and nutrient loss of silage that were made of different ages Red Napier grass (*Pennisetum purpureum* cv. Red) and the addition of *Lactobacillus plantarum*. The experiment employed Nested Completely Randomized Factorial Design consisted 2 treatment factors i.e. the grass of 50, 60, 70 and 80 harvested days after planting as first factor and the addition of three levels of *Lactobacillus plantarum* i.e. 0, 10^3 and 10^6 cfu/g as second factor. Each factor combination was replicated 3 times. Collected data were subjected to analysis of variance followed by Duncan's Multiple Range Test (DMRT). It was found that harvest ages did not affect pH ($p>0.05$) but level of *L plantarum* significantly ($p<0.05$) affect silage pH measured at day 7 and highly significantly ($p<0.01$) affect pH at day 14, and 21. *L plantarum* significantly ($p<0.05$) affect DM loss, and highly significant ($P<0,01$) affect OM loss, while the different harvesting ages gave highly significant ($P<0,01$) affect CP loss. The higher level of *L. plantarum* can increase the DM, CP and decrease levels of CF, NDF and ADF. It can be concluded that interaction between Red Napier grass of 50 harvested days after planting and addition of *L. plantarum* 10^6 cfu/g produced the good quality silage based on the relatively low weight loss of DM, OM and CP.

KEYWORDS: harvest ages, *Lactobacillus plantarum*, red napier grass, silage, weight loss

INTRODUCTION

Red napier grass (*Pennisetum purpureum* cv Red) is one of the forages that can be chosen as a source of fiber needs for ruminants because of its high production. The dry matter biomass production of red napier grass harvested at 56 days was 6.09 tons/ha/cut, higher than the Common, Silver and Dwarf varieties of napier grass (Zailan et al., 2016). Red napier grass has a lot of potential as animal feed, but has not been widely developed in Indonesia. This variety has the advantage of containing anthocyanins which range from red to purple in the leaves and stems. The potential of anthocyanins as a substitute for antibiotics in livestock by reducing deaths due to pathogenic bacteria (antibacterial), encouraging growth, improving meat quality and immunity (Guo and Shahidi, 2024). The total anthocyanin of red napier grass decreases as harvest age increases, where at 40, 60 and 70 days it contains total anthocyanin respectively, namely 2.52 mg/g, 1.32 mg/g and 0.57 mg/g (Onjai -ua et al., 2022).

The main problem faced by farmers in providing forage needs is fluctuating availability. According to Hilmi, et al. (2016) during the rainy season, forage production is abundant, whereas during the dry season forage availability is very limited. This problem can be overcome with alternative feed preservation technologies, one of which is making silage. Making silage aims to preserve forage which is very abundant in the rainy season so that it can be used in the dry season, increasing palatability and minimizing loss of forage nutrient content during storage (Natsir et al., 2019). There is still little research regarding red napier grass, especially in the preservation process. The anthocyanins contained in red napier grass are only suitable in acidic conditions where their content decreases as the pH value increases. This is supported by research by Fathinatullabibah, et al. (2014) where the total percentage reduction in anthocyanin extracted from teak leaves with pH 3, 5 and 7 treatment was respectively 4.11%, 10.56% and 19.06%, so it is very appropriate if red napier grass is processed into silage.

The principle of making silage is fermentation by lactic acid bacteria of forage so that it can accelerate the production of lactic acid (Utomo et al., 2021). One of the bacteria that only focuses on producing lactic acid is *Lactobacillus plantarum* (homofermentative bacteria). The addition of *L. plantarum* 1 x 10^5 cfu/g and citric acid was able to reduce the pH of king grass silage on day 14, it was 4.2, which was lower than the control treatment (pH = 4.6) (Zi et al., 2021) . A rapid decrease in silage pH will inhibit the growth of harmful bacteria such as *Clostridia* and *Enterobacteria*, and minimize protein degradation (Yuliatun and

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Triantari, 2021). There is an effect of the age at which plants are harvested on the fermentation process by LAB, where the older the harvest, the crude fiber content increases, but the crude protein and water content decreases (Heriyanti et al., 2023). High crude fiber content causes a decrease in LAB's ability to degrade crude fiber (Septian et al., 2022), while low protein inhibits the increase in cell number (Holik et al., 2019).

Based on the description above, research is needed regarding the evaluation of red napier grass silage with different harvest ages and addition levels of *Lactobacillus plantarum* in terms of pH, nutritional content, weight loss of DM, OM and CP.

MATERIALS AND METHODS

The research was carried out in September - December 2023 at the Sumber Sekar Field Laboratory, Animal Nutrition and Feed Laboratory, Faculty of Animal Science, Universitas Brawijaya. The materials used were red napier grass (*Pennisetum purpureum* cv. Red) which was harvested at the age of 50th, 60th, 70th and 80th days obtained from the Dau District area, Malang Regency, molasses as Water Soluble Carbohydrate (WSC), corn hull as binder as well as *Lactobacillus plantarum* bacteria with levels of 1x10^{3} cfu/g and 1x10^{6} cfu/g as starter obtained from the Faculty of Medicine, Universitas Muhammadiyah Malang. Nutrient content of materials before ensiled presented in Table 1.

Table 1. Nutrient content of materials before ensiled

	Nutrint content					
		OM	CP	CF	EE	NFE
Harvesting age	DM (%)	%DM				
A1	22,31	89,44	8,4	31,75	2,46	49,77
A2	22,59	87,54	7,56	32,47	1,74	44,01
A3	24,29	84,12	6,61	35,81	1,50	38,82
A4	25,34	85,02	5,43	37,07	1,94	36,16

METHODS

The research method used was an experimental method using Nested Completely Randomized Factorial Design consisted 2 treatment factors The first factor treatment was 4 different harvest ages for red napier grass: A1 = 50th day, A2 = 60th day, A3 = 70th day and A4 = 80th day. The second factor treatment was 3 levels of addition of *L. plantarum*: B0 = 0, B1 = 1x10^{3} cfu/g and B2 = 1x10^{6} cfu/g. The research design is presented in Table 2.

First factor:
A1 = Red napier grass with an age of 50th days after planting
A2 = Red napier grass with an age of 60th days after planting
A3 = Red napier grass with an age of 70th days after planting
A4 = Red napier grass with an age of 80th days after planting
Second factor:
B0 = no *Lactobacillus plantarum*
B1 = *Lactobacillus plantarum* 1x10^{3} cfu/g
B2 = *Lactobacillus plantarum* 1x10^{6} cfu/g

Table 2. Research design in making silage

Harvest age	Bacteria level	Block		
		1	2	3
A_1	B_0	A_1B_01	A_1B_02	A_1B_03
	B_1	A_1B_11	A_1B_12	A_1B_13
	B_2	A_1B_21	A_1B_22	A_1B_23
	B_0	A_2B_01	A_2B_02	A_2B_03

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A_2	B_1	A_2B_11	A_2B_12	A_2B_13
	B_2	A_2B_21	A_2B_22	A_2B_23
	B_0	A_3B_01	A_3B_02	A_3B_03
A_3	B_1	A_3B_11	A_3B_12	A_3B_13
	B_2	A_3B_21	A_3B_22	A_3B_23
	B_0	A_4B_01	A_4B_02	A_4B_03
A_4	B_1	A_4B_11	A_4B_12	A_4B_13
	B_2	A_4B_21	A_4B_22	A_4B_23

Procedure for making red napier grass silage

The procedure for making red napier grass silage in this research was as follows.

1. Preparation of tools and materials to be used.
   - Prepared red napier grass with a harvest age of 50th, 60th, 70th and 80th days after planting, molasses, corn hull, *Lactobacillus plantarum* starter and barrels as silos.
   - Red napier grass was chopped to a size of around 1-3 cm and withered/aired for around 1-1.5 hours
   - Red napier grass was weighed for each treatment
2. Used 5% molasses and 5% corn hull for the fresh weight of forage (1kg molasses and 1 kg corn hull if 20 kg/barrel of red napier grass was used).
3. Mix red napier grass, a mixture of molasses and corn hull, and add a LAB starter of 0.1 % of the fresh weight of the grass (20kg grass = 20 ml bacteria) according to the treatment.
4. The stage for mixing molasses and corn hull was to slowly spread each layer of red napier grass into the barrel, then spray LAB on each layer.
5. The top of the barrel was coated with 2 layers of plastic and then closed tightly.
6. Incubate for 21 days.

Red napier grass silage sampling procedure

The stages of taking samples for analysis in the laboratory are as follows.

1. Samples of ± 300 g/repetition were taken when the silage was harvested (36 samples)
2. The samples were dried in an oven at 60°C for ± 24 hours
3. The weight was weighed and recorded after the oven, then ground using a *grinding machine* to a size of ±1 mm
4. A subsample (composite) was taken from each replication so that 12 samples were obtained for analysis in the laboratory
5. Put the sample in a plastic clip and analyze it in the laboratory

Research variable

The research variables measured in this study include:

1. pH. pH measurements were carried out based on the method of Bernardes et al. (2019) where silage was extracted using water in a ratio of 1:4 (25 g of silage for 100 mL of water) with manual homogenization for 15 minutes, then the pH was measured using a pH meter.
2. The nutritional content of red napier grass silage on days 0 and 21 which includes Dry Matter (DM), Organic Matter (OM), Crude Protein (CP) and Crude Fiber (CF) were analyzed using the AOAC method (1995)
3. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) content analyzed using the Van Soest (1994) method
4. Total weight loss of dry matter, organic matter and crude protein. The formula for measuring the weight loss according to Hernaman *et al*. (2007) are:
   - ✓ Dry Matter (DM) Weight Loss (%) = [(DM weight before ensiling – DM weight after ensiling)/DM weight before ensiling)] x 100%
   - ✓ Organic Matter (OM) Weight Loss (%) = [(OM weight before ensiling – OM weight after ensiling)/OM weight before ensiling)] x 100%

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- ✓ Crude Protein (CP) Weight Loss (%) = [(CP weight before ensiling – CP weight after ensiling)/CP weight before ensiling)] x 100%

Research Data Analysis

The collected data were analyzed using analysis of variance (ANOVA) with Nested Completely Randomized Factorial Design. Collected data were subjected to analysis of variance followed by Duncan's Multiple Range Test (DMRT). The analysys model used according to Azizatin (2015) is as follows.

$$Yij = \mu + A_i + B_j + \varepsilon ij$$

Information:
i = 1 ,2 , … a (harvesting age factor)
j = 1 ,2 , … b (level of *L. plantarum*)
Yij = observations from factor A at level i, factor B at level j
μ = middle value
Ai = effect of factor A at level i
Bj = effect of factor B at level j
εijk = experimental error for the level i (factor A), level j (factor B)

RESULT AND DISCUSSION

pH of Silage

The analysis of varianvce found that the level of *L. plantarum* gave significant effect (P<0,05) to pH on day 7 and highly significant effect (P<0,01) to pH on day 14 and 21. The data is presented in Table 3.

Table 3. The effect of differences level of *L. plantarum* to pH on day 7, 14 and 21

Harvesting age	Bacteria level	pH		
		7th days	14th days	21st days
A_1	B_0	$4,83^b \pm 0,06$	$4,47^b \pm 0,06$	$3,87^b \pm 0,06$
	B_1	$4,57^a \pm 0,16$	$4,13^a \pm 0,16$	$3,57^a \pm 0,06$
	B_2	$4,57^a \pm 0,16$	$4,17^a \pm 0,06$	$3,57^a \pm 0,06$
	Mean	4,66 ± 0,16	4,26 ± 0,17	3,67 ± 0,16
A_2	B_0	$4,67^b \pm 0,06$	$4,43^b \pm 0,06$	$3,83^b \pm 0,06$
	B_1	$4,53^a \pm 0,06$	$4,10^a \pm 0,10$	$3,53^a \pm 0,06$
	B_2	$4,60^a \pm 0,00$	$4,03^a \pm 0,06$	$3,53^a \pm 0,06$
	Mean	4,60 ± 0,07	4,19 ± 0,20	3,63 ± 0,16
A_3	B_0	$4,67^b \pm 0,16$	$4,33^b \pm 0,06$	$3,83^b \pm 0,06$
	B_1	$4,57^a \pm 0,06$	$4,13^a \pm 0,06$	$3,60^a \pm 0,00$
	B_2	$4,53^a \pm 0,06$	$4,00^a \pm 0,00$	$3,60^a \pm 0,10$
	Mean	4,59 ± 0,09	4,16 ± 0,15	3,68 ± 0,13
A_4	B_0	$4,73^b \pm 0,06$	$4,30^b \pm 0,10$	$3,80^b \pm 0,10$
	B_1	$4,63^b \pm 0,06$	$4,27^b \pm 0,06$	$3,60^a \pm 0,00$
	B_2	$4,47^a \pm 0,06$	$4,03^a \pm 0,06$	$3,50^a \pm 0,00$
	Mean	4,61 ± 0,13	4,20 ± 0,14	3,63 ± 0,14
P value		A : P > 0,05 B : P < 0,05	A : P > 0,05 B : P < 0,01	A : P > 0,05 B : P < 0,01

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Table 3. shows that the average pH value on day 7 in this study ranged from 4.47 to 4.83, then decreased on day 14 to between 4.00 and 4.47 and further decreased by day 21 to between 3.50 and 3.87 (Table 3). Such pH reduction occurs along with the progression of the fermentation phase. The pH on day 21 reaches its lowest value because lactic acid bacteria (LAB) exhibit rapid growth patterns at the beginning of incubation, then reach peak production and accumulate lactic acid. This is in line to Wahyudi (2019) who mentioned that there are fermentation phases that fresh materials undergo to become silage over 21 days, including the respiration phase, acetic acid production, initial lactic acid production, peak lactic acid production and accumulation.

The pH of silages obtained under B1 and B2 treatments lower pH at all four grass harvest ages compared to B0 across all observation days. This indicates that *L. plantarum* can produce a larger amount of lactic acid, thereby lowering the pH. Irsyammawati et al (2024) also mentioned that homofermentative LAB like *L. plantarum* can accelerate fermentation, lowering pH, increase lactic : acetic acids ratio, decrease ethanol and ammonia concentrations. Data in Table 3 shows that pH of silages at day 21 under B2 and B3 treatments ranged from 3,53 to 3,60. As their pH were below 4.2, they can be classified as good silage as outlined by Anonimous (2024). Such pH level will ceased pathogenic bacterial and thus the silage can be conserved for long period. The pH values obtained in this study was better as compared to the Napier grass silage obtained by Marawali et al. (2022) using 5% molasses as additive. In this study, it is found that the average pH decreases with the addition of *L. plantarum* (Table 3). The addition of the bacteria increase the number of LAB during fermentation process, accelerating the degradation of digestible carbohydrate such as sugars, hydrolyzed hemicellulose and WSC (Water Soluble Carbohydrate) in silage material and converting them into lactic acid and thereby decrease pH (Muhamad et al.; 2022 and Yusren et al., 2023). Referring to results given in Table 3, the addition of *Lactobacillus plantarum* $1x10^3$ cfu/g is enough in making silage of 50 to 80 days old of Red Napier grass.

Nutrient Content of Red Napier Grass Silage

Table 4. Nutrient content of red napier grass after ensiling

Harvest age	Bacteria Level	Nutrient Content								
		DM (%)	OM %DM	CP	EE	CF	NDF	ADF	Hemicelulose	NFE
A_1	B_0	15,89	77,11	8,44	2,33	29,71	54,52	36,70	17,82	36,63
	B_1	17,82	80,41	8,66	3,66	30,34	53,06	36,03	17,03	40,43
	B_2	18,35	80,22	9,55	2,49	28,85	53,19	35,33	17,86	36,40
A_2	B_0	16,18	81,39	8,11	2,29	30,57	57,11	39,85	17,25	39,05
	B_1	18,04	80,71	8,92	2,55	28,85	54,61	38,35	16,26	37,76
	B_2	17,79	80,22	8,32	2,33	29,28	55,60	38,20	17,40	40,40
A_3	B_0	17,45	81,04	6,66	2,64	35,34	58,14	44,18	13,96	33,34
	B_1	17,34	77,82	6,47	2,45	33,56	58,21	44,21	14,00	42,05
	B_2	18,49	79,38	9,87	2,33	35,89	58,97	44,61	14,36	39,32
A_4	B_0	18,01	84,01	6,72	1,96	36,29	56,32	44,24	12,09	38,67
	B_1	18,35	83,76	6,48	2,49	33,65	60,53	46,56	13,97	34,75
	B_2	18,80	81,18	7,97	2,52	33,92	56,19	44,07	12,12	36,77

Table 4. show that the average DM and CP content of silage under B1 and B2 treatments tend to be higher than under the B0 treatment at all the grass harvest ages. It indicating that the addition of *L. plantarum* to Red Napier grass of 50 to 80 days old could increase the DM and CP content of the resulted silage. At all the grass harvest ages, addition of highest level of *L. plantarum* $1x10^6$ cfu/g gave highest level of CP which is reasonable considering that the bacteria is a single cell proteins organism. Moreover, as discussed earlier, addition of *L. plantarum* decreased pH that prevent the growth of pathogen bacteria such as *Clostridia* and *Enterobacteria* that may degrade the available CP and DM (Ridwan et al.,2020; Yuliatun and Triantari, 2021).

Table 4. show that the average CF, NDF and ADF content under B1 and B2 treatments tend to be lower than under B0 treatment at all grass harvest ages. This indicates that *L. plantarum* can decrease the CF, NDF and ADF content of the resulted

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silage from Red Napier grass of 50 to 80 days. The decrease CF, NDF and ADF also occurred in *Musa paradisiaca* L. peel silage with increasing levels of LAB by Nurkholis et al. (2018) using *L. plantarum* at levels of 0, 10^4 cfu/g, 10^5 cfu/g and 10^6 cfu/g. The decrease in CF was due to the ability of *L. plantarum* to convert simple sugars of red napier grass. Accordimg to Karyono et al. (2022), cellulolytic bacteria can degrade lignocellulose and lignohemicellulose bonds, so that the fiber components become simpler. These simple sugars were then converted by *L. plantarum* to organic acids during ensilation. Organic acids formed during ensiling can also degrade fiber fractions, especially cellulose and hemicellulose (Pratiwi et al., 2015).

Treatment B1 produced lower average CF, NDF and hemicellulose contents than B2, but ADF contents were higher at each harvest age. This is in line with research by Zhu, et al. (2022) the NDF content of *Pennisetum hybrid* fermented with *L. plantarum* 10^7 cfu/g was 68.32%, higher than *L. plantarum* 10^5 cfu/g (67.70%). The lower CF and NDF contents in B1 occurs due to *L. plantarum* 10^3 cfu/g degrades more hemicellulose components than cellulose and lignin. The breakdown of hemicellulose occurs due to degradation by plant enzymes, bacterial enzymes and hydrolysis by organic acids during ensilation (Chalisty, 2021). *L. plantarum* 10^6 cfu/g (B2) degrades more ADF components including cellulose and lignin, so the hemicellulose content is higher than B1. The decrease in ADF contents followed by an increase in hemicellulose proves the breakdown of the lignocellulosic fraction (which is difficult to digest) from silage into components that are relatively easy to digest (hemicellulose) (Usman et al., 2022).

Weight loss of dry matter, organic matter and crude protein

The analysys of variance found that the *L plantarum* significantly ($p<0.05$) affect DM loss and highly significant ($P<0,01$) affect OM loss, while the different harvesting ages gave highly significant ($P<0,01$) affect CP loss. The data is presented in Table 5.

Table 5. Weight loss of dry matter, organic matter and crude protein

Harvesting ages	Bacteria levels	DM weight loss (%)	OM weight loss (%)	CP weight loss (%)
A_1	B_0	$32,32^c \pm 0,58$	$41,65^c \pm 0,50$	$32,03 \pm 0,59$
	B_1	$28,09^b \pm 0,78$	$35,35^b \pm 0,70$	$25,89 \pm 0,80$
	B_2	$21,96^a \pm 1,26$	$30,01^a \pm 1,13$	$11,23 \pm 1,43$
	Mean	$27,46 \pm 4,58$	$35,67 \pm 5,10$	$23,05^a \pm 9,30$
A_2	B_0	$32,23^c \pm 0,11$	$36,99^b \pm 0,10$	$27,33 \pm 0,12$
	B_1	$27,38^b \pm 1,10$	$33,04^a \pm 1,01$	$14,34 \pm 1,30$
	B_2	$23,91^a \pm 1,46$	$31,69^a \pm 1,31$	$16,26 \pm 1,61$
	Mean	$27,84 \pm 3,73$	$33,91 \pm 2,53$	$19,31^a \pm 6,16$
A_3	B_0	$32,01^b \pm 0,09$	$34,50^a \pm 0,09$	$31,49 \pm 0,09$
	B_1	$32,97^b \pm 0,74$	$37,99^b \pm 0,68$	$34,39 \pm 0,72$
	B_2	$28,60^a \pm 0,65$	$32,63^a \pm 0,62$	$25,76 \pm 0,68$
	Mean	$31,19 \pm 2,05$	$35,04 \pm 2,40$	$30,55^b \pm 3,83$
A_4	B_0	$32,03 \pm 0,40$	$32,84 \pm 0,40$	$15,94 \pm 0,50$
	B_1	$30,99 \pm 1,47$	$32,02 \pm 1,45$	$17,67 \pm 1,76$
	B_2	$30,33 \pm 0,41$	$33,48 \pm 0,39$	$-2,23 \pm 0,60$
	Mean	$31,12 \pm 1,08$	$32,78 \pm 1,00$	$10,46^a \pm 9,59$
P value		A : P > 0,05 B : P < 0,05	A : P > 0,05 B : P < 0,01	A : P < 0,01 B : P > 0,05

Dry matter and organic matter weight loss

The percentage of dry matter loss in red napier grass silage with the addition of *L. plantarum* shows significant differences ($P<0.05$). B2 significantly minimizes dry matter loss in A1, A2, and A3 compared to B1 and B0. Table 5. shows the higher level of bacterial addition, the smaller the loss. Marawali et al. (2022) also stated that there were significant differences

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in dry matter loss of napier grass fermented with the addition of 3% EM4 and 5% molasses compared to the control treatment, which was 19.91% and 44.67%, respectively. A s discussed earlier, addition of *L. plantarum* decreased pH that prevent the growth of pathogen bacteria which degrade carbohydrates, proteins, and lactic acid as energy sources to produce butyric acid, causing spolage in the silage (Karmila et al., 2020).

The addition level of *L. plantarum* shows highly significant differences (P<0.01) in organic matter loss. B2 significantly minimizes organic matter loss in A1 and A2 compared to B0. The decrease in organic matter was in line with the decrease in dry matter because organic matter is a component of dry matter. This is supported by Marawali et al. (2022), where the dry matter and organic matter loss in fermented red napier grass are 44.67% and 45.77%, respectively. The factors affecting organic matter loss are the same as those causing dry matter loss. LAB utilize organic compounds to grow and function. The longer the ensiling time or reaching of acidic conditions (pH<4,2), so bacteria will degrade more organic compounds, causing substrate loss (Raguati et al., 2022). The addition of *L. plantarum* accelerates the silage reaching acidic conditions and made stabilizing bacterial growth. The fermentation process stops when the substrate environment reaches an acidic pH (4.0 - 4.5), microbial activity will stop (Kung et al., 2018).

DM and OM loss also occurs due to the respiration process in the first fermentation phase, where glucose was converted into CO2, H2O, and heat when oxygen was still available in the silo (Nurkholis et al., 2018). Increased water content in silage reduces dry matter content, leading to higher dry matter loss. Higher dry matter content in forage increases the respiration rate due to more substrates available for oxidation. This is supported by Setyoaji and Setiawan (2021), stated that carbohydrates, fats, and proteins are substrates in the forage respiration process that generate energy for plant organ growth. The higher the harvesting age, the higher the dry matter content (Table 1) and the respiration rate, causing increased dry matter loss. This is shown in Table 5. that the lowest dry matter loss is in A1 (27.46 ± 4.58%), followed by A2, A4, and A3.

CP weight loss

The percentage of crude protein loss in red napier grass silage with different harvesting ages shows highly significant difference (P<0.01). The lowest average crude protein loss occurred in A4, followed by A2, A1, and A3. This indicates that The younger the harvesting age, the higher the CP content (Table 1), thus increasing the loss of CP as well. Thaariq (2017) stated that the higher the CP content, the more pathogenic bacteria growing to degrade CP. *Clostridium* Sp. bacteria break down amino acids and peptides into ammonia and amines, then convert lactic acid into butyric acid (Sadarman et al., 2019). This type of bacteria will grow if the pH reduction during the ensiling process was slow. The activity of spoilage and butyric acid bacteria were more optimal if the environmental pH exceeds 4.4 (Zhang et al., 2021).

Table 5. shows the lowest average loss in treatment A4B2 (-2.23 ± 0.60%), while the highest is in A3B1 (34.39 ± 0.72%) and A1B0 (32.03 ± 0.59%). The incerasing crude protein in A4B2 due to additional protein from bacterial carcasses and more dominant degradation of soluble carbohydrates. This is in line with Sariri and Sukaryani (2021), the increase in CP occurs from bacterial carcasses (free N) and residual VFA compounds that lose O, N, and H ions during fermentation. The level of *L. plantarum* addition did not affect CP loss (P>0.05), but the percentage of loss showed minimize decrease with increasing levels of *L. plantarum*. B2 provided the smallest loss value, followed by B1 and B0. A high level of *L. plantarum* increases the lactic acid production rate, making the silage pH very acidic. Lactic acid production increased until day 11 (pH = 3.6), then decreased, forming a quadratic curve (Herawati and Royani, 2022). The higher the level of *L. plantarum*, the faster the environment becomes acidic, minimizing crude protein.

CONCLUSION

The higher level of *L. plantarum* can increase the DM, CP and decrease levels of CF, NDF and ADF by accelerating the decrease in pH. Interaction in the interaction between Red Napier grass of 50 harvested days after planting and addition of *L. plantarum* 10^6 cfu/g produced the good quality silage based on the relatively low weight loss of DM, OM and CP.

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