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Silage quality based on the physical and chemical of several napier grass varieties (*Pennisetum purpureum*) supplied with different levels of pollard

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Abstract. Making silage is one method for storing feed during the dry season. To preserve the ingredients for feed, silage is made by carefully controlling the fermentation of forage with a high water content. The idea behind making silage is to create anaerobic conditions in which lactic acid bacteria can grow in order to stop the decay process. Fresh fodder, such as grasses, is commonly used in silage. The goal of this study was to assess the physical and chemical properties of various types of *Pennisetum purpureum sp.* that had been supplemented with varying amounts of pollard. Factorial Completely Randomized Design (CRD) in 2 x 3 Factorial is the design used in this experiment. The first factor involves two kinds of *Pennisetum purpureum sp*., specifically *Pennisetum purpureum* cv. Mott and *Pennisetum purpureum* with gamma radiation (cv. GU). The second element is the level of pollard supplementation, which consists of 0%, 3%, and 6% with 5 replications. The results were then examined for the physical qualities of the silage quality, including the silage's color, flavor, texture, and fungus content. The silage's detected chemical properties included pH, DM, OM, CP, CF, and EE silage. The outcomes demonstrated that silage with good physical quality was produced by the types and pollard supplementation treatments. Increased pollard supplementation levels were negatively correlated with pH (P 0.05). When pollard supplementation was increased, the levels of lactic acid, DM, and CP considerably increased linearly (P 0.05), CF and EE levels dramatically decreased (P 0.05), while OM levels did not significantly differ (P > 0.05). It was determined that adding pollard at a level of 6% and using gamma radiation on *Pennisetum purpureum* in combination are treatments that result in crops with a color that is similar to silage material, an acidic odor, a non-slimy texture, and no fungus.

1. Introduction

Napier grass has many varieties spread around the world. Napier grasses such as *Pennisetum purpureum gamma radiation (*cv GU*)* and *Pennisetum purpureum* cv. Mott. *Pennisetum purpureum* cv.GU is the latest variety of research results given gamma ray treatment [1]. This grass already has a registration mark in the Ministry of Agriculture number 889/PVHP/2020 of 2021. [2] Mutations can increase the genetic diversity of plants. In its application, the use of Gamma rays as a mutagen is popular because the frequency of mutation results is higher and the application is easier. Such mutations can alter the nature of genotypes and phenotypes that are inherited. [3] explained that Odot grass (*Pennisetum purpureum* cv. Mott) is a grass that excels in productivity and nutrient content, therefore many breeders use them as raw materials for making silage.

This is because the nutrient content is quite sufficient, such as DM, OM, and CP on napier grass, hence conservation is needed to maintain the forage quality for several months and given when the accessibility of fresh forage is limited in the dry season. Silage is a potential method of forage preservation to maintain the accessibility of forage, especially in the dry season. Forages are preserved at high water levels anaerobically. Anaerobic storage of preserved feed can be a solution to maintain the forage quality in tropical climates with high humidity such as in Indonesia. Central Agency on Statistics in 2020 [4] noted that the average humidity in Indonesia in 2020 was 84.3.

The obstacle encountered in making silage is the fairly low water-soluble carbohydrate (WSC) content in napier grass. The material addition (supplementation) is widely utilized for making silage as a source of water-soluble carbohydrates. [5] lactic acid bacteria require water-soluble carbohydrates as the substrate source. The small amount of water-soluble carbohydrates causes lactic acid bacteria to use the nutrient content of forage as a substrate source. Therefore, supplementation of water-soluble carbohydrates is needed to ensure success in making silage.

Water-soluble carbohydrate supplementation can help in providing an easily fermented substrate source for lactic acid bacteria. Water-soluble carbohydrate supplementation can also be used for preservation in silage. Pollard is often used as a supplementation in the preparation of silage because it contains 12.5% water-soluble carbohydrates [6].

Based on this, it is necessary to observe silage quality based on the physical and chemical in several Napier grass varieties (Pennisetum purpureum) supplied with different levels of pollard, so that later the quality of each variety can be identified.

2. Materials and methods

2.1. Design and preparation of the study

This study was designed in a complete randomized design (CRD) with a 2 x 3 factorial pattern. Two varieties of *Pennisetum purpureum sp.* consisting of *Pennisetum purpureum* cv. Mott and *Pennisetum purpureum* cv GU was the first factor and the second factor was 3 levels ofsupplementation of pollards consisting of 0%, 3%, and 6%.

Pennisetum purpureum grass obtained from the field of Laboratory of Forage and Pasture, Animal Science Faculty, UGM, was chopped using choppers up to a size of 3-6 cm and wilted. Wilting was carried out by being aired for ±6 hours by being spread thinly in a shade. As much as 800 grams of grass was put in a thick plastic bag. Pollard was added according to treatment. Then, the plastic bag that already contained forages and pollards was vacumed until there is no air. The plastic end was tied with a rope and interlaced.

2.2. Sample analysis

2.2.1. Physical characteristics analysis of silage. The organoleptic test was carried out by including color, odor, texture, and presence of fungi by filling out a questionnaire assessing the characteristics of color, odor, texture, and presence of fungi by panelists by using the senses of sight, odor, and touch. The panelists were limited to those who had taken a course in forage and crop development and feed technology. The number of panelists was determined to be 10 people so each silage sample received 10 replicates.

2.2.2. pH determination *of the silage.* The pH analysis followed the procedure of Nahm (1992) [7].

2.2.3. Chemical composition analysis of silage. Proximate testing of organic matter, dry matter, crude protein, and crude fiber content in silage was following the AOAC method and procedure [8].

2.3. Research Parameter

The parameters of this study included the physical characteristics of the silage (color, texture, odor, presence of fungi), the chemical composition of the silage (dry matter, organic matter, crude protein, crude fiber, ether extract, nitrogen-free extract) and pH of silage.

2.4. Data Analysis
The results of statistical analysis with significant differences between treatments were further followed by Duncan's multiple range test. The physical quality characteristics of the silage were analyzed descriptively. Chemical characteristics were analyzed and calculations were performed using the help of SPSS version 16 software [9].

3. Results and Discussion

3.1. General condition of silage component materials
The results showed that the chemical composition of napier grass with a shearing life of 60 days had a fairly good nutrient content (Table 1). One of the potentials of napier grass is its good nutrient content to meet the nutrient needs of forage for ruminants as showed in Table 1.

Table 1. The chemical composition analysis results of silage components.

Component materials	Chemical composition (%DM)					
	DM	OM	CP	CF	EE	NFE
P. purpureum cv. Mott	13.96	84.35	12.58	28.01	2.93	40.83
P. purpureum cv. GU	14.62	88.66	8.55	34.86	3.23	42.20
Pollard	89.66 *	96.51 *	15.55 *	2.31 *	5.11 *	67.60 *

The final quality of forage conservation depends on the forage initial quality. One of the factors that affect the plants' nutrient content is the plant age or the age of maturity when the crop is harvested [10]. Cutting was carried out at the age of 60 days to keep high NFE content in the plant, on the other hand [11] the older the plant, the proportion of non-structural carbohydrates decreases, hence the proportion of structural carbohydrates increases. The low proportion of non-structural carbohydrates (soluble carbohydrates) is not advantageous in the production of silage. It is because non-structural carbohydrates are the main components in the formation of lactic acid.

The water-soluble carbohydrate content is needed by lactic acid bacteria as their source of a substrate. The small amount of water-soluble carbohydrates causes lactic acid bacteria to use the nutrient content of forage as a source of the substrate. Therefore, supplementation of water-soluble carbohydrates is needed to ensure success in making silage. The NFE of Napier grass is ranging from 37-44%, so it is necessary to add water-soluble carbohydrates in making napier grass silage. The source of water-soluble carbohydrates needed to make napier grass-based silage should contain more than 45% in the form of NFE. Pollard can be substituted as a source of NFE where it has [12] 67.6% NFE.

Physical quality characteristics of silage
3.1.1. Silage color
Colour in silage is one of the indicators in determining its physical quality [12]. The increasing pollard supplementation level did not affect the average value of the silage color score (Table 2). Good quality silage has a color close to the original color of the forage used. The napier grass is dark green with a silage score of bright green close to the silage's original color. [12] The good quality silage's color is close to the original forage. Moreover, the color deviates from the original color in low-quality silage [27]. Silage quality is reviewed based on color because this condition will inhibit the process of respiration, and proteolysis, and prevent the active Clostridia bacteria. [3] The normal color for silage from grass, seeds, and corn is bright green to brownish; while the normal color of silage for treated grass is pale green or yellow.

Table 2. Colour characteristic score on *P. purpureum* silage supplemented with pollard

Pollard level	*Pennisetum* variety	Average score	Color
0%	*P. purpureum* cv. Mott	3.00	Light Green
	P. purpureum cv. GU	3.12	Light Green
3%	*P. purpureum* cv. Mott	3.00	Light Green
	P. purpureum cv. GU	3.15	Light Green
6%	*P. purpureum* cv. Mott	3.00	Light Green
	P. purpureum cv. GU	3.09	Light Green

3.1.2. Silage Odor. Silage odor is one of the indicators of the good and poor quality of the silage produced. The results showed that *Pennisetum purpureum cv Mott* silage with sour odor and *P. purpureum* cv. GU silage with fresh sour odor in all treatments (Table 3). This showed that the produced silage had the silage typical odor. Overall results of silage with different varieties and different levels of pollard supplementation had a sour odor, so silage was good in physical quality. Good silage has an acidic (sour) odor [3].

Table 3. Odor characteristic score on *P. purpureum* silage supplemented with pollard

Pollard level	*Pennisetum p* variety	Average score	Odor
0%	*P. purpureum* cv. Mott	2.80	Sour
	P. purpureum cv. GU	1.36	Fresh sour
3%	*P. purpureum* cv. Mott	2.74	Sour
	P. purpureum cv. GU	1.32	Fresh sour
6%	*P. purpureum* cv. Mott	2.69	Sour
	P. purpureum cv. GU	1.21	Fresh sour

The availability of pollard as water-soluble carbohydrates as much as 3% and 6% were able to provide a substrate for Lactic Acid Bacteria (LAB) to produce lactic acid. The conversion process of dissolved carbohydrates to organic acids, especially lactic acid, runs optimally as supported by the 21-day silage pH range produced in this study (Table 14). The treatments with pollard supplementation with levels of 0%, 3%, and 6% can provide an acid odor to the physical quality of silage odor.

*3.1.3. Silage texture.*The texture is one of the indicators In the examination of silage physical quality. The dense texture of silage shows good quality silage [6]. The physical quality test results of the napier grass silage texture showed that the overall silage texture was dense and intact with clumps in *P. purpureum cv. Mott* at all levels of pollard supplementation silage (Table 4). The addition of pollard as much as 3% and 6% contributed to the silage texture characteristics in terms of silage texture score (Table 10), which helps to reduce water content during the silage fermentation process, this is related to the osmotic dehydration properties of pollard. The results [6] showed that silage supplemented with silage (rice bran, sweet potato flour, and pollard) had an intact, dense, and non-slippery texture.

Table 4. Colour characteristic score on *P. purpureum* silage supplemented with pollard

Pollard level	*P. purpureum* varieties	Average score	Texture
0%	*P. purpureum* cv. Mott	2.80	Solid, whole, slightly clumped
	P. purpureum cv. GU	1.22	Solid, intact, not clumping
3%	*P. purpureum* cv. Mott	2.72	Solid, whole, slightly clumped
	P. purpureum cv. GU	1.15	Solid, intact, not clumping
6%	*P. purpureum* cv. Mott	2.62	Solid, whole, slightly clumped
	P. purpureum cv. GU	1.11	Solid, intact, not clumping

3.1.4. Presence of silage fungi. No fungus was found on napier grass silage with pollard supplementation. Napier grass silage with pollard supplementation in different levels ranging from 0%, 3%, and 6% can prevent silage from fungal contamination. Overall, the silage produced in the study was in good condition and not grown by any fungi (Table 5). This was due to the effect of pH from silage (Table 6).[13] Silage with a low pH will produce products in the form of lactic acid from the dominant LAB and will inhibit other bacteria growth.

Table 5. The characteristic score of the presence of silage fungi in *P. purpureum* varieties supplemented by pollard

Pollard level	*P. purpureum* varieties	Score	Presence of fungi
0%	*P. purpureum* cv. Mott	1	No fungus
	P. purpureum cv. GU	1	No fungus
3%	*P. purpureum* cv. Mott	1	No fungus
	P. purpureum cv. GU	1	No fungus
6%	*P. purpureum* cv. Mott	1	No fungus
	P. purpureum cv. GU	1	No fungus

The growth of fungus on silage can be caused by incomplete compaction during the filling process of the silo, the compaction process is not perfect or because there is a silo leakage so contamination occurs. However, the overall silage produced in this study occurred in anaerobic conditions, therefore the silage was formed in good condition and was not grown by fungi. [14] stated that fungi will grow up when the anaerobic conditions in the silo are not achieved.

Chemical characteristics of silage

3.1.5. pH of silage. The results showed that the pH value of silage noticeably decreased with the addition of pollard. Pollard supplementation treatment at the level of 6% resulted in the lowest pH and showed a significant difference with supplementation treatment. Decreased silage pH was inversely proportional to the increase in pollard supplementation level (Table 6). Pollard functions as a water-soluble carbohydrates enhancer, thereby the presence of more water-soluble carbohydrates, the microbes get more substrate for their growth following [15] the production of lactic acid by LAB so that there is a decrease in pH in the silage.

Table 6. Average pH of 21 days of silage fermentation of *P. purpureum* varietiessupplemented by pollard.

P. purpureum varieties	Pollard level			Average
	0%	3%	6%	
P. purpureum cv. Mott	$4.74^{a} \pm 0.072^{a}$	$4.41^{b} \pm 0.100$	$4.33^{b} \pm 0.015$	$4.49^{x} \pm 0.061$
P. purpureum cv. GU	$4.44^{a} \pm 0.058$	$4.25^{b} \pm 0.021$	$4.13^{c} \pm 0.022$	$4.27^{y} \pm 0.033$
Average	$4.59^{a} \pm 0.06$	$4.33^{b} \pm 0.06$	$4.23^{b} \pm 0.017$	

^{abc} the different superscript in the same line showed significant different (P< 0.01)
^{xy} the different superscript in the same coloum showed significant different (P<0.01)

The occurrence of buffers from pollard and silage compositions contributed to the pH value of the silage. Buffer capacity is a measure of how much acid is needed for successful silage conservation. The high buffer capacity of material will require high amounts of acid as a conservation agent; and vice versa [16]. Similar results were reported by [17] obtained a pH of 4.5 in cornhusk and wild tamarind mixed silage (75: 25 ratio) and showed pH buffering activity by protein due to the additionof wild tamarind.

The low pH in the *P. purpureum* cv. GU was due to the low CP content and the high content of nitrogen-free extract (NFE) in *P. purpureum* cv. GU pre-silage (Table 1). It is known that ammonia is an indicator of the proportion of proteins that degrade duringthe ensilage process. Proteins are degraded by microbial enzyme activity and secondary fermentation occurs. The occurrence of secondary fermentation will affect lactic acid production and then will affect the silage pH. Furthermore, [18] also stated that the WSC content in napier grass can be estimated and viewed from the nitrogen-free extract (NFE)/100 g of total N [19].

3.1.6. Dry matter content of silage. The results performed that pollard supplementation did not affect the dry matter content (DM), but the forage variety treatment showed a significant effect on the DM content of napier grass silage. The content of lactic acid in silage was markedly increased following the increase in pollard supplementation level. Pollard supplementation treatment at the level of 6% resulted in the highest DM content compared to 0% and 3% treatments (Table 7).

Table 7. Average dry matter (DM) in silage of *P. purpureum* variety supplemented with pollard

P. purpureum Variety	Pollard			Average
	0%	3%	6%	
P. purpureum cv. Mott	10.34± 0.54	10.32± 0.52	10.85± 0.32	10.50± 0.46^{x}
P.purpureum cv. GU	12.81± 0.06	13.22± 0.03	13.68± 0.07	13.24± 0.53^{y}
Average ^{ns}	11.57±0.3	11.77±0.27	12.24±0.195	

^{xy} the different superscript in the same coloum showed significant different (P<0.01)
^{ns} non significant different

The accessibility of soluble carbohydrate sources made possible by pollard supplementation contributed to the high DM content of silage in the 6% pollard supplementation treatment. Because LAB colonies develop and create lactic acid, it is able to maintain an acidic climate and prevent harmful bacteria from degrading the DM in silage. [20] claimed that the breakdown process stopped and the silage became stable when the pH was acid because of the presence of lactic acid produced by LAB. According to various reports from [21], adding homofermentative LAB can enhance the loss of dry matter (DM). This could be because inoculated LAB uses nutrients more efficiently.

The loss of DM content in silage is caused by LAB related to the utilization of nutrients by LAB to produce lactic acid. However, the DM loss in the study was in the normal range (below 5%). [22] states that if the fermentation process proceeds normally, the loss or decrease will reach 3 – 5%.

3.1.7. Organic matter content in silage. The organic matter (OM) content of silage was not influenced by the level of pollard supplementation but was markedly influenced by the napier grass variety (Table 8). The OM content of the silage was not affected by the level of pollard supplementation. Pollard supplementation level showed no significant difference in the organic matter content of silage but showed a non-significant decrease in line with the decrease in supplementation level. Organic materials, especially from soluble substrates, will be used by inoculants and/or epiphytic LAB for its growth. Even though the silage was addedwith pollard as much as 6%, it seems to be insufficient for the ideal needs of epiphytic LAB inoculants against organic material sources in producing organic acids, especially lactic acid. [11] states that the loss of organic material in the producing silage mainly comes from the carbohydrate fraction, namely NFE with the main components of starch and sugar used by bacteria to produce organic acids, especially lactic acid.

Table 8. The average organic matter (OM) content in silage of *P. purpureum* varietiessupplemented with pollard

P. purpureum Variety	Pollard level			Average
	0%	3%	6%	
P. purpureum cv. Mott	75.12^{a}± 0.50	75.48^{a}± 0.95	72.50^{b}± 0.87	74.36^{x}± 0.773
P. purpureum cv. GU	81.14± 0.55	82.24± 0.07	83.92± 0.16	82.42^{y}± 0.260
Average ^{ns}	78.13±0.33	78.86±0.51	78.21±0,51	

^{ab} the different superscript in the same line showed significant different (P< 0.01)
^{xy} the different superscript in the same coloum showed significant different (P<0.01)
^{ns} non significant different

3.1.8. Crude protein content of silage. The results showed that the pollard supplementation and forage variety in making napier grass silage had a significant influence on the crude protein (CP) content of the silage. The CP content of silage increased following the increase in the pollard supplementation level and showed a significant difference between supplementation levels (Table 9). Pollard contributes high water-soluble carbohydrates which will be utilized to produce lactic acid by LAB. Higher LAB colonies and lactic acid production will suppress the growth of decaying bacteria that can damage or degrade chemical compositions such as proteins in silage. This was following the opinion of [23] which stated that the main principle of making silage are 1) stopping the respiration and evaporation of plant cells; 2) converting carbohydrates into lactic acid by LAB; 3) withstanding the activity of enzymes and rotting bacteria.

Table 9. Average crude protein (CP) content in silage of *P. purpureum* varietiessupplemented with pollard

P. purpureum Variety	Pollard level			Average
	0%	3%	6%	
P. purpureum cv. Mott	9.64± 0.13	9.56 ±0.25	9.98 ±0.19	9.72^{x}± 0.28
P. purpureum cv. GU	9.47^{a}± 0.03^{e}	9.61^{bc}± 1.02	10.09^{c}± 0.42	9.72^{y}± 0.49
Average	9.55^{h}± 0.08	9.58^{i}± 0.63	10.03^{j}± 0.302	

$^{abc, hij}$ the different superscript in the same line showed significant different (P< 0.01)
^{xy} the different superscript in the same coloum showed significant different (P<0.01)

Silage with the *P. purpureum cv. Mott* had the highest CP content compared to *P. purpureum* cv. GU (Table 9). The silage with the best CP content was the *P. purpureum cv. Mott* while the lowest CP content was the *P. purpureum* cv. GU. The study of [24] elaborated that the crude protein content in the pre-silage treatment had a significant effect on the crude protein yield of silage. Wild tamarind supplementation with the addition of levels of 10, 20, and 30% had a significant effect on the CP content of the silage. This was because wild tamarind has a fairly high protein content.

3.1.9. Crude fiber content of silage. The level of pollard supplementation and various napier grass kinds had a big impact on the amount ofcrude fibers (CF). The findings indicated that the CF content of napier grass silage was affected by interaction. Lower CF was created by pollard supplementation at the 6% level than at the 3% and 0% levels (Table 10). The decomposing bacteria's degradation of the fiber during the fermentation process resulted in a simpler crude fiber structure, which was the cause of the low CF at the 6% pollard supplementation level. This came after [25], which described how cellulose fibers were fermented before being polymerized into simpler polymers, which were then converted into sugars.

Table 10. Average crude fiber (%DM) content of *P. purpureum* varieties supplemented withpollard silage

P. purpureum varieties	Pollard level 0%	3%	6%	Average
P. purpureum cv. Mott	30.60^{a}± 0.04	28.81^{b}± 0.05	28. 21^{b}± 0.02	29.20^{x}± 0.03
P. purpureum cv. GU	32.06^{a}± 0.08	31.28^{ab}± 0.08	30.54^{b}± 0.06	31.29^{y}± 0.07
Average ^{ns}	31.33^{a}± 0.06	30.04^{b}± 0.06	29.37^{c}± 0.04	

^{abc} the different superscript in the same line showed significant different (P< 0.01)
^{xy} the different superscript in the same coloum showed significant different (P<0.01)

Napier grass variety had a significant effect on the CF content of the silage. *P. purpureum* cv. Mott noticeably produced the lowest CF compared to *P. purpureum* cv. GU (Table 10). The low CF content of silage in the treatment of *P. purpureum* cv. Mott compared to the other treatment variety because of CF content pre-silage in *P. purpureum* cv.Mott was lower compared to *P. purpureum* cv. GU(Table 1.)

3.1.10. Content of ether silage extract. The results of variance analysis showed that silage with different Napier grass varieties treatment and pollard supplementation levels had a significant effect on the ether extract (EE) content of the silage. The result showed that there was an interaction effect on the EE content of Napier grass silage. A higher pollard supplementation level provides more soluble carbohydrates available which will beused as substrates to form LAB colonies. Decreased EE of Napier grass silage was used by LAB during the ensilage process. [23] and [26] reported that the reduced content of unsaturated fatty acidsin silage is caused by the biohydrogenation process that occurs during ensilage.

Table 11. The average ether extract (% DM) content of *P. purpureum* varieties supplemented with pollard silage

P. purpureum Varieties	Pollard level 0%	3%	6%	Average
P. purpureum cv. Mott	1.84 ±0.34	2.43± 0.24	2.46± 0.33	1.63^{x}± 0.30
P. purpureum cv. GU	1.70^{a}± 0.23	2.82^{c}± 0.25	2.61^{b}± 0.25	2.37^{y}± 0.24
Average	1.77^{h}± 0.28	2.62^{i}± 0.24	2.53^{j}± 0.29	

$^{abc, hij}$ the different superscript in the same line showed significant different (P< 0.01)
^{xy} the different superscript in the same coloum showed significant different (P<0.01)

Napier grass varieties had a significant effect on the EE content of silage (Table 11). Organic matter from the *P. purpureum* cv. GU was higher than *P. purpureum cv. Mott.* Organic matter is the chemical composition of Napier grass that is used as a substrate by LAB toproduce lactic acid. High organic matter will produce a high LAB population, so the use of EE as one of the organic matter will be more utilized. Other findings [27-29] shows that the presence of lactic acid bacteria, whether due to addition or not, will cause a decrease in the content of unsaturated fatty acids in the silage.

4. Conclusions

The best treatment was found in *P. purpureum* gamma cv. GU with 6% pollard supplementation. The silage had good physical quality in colors that were close to silage material, sourodor, non-slimy texture, not overgrown with fungi, low pH, and CF, as well as DM, OM, and CP content of the silage.

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