

Enhancing Physical-chemical Quality and Palatability of King Grass (*Pennisetum Hybrid*) Silage Treated by Combination of Water Soluble Carbohydrate and Legume Sources

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Keywords: King grass, legumes, palatability, silage, WSC

Abstract: Silage is alternative technology is needed in the preservation of feed that is easily applied by farmers to needs forage in the dry season. The grass silage combined with WSC and legume are expected a solution in the provision of forages that can available throughout the year and improve silage quality. This study was conducted to examine the effect of combining the addition of various WSC and local legume sources on physical-chemical quality and palatability of king grass (*Pennisetum hybrid*) silage. The observed variables were physical-chemical quality (color, flavor, texture, fungal contamination, DM, OM, pH, and lactic acid), lactic acid bacteria population and palatability of silage. The experiment was arranged on a completely randomized of factorial design (4x5) with a treatment factor of king grass with addition of 5% WSC source (control, molasses, cassava flour, sweet potato flour) and addition of legume source (control, Cassava leaves, *Bauhinia* leaves, *Leucaena* leaves, *Gliricidia* leaves) and each treatment consisting of 3 replications. The results showed that the combination of water-soluble carbohydrate and legume source improved the color, flavor, and silage texture. The addition of legume source had significant effect ($P < 0.01$) on the decrease of pH silage followed by increasing lactic acid concentration. The combination of WSC and legume had significant effect ($P < 0.01$) on organic matter. The conclusion of this research is that the addition of WSC and legume source can improve the physical-chemical quality and can be used as a solution for the farmers in the provision of forage throughout the year.

1 INTRODUCTION

Currently, Indonesia's problem are decline in population and livestock productivity, scarcity of feed quality and quantity, and still weak farmers about technology. Low quality of feed and availability of fluctuating feed were resulted to low of cattle productivity. The one of forage was used by farmers is king grass. King grass is easy to grow in high and low land, and higher production than elephant grass (Budiman and Djamal, 1994). The availability of king grass is currently affected by the season. When the rainy season arrives, the availability of forage is very high while in the dry season the availability of forage is very low. So that, the alternative technology is needed in the preservation of feed that is easily applied by farmers for fulfill their requirement in the dry season is silage technology. According to Despal *et al.*

(2011), silage is anaerobic forage preservation with spontaneous work of lactic acid fermentation by epiphytic lactic acid bacteria (LAB). Lactic acid bacteria will convert water-soluble carbohydrates to organic acids especially lactate (Chen and Weinberg, 2009). The addition of water-soluble carbohydrate sources such as molasses is used in silage to increase dry matter content, lactic acid content, and reduce pH silage (Tjandraatmadja *et al.*, 1994; Bilal, 2009). Research conducted by Hidayat (2014), king grass (*Pennisetum purpureoides*) silage with the addition of molasses 1-3% can maintain the characteristics and nutrient content. Cassava and sweet potatoes are can be used as local feed ingredients because they contain highly fermentable carbohydrates that can be used as a source of water-soluble carbohydrates in silage process but, they are low in protein, while protein is needed in the silage process to increase the crude

protein content and increase the nutritional value of silage. Therefore, protein sources need to be added in silage process such as the addition of a legume source.

Tree legumes supplementation such as Lamtoro (*Leucaena leucocephala*), Gamal (*Gliricidia sepium*), Tayuman (*Bauhinia purpurea*), and Cassava leaves (*Manihot esculenta* Crantz) can be used in silage process based on availability and easy to find by farmers. According to Copani *et al.* (2014), the addition of legumes to improve the quality of grass silage with increased protein and WSC content. Research conducted by Halim (2000) shows that king grass given an additional of more than 30% legume can have a beneficial effect on king grass silage quality. Combination of grass with leguminous and water-soluble carbohydrate (WSC) on silage making has the potential to used as a silage that can improve the nutritional quality. This is supported by Jusoh *et al.* (2016), inclusion of molasses and legumes in silage grass can increase the nutritional value of silage, improve the quality of silage, can supply a good quality for animal feed, and lower the cost of concentrate on animal feed. Thus, the grass silage combined with WSC and leguminous sources is expected to be a solution for farmers in the provision of forages that can be available throughout the year. This study aims to examine the effect of combining the addition of water soluble carbohydrate sources and legume trees sources to physico-chemical quality and palatability of king grass silage.

2 MATERIALS AND METHODS

This research was conducted at the Laboratory of the Research Unit for Natural Product Technology (BPTBA), the Indonesian Institute of Sciences (LIPI) in Yogyakarta and Food and Nutrition Laboratory, Faculty of Agricultural Technology, Gadjah Mada University in September 2017 until January 2018.

2.1 The Procedure of Making Silage

The procedure of making silage consisted of 3 stages was preparing raw materials, mixing, packing and incubation. King grass (*Pennisetum hybrid*) was harvested at 60 days, which was planted in the forage collection field of the Research Unit for Natural Product Technology (BPTBA), the Indonesian Institute of Sciences (LIPI) in Yogyakarta. King grass was chopped by chopper in 1-3 cm. Sample of legume like cassava leaves, *Leucaena* leaves, *Bauhinia* leaves, and *Gliricidia* leaves was wilted for 24 hours in room temperature. Cassava and sweet potatoes

peeled and then cut and put into 55 °C oven to dry. After dried, cassavas and sweet potatoes are ground into flour.

King grass was thoroughly mixed with either 5% water soluble carbohydrate sources and 5% tree legume sources. After the ingredients mixed homogeneously, the mixture silage put into a 1.5 liter capacity of jar and silage incubated for 21 days at room temperature. Anaerobic condition was made by removing free air with manually pushed and strengthfully tied by a transparent tape. After 21 days of fermentation, the jars were opened and quality of silages were assessed.

2.2 Physical Assessment of Silage

The physical characteristics of the silage include texture, color, flavor, and fungal contamination. The level of silage flavor was measured by the method of assessment has been done by Sofyan *et al.* (2017) : off-flavor (score 0), less fragrant (score 1), medium fragrant (score 2), and heavy fragrant (score 3). Observations level of fungal contamination in silage was conducted by observing at the presence of mold. The percentage of fungal contamination on the surface area by categories : no contamination (0%), mild (<5%), medium (5-15%), and severe (> 15%).

2.3 Chemical Assessment of Silage

The chemical characteristics of the silage include dry matter, organic matter, pH and lactic acid concentration. Dry matter (DM) and organic matter (OM) according to the procedure of AOAC (2005). Measurement of acidity degree (pH) by using a pH meter. The concentration of lactic acid was determined by acid titration method (AOAC, 2005). Sample of silage was taken 10 g from each treatment, added by sterile aquadest (90 ml) then stirred until homogeneously. The supernatant of samples was taken (10 ml) to measure pH and lactic acid concentration. The concentration of lactic acid as followed the equation :

$$\%LA = \frac{(V_{ts} - V_{to}) \times N \times MW \times Df}{V_s \times 1000} \times 100\%$$

Note :

- LA = concentration of crude lactic acid
- V_{ts} = volume of sample titrant (ml)
- V_{to} = volume of blank titrant (ml)
- N = normality of titrant (NaOH = 0,097 N)
- MW = molecular weight of lactic acid = 90 (g/mol)
- Df = dilution factor = 10x
- V_s = volume of sample (ml)

2.4 Microbiological Assessment of Silage

The number of colonies of lactic acid bacteria was calculated by the Total Plate Count (TPC) method. Sample of silage was taken 10 g from each treatment, added by sterile aquadest (90 ml) then stirred until homogeneously in aseptic condition. The supernatant of samples was taken 1 ml and homogenized in aseptic conditions. Supernatant was grown on selective media MRSA with serial dilution at 10^2 , 10^3 , 10^4 , 10^5 , and 10^6 in which incubated at 37 °C for 24 hours and 48 hours.

2.5 Palatability Test of Silage

Palatability test of silage using previous method by Sofyan *et al.* (2017). Amount 1000 g of silage which was harvested, was taken and given to cows that are in the farms. Each sample of the silage given to the cow is randomized sample position to minimize the bias in the sample sequence. Silages that were consumed more were considered to be more palatable.

2.6 Data Analysis

Data analysis for physical characteristics of silage were analyzed descriptively. Data of chemical characteristic such as pH, lactic acid concentration, lactic acid bacteria population and palatability were analyzed by ANOVA (analysis of variance), if among treatments showed significant difference followed with Duncan test performed by the CoSTAT statistical software (Cohort, 2008)

3 RESULT AND DISCUSSION

3.1 Silage Quality

The physical qualities of the silage include color, aroma, texture, and fungal contamination are shown in Table 1. King Grass silage on the combination of 5% C + LL and 5% MS + 5% LL showed a yellowish green, while others showed a brownish green. This is due to the lack of compaction on silos so that there is still air. According Reksohadiprodjo (1988), the color changes that occur in the ensilage process caused by aerobic respiration process that lasts for oxygen supply is still there until the sugar contained in the plant runs out. Sugar will oxidize to CO₂ and water and heat up until the temperature rises. The fragrant test showed the treated silage 5% C + GL and 5% MS

+ 5% GL had a slightly fragrant compared to other treatments. Heavy fragrances showed the treated silage combination 5% CF + 5% CL and 5% CF + 5% LL. Contrastly, other study (Sudarman *et al.*, 2016), the combination of cassava leaves with addition of 5% cassava flour (tapioca flour) showed mild sour flavor on silage. Gallaher and Pitman (2000) reported that good silage has a characteristic yellowish green up to brownish green color, depending upon silage material and has pleasant, sour, and sweet smell (Kaiser *et al.*, 2004). Silage texture showed soft texture except for 5% C + CL and 5% CF + 5% LL silage combination showed fresh texture. Most numerous fungal contamination in silage are treatment combinations 5% MS + 5% BL and 5% CF + 5% BL than other treatments. This may be due to presence of air in the silo that causes silage contaminated by fungi. Silage treated by the 5% LL and 5% GL not found fungal contamination. In this study, generally fungal contamination on the surface of the silo. According to Johnson *et al.* (1998), the level of silage damage below 5% can still be classified as a good silage.

Table 1: Physical characteristic of king grass silage with combination of water soluble carbohydrate and legumes

WSC ¹	Variables	Legumes ²				
		C	CL	BL	LL	GL
C	Color	Brownish green	Brownish green	Brownish green	Yellowish green	Brownish green
	Flavor	Fragrant	Fragrant	Fragrant	Fragrant	Slightly fragrant
	Texture	Soft	Fresh	Soft	Soft	Soft
	LFC	Slightly ++	Slightly +	Slightly +	Not found	Not found
MS	Color	Brownish green	Brownish green	Yellowish green	Yellowish green	Brownish green
	Flavor	Fragrant	Fragrant	Fragrant	Fragrant	Slightly fragrant
	Texture	Soft	Soft	Soft	Soft	Soft
	LFC	Slightly +	Slightly +	Medium +++	Slightly ++	Not found
CF	Color	Brownish green	Brownish green	Brownish green	Brownish green	Brownish green
	Flavor	Fragrant	Heavily fragrant	Fragrant	Heavily fragrant	Fragrant
	Texture	Soft	Soft	Soft	Fresh	Soft
	LFC	Slightly ++	Slightly ++	Medium +++	Not found	Not found
SPF	Color	Brownish green	Brownish green	Brownish green	Brownish green	Brownish green
	Flavor	Fragrant	Fragrant	Fragrant	Fragrant	Fragrant
	Texture	Soft	Soft	Soft	Soft	Soft
	LFC	Not found	Not found	Slightly ++	Not found	Slightly ++

LFC: Level of Fungal Contamination

¹WSC Sources: C (Control); MS (Molasses); CF (Cassava flour); SPF (Sweet potato flour).

²Legume Sources : C (Control); CL (Cassava leaves; *Manihot esculenta* Crantz); BL (*Bauhinia purpurea* leaves); LL (*Leucaena leucocephala* leaves); GL (*Gliricidia sepium* leaves)



Figure 1. Colour of silages with combination of WSC and legume sources after 21 days ensilage

Table 2. Chemical composition of silages treated by WSC and legume sources after 21 days ensilage

WSC ¹	Legumes ²					Average
	C	CL	BL	LL	GL	
----- Dry Matter (%) -----						
C	88.29	89.61	88.62	89.99	88.94	89.09
MS	89.95	89.98	89.25	90.03	89.42	89.72
CF	89.73	90.02	89.85	89.20	89.06	89.57
SPF	89.63	89.48	89.68	88.98	89.36	89.43
Average	89.40	89.77	89.35	89.55	89.19	
----- Organic Matter (%) -----						
C	84.17ef	84.76cdef	84.43def	85.67bc	84.05f	84.62c
MS	84.19ef	84.02f	84.41def	85.56bc	84.72cdef	84.58c
CF	85.33bcd	87.03a	84.89cdef	86.13bc	85.68bc	85.81a
SPF	84.96cdef	85.60bc	85.08cde	85.24bcd	84.85cdef	85.15b
Average	84.66b	85.35a	84.70b	85.65a	84.82b	

Different superscript in same column or row showed a significant difference (P<0.01)

¹WSC Sources: C (Control); MS (Molasses); CF (Cassava flour); SPF (Sweet potato flour).

²Legume Sources : C (Control); CL (Cassava leaves; *Manihot esculenta*); BL (*Bauhinia purpurea* leaves); LL (*Leucaena leucocephala* leaves); GL (*Gliricidia sepium* leaves)

Chemical characteristics of silage include dry matter, organic matter, pH value and lactic acid concentration, whereas biological characteristics of silage ie population of lactic acid bacteria are shown in Table 3. Silage added by WSC and legume sources was not significant difference (P>0.01) with dry matter content. This shows the king grass silage with the addition of WSC and legumes had no effect on dry matter content. Addition of WSC and legume sources had significant effect (P<0.01) on organic matter content. Addition of 5% CF higher organic matter content than other WSC sources. The content of theorganic matter is high with the addition of 5% CF probably due to the high ash content in the sample. Addition of 5% CL and 5% LL higher organic matter

content than other legume sources. The highest content of organic matter in the treatment of silage combination 5% CF + 5% CL.

The addition of legume sources significantly (P<0.01) on the pH and lactic acid concentration. The pH value of king grass silage without legume (control), silage given 5% BL and 5% LL was lower than silage given 5% CL and 5% GL. The addition of legume sources increases the pH value compared to without the addition of legume. According to Heinritz *et al.* (2012) that the pH of the leguminous plant silage after a 92 day fermentation ranged from 4.3 to 6.3. Even if legumes present higher buffering power in relation to grasses, tending to maintain the pH at higher values (McDonald *et al.*, 1991). The highest pH value occurred in the silage combination 5% CF + 5% GL compared to other treatments. The pH of silage in this study was within the normal range of McDonald *et al.* (1991), good quality silage has a pH <4.2. For Kung and Shaver (2001), the pH of legume silages with 30% DM and perennial temperate grass silage ranges from 4.3 to 4.7.

Decreasing pH silage value is followed by increasing the concentration of lactic acid produced. Decreased pH is accelerated due to the increasing number of lactic acid produced by lactic acid bacteria. According to Henderson (1993), the acidity of the silage is very important in the success of making silage will prevent forage from spoilage by microbial spoilage. Total lactic acid in silage with addition of legume source gave a very significantly (P<0.01). The highest concentration of lactic acid in the combination treatment of 5% MS + C and 5% SPF + C. It is caused by molasses and sweet potato flour to be a source of water-soluble carbohydrates which is a substrate source for LAB. In accordance with the opinion of Sofyan *et al.* (2017), increased lactic acid indicated that soluble carbohydrates may support microbial growth. Other study reported that concentration of lactic acid in king grass silage with addition of WSC source (rice bran) resulted in lactic acid was 7.59-13.40% (Sofyan *et al.*, 2017) and Li *et al.* (2014) observed that king grass silage with addition of WSC sources (molasses, sucrose, and glucose) resulted in lactic acid was 4.36-4.56%. Compared those studies, concentration of lactic acid in this study lower than other studies. Increased lactic acid followed by a decrease in pH and an increase in the population of lactic acid bacteria. Ridwan *et al.* (2005), the content of lactic acid in silage will affect the amount of LAB and the degree of acidity.

The population of lactic acid bacteria was calculated after 21 days ensilage. The addition of water soluble carbohydrate and legume sources to the

king grass silage did not significantly affect the population of lactic acid bacteria. Increased LAB population occurs from 24-hour incubation to 48-hour incubation. The addition of WSC and legume sources has a higher LAB population than controls. According to Ridwan *et al.* (2005) that the activity of lactic acid bacteria is strongly influenced by the availability of WSC sources in plants because WSC is a substrate source for bacteria growth. In the legume itself the source of WSC is low so that in addition to the source of legume will decrease the population of LAB and low activity that cause additive does not give positive effect to activity of lactic acid bacteria.

Table 3. pH, crude lactic acid, and lactic acid population of silage with combination of WSC and legume sources

WSC ¹	Legumes ²					Average
	C	CL	BL	LL	GL	
-----pH (%)-----						
C	3.81b	4.15b	3.93b	3.89b	3.85b	3.92ab
MS	3.64b	4.66ab	3.81b	3.74b	3.94b	3.96ab
CF	3.69b	3.89b	4.05b	3.80b	3.92b	3.87b
SPF	3.74b	4.09b	3.88b	3.97b	4.77a	4.09a
Average	3.72c	4.20a	3.92bc	3.85c	4.12ab	
-----Crude lactic acid (%)-----						
C	2.34ab	1.79bc	1.71bc	2.13abc	2.01bc	1.99a
MS	2.93a	0.82d	2.41ab	2.45ab	1.88bc	2.10a
CF	2.25ab	2.16abc	1.65bc	2.47ab	2.40ab	2.19a
SPF	2.90a	1.72def	2.19abc	1.92bc	1.38cd	2.02a
Average	2.60a	1.62d	1.99bc	2.24b	1.92c	
-----Lactic acid bacteria population 24 hours (Log CFU ml ⁻¹)-----						
C	6.68	6.86	6.94	6.52	6.31	6.66a
MS	6.62	6.74	6.47	6.49	7.13	6.69a
CF	6.67	6.38	6.29	7.10	7.02	6.69a
SPF	7.09	6.42	6.77	7.20	7.07	6.91a
Average	6.76a	6.60a	6.62a	6.83a	6.88a	
-----Lactic acid bacteria population 48 hours (Log CFU ml ⁻¹)-----						
C	6.94	6.96	6.95	6.74	6.62	6.84a
MS	6.72	6.93	6.53	6.73	7.20	6.82a
CF	6.79	6.78	6.52	7.11	7.06	6.85a
SPF	7.12	6.60	6.86	7.21	7.16	6.99a
Average	6.89a	6.82a	6.71a	6.95a	7.01a	

Different superscript in same column or row showed a significant difference (P<0.01)

¹WSC Sources: C (Control); MS (Molasses); CF (Cassava flour); SPF (Sweet potato flour).

²Legume Sources : C (Control); CL (Cassava leaves; *Manihot esculenta* Crantz); BL (*Bauhinia purpurea* leaves); LL (*Leucaena leucocephala* leaves); GL (*Gliricidia sepium* leaves)

3.2 Palatability of Silage

Palatability is an illustration of the attraction of livestock and stimulation of smell, taste and texture to consume the feed. According to Scharenberg *et al.* (2007), feed palatability involves feedstuffs characteristics that stimulate sensorial acceptance by olfactory, gustatory, and tactile stimuli in animal. Palatability test silage to cattle is shown in Table 4. King grass silage treated with addition of WSC

significantly (P<0.05) on palatability level. Influenced without addition of WSC source (control) was not different from the addition of 5% MS but higher consumption than the addition of 5% SPF and 5% CF. It shows that without the addition of WSC (control) and 5% MS has a high level of palatability. The addition of WSC and legume sources improved the physical quality by improving silage flavor. In accordance with the physical parameters in Table 1. The silage with the addition of 5% MS has a sweet smell and flavor making it was more palatable. According Keady (1996) that used molasses as silage additive improve silage preservation and silage dry matter intake.

Table 4. Palatability of silage treated by combination of WCS and legume sources (%)

WSC ¹	Legumes ²					Average
	C	CL	BL	LL	GL	
C	33.24	40.87	38.02	39.32	29.59	36.21a
MS	36.67	33.87	22.50	36.59	17.82	29.49ab
CF	17.28	8.23	23.41	17.99	20.47	17.48c
SPF	19.10	26.26	11.40	16.41	25.30	19.69bc
Average	26.57a	27.31a	23.83a	27.58a	23.30a	

Different superscript in same column or row showed a significant difference (P<0.05)

¹WSC Sources: C (Control); MS (Molasses); CF (Cassava flour); SPF (Sweet potato flour).

²Legume Sources : C (Control); CL (Cassava leaves; *Manihot esculenta* Crantz); BL (*Bauhinia purpurea* leaves); LL (*Leucaena leucocephala* leaves); GL (*Gliricidia sepium* leaf)

4 CONCLUSIONS

The addition of a water-soluble carbohydrate and legume sources improve the physical characteristics of king grass silage. Therefore, adding water soluble carbohydrate can be practiced by the farmers to enhance the quality of feed based on king grass silage. Further study is needed conduct to evaluate fermentability of silage.

ACKNOWLEDGEMENTS

The authors would like gratefully appreciate to the Biofeed Additive Research Group, Research Unit for Natural Product Technology (BPTBA), the Indonesian Institute of Sciences (LIPI) for supporting the experiment.

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