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Technical Note: Silo Type for Laboratory Scale Experiment on the Silage Quality

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ABSTRACT

The present study was aimed to investigate the effects of silo type for laboratory scale on chemical compositions, fermentation characteristics, and microbial counts of silage. Four typical silos use on a laboratory scale, consisting of transparent plastic bags (Silo A), black plastic bags (Silo B), transparent plastic bags covered with a bucket (Silo C), and transparent plastic bags covered with a sack (Silo D). All silo types were used to ensilage 5 kg Elephant grass (*Pennisetum purpureum* cv. Mott) for 21 d. Each Silo was conducted in triplicate. After ensiling, Silo C had higher crude protein with lower ammonia compared to other Silos ($p<0.05$). In addition, the Silo C resulted in lower pH, butyrate, and yeast with higher lactate and lactic acid bacteria ($p<0.05$) compared to other Silos. Silo C had the lowest bulginess, which indicated the optimum ensiling process. The present study concluded that ensiling forage with Silo C is more suitable and recommended for laboratory scale, which can reduce the errors, especially in the nutrient loss, production of ammonia, lactate, and butyrate, and also the counts of microbes in the silage.

Keywords: Elephant grass, Fermentation, Silo type, Silage

Introduction

The quality of silage could be influenced by several factors consisting of density, anaerobic conditions, chemical compositions, and water-soluble carbohydrates (McDonald *et al.*, 1991). Density directly affects the fermentation quality, which can help reduce nutrient loss during ensiling. Silage with high density has a lower dry matter (DM) loss and a temperature change during ensiling (Borreani *et al.*, 2018; Kung *et al.*, 2018). The increase of temperature during ensiling indicates respiration, which results in nutrient loss (McDonald *et al.*, 1991; Borreani *et al.*, 2018; Kang *et al.*, 2018). In addition, the production silage in industry has a high consideration with density and silo type. The silo must be able to maintain the density of silage during ensilage. For example, the bale silo must add a round net before wrapping plastic. Without a round net, the silage has a low density and then bulges due to CO_2 during respiration. For the same reason, the addition of ballast is necessary for the appearance of a bunker silo. At the level of farmers, the silage is ensiled in aerobic conditions, commonly using a plastic bag. The use of plastic bags increases the failure of ensilage since the silo cannot maintain its density. Moreover, the transparent plastic bag as a silo could increase the potential growth of photosynthetic microorganisms that decrease the quality of silage. The organic acid profiles of silage consisting of lactate, acetate, propionate, and butyrate indicate the success of ensilage, which also influences the physical appearance of silage (Muck *et al.*, 2018; Kung *et al.*, 2018; Paradhipta *et al.*, 2021).

Generally, the research on silage in the laboratory is conducted on a small scale around 3-6 kg (Rafiauddin *et al.,* 2018; Kim *et al.*, 2018; Paradhipta *et al.*, 2021). The silo commonly uses a plastic bag to maintain a small amount of forage. However, on a small scale, the density of silage is not considered during ensiling, especially if using a plastic bag as a silo. Moreover, the use of transparent plastic bags in small-scale production may increase the growth of fungi. Without any high consideration of silage density, the result of the laboratory research scale will not be applicable to the industry scale. Therefore, the present study, as the technical note, was purposed to recommend the silo types for small-scale experiments considering the density of silage. The present study uses a high moisture forage to highlight the effect of silo type. According to Paradhipta *et al.* (2019), the high moisture forage could increase butyrate concentration. In addition, high moisture content during fermentation could increase the gas production in the early phase of fermentation,

which has a direct effect on the density of plastic bag silo. The present study aimed to investigate the effects of silo types on a laboratory scale on the chemical compositions, fermentation characteristics, and microbial counts of elephant grass silage.

Materials and Methods

Silage production

Dwarf elephant grass (*Pennisetum purpureum* cv. Mott) was harvested at 55 d after regrowth, containing moisture around 77.3%. The forage was chopped to a length of about 3-5 cm. The chopped forage was sub-sampled at 500 g for chemical composition analysis. The chopped forage was ensiled into a 5 kg mini-silo for 21 d with four different types of mini silos, the following: Silo A, transparent plastic bag silo; Silo B, black plastic bag silo; Silo C, transparent plastic bag covered with a bucket; and Silo 4, transparent plastic bag covered with a sack. All treatments were vacuumed and ensiled for quadruplication. After 21 d, each silage was sub-sampled at 500 g for chemical composition analyses and 20 g for silage extraction. Sillage extraction was used to analyze fermentation characteristics and microbial counts.

Chemical compositions

The sub-sampled forage and silage were placed into a dry oven (Memmert UN55, Germany) at 55°C for 72 h. The dried samples were ground to pass a 1-mm screen using a Wiley mill (Thomas Wiley Cutting Mill, Model 4, USA). The concentration of dry matter (DM) was measured by drying 1 g of the sample in a dry oven (Memmert UN55, Germany) at 105°C for 24 h (method number 934.01; AOAC, 2005). The organic matter (OM) concentration was analyzed using a muffle furnace (Advantec KM-420, Japan) at 550ºC for 5 h (method number 942.05; AOAC, 2005). The crude protein (CP) was determined by the Soxhlet method (method number 920.39; AOAC, 2005), respectively. The neutral detergent fiber (NDF) (method number 2002.04; AOAC, 2005) and acid detergent fiber analyzed (ADF) (method number 973.13; AOAC, 2005) were determined using ANKOM 200 fiber analyzer (Ankom Technology, Macedon, NY, USA).

Fermentation characteristics

A total of 20 g of sub-sampled silage was blended with 180 mL of ultra-pure distilled water for 30 sec to make silage extraction. This silage extraction was used to analyze pH and microbial counts. The pH was measured using a pH meter (Ohaus AB23PH-F, China). The concentration of ammonia-N was determined following the procedure of Chaney and Marbach (1962). Meanwhile, the lactate concentration was determined according to the procedure of Barker and Summerson (1941) using a UV-VIS spectrophotometer. The concentrations of volatile fatty acid (VFA) profiles consisting of acetate, propionate, and butyrate were determined using gas chromatography (GC 8A, Shimadzu Crop., Japan) according to the procedure of Filipek and Dvorak (2009). The data of ammonia-N, lactate, acetate, propionate, and butyrate were expressed as % of DM (%, DM).

Microbiological counts

Silage extraction was continued into several dilution series consisting of 10^{-2} to 10^{-8}. Several mediums were prepared to analyze the enumeration of LAB, yeast, mold, and clostridia. The LAB was cultivated on de Man, Rogosa, and Sharpe agar (MRS; Sigma Aldrich, USA). Yeast and mold were grown on Potato Dextrose Agar (PDA; Sigma Aldrich, USA. All plates of MRS agar were incubated anaerobically at 30°C for 48 d, while all plates of PDA were incubated aerobically at 30°C for 48 d according to previous studies (Lee *et al.*, 2019; Paradhipta *et al.*, 2021). Colony counts were presented as colony forming unit (cfu) per gram of silage and then transformed to Log10.

Silo bulginess

On the 21st day of ensiling, before opening the silo, silage was observed in the density appearances. A total of 20 non-expert respondents observed the bulging condition of the silo. The observation condition consisted of not bulge, slightly bulge, bulge, and highly bulge. The bulginess data would be used to support the Principal Component Analysis (PCA).

Statistical analysis

The research was conducted based on a completely randomized design (CRD) with four treatments and three treatment replications. Data were analyzed using one-way analysis of variance (ANOVA) with SPSS IBM 20; if there was a significant difference between treatments ($p<0.05$), continued with Duncan's test with a significance level of 5%. Interrelationships among various variables of silage quality were investigated using PCA. The silage variables comprised bulginess, organic acids, pH, ammonia, and microbe profiles. The direction and length of the vector are interpreted as the relationship among silage variables. The PCA was performed by R software using a Facto Mine R Package.

Results and Discussion

Chemical compositions

Nutrient concentrations of DM, OM, CP, NDF, and ADF from elephant grass before ensiling were 18.7%, 81.8%, 10.2%, 52.2%, and 32.4%, respectively (Table 1). After ensiling, concentrations of DM, OM, NDF, and ADF were not affected by silo types (Table 2). However, silo type affected CP concentration. Silo C resulted in a higher CP concentration than Silo A, Silo B, and Silo D ($p= 0.022$; 8.93% vs. 8.33%, 8.37%, and 8.32%).

Table 1. Chemical compositions of Elephant grass before ensiling (%, DM)

Chemical compositions	
Dry matter	18.7
Organic matter	81.8
Crude protein	10.2
Neutral detergent fiber	52.2
Acid detergent fiber	32.4

Fermentation characteristics

The use of Silo C had a lower pH (p= 0.003; 5.38 vs. 5.47, 5.54, and 5.52), ammonia concentration (P= 0.003; 0.12% vs. 0.16%, 0.17%, and 0.18%), and butyrate concentration (p= 0.001; 0.21% vs. 0.36%, 0.38%, and 0.36%), but a higher lactate concentration (p= 0.001; 1.35% vs. 1.08%, 1.03%, and 1.08%) than Silo A, Silo B, and Silo D (Table 3). Acetate concentration was not affected by silo types. Propionate concentration was not detected in the present study. In addition, lactate to acetate ratio also was not affected by silo types.

Microbial count

The use of Silo C resulted in a higher LAB count (p= 0.001; 7.06 Log10 CFU/g vs. 6.74, 6.70, and 6.52 Log10 CFU/g) and a lower yeast count (p= 0.010; 6.62 Log10 CFU/g vs. 6.85, 6.83, and 6.82 Log10 CFU/g) than Silo A, Silo B, and Silo D (Table 4). The molds were not detected in all silage. The count of mold might be lower than 3.00 Log10 CFU/g.

Silo bulginess

Based on appearance, the majority of respondents were observed slightly bugle in Silo A and Silo B, with the presentations being 75% of the respondents, respectively. In Silo C, 65% of total respondents were observed not bugle. In Silo D, 60% of total respondents were observed bulge. The interrelationship between variables was analyzed using PCA (Figure 1). The result of the first component (Dim 1) was 70.75% of the total variation. In Dim 1, there was a high positive value (>0.6) for pH, ammonia, butyrate, bulging (bulginess), and yeast. In addition, lactate and LAB had a negative value (>-0.6).

According to previous studies, concentrations of DM, OM, CP, NDF, and ADF from dwarf elephant grass (*Pennisetum purpureum* cv Mott.) in the present study were similar to previous studies (Sanjaya *et al.*, 2022 and Wodebo *et al.,* 2023). In some cases, the nutrient content of elephant grass might be varied. The differences in nutrient content from forage could be affected by various factors, including the age of harvest and soil nutrients (Umami *et al.*, 2023).

In the present study, Silo C had the highest CP concentration after ensiling, which could be a recommendation for in laboratory scale. Application of Silo C could decrease protein loss during ensiling in the present study. The Silo C was covered by a mini bucket that helped to maintain

Table 2. Effects of silo types on chemical compositions of elephant grass silage (%, DM)

Chemical compositions	Treatments[1]				SEM	p-value
	Silo A	Silo B	Silo C	Silo D		
Dry matter	15.6	15.70	15.31	15.78	1.173	0.962
Organic matter	78.95	78.42	79.03	78.43	0.634	0.518
Crude protein	8.33[b]	8.37[b]	8.93[a]	8.32[b]	0.147	0.002
NDF	57.0	56.07	58.56	55.5	1.738	0.225
ADF	34.0	33.18	35.15	33.71	0.730	0.056

SEM: standard error of mean; NDF: neutral detergent fiber; ADF: acid detergent fiber.
[1]Silo A: transparent plastic bag silo; Silo B: black plastic bag silo; Silo C: transparent plastic bag covered with a bucket; Silo 4: transparent plastic bag covered with a sack.
[a,b]Different superscripts show significant results (p<0.05).

Table 3. Effects of silo types on fermentation characteristics of elephant grass silage

Item	Treatments[1]				SEM	p-value
	Silo A	Silo B	Silo C	Silo D		
pH	5.47[a]	5.54[a]	5.38[b]	5.52[a]	0.038	0.003
Ammonia, % DM	0.16[a]	0.17[a]	0.12[b]	0.18[a]	0.014	0.003
Lactate, % DM	1.08[b]	1.03[b]	1.35[a]	1.08[b]	0.058	0.001
Acetate, % DM	0.43	0.41	0.43	0.39	0.038	0.510
Propionate, % DM	nd	nd	nd	nd	na	na
Butyrate, % DM	0.36[b]	0.38[b]	0.21[a]	0.36[b]	0.020	0.001
Lactate: Acetate	2.50	2.55	3.15	2.76	0.320	0.127

SEM: standard error of mean; NDF: neutral detergent fiber; ADF: acid detergent fiber.
[1]Silo A: transparent plastic bag silo; Silo B: black plastic bag silo; Silo C: transparent plastic bag covered with a bucket; Silo 4: transparent plastic bag covered with a sack.
[a,b]Different superscripts show significant results (p<0.05).

Table 4. Effects of silo types on microbial counts of elephant grass silage (Log10 CFU/g)

Item	Treatments[1]				SEM	p-value
	Silo A	Silo B	Silo C	Silo D		
LAB	6.74[b]	6.70[b]	7.06[a]	6.52[b]	0.100	0.001
Yeast	6.85[a]	6.83[a]	6.62[b]	6.82[a]	0.070	0.010
Molds	nd	nd	nd	nd	nd	nd

SEM: standard error of mean; NDF: neutral detergent fiber; ADF: acid detergent fiber.
[1]Silo A: transparent plastic bag silo; Silo B: black plastic bag silo; Silo C: transparent plastic bag covered with a bucket; Silo 4: transparent plastic bag covered with a sack.
[a,b]Different superscripts show significant results (p<0.05).

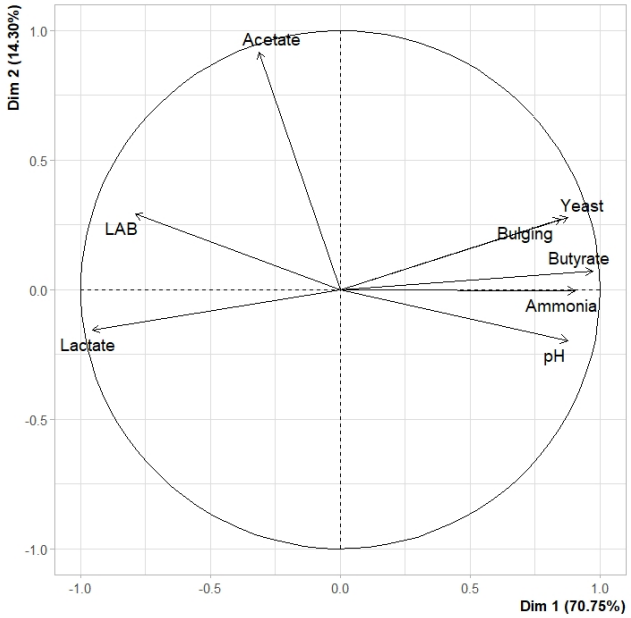

Figure 1. Principal component biplots of silage variables in the present study. Dim 1 is mean of the first principal component, and Dim 2 is mean of the second principal component.

the density during ensiling. On the other side, the transparent and non-transparent plastic had no effects on the chemical compositions of silage. Packing density is a key factor during the ensiling process to get good silage quality.

Sun *et al.* (2021) found that packing density had a positive correlation with silage quality. Packing density could improve silage quality by maintaining the anaerobic condition of the ensiling process. In the early stage of the ensiling process, a silo with low packing density will have high residual oxygen that leads to an increment of undesired microorganisms and an increase in nutrient loss, which might be a protein in this case of the present study—the result of high CP concentration. A high concentration of CP is also supported by a low concentration of ammonia (Table 4). Ammonia during ensiling indicated protein loss (McDonald *et al.*, 1991; Paradhipta *et al.*, 2019).

The present study used a high moisture forage to evaluate the effects of silo type in the reduction of fermentation quality. Generally, Silo C had the best fermentation quality in the present study. This could be indicated by the low production of butyrate and ammonia-N. The presence of butyrate indicates a clostridial fermentation, especially in high moisture silage (McDonald *et al.*, 1991; Paradhipta *et al.*, 2019; Kung *et al.*, 2018). The lactate production was also the highest in Silo C, which supported the lowest pH condition. Kung *et al.* (2018) and Wahyudi *et al.* (2019) mention that lactate is the most vital organic acid in the silage and the main contributor to the pH decline during fermentation. The high production of lactate was supported by high LAB count and low yeast count in Silo C. During the fermentation process, the microbial population will change according to the condition in a silo. Silo that has good anaerobic conditions can support the growth of LAB and suppress the growth of undesirable microbes (yeast and molds). The LAB produces lactate acid that makes a silage pH low and preserves well. On the other hand, undesirable microbes can convert lactate to butyrate, leading to high silage pH, and some of them have proteolytic activity that reduces silage protein to produce ammonia. Sun *et al.* (2021) mention that low silo packing density contributed to a high yeast population during the ensiling process. This also supported the result of the present study that the bulging of the silo had a positive correlation with yeast, butyrate, and ammonia (Figure 1). In addition, bulging had a negative correlation with LAB and lactate. High bulginess could increase the production of butyrate and ammonia and also stimulate the growth of yeast due to much aerobic space on the inside of the silo. On the contrary, low bulginess could lead to good ensiling conditions by increasing lactate and growth of LAB. Chotimah *et al.* (2024) also reported that clostridia and yeast growths positively correlate with ammonia-N production. In addition, previous studies (Santos *et al.*, 2014; Zhang *et al.*, 2018; Kung *et al.*, 2018) found that yeast mostly becomes the initiator of aerobic spoilage by degrading lactic acid, leading to pH increment and allowing other bacteria to grow (e.g., *Clostridia* organism). Clostridia can convert water-soluble sugar and lactic acid into butyric acid and break down protein into ammonia (McDonald *et al.*, 1991).

Conclusion

The type of silo could affect CP, pH, ammonia, and butyrate of silage ensiled in the laboratory scale. The present study concluded that Silo C is suitable and recommended for making

silage on a laboratory scale. The Silo C can maintain the density, which reflects the condition in the industry/field and reduce an error during experimenting. It could be seen that Silo C can reduce the errors in nutrient loss and the fermentation characteristics, such as lactate, organic acids, and microbial counts.

Conflict of interest

No potential conflict of interest relevant to this article was reported. All authors have agreed with the contents of the manuscript.

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Author's contribution

The authors confirm their contribution to the paper as follows: study conception and design: DHVP, KTH, PCS; data collection: DHVP, KTH, PCS; analysis and interpretation of results: DHVP, KTH, PCS, NF; draft manuscript preparation: DHVP, NF, AA, YHJ.

Ethics approval

This article does not involve animal subjects, so ethical approval for animal studies is not necessary in the present study.

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